

**Conservation ecology and human disturbance
of Australian sea lions (*Neophoca cinerea*)
in Western Australia**

Sylvia Karin Osterrieder, Dipl.-Biol.

College of Engineering and Science, Victoria University
in association with
Centre for Marine Science and Technology (CMST),
Curtin University

Submitted in fulfillment of the requirements of the degree
of Doctor of Philosophy

September 2016

Abstract

Effective conservation and management are paramount to long-term recovery of endangered species. Achieving recovery relies on knowledge of their behaviour and habitats. *Neophoca cinerea* (Australian sea lion) is an endangered species endemic to Australia. This study focused on three key questions aimed at improving its management near Perth (Western Australia): testing a sea lion identification tool, describing haul-out patterns, and identifying human disturbance sources. *N. cinerea* whisker spot patterns as a tool for individual identification were tested using Chamfer distance-transform. Patterns contained sufficient information to reliably (99%) identify individuals in populations of 50, matching 90% correctly when testing known captive animals photographed at 90°. Off-angle photographs resulted in 48% correct matches. Resighting in the wild proved unfeasible in this study. However, resights of four scarred *N. cinerea* at Carnac and Seal Islands (the main study sites) confirmed returns and visitations to both islands. To describe haul-out patterns, generalized additive models were applied to hourly counts between 0800h-1600h. *N. cinerea* numbers followed 17-18 month cycles, inversely aligned with the breeding cycle. During non-breeding seasons, hauled-out numbers increased throughout the day, and were associated with air temperature and tide. Research investigating human disturbance indicated that all human activity types elicited responses, which varied between islands depending upon stimulus types (vessel types, people), ranges to stimuli, and activities at each islands. People at close range elicited most elevated responses, including aggression and retreating. This occurred mainly at Carnac Island since direct beach access is allowed. Significant rates of lower-level disturbances were also elicited by vessels within close proximity, including paddlers and those undertaking noisy activities. Resulting recommendations include further restriction and enforcement in approach distances allowed. Also, ongoing monitoring of abundance and behaviour is required for long-term trend estimation. Because of high variability in haul-out behaviour, surveys undertaken at comparable times are recommended.

Student declaration

“I, Sylvia Karin Osterrieder, declare that the PhD thesis by Publication entitled ‘Conservation ecology and human disturbance of Australian sea lions (*Neophoca cinerea*) in Western Australia’ is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work”.

Signature:



Date: 26 September 2016

Details of publications

List of peer-reviewed publications resulting from this thesis:

Osterrieder S. K., Salgado Kent C., Anderson C. J. R., Parnum I. M., Robinson R. W. (2015). Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (*Neophoca cinerea*). *Journal of Mammalogy*, 96(5), 988–997.

Osterrieder S. K., Salgado Kent C., Robinson R. W. (2015). Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia. *Endangered Species Research*, 28, 259-274.

Osterrieder S. K., Salgado Kent, C., Robinson, R. W. (2016). Responses of Australian sea lions, *Neophoca cinerea*, to anthropogenic activities in the Perth metropolitan area, Western Australia. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Osterrieder S. K., Parnum I. M., Salgado Kent C., Robinson R. W. (2016). Difficulties identifying Australian sea lions (*Neophoca cinerea*) in the wild using whisker spot patterns. *Australian Mammalogy*.

Table of Contents

Title	i
Abstract	ii
Student declaration	iii
Details of publications	iv
Table of Contents	v
List of Figures	x
List of Tables	xix
Acknowledgments	xxvi
Chapter 1 Introduction	1
1.1 Conservation Ecology and Management	2
1.2 Anthropogenic impacts	4
1.3 Pinnipeds	7
1.4 Pinniped population estimates	10
1.4.1 Pup counts	10
1.4.2 Adult male counts	10
1.5 Threats to <i>N. cinerea</i>	13
1.5.1 Human disturbance in <i>N. cinerea</i>	14
1.6 Structure of thesis	15
Chapter 2 Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (<i>Neophoca cinerea</i>)	17
2.1 Abstract	20
2.2 Keywords	20
2.3 Introduction	21
2.4 Methods	22

2.4.1	Study areas and collection of photographs	22
2.4.2	Preparation of photographs for reliability testing and matching	26
2.4.3	Variability of whisker spot patterns in <i>N. cinerea</i>	29
2.4.4	Pattern recognition using Chamfer distance transform	30
2.4.5	Software settings and pairwise matching	31
2.5	Results	32
2.5.1	Variability of whisker spot patterns in <i>N. cinerea</i>	32
2.5.2	Pattern recognition algorithm and application	34
2.6	Discussion	36
2.6.1	Variability of whisker spot patterns in <i>N. cinerea</i>	36
2.6.2	Pattern recognition algorithm and application	37
2.6.3	Application and recommendations	38
2.7	Acknowledgments	39

**Chapter 3 Difficulties identifying Australian sea lions (*Neophoca cinerea*)
in the wild using whisker spot patterns** **40**

3.1	Abstract	43
3.2	Keywords	43
3.3	Introduction	44
3.4	Material and Methods	46
3.4.1	Study sites	46
3.4.2	Collection and selection of photographs	47
3.4.3	Application of the Chamfer distance transform method for identification	48
3.4.3.1	Preparation of photographs for matching using the Chamfer distance transform method	48
3.4.3.2	Pairwise matching of photographs method	49
3.4.3.3	Thresholds to identify matching pairs of photographs using the Chamfer distance transform method	49
3.4.3.4	Variability between photographs of the same indi- vidual using the Chamfer distance transform method	50
3.4.3.5	Manual verification of photographs to identify cor- rect matches	50
3.4.4	Identification using row-column locations	51
3.4.4.1	Processing of photographs, analysing occurrence of whisker spots	51
3.4.5	Observer variability	53
3.4.6	Analysing uncertainties of marking whisker spots	53

3.5	Results	55
3.5.1	Composition of whisker spot patterns	55
3.5.2	Application of the Chamfer distance transform method for identification	55
3.5.2.1	Identifying similarity thresholds for matches	56
3.5.2.2	Variability between photographs of the same indi- vidual	57
3.5.2.3	Manual verification	58
3.5.2.4	Variability between photographs of wet and dry individuals	64
3.5.3	Identification using row-column locations	65
3.5.4	Observer variability	68
3.5.5	RGB levels of fur and whisker spots	72
3.6	Discussion	72
3.7	Acknowledgments	78

Chapter 4 Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia **79**

4.1	Abstract	82
4.2	Keywords	82
4.3	Introduction	83
4.4	Methods	85
4.4.1	Study sites	85
4.4.2	Data collection	87
4.4.2.1	<i>N. cinerea</i> counts	87
4.4.2.2	Environmental data	89
4.4.3	Statistical analysis	90
4.4.3.1	Modelling	90
4.4.3.2	Data exploration and model validation	91
4.5	Results	93
4.5.1	Peak periods	98
4.5.2	Age classes	99
4.6	Discussion	103
4.7	Acknowledgements	109

Chapter 5 Responses of Australian sea lions, <i>Neophoca cinerea</i>, to anthropogenic activities in the Perth metropolitan area, Western Australia	110
5.1 Abstract	113
5.2 Keywords	114
5.3 Introduction	115
5.4 Methods	118
5.4.1 Study sites	118
5.4.2 Experimental design	119
5.4.2.1 Counts of vessels, ‘People’ and <i>N. cinerea</i>	122
5.4.2.2 Behavioural responses to human activities	122
5.4.3 Analytical approach	128
5.4.3.1 Number of vessels and ‘People’	128
5.4.3.2 Behavioural responses to anthropogenic activities	129
5.4.3.3 Response distances	130
5.5 Results	130
5.5.1 Numbers of vessels, ‘People’ and <i>N. cinerea</i>	130
5.5.2 Number of responses	134
5.5.3 Response distances	138
5.5.4 Response behaviours	142
5.6 Discussion	146
5.6.1 Suggestions for management	152
5.7 Acknowledgments	154
 Chapter 6 Discussion	 155
 Bibliography	 166
 Appendix A	 196
Chapter 2 publication	196
Chapter 3 publication	207
Chapter 4 publication	224
Chapter 5 publication	241
 Appendix B	 264
Chapter 4 tables	264
 Appendix C	 267
Chapter 5 figures	267

Appendix D	270
Chapter 6 table and figure	270

List of Figures

Figure 1.1	Historic and present distribution of <i>N. cinerea</i> (original from Ling (1992); adaptation by Campbell (2003)).	8
Figure 1.2	<i>N. cinerea</i> breeding and haul-out sites. Temporally asynchronous timing of breeding is indicated in positive or negative months (in brackets) posterior or prior in relation to North Fisherman Island, Western Australia (reference at 0 months). High concentration of colonies in sections A and B, not named here (from Gales <i>et al.</i> (1994)).	9
Figure 2.1	Locations of islands where photographs of <i>N. cinerea</i> in the wild were obtained.	24
Figure 2.2	Adapted software interface to build a library and match whisker patterns using Chamfer distance transform. Whisker spots in the image are marked with black circles and reference points with white circles. The matching scores with other marked photographs are displayed on the left.	27
Figure 2.3	Example of marked cells where whiskers are present on grids overlaid over the muzzles of six captive individual <i>N. cinerea</i> . Black cells are where whisker spots are present and empty cells where spots are absent. The coordinate [0,0] is the position of the inner corner of the eye, and [1,0] the reference point on the nostril.	27
Figure 2.4	Flow chart presenting the entire process of testing the method of using whisker spot patterns for individual <i>N. cinerea</i> identification.	28
Figure 2.5	Pairwise probabilities of cells having whiskers present a) within columns (cells above and below each other) and b) within rows (cells right and left of each other). Pairwise probabilities of cells having whiskers absent c) within columns and d) within rows.	33

Figure 2.7	Percentage of reliable whisker spot patterns estimated from 50 repeated simulations for a population of 50, 100, 500, and 1,000 individuals, with SD (whiskers).	33
Figure 2.6	a) Frequency of occurrence and information content of whisker spots in grid cells after the removal of dependant cells, b) without removal of dependent cells, based on 53 individual <i>N. cinerea</i> , visually illustrating to the reader the locations on the muzzle where dependent cells were predominantly present.	34
Figure 2.8	Box and whisker plots of averaged similarity scores of “matches” and “non-matches” of whisker spots of 16 individual captive <i>N. cinerea</i> for a) 90° angle, b) 70°, 90°, and 110° angles, c) 1- and 2-m distance at 90° angle, and d) 10, 30, 60, 180, and 360 days from the first session at a 90° angle. “Matches” include comparisons of different photographs of the same individuals, whereas “non-matches” are comparisons of photographs from an individual to those from all other individuals. The median is displayed as a black line, 25 th and 75 th percentiles as vertical boxes, and 90 th percentiles as range bars, and outliers as black crosses.	35
Figure 2.9	Probability of false positive matches based on the similarity scores of whisker spot patterns of 16 captive <i>N. cinerea</i> at a 90° angle ($n = 90$).	36
Figure 3.1	General whisker spot sketch based on 20 individual wild <i>N. cinerea</i> , ordered from row A (RA) to G (RG) and columns 1 (C1) to 10 (C10). Black spots indicate those present in >80% of photographs, grey spots indicate those in 30-80% of photographs. Rows A to G are illustrated by red lines and Columns 1 to 10 by light blue lines. Whiskers may also occur above the eye.	52
Figure 3.2	Box-whisker plots of the distance scores of confirmed ‘matches’ (same individual, number of scores = 332) and confirmed ‘non-matches’ (different individuals, number of scores = 1080) of whisker spot patterns photographed on the same day. The median is shown as a black line, 25 th and 75 th percentiles as vertical boxes, 90 th percentiles as range bars, and outliers as black crosses.	56

Figure 3.3	Probability of confirmed matches and confirmed non-matches based on the distance scores of whisker spot patterns calculated using the Chamfer distance transform algorithm. Distance scores of pattern comparisons are based on multiple photographs of an individual taken on a single day and of known different individuals on the same day (n = 135 photographs of 66 individual sea lions).	57
Figure 3.4	Distance scores of whisker spot patterns between pairs of photographs of the same individuals taken within 70 s of each other. 10 scores beyond 70 s were excluded from the figure for display purposes (four data points around 150-250 s, four data points around 800 s, and two data points around 1400 s).	58
Figure 3.5	Standardised whisker spot patterns of individuals A – F, index numbering the good photographs available for each individual. Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Example of individual A (bold) pairwise matched with all individuals in the final library. B – F are individuals, each returning at least one spot pattern matched with a distance score ≤ 0.02 (numbers in parentheses show distance scores to A ₁ , the lowest score resulted for the combination of individuals). Panels without distance scores show spot patterns of other photographs available to aid the matching process. Individuals C and E are unconfirmed matches and individuals B, D, and F confirmed non-matches with individual A (based on age classes). Only spots between reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth 0.5,0.5] are shown.	60
Figure 3.6	‘Unconfirmed’ matches of A ₁ (top photograph) with C ₂ (middle photograph) and E ₁ (bottom photograph) after visual inspection of all potential matches (distance score ≤ 0.02) in the library (Figure 3.4). Standardised spot patterns were plotted on top of each other to aid visual comparison of A ₁ and C ₂ (top grid) and A ₁ and E ₁ (bottom grid), including spots beyond the reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5].	61

- Figure 3.7** Example of a confirmed match of two photographs of the same individual taken 1 s apart with a distance score of 0.0182 (top, ID1₁ and bottom, ID1₂, photographs). The standardised marked cells of ID1₁ and ID1₂ indicate where whiskers are present on grids overlaid over the muzzle (top right). Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Only spots between the reference points are displayed. The bottom right grid shows the standardised spot patterns of ID1₁ and ID1₂ plotted on top of each other, including spots beyond the reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5]. 62
- Figure 3.8** Example of a confirmed non-match of two photographs of different individuals with a distance score of 0.0188 (top ID1₂ and bottom ID2₁ photographs). The standardised marked cells of ID1₂ and ID2₁ indicate where whiskers are present on grids overlaid over the muzzle (top right). Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Only spots between the reference points are shown. The bottom right grid shows the standardised spot patterns of ID1₂ and ID2₁ plotted on top of each other, including spots beyond reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5]. 63
- Figure 3.9** Box-whisker plots of the distance scores of ‘Non-matches’ (confirmed rejections) and ‘Unconfirmed’ (no confirmed resightings or rejections) of whisker spot patterns with distance scores of ≤ 0.02 . The median is shown as a black line, 25th and 75th percentiles as vertical boxes, and 90th percentiles as range bars, and outliers as black crosses. 64
- Figure 3.10** Box-whisker plots of the distance scores of ≤ 0.02 between whisker spot patterns of wet and dry individuals and their combinations, which resulted in ‘Unconfirmed’ (no confirmed resightings or rejections) and ‘Non-matches’ (confirmed rejections). The median is shown as a black line, 25th and 75th percentiles as vertical boxes, 90th percentiles as range bars, and outliers as black crosses. Numbers on top of each box show the number of distance scores for each category. 65

Figure 3.11	Top and middle panels: a) number of whisker spots' presence based on 20 wild individuals ('x' indicates locations in which whiskers are absent), and b) uncertainty of whisker spots' presence (white indicates 0 uncertainty, and 'x' indicates locations in which whiskers were absent with 0 uncertainty). The bottom row (Eye) represents the number of whiskers occurring above the eye. Bottom panel: c) uncertainty of a whisker spot's presence Frequency of mystacial whisker spots occurring (e.g. uncertainty of 10 means that in 10 individuals it was uncertain if a whisker spot was present) as a function of mystacial whisker spots occurring (e.g. an occurrence of 15 means that 15 individuals had this whisker spot) plotted for 20 individuals (excluding superciliary whiskers).	67
Figure 3.12	Number of observers (n = 6) that marked whisker spots in rows A to G in columns 1 to 10; where 10 whisker spot patterns (IDs 1 to 10) were marked by the first author and five observers experienced in marine science, and the numbers of selected whisker spot locations summed (i.e. 1: one of the observers marked a whisker spot at this location, 6: all of the observers marked a whisker spot at this location). Note that panels 4 and 9 were patterns from two different photographs of the same individual.	69
Figure 3.13	a) Average (dot), and standard deviation (lower and upper error bars) of whisker spots marked by six observers on 10 different photographs (IDs 4 and 9 are different photographs from the same individual). b) Box-whisker plot of distance scores for selected whisker spot patterns by six different observers of the same photographs (IDs 1 to 10). 'Total' shows the distribution of distance scores from all pairs (IDs 1 to 10). IDs 4 and 9 are photographs of the same individual, taken on the same day, and '4:9' shows the distribution of distance scores from each observer matching the two photographs of the same individual.	70
Figure 4.1	Haul-out sites of <i>N. cinerea</i> in the Perth metropolitan area.	86
Figure 4.2	Maximum number of <i>N. cinerea</i> observed on Carnac (○) and Seal Islands (●) during 166 survey days between June 2012 and April 2014, including the exceptionally high and low observations (△) on Carnac Island. Dashed lines mark the survey periods (survey intervals 1 to 8).	95

Figure 4.3	Number of <i>N. cinerea</i> observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represent counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) was added as visual aid. (The two exceptionally high and low observation days on Carnac Island were excluded.)	96
Figure 4.4	Smoothing function (solid line) with 95% confidence intervals (dashed lines) fitted to air temperature (°C) estimated in the final generalised additive model (GAM) model to show the influence of air temperature on the number of <i>N. cinerea</i> hauling out. The small ticks above the x-axis represent air temperature values of the observations.	97
Figure 4.5	Relationship between number of <i>N. cinerea</i> and tide level (m) observed on Seal and Carnac Islands. A LOESS smoother (blue line) with 95% confidence intervals (grey) was added to aid visual interpretation.	98
Figure 4.6	Number of sub-adult male <i>N. cinerea</i> observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represent counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The two exceptionally high and low observation days on Carnac Island were excluded.)	100
Figure 4.7	Number of adult male <i>N. cinerea</i> observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represent counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The two exceptionally high and low observation days on Carnac Island were excluded.)	101

Figure 4.8	Age classes of <i>N. cinerea</i> observed at time of maximum count on 166 days on Carnac and Seal between September 2012 and April 2014 (after the first non-breeding season, i.e. high numbers in Perth). Top: Ratio of sub-adults to adults on Carnac (black crosses) and Seal (diamonds) Islands. The dashed horizontal line marks the ratio of sub-adults to adults at 1:1. Three data points are missing due to zero sub-adults or adults present and no ratio could be calculated. Bottom: Number of <i>N. cinerea</i> observed in each age classes (adults: grey triangles; sub-adults: black, solid circles; juveniles: white-filled circles; unknown: black asterisks) (bottom graph).	102
Figure 5.1	Seal Island and Carnac Island, largest <i>N. cinerea</i> haul-out sites in the Perth metropolitan area, Western Australia.	119
Figure 5.2	a) Sampling frequency with number of counts conducted per sampling day (Carnac Island is demarcated in black, Seal Island in blue, and dashed line at end of November 2013 indicates the start of the collection of disturbance data). b) Sampling method used throughout the sampling period displayed in Figure 2a (black stripes = remote, including Seal Island observations from the vantage point in Shoalwater using the telescope or spotting scope, and Carnac Island observations with the remote controlled, live camera). c) Maximum number of vessels (○) and <i>N. cinerea</i> (+) observed on Carnac Island (black) and Seal Island (blue) each day during 166 survey days between June 2012 and April 2014.	121
Figure 5.3	Percentage of groups of vessels observed visiting Carnac Island and Seal Island. Percentages are of the total at each island rather than the total combined at both islands. Values on top of each bar display the number of times each vessel type was observed (with Carnac Island having 134 h, and Seal Island 142 h of sampling effort between November 2013 and end April 2014). . . .	133

Figure 5.4	a) Duration (h) and b) minimum approach distance (m) of ‘People’ and vessels staying in the vicinity of Carnac Island and Seal Island. Values next to each bar display sample size of recorded approach and departure times for groups of vessels and ‘People’ observed (with Carnac Island having 134 h and Seal Island having 142 h sampling effort between November 2013 and end April 2014). * = significant differences between the islands.	135
Figure 5.5	Percentage of groups for the different stimulus types (different vessels and ‘People’) that elicited one or more responses from one or more <i>N. cinerea</i> (with Carnac Island having 134 h and Seal Island having 142 h observation effort between November 2013 and end April 2014). Values on top of each bar display the sample size of groups of vessels or ‘People’	137
Figure 5.6	Number of <i>N. cinerea</i> responses occurring at 5 m binned distances at Carnac Island (n = 280) and Seal Island (n = 202), displayed on log 10 transformed axis. Loess smoothers for Carnac Island (black) and Seal Island (gray) with 95% confidence intervals were added to aid visual interpretation.	139
Figure 5.7	Number of <i>N. cinerea</i> responses elicited by groups of ‘People’ and vessels at Carnac Island and Seal Island, in 5 m bins and truncated at 100 m.	140
Figure 5.8	Frequency of a variety of <i>N. cinerea</i> responses elicited by ‘People’s’ activities on board, in the water and on the beach at Carnac Island and Seal Island, in 5 min bins and truncated at 100 m.	141
Figure 5.9	Number of <i>N. cinerea</i> responses per hour of sampling elicited by a) ‘People’ and b) vessels at Carnac Island and Seal Island (excluding ‘Aircrafts’). Numbers above each bar indicate the total number for each behaviour observed (Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).	143
Figure 5.10	Number of <i>N. cinerea</i> responses elicited per hour as a result of groups of ‘People’ undertaking different activities at Carnac Island and Seal Island (Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).	144

Figure 5.11	Number of <i>N. cinerea</i> responses per hour of sampling elicited by different vessel types at Carnac Island and Seal Island (excluding ‘Aircrafts’; Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).	145
Figure A1	The interquartile ranges in the numbers of <i>N. cinerea</i> responses per hour per group for each vessel type and ‘People’ (with Carnac Island having 134 h and Seal Island having 142 h observation effort between November 2013 and end April 2014). The horizontal dotted line corresponds with 10 responses per hour. . . .	268
Figure A2	Number of <i>N. cinerea</i> responses per hour of sampling elicited by vessels at Carnac and Seal Island (including ‘Aircrafts’), divided into vessel activities and ‘People’ activities onboard. . . .	269
Figure A3	Photographs of resighted, scarred individuals (1-4 in Table A3) including the location and date of sighting.	273

List of Tables

Table 2.1	Number of individuals and number of photographs taken of the right muzzle of captive <i>N. cinerea</i> on different days throughout 1 year.	22
Table 2.2	Number of field days and photographs, which were taken of the right side of wild <i>N. cinerea</i> muzzles on various islands in Western Australia.	23
Table 2.3	The probability (P) of a spot pattern occurring once at most, calculated as: $(1 - P)^M + MP(1 - P)^{M-1}$ and the corresponding information content (I) for a range of population sizes (M).	30
Table 2.4	Sample sizes of photographs from 16 captive <i>N. cinerea</i> used for testing matches for photographs taken: 1) during the same day at 90°; 2) during the same day at 70°, 90°, and 110°; 3) during the same day at 1- and 2-m distances; and 4) during different sessions at 90°.	32
Table 3.1	Number of whiskers marked by six different observers on 10 photographs (IDs 1 to 10): range (min - max), mode, mean (\pm SD) as well as the number of whiskers marked above the eye, and additional whiskers marked outside of the row/column arrangement. Characteristics for each <i>N. cinerea</i> are given as the fur and whisker spot colour as well as wet or dry condition of the fur and any other notable feature (and whisker colours generally light to dark brown unless stated otherwise). Note that IDs 4 and 9 were two photographs of the same individual.	71
Table 5.1	Definition of stimulus categories.	123
Table 5.2	List of categories of activities associated with vessels ordered from highest to lowest anticipated impact.	124

Table 5.3	List of categories used for recording anthropogenic activities in the order of the highest to lowest anticipated impact levels (if different activities were performed at the same time, the highest activity was recorded). Abbreviated activity names used in text and figures are marked in bold.	125
Table 5.4	Definitions of response types of <i>N. cinerea</i> responses to vessel and human activities, in order from highest to lowest level anticipated responses (if different responses occurred in combination with each other, the most severe was recorded).	127
Table 5.5	Results of Chi ² and Kruskal-Wallis tests comparing the number of groups of vessels of different types and ‘People’ visiting, the duration of visits, and the minimum approach distances to <i>N. cinerea</i> at Carnac and Seal Islands. Numbers in bold represent significant values, type of test added as + Chi ² test or * Kruskal-Wallis rank sum test, and X ² or KW-X ² , respectively, in brackets following the <i>p</i> -value. (Sample size for ‘Large vessels’ were too small for calculations and not included.)	132
Table 5.6	Results of Chi ² and Fisher’s exact tests comparing the number of <i>N. cinerea</i> responses elicited by groups of vessels and ‘People’ at Carnac and Seal Islands. Numbers in bold represent significant values.	136
Table A1	Results of the final GAM determining effects of temporal and environmental factors on <i>N. cinerea</i> haul-out numbers.	265
Table A2	Proportion of juvenile, sub-adult, adult and unknown <i>Neophoca cinerea</i> hauling out on Seal and Carnac Islands in Period 1 to 8 (n = sample size of field days on the particular island and season).	266
Table A3	Resighted individuals based on their scarring, including the date and location as well as the number of sightings and age of the individual <i>N. cinerea</i> . Location in brackets after the date: C – Carnac Island, S – Seal Island.	271

Acknowledgments

This PhD would have not been possible to undertake and complete without the support and help of my family, friends, supervisors, the collaboration with the Department of Parks and Wildlife (DPaW) and so many volunteers :-)!

First of all, an enormous thank you to my main supervisors Dr Chandra Salgado Kent and Dr Randall Robinson for taking me on as one of your students, at more or less the last minute! I am very grateful that you didn't even hesitate to take me on, even though I am not even in the same field as you, Randall; and Chandra, I wasn't even in the same state. Without both of you, I would have never persevered to start a new project, let alone to complete this thesis. Thank you, Randall, for letting me move to Perth, for withstanding all the head-butting that came with that decision, and thanks for actually driving over with me too, making it a very nice and fun trip. I would have not been able to extend my thesis without your support either, and there were several more hurdles to clear to keep this PhD going.

Chandra, I loved the project we came up with and the fact that the information is useful and actually important beyond this study. While the sea lions know how to make it so difficult to study them, they are a fascinating bunch and do choose to stay in amazing places that I was able to visit. I am so grateful to you for letting me go on all those various field trips! You have taught me so much over the past years, shared your knowledge, advised me how to conduct a project and to deal with the difficulties coming with it. You always supported me in anything I wanted to try and never stopped believing in me or losing your patience, even when I didn't have any. There is no way I could list all the amazing things you did for this study, often in your free time and I am truly thankful for all of it! I am very lucky having such an amazing and knowledgeable supervisor!

Thanks for agreeing to be on board, Prof Dr Bernd Würsig. It was always great to know that I can ask you pretty much anything when I needed or wanted another opinion, since you just know everything. You are a great mentor, and I will never forget (hopefully none) what you have taught me!

Thanks also to Carol Scarpaci for enabling me to receive the IPRS scholarship, without which I would have not moved to Australia in first place and to Dr Patrick-Jean Guay, who stepped in when the first project went pear-shaped, and who I am also thankful to for help with some funding applications.

Dr Iain Parnum, you taught me my words in Matlab, writing some neat, magical codes to save me fighting through a combination of PhotoShop and PowerPoint. Thanks for all the discussions we had about various aspects of the project and also continuing on the Groth algorithm.

The two years of field work would have not been possible without the immense help of so many volunteers/friends. I am still amazed and so thankful for all the time and effort you have invested in this project. I would not have had a smooth field work season without your endurance throughout long days, often either super cold, hot or wet, and literally tonnes of sea lion data to collect. In alphabetical order: Ángel Garcia Fernandez , Anthony Cole, Benjamin Moon, Cara Bourne, Carrie Skorcz, Cathryn O’Sullivan, Charlotte Barclay, Claire Taylor, Damien Morales, Eloisa Wainright, Estenio Paiva, Florian Mayer, Holly Raudino, Jacklyn Buchanan, Jaime Hernandez, Jana Kuroпка, Jessica Leask, Jom Acebes, Kelly Campbell, Klaus Lucke, Laura Debry, Maria Garcia, Marta Galindo Romero, Matt Long, Matt Koessler, Matt Rohner, Meagan Cundy, Megan Burke, Michael Bittle, Mick Todd, Miles Parsons, Mirco Bortolozzo, Natalie Prinz, Nick Riddoch, Nicole De Campe, Nicole Fiasco, Olga Titova, Reilly Evetts, Rhianne Ward, Roanna Goater Samuel Montague, Sarah Marley, Shyam Madhusudhana, Steffi Balzalesto, Terence Quah and Tina Berry. Thank you!! There is no way of repaying the favour, but I hope I can help in your projects you will be doing soon yourself.

Special thanks for your committed field work over extended periods, and entering and double checking a huge amount of data, especially towards the end of this study: Damien Morales, Laura Debry, Kelly Campbell, Matt Long and Natalie Prinz. Thanks for going through photographs and marking whisker spots as well: Leila Fouda, Matt Long, Montse Landero, Nick Riddoch, and Sarah Marley. Mick

Todd, thank you for lending me your telescope. Sarah, thanks so much for setting up the blog, and updating blogs and writing such nice posts :-).

This project was conducted in cooperation with DPaW, many of whose staff have put in a huge effort to make this study possible. The marine park coordinators and rangers provided transportation to the islands whenever it was possible, even when it meant rising earlier and putting in extra hours. I truly appreciate all your effort. Thank you for allowing me to use the remotely controlled camera in your office; it was fun to share the experience, and also the frustrations with failing technology. Apart from marine park coordinators and rangers, several others from DPaW have been involved in this project in various ways. Whether it was the organisation of field work or conferences, loan of a spotting scope, discussions on sea lions, providing long-term datasets or developing data sheets for tablets, everything was always made easy, motivating and it's been great fun to work with every one of you! I have had amazing field trips, and in fact, would have not been able to conduct this project without your help, and I hope there will be more field work days and projects to come. Thank you Adrian Pinder, Alison Sampey, Anthony Desmond, Chris Phillips, David Holley, Erin Clitheroe, Florian Meyer, Dr Holly Raudino, John Edwards, Jon Pridham, Dr Kelly Waples, Kevin Crane, Dr Kim Friedman, Matt Dasey, Melissa Evans, Miecha Bradshaw, Murray Banks, Pam Sutton, Paul Jennings, Peter Collins (thanks heaps for everything Peter!!), Robin Jordan, Rosalba Lingwood, Dr Richard Campbell, Sean Emmett, Sophia Nolan, Stephen Goodlich and Vaughn Chapple.

All staff and students from CMST made this a much more fun journey. A huge thank you for letting me use all your facilities, the dinghy, the ute and all the equipment I needed, even though I was enrolled at another university. I loved being part of CMST, and Dr Christine Erbe, you are just fabulous. Thank you for having me print my first official photo booklet, they made quite few people happy. Ann Smith, thanks for sorting out all the receipts so quickly, dealing with a heap of paperwork on all sorts of things, and always being there when needed. Thank you Malcolm Perry for teaching me how to back-up the trailer at the boat ramp. The first few attempts were hilarious! Thanks for sorting out the boat and making sure we were always safe out there. It was great to share the PhD experience and have all the valuable discussions with other post-graduate students. I also enjoyed the various nice coffee and lunch breaks with Angela Recalde Salas, Arti Verma, Bec Wellard, Jamie McWilliam, Leila Fouda, Marta Galindo Romero, Matt Kössler, Michael

Caley, Montse Landero, Sarah Marley, Shyam Madhusudhana, Sue Mason, Sven Gestauer and Tristan Lippert. It was great fun to share an office with you, Michael Bittle, whom has made me remember to always keep Nerf guns close by at any time of day or night and increased my swearing vocabulary by 1000%. I will have to make sure I am in few more mornings to hear your lovely morning greeting :-D.

It was a pleasure to be part of the Applied Ecology Research Group at VU and to learn about and join various other projects, and have you as good friends. Thanks for keeping me part of the group and keeping in touch. You also always provided me with all the information I needed for all sorts of procedures at VU. It was always nice to come back and see everyone again: Dr Alice Taysom, Annett Finger, Dr Debbie Reynolds, Kirby Smith, Dr Mark Scarr, Mary Skram (Cowling), Dr Megan O'Shea, Dr Nazim Uddin, Dr Randall Robinson, and Richard Stafford-Bell.

To the staff and of course the participating sea lions at Adelaide Zoo: Steve McKee, Jason Hakof, and David Mattner; at Pet Porpoise Pool in Coffs Harbour: Aaron Tolley; at SEALIFE (previously UnderWater World) in Mooloolaba: Mark Ryan, Katie Miers, and Rebecca Weston; and at Taronga Zoo in Sydney: Elly Neumann, Elle Bombonato, and Ady D'Ettore. Thanks for the incredible amount of photographs you were providing, covering all different settings I asked for. Without your patience, training the sea lions, and taking all the photographs, we would have not been able to conduct a part of this study. It was so nice to also receive e-mails about the sea lions undertakings; it was definitely a motivational kick to keep me going marking spot after spot. I hope I can thank you in person one day, and of course the sea lions too. Thank you Matthew Gogoll and Natasha for picking us up from Carnac Island when it was not possible for anyone else. You provided an epic trip back to shore; we were never as quickly on our way out there than with you, it was great fun! I also appreciated the transportation to Carnac Island with the Cockburn Volunteer Sea Search and Rescue started their day earlier and finished later to be able to help us out. Thanks to the Fremantle Sailing Club for providing access to their boat ramp.

Wild Encounters Rockingham has always been very open to the project and thanks for letting people know about the project as well as the blog, and keep counting sea lions on each of your trip. Your counts are invaluable.

Your data set is amazing, Francis Merchant. Thank you for sharing your data as well as your experiences you have had with the sea lions. Thanks Sabine for continuing the counts, the data will definitely be helpful and fill in the gaps when the weather is not too pleasant to be in the field.

This project would have been so much harder without the support of so many friends around me, knowing that I can count on your help made all the difference. Thanks so much for helping me with the coding in Matlab Michael Bittle and Shyam Madhusudhana; and fixing up all my funny codes in R Sven Gastauer. It would have taken me many days longer to sort through all the data by hand, or use a combination of R, copy-paste and Excel (and do it all over again when one number changes). I am glad I could make you laugh with my way of writing codes ;-). Thanks Mike for attempting to teach me using Matlab properly so many times, at least now I know how some of it is supposed to work. I doubt the white board will get that much use again!

It was very helpful to discuss one of my models with you, Phil Bouchet. Thank you for coming over and looking through it with me. I would also like to thank Alain Zuur and Julie Sims for their advice in the application of models to multivariate data.

Getting everything into a nice format in the end wasn't quite as straight forward to start with. Tristan Lippert, Florian Meyer and Carlos Anderson, thanks heaps for the Latex examples, coding lines and tips to get it all right in the end. Thanks for setting me up with Github to have everything saved properly, Flo. I can't thank you enough for proof-reading my whole thesis in the end, Bethan Parnum and Sarah Marley. You are fabulous friends to have around :-)!

Having me work in your apartment on several weekends was the perfect carpet change, Dion Bullen. Loved all the mochas, breakfast/lunches and dinners we had and the perfect silliness distractions. Looking forward to having more time for more of all these. Thank you Flo, Kristin and Sophie for keeping me sane in the last several months. I am really glad you live so close and although we were all so busy, we were still able to catch up and manage dinners, drinks and cakes. Can't wait for more to come :-D. Thanks to my friends at gymnastics, motivating me to get me off the desk and tumble in for a break. Always fun to hang out with you Adam Brown, Alana French, Andre Dias Da Silva, Benjamin Moon, Cathryn O'Sullivan, Ngaire

Mulholland, Katie Salamone, and Roslyn-Lee Hughes. I loved the chocolate coated coffee beans, Cath, they kept me going for quite few more hours. Ben, your grading and drawing is perfect :-)! Thank you for swapping a gym class with reading my introduction! My not-uni friends in Melbourne, thanks for having me stay with you when I came back to visit and organise parties with the whole crew so I could see everyone Camil, Jaclyn, Maria, Marie and Mhaz and your families. Looking forward to catching up properly once I submitted :-)!

My best friend at home, Sarah Feurle, thank you for all your support and encouragement throughout the past few years (should say 20 years). You were always there for me, didn't matter what time or how big or small the problem was, you made Skype, texts and calls always possible. You always had an open ear for me and I know that I will always be able to count on you. I hope I can be there for you when you need me too. Though you are half a world away, you have always been supporting, funding, and encouraging me to continue studying, Siegfried and Annie. You made it possible that I have been able to travel a lot throughout the past 10 years of studying. Without your love, faith and inspiration, I would have never gotten this far. You are a great little brother, Michael! Loved your e-mails cheering me on and up, and making me smile and cry at the same time, your support definitely worked. It was always fun to come home for games, Schafkopf, all the laughter and wrestles in between all the PhD work. I do miss you all here but I can't wait to show you and my soon-to-be sister-in-law, Karin, around Western Australia in a few months' time :-)!

Miles, I think you are the most patient person I have ever met, still can't believe you never punched back ;-). In whichever mood I was in, you still brought me food, wine (or sometimes something with more serious percentage), and chocolates, at pretty much any hour of the day or should better say night; and I am looking forward to more of everything except the PhD stress. You are very cool to have on my side and I'm looking forward to having more time so we can do some epic dives and I can drag you to gymnastics lots more (basically just anything super fun is what I mean), instead of looking at weird English words and phrases which would never make sense anyway. I am also looking forward to only communicating in German with you soon :-D. Anyway, thank you for not giving up, always supporting me and being there whenever I needed you.

Having said all of this, happy reading. . .

Chapter 1

Introduction

1.1 CONSERVATION ECOLOGY AND MANAGEMENT

Biodiversity of species in an ecosystem is important for its health, i.e. its balance and sustainability. Removal of species from an ecosystem can initiate the increase of others, but may have adverse effects on reliant species, and ultimately change an ecosystem's biodiversity and productivity overall (e.g. Hooper *et al.*, 2005; Worm *et al.*, 2006; Stachowicz *et al.*, 2007). The conservation of species is therefore fundamental to maintaining a robust and highly diverse environment for inhabiting species (e.g. Roberts *et al.*, 2002; Dudgeon *et al.*, 2006; Jones *et al.*, 2007).

The expansion of commercial and recreational anthropogenic activity such as resource harvesting and tourism in the marine environment has substantially increased the level of interaction between humans and marine fauna, often to the detriment of the ecosystem as a whole as well as the individual species involved. In many cases, this human/animal interaction has a direct impact on a species of particular ecological or economic importance (e.g. Topelko and Dearden, 2005; Fergusson *et al.*, 2009; Mejer *et al.*, 2011; Rosenbaum *et al.*, 2014). When impacts are severe, they need to be controlled through clear legislation and general guiding principles such as effective species-specific management strategies that ensure long-term species conservation and survival. With many species and populations in decline and an increasing number of fauna listed as vulnerable or endangered, the management of anthropogenic activity and their impacts is under increasing pressure to improve, and is of growing importance to avoid extinction of key ecosystem species (e.g. Sala and Knowlton, 2006; Slooten, 2007; Van Der Hoop *et al.*, 2013; Notarbartolodi Sciara, 2014).

Knowledge on the distribution, abundance, and demographic characteristics, including survival and reproductive rates, of a population are needed to determine the risk of reduction or loss of a species. Effective conservation management strategies require accurate baseline and ongoing information if their performance is to be properly monitored and determined. For the collection of this information to be possible and accurate, a basic knowledge of species behaviour, essential habitats and responses to external stressors, such as environmental changes and anthropogenic activities are required (e.g. Boehme *et al.*, 2012; Arcangeli *et al.*, 2013; Balmer *et al.*, 2013).

Together with a developed understanding of potential impacts of anthropogenic activities, as well as the particular species' status and necessities for survival and recovery, appropriate and timely management may reduce or prevent further vulnerability and constriction to the species. Conservation management can be applied in many forms, the most direct of which is the safety of the animals. Humpback whale (*Megaptera novaeangliae*) as well as southern right whale (*Eubalaena australis*) numbers, for example, were dramatically reduced by commercial whaling, and have been recovering to varying degrees, since whaling was prohibited (e.g. Best, 1990; Payne *et al.*, 1990; Paterson *et al.*, 1994; Bannister, 2001). Similarly, New Zealand fur seal (or long-nosed fur seal, *Arctocephalus forsteri*) populations have been increasing considerably after hunting ceased (e.g. Lalas and Bradshaw, 2001; Campbell *et al.*, 2014).

In contrast to directly managing the safety of individuals, habitat management makes use of sanctuaries and Marine Protected Areas (MPAs) which are designated areas used to protect species within species-important, and/or ecologically important (e.g. highly biodiverse or productive) habitats (e.g. Gray, 1997; Allison *et al.*, 1998; Hoyt, 2011). The Great Barrier Reef Marine Park in Queensland, Australia, is one of the most famous MPAs. Protecting essential habitat for a species to conduct fundamental life functions, such as seasonal restrictions to fishing for spawning fish, have been shown to help the recovery of several species (Bohnsack, 1996; Halpern and Warner, 2002), e.g. red hind (*Epinephelus guttatus*; Nemeth, 2005).

For highly mobile species, such as cetaceans and many pinnipeds, sanctuaries and MPAs may only protect part of their home ranges (Allison *et al.*, 1998; Slooten *et al.*, 2006; Slooten, 2013). Thus, sanctuaries and MPAs need to be appropriately placed, sized and managed to be effective (Slooten *et al.*, 2006; Jones *et al.*, 2007; Gormley *et al.*, 2012; Slooten, 2013). The Banks Peninsula Marine Mammal Sanctuary, New Zealand, for example, was established to reduce the unsustainable numbers of by-caught Hector's dolphins (*Cephalorhynchus hectori*) by restricting gillnet and trawl fishing within the sanctuary (e.g. Dawson, 1991; Dawson and Slooten, 1993). Harbour porpoises (*Phocoena phocoena*) are also by-caught in high, most likely unsustainable numbers in several areas (Jefferson and Curry, 1994), e.g. in the Celtic Sea (Tregenza *et al.*, 1997; Hammond *et al.*, 2002), Black Sea (subpopulation *P. p. relicta*; e.g. Birkun, 2002; Birkun and Frantzis, 2008), Baltic Sea (Berggren, 1994; Koschinski, 2001), and the Gulf of Maine (e.g. Palka *et al.*, 1996). A successful example of the mitigation of such by-catch through spa-

tial and temporal management is the Gulf of Maine, under The Harbor Porpoise Take Reduction Plan (Read, 2013). Changes to fishing practices included time and area closures in these areas, prohibiting fishing altogether or at certain times, and the use of ‘pingers’, acoustic alarms on fishing nets to deter *P. phocoena* and prevent entanglement (e.g. Kraus *et al.*, 1997; Palka *et al.*, 2008). By-catch numbers of *Cephalorhynchus hectori* and *Phocoena phocoena* in these protected areas have considerably decreased following the management measures (Palka *et al.*, 2008; Slooten and Dawson, 2010; Gormley *et al.*, 2012; Orphanides and Palka, 2013). However, while these strategies may limit further population loss, stronger efforts may be required for species such as *Cephalorhynchus hectori* to begin recovery (e.g. Slooten and Dawson, 2010; Slooten and Davies, 2012; Slooten, 2013).

The responses of animals to external pressures can be species-specific and quantifying the source, impacts, responses, and resulting implications for the health of the species due to each pressure, can be highly involved and complicated to clearly delineate. Despite this, it has been achieved for some species, e.g. in killer whales (*Orcinus orca*; Morton and Symonds, 2002), grey whales (*Eschrichtius robustus*; Gailey *et al.*, 2007), and ringed seals (*Phoca hispida*; Harris *et al.*, 2001). However, many behaviour studies for many species and populations are limited by a fundamental lack of studies on basic life histories, population dynamics and abundance. Management of a species can only be effective when monitoring and management studies target questions that are fundamental to its reproduction and survival, including interactions with its environment, other organisms and humans.

1.2 ANTHROPOGENIC IMPACTS

In marine environments, a wide range of environmental pressures, such as climate change or variations in water temperature, can lead to shifts in the distribution of prey species, and currents may have widespread and general ecosystem impacts. Anthropogenic activities and general degradation of habitat resulting from these activities are adding to environmental pressures and have been increasingly identified as directly impacting various species and communities including apex predators (e.g. Perry *et al.*, 2005; Kovacs *et al.*, 2012; Bester, 2014). For example, direct human pursuits, such as hunting and unintentional take (e.g. by-catch) or activities that deter fauna from a food source, may have severe impacts on behaviour and reproductive success of a species (e.g. Cox *et al.*, 2003; Kyhn *et al.*, 2015). Indirect activities, such as habitat destruction, recreational or commercial use of habitats and their surroundings, or noise pollution (both in and out of the water) may have

impacts that are equal to or even greater than direct impacts (e.g. Kelly *et al.*, 1988; Airoidi *et al.*, 2008; McDonald *et al.*, 2008; Demarchi *et al.*, 2012; Bester, 2014). To measure these impacts, it is imperative to have baseline data and adequate monitoring to determine and quantify how both direct and indirect impacts that affect species, populations and even individual animals.

Some anthropogenic activities have been quite detrimental at a species level, leading to severe medium to long-term decline and even extinction of species. Hunting and harvesting, for example, have decimated a range of marine mammal species populations, including several well recorded instances of cetacean and pinniped species. While some of these species numbers have recovered substantially, e.g. *M. novaeangliae* and *A. forsteri* (e.g. Paterson *et al.*, 1994; Gales *et al.*, 2000; Campbell *et al.*, 2014; Bejder *et al.*, 2016), others have only shown very slow recovery rates or no sign of recovery at all, such as blue whales (*Balaenoptera musculus*), Australian (*Neophoca cinerea*) and Hooker's sea lions (*Phocarctos hookeri*) (e.g. Gales and Fletcher, 1999; Campbell, 2003; Branch *et al.*, 2007; Childerhouse *et al.*, 2014; Robertson, 2015). *A. forsteri* and *N. cinerea* live sympatrically (i.e. their occurrence overlaps) at several locations (Ling, 1992). While *A. forsteri* have been increasing in numbers and expanding their range, numbers of *N. cinerea* continue to decline (Ling, 1992; Campbell *et al.*, 2014; Goldsworthy, 2015).

In the last few decades, there has been a substantial increase in wildlife viewing and interacting activities, that involve marine mammals (Kirkwood *et al.*, 2003). While these anthropogenic activities have been shown to influence a range of animal species, long-term impact at a population level is generally unknown (e.g. Constantine, 1999; Newsome and Rodger, 2008; Bejder *et al.*, 2009; Parsons, 2012). Long-term impacts are relatively hard to study, but can include:

- habituation to humans/human activities (e.g. Connor and Smolker, 1985; Watkins, 1986; Higham and Shelton, 2011);
- displacement or avoidance of preferred habitat (e.g. Gerrodette and Gilmartin, 1990; Stevens and Boness, 2003);
- females leaving pups or calves unattended, potentially increasing pup or calf mortality (e.g. Kovacs and Innes, 1990; Jansen *et al.*, 2010);
- decreased reproductive rate (e.g. French *et al.*, 2011), and;

- a higher risk of boat strikes with increased numbers of vessels in the vicinity (e.g. Goldstein *et al.*, 1999; Stone and Yoshinaga, 2000; Donaldson *et al.*, 2010).

A range of short-term impacts, which are easier to study and more readily observed, have been documented and include:

- behavioural changes, such as disruption of foraging or resting (e.g. Nowacek *et al.*, 2001; Dans *et al.*, 2012);
- physiological responses, like increased stress levels and suppressed immune system (e.g. Wright *et al.*, 2007; Seuront and Cribb, 2011);
- increased aggressive behaviours amongst each other, but also directed towards humans (e.g. Lovasz *et al.*, 2008; Tripovich *et al.*, 2012).

Due to the nature of responses, the varying levels of response, and the unknown impact on long-term behaviours and health (for example, whether consistent disturbance impacts future flight responses from predators), Frid and Dill (2002) suggested responses to anthropogenic disturbance should be considered as a type of predator risk. Responses elicited not only vary considerably between age and sex classes, among breeding, moulting and pupping seasons, and among individuals; but also vary among the type of approach and stimuli (e.g. vessels, people, helicopters/planes), the number of vessels, people, etc. that form the stimulus (group size), approach distance, and the activity carried out (e.g. Constantine, 1999; Cassini, 2001; Boren *et al.*, 2002; Labrada-Martagón *et al.*, 2005; Lovasz *et al.*, 2008; Cowling *et al.*, 2014). Determining the individual and collective impact of this wide range of variables is not trivial and can be complicated, but must be addressed to adequately determine their effects and the magnitude of their overall impact.

Enforced regulations and voluntary-based codes of conduct have been implemented at several locations to control known anthropogenic disturbance, but these tend to be prescriptions of generalised behaviour, applied mostly to general ‘area-of-influence’ in management assessments (Orams, 1999). In a more controlled environment, human disturbance would be controlled through management of viewing and interacting sites, and separate no-access sanctuary zones. Information on population-specific responses to specific anthropogenic activities is beneficial for optimising guidelines for public approach behaviour and distances to minimise local disturbance to help manage such viewing sites.

1.3 PINNIPEDS

The clade Pinnipedia comprises the families Phocidae (true, earless seals), Otariidae (eared seals: fur seals and sea lions) and Odobenidae (walrus, *Odobenus rosmarus*). Several of the pinniped species reside in proximity to humans, sometimes sharing habitat, and may often be in varying levels of competition for food resources (e.g. Gerrodette and Gilmartin, 1990; Wickens *et al.*, 1992; Childerhouse *et al.*, 2001; Kemper *et al.*, 2003; Anderson *et al.*, 2007b). More specifically, there are six extant species of sea lions of which three species are listed as endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. In contrast to the declining Australian *N. cinerea*, the California sea lion (*Zalophus californianus*) has been increasing in numbers despite residing along a coastline with several major cities and large human populations and their activities (Auricles and Trillmich, 2008; Goldsworthy, 2015). This strong contrast in population trends for closely related species illustrates that species and location specific studies are needed to understand the particular factors influencing abundance and the challenges the respective species are facing.

N. cinerea is the only endemic pinniped species in Australia and of all Australian pinnipeds it is the species with the smallest overall population size. Its current range extends from the Abrolhos Islands (28°51'S 114°03'E) in Western Australia to the Pages (35°46'S 138°18'E) in South Australia (Figure 1.1; Ling, 1992). *N. cinerea* was listed as vulnerable under the Commonwealth Environment Protection and Biodiversity Conservation (EPBC) Act 1999 in 2005. In 2008, *N. cinerea* was listed as endangered on the IUCN 'Red List' due to its overall relatively small and declining population, which was estimated at 12,690 (estimate from 20013/14; Goldsworthy *et al.*, 2014; Goldsworthy, 2015). The species' population is divided into mostly small and widely scattered colonies with several of these declining in numbers, putting smaller colonies at risk of local extinction (Goldsworthy *et al.*, 2009a; Goldsworthy, 2015).

Numbers of *N. cinerea* prior to European hunting in Australia are unknown, but were likely considerably greater than present (cf. Abbott, 1979; Ling, 1992; Campbell, 2005). It is known however, that prior to European hunting, the species inhabited the Bass Strait and Tasmania before local extinction occurred in these areas (Figure 1.1; Ling, 1992).

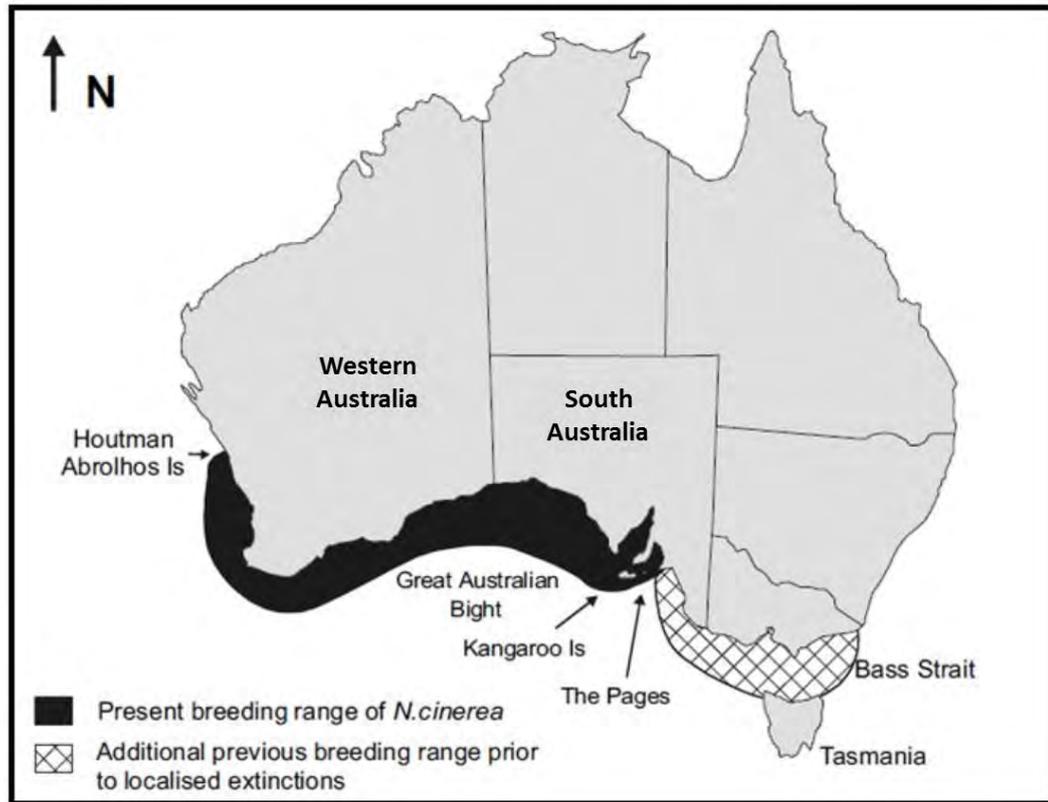


Figure 1.1: Historic and present distribution of *N. cinerea* (original from Ling (1992); adaptation by Campbell (2003)).

N. cinerea have an unusual breeding cycle of 17-18 months (range: 16.0-19.9 months) which is unique compared with other pinnipeds, that typically have annual cycles (Ling and Walker, 1978; Higgins, 1990; Kovacs and Lavigne, 1992; Gales *et al.*, 1994; Atkinson, 1997). Individual breeding sites for *N. cinerea* exhibit asynchronous breeding cycles, i.e. while the cycle period/length appears to be the same, breeding may occur at different times at different sites (Figure 1.2; Gales *et al.*, 1992, 1994; Campbell, 2003). Pupping, or birthing, takes place about 10 days before mating and the overall pupping period is elongated, occurring over ~6 months (Ling and Walker, 1978; Higgins and Gass, 1993; Gales and Costa, 1997). Males display sequential polygyny with a high turnover rate of male territories, due to their foraging trips during mating season (Higgins, 1990). Otariids generally, show protracted lactation with females investing in their pups over a considerable period (Kovacs and Lavigne, 1992; Atkinson, 1997). *N. cinerea* exhibit the second longest lactation period amongst otariids, weaning their pups after ~17 months, just before the successive pup is born, with only the Galapagos fur seal (*Arctocephalus galapagoensis*) nursing for longer (Gales *et al.*, 1992; Kovacs and Lavigne, 1992; Higgins

and Gass, 1993; Atkinson, 1997). Females that do not give birth in the consecutive breeding season often suckle their pup for an additional whole cycle (Higgins, 1990; Higgins and Gass, 1993).

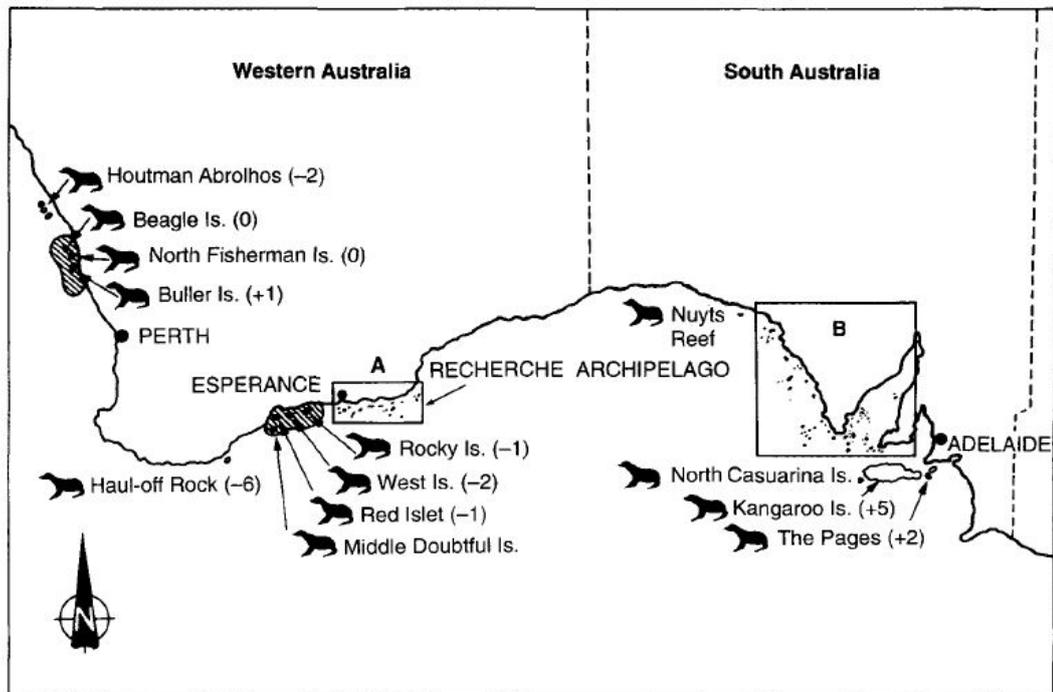


Figure 1.2: *N. cinerea* breeding and haul-out sites. Temporally asynchronous timing of breeding is indicated in positive or negative months (in brackets) posterior or prior in relation to North Fisherman Island, Western Australia (reference at 0 months). High concentration of colonies in sections A and B, not named here (from Gales *et al.* (1994)).

Female *N. cinerea* display high natal site fidelity (Higgins and Gass, 1993; Campbell *et al.*, 2008b), which, combined with a temporally asynchronous breeding cycle, may contribute to the lack of recovery in species numbers and the lack of re-inhabiting extinct locations. There is high genetic diversity and segregation between females of different colonies which aligns with the behaviour of high site fidelity in females, a finding that indicates that re-colonisation of an extinct breeding location is highly unlikely (Campbell *et al.*, 2008b; Lowther *et al.*, 2013).

Most of the extant breeding colonies of *N. cinerea* are located in South Australia (SA), with 84% of the total pup production and by proxy, overall population being presently confined to that state (Goldsworthy *et al.*, 2009b; Goldsworthy, 2015). The remaining 16% of the extant population is found in Western Australia (WA),

with breeding locations along the south coast (approximately between the coast from Recherche Archipelago to Albany) and the west coast (around Jurien Bay and Abrolhos Islands; Gales *et al.*, 1992; Campbell, 2003; Goldsworthy, 2015). Most breeding islands are small with a pup production of <25 pups per breeding cycle. There are 81 known breeding sites (47 in SA, 34 in WA) with the largest eight breeding sites (all in SA) producing 61% of the total pups (3204 pups per breeding season; Campbell, 2003; Goldsworthy *et al.*, 2009b; Shaughnessy *et al.*, 2011; Goldsworthy, 2015).

1.4 PINNIPED POPULATION ESTIMATES

1.4.1 Pup counts

Population estimates in pinnipeds are usually based on pup counts (e.g. Berkson and DeMaster, 1985; Gales *et al.*, 1992, 1994; Gales and Fletcher, 1999; Kirkman *et al.*, 2007). The methods for population estimates in *N. cinerea* have been established at breeding sites in South Australia and are still being tested for use as reliable abundance indices at most places in Western Australia (Goldsworthy *et al.*, 2009b). In the last several years new methods have been developed, mainly including marking of pups, counts of live and dead pups and recording of re-sightings (Goldsworthy *et al.*, 2007b, 2009b). For large *N. cinerea* colonies (>40 pups), analyses involved mark-recapture models, specifically Cormack-Jolly-Seber models and Peterson estimate methods. In small colonies (<40 pups), a cumulative mark and count approach has been developed, consisting of counting marked, unmarked and dead pups at each survey to calculate the number of pups born in a given season (Goldsworthy *et al.*, 2007b, 2009b). Due to the protracted pupping season, pups need to be counted in a minimum of three surveys at appropriate times to provide a relatively accurate number of pup production (Goldsworthy *et al.*, 2007b, 2009b). The multiplier to calculate population size based on pup counts was estimated to be 3.81 – 4.81 (Gales *et al.*, 1994).

1.4.2 Adult male counts

Abundance estimates based on counts at haul-out sites, which are not based on the number of pups, are considered inaccurate, due to the lack of information on numbers of sea lions foraging at any one time. Abundance estimates at non-breeding haul-out locations, however, cannot be based on pup counts. In Western Australia, there are six haul-out islands off the Perth metropolitan area only used by male *N. cinerea* (Gales *et al.*, 1992). In the past, *N. cinerea* also hauled out on Rottneest and

Garden Islands where they faced local extinction, presumably due to being hunted (Abbott, 1979; Campbell, 2005). According to historical observations, it is likely that breeding also occurred in this area prior to hunting, probably on Rottnest, Garden and Carnac Islands (Campbell, 2005).

Considering that only 16% (≈ 2030 individuals) of the total *N. cinerea* population occurs in Western Australia, the Perth metropolitan area may be inhabited by a significant proportion of the Western Australian male *N. cinerea* population (Goldsworthy, 2015). Seal and Carnac Islands are used by the largest number of *N. cinerea*, with up to 28 and 45 *N. cinerea* recorded to be hauled out during the Perth peak season, on each of the respective islands (Gales *et al.*, 1992). Approximately 15 *N. cinerea* have been found hauled out at Burns Rocks and Little Island combined, mostly less than 10 at Dyer Islands during the Perth peak season, and on Penguin Island one or two *N. cinerea* have been recorded infrequently (Department of Parks and Wildlife, unpublished data).

During the breeding season, male *N. cinerea* migrate from the Perth metropolitan islands to the closest breeding islands ca. 250 km north at Jurien Bay, (Gales *et al.*, 1992). The closest breeding islands to the south/south-east of Perth, are located off Albany, Western Australia, and are ca. 1000 km swimming distance from the Perth male-only haul-out islands (Campbell, 2003). It is unknown if some *N. cinerea* hauling out in the Perth metropolitan area migrate to the southern islands for breeding. The distinct breeding patterns associated with Albany in the south and Jurien Bay in the north are six months apart (Figure 1.2; Campbell, 2003). The fluctuations in numbers of *N. cinerea* hauling out off Perth inversely align with the breeding season in Jurien Bay but no such correlation exists for the islands off Albany. Small numbers of *N. cinerea* are found hauling out off Perth during the breeding season at Jurien Bay and reach a maximum during the non-breeding season (Gales *et al.*, 1992). This suggests that most males in Perth metropolitan waters do not regularly migrate to the southwest breeding colonies. The population size of the *N. cinerea* residing in the Perth metropolitan area is, however, unknown.

Estimating population size and monitoring population trends of a male-only population based on count data needs to be carefully planned on a robust survey design. For an accurate estimate, it is important to consider daily haul-out pattern separately from the overall fluctuations due to the breeding cycle (Southwell, 2005). To obtain more accurate trend analyses, recommendations have been made suggest-

ing that counts should be conducted at times of least variance in numbers of the subject species and when the maximum proportion of the population is hauled out (Thompson and Harwood, 1990; Southwell, 2005). To achieve maximum reliability, effective surveys for accurate abundance and trend estimates must be designed based on known daily and seasonal patterns in haul-out behaviour.

Simplistic assumptions of abundance estimates based on sparse daily or seasonal surveys, may not accurately reflect true trend data. Haul-out patterns can vary significantly throughout the day, with several species of pinniped showing peaks at species-, location- and season-specific times of day (e.g. Stirling, 1968; Thompson *et al.*, 1989; Lake *et al.*, 1997; Sepúlveda *et al.*, 2001; Reder *et al.*, 2003; Carlens *et al.*, 2006; Cunningham *et al.*, 2009; Sepúlveda *et al.*, 2012). Apart from season and time of day, variations in numbers hauling out within a species have been shown to be influenced by differences in age of the individual and sex classes, presence of pups or timing of the breeding or moulting cycle (e.g. Thompson *et al.*, 1989; Lake *et al.*, 1997; Reder *et al.*, 2003; Southwell, 2003, 2005). Furthermore, environmental conditions such as air temperature, wind speed, cloud cover, tidal heights as well as currents may alter haul-out patterns in some populations (e.g. Schneider and Payne, 1983; Pauli and Terhune, 1987a,b; Watts, 1992; Carlens *et al.*, 2006; Andrews-Goff *et al.*, 2010). These fluctuations may introduce high daily variability in numbers of pinnipeds hauling out, and therefore need to be accounted for to avoid excessive inaccuracies in count-based population estimates.

Alternatively, abundance estimates may be based on mark-recapture models, analysing the resighting rate of individuals (Nichols, 1992). Individual identification is beneficial for studies on population estimates and demographics, as well as to investigate movement and residency patterns. Non-invasive identification is based on using natural marks which are unique to the individuals, like fur patterns (for example stripes in tigers (*Panthera tigris*) or zebras (*Equus sp.*), or spots in harbour seals (*Phoca vitulina*)) or the shape or outline of some part of the body, like the dolphin's dorsal fin (e.g. Peterson, 1972; Hiby and Lovell, 1990; Whitehead, 1990; Würsig and Jefferson, 1990; Agler, 1992; Ullas Karanth and Nichols, 1998; Friday *et al.*, 2000; Hastings *et al.*, 2001; Kelly, 2001; Parra and Corkeron, 2001; Dixon, 2003; Karlsson *et al.*, 2005).

N. cinerea do not have any obvious marks or fur pattern which could be used, thus invasive methods like tagging, microchipping or branding individuals have been used in this and similar species (Summers and Witthames, 1987; Walker *et al.*, 2012). These methods involve capturing, handling, potentially anaesthetising the animals and applying the mark. These procedures can be risky for the animals and also involves some dangers for researchers themselves (Troy *et al.*, 1997; Walker *et al.*, 2012). Scars may be useful to assist identification also in pinnipeds, but often change over time, e.g. when animals moult (Forcada and Aguilar, 2000; Vincent *et al.*, 2001). Lions (*Panthera leo*) and polar bears (*Ursus maritimus*), however, have successfully been identified using their whisker spot patterns, a method yet to be tested on pinnipeds for re-identification (Pennycuick and Rudnai, 1970; Anderson *et al.*, 2005, 2007a). Their whisker patterns appear to be similarly visible as in *P. leo* and *U. maritimus*, and have aided in individual identification in a previous study in *P. hookeri* (Beentjes, 1989).

1.5 THREATS TO *N. CINEREA*

The most serious readily apparent known threat to *N. cinerea* is bycatch, primarily in gillnets, which are the most prominent fishing method in South Australia, rock lobster pots (throughout the range) and entanglement in marine debris (e.g. c.f. Abbott, 1979; Gales *et al.*, 1994; Page *et al.*, 2004; Goldsworthy and Page, 2007; Hamer *et al.*, 2007, 2011, 2013). A steady, low number of *N. cinerea* bycatch would be capable of instigating a decline in population size and has been implicated in and predicted to ultimately result in quasi-extinction of several *N. cinerea* populations in 1.5 - 43.1 years with current bycatch rates (Goldsworthy and Page, 2007; Hamer *et al.*, 2011). As a consequence, closure of gillnet fisheries in an extended area and low by-catch limits were suggested for management regulations (Hamer *et al.*, 2011). After closing gillnet fisheries in Spencer Gulf, South Australia, the colony appeared to recover (Goldsworthy *et al.*, 2007a). In parts of Western Australia, rock lobster pots in the vicinity of *N. cinerea* colonies are required to have Sea Lion Exclusive Devices (SLED) to prevent their entry into the trap (Campbell *et al.*, 2008a; Department of Fisheries, 2012).

Such anthropogenic pressures to *N. cinerea* occur in addition to lower impact, natural pressures, which include interspecies resource competition, risk of predation by great white sharks (*Carcharodon carcharias*), diseases and parasites, as well as environmental changes which may cause prey distributions to shift (Campbell, 2005; Shaughnessy *et al.*, 2007; Linnane *et al.*, 2010; Booth *et al.*, 2011; Hamer

et al., 2013; Schumann *et al.*, 2013; Marcus *et al.*, 2014). Availability of large schools of fish appeared to influence the haul-out behaviour in Steller sea lions (*Eumetopias jubatus*; Womble *et al.*, 2005; Marcotte, 2006; Womble *et al.*, 2009).

N. cinerea faces indirect pressures from various types of anthropogenic disturbance. *N. cinerea* is one of the pinniped species increasingly targeted by marine tourism in Australia, and pinniped tourism has developed into an activity with a high economic value (Kirkwood *et al.*, 2003). In the Southern Hemisphere alone, 1.3 million people visit pinnipeds every year and support this tourism branch with US\$ 12.6 million per year through seal and sea lion watching from boats, planes or land and swimming with pinnipeds (Kirkwood *et al.*, 2003; c.f. Newsome and Rodger, 2008). Seal Bay, Kangaroo Island, South Australia is the most popular location to watch *N. cinerea* in the wild with 110,000 visitors per year contributing to the local economy (Kirkwood *et al.*, 2003). Other popular places to view or interact with *N. cinerea* are Carnac and Seal Island off metropolitan Perth and Jurien Bay in Western Australia (Orsini, 2004; Orsini and Newsome, 2005). A limited amount of research on *N. cinerea* disturbance in relation to tourism or indeed to any human-influenced disturbance activity, has been conducted (Orsini, 2004; Orsini *et al.*, 2006; Lovasz *et al.*, 2008). However, preliminary results on the preferred haul-out locations near Perth may no longer be applicable today with *N. cinerea* using the part of the beach outside the sanctuary as much as within the sanctuary (Salgado Kent and Crabtree, 2008). Furthermore, data on the overall impacts of the different types of activity were insufficient to draw conclusions regarding *N. cinerea* responses. For local populations in the Perth metropolitan area, disturbance has become of increasing concern in recent years (CALM, 2003; DEC, 2007).

1.5.1 Human disturbance in *N. cinerea*

N. cinerea invest substantial effort and can quickly diminish energy reserves during foraging trips. As benthic foragers, they conduct long and deep dives. They spend most of their time (58%) diving during foraging trips, spending on average 61% of their dive time close to the sea floor and regularly exceeding their aerobic dive limits (Costa and Gales, 2003). *N. cinerea* prey on a range of benthic fish (including sharks), cephalopods and crustacean species, and have also been documented to include penguins and cormorants in their diets, and potentially even sea urchins (Richardson and Gales, 1987; Gales *et al.*, 1992; McIntosh *et al.*, 2006; Baylis *et al.*, 2009; Berry, 2013; Peters *et al.*, 2015). Females display foraging specialisation with large inter-individual variation (Lowther *et al.*, 2011). Regular visits to haul-out ar-

as serve as periods of recuperation and preparation for subsequent foraging trips. Disruption of their rest and recuperation may influence an individual's energy budget as more energy is needed if increased time is spent at higher activity levels, such as escaping human disturbance. This would require *N. cinerea* to feed more during foraging trips and potentially increase trip length or increase the number of foraging trips which would likely reduce time spent resting. If *N. cinerea* leave their haul-out sites to forage in a tired or weakened state, they may present an easier target for predators. The haul-out locations in the Perth metropolitan area are close to the coast and easily accessible, potentially exposing *N. cinerea* to a wide range of anthropogenic activities in their vicinity (Orsini and Newsome, 2005; Orsini *et al.*, 2006). For *N. cinerea* in the Perth metropolitan area (a potentially significant proportion of the male *N. cinerea* population), it is therefore important to determine the influences that anthropogenic activities have on their behaviours. This will begin to build up the necessary information to identify the possible deleterious effects they have on the population.

1.6 STRUCTURE OF THESIS

This thesis addresses significant gaps in current knowledge on *N. cinerea*. It tests tools to improve monitoring practices of *N. cinerea* into the future, investigates behavioural patterns and *N. cinerea*' responses to human disturbance at two main haul-out islands in the Perth metropolitan area. It also makes recommendations for improved management of human disturbance at these locations. *N. cinerea* in the Perth metropolitan area haul out in proximity to what is a highly populated area of coastline. They are exposed to considerable visitation by tourists and the broad community, making them highly vulnerable to the impacts of human disturbance. The population is of significant economic and ecological value, yet data on their numbers, movement and haul-out patterns are sparse. Thus, this study is significant in gathering the necessary information to provide managers with a better understanding of the species' ecology to use as a basis for improving current guidelines. Ultimately, the work is aimed to feed directly into management outcomes through maintaining or improving the species' current status. With this in mind, this thesis has been organised with the following main objectives and chapters:

Following this first introductory chapter, the second and third chapters of this thesis describe the testing of a non-invasive method to identify individual *N. cinerea* based on their whisker spot patterns, following its successful application in *P. leo* and *U. maritimus* (Pennycuick and Rudnai, 1970; Anderson *et al.*, 2007a). The aim

of this work was to develop a method of individual identification that can be used by researchers and managers to obtain key information on residency, movement and abundance. The second chapter tested the method using photographs of captive individuals in controlled environments, while the third chapter extended the tests to wild individuals. The third chapter uses photographs taken at the two main haul-out islands in the Perth metropolitan area. In this chapter, resighting wild individuals is trialled, and limitations of the method with their origins identified.

The fourth chapter evaluates haul-out patterns of *N. cinerea* residing in the main haul-out locations in the Perth metropolitan area. The numbers of *N. cinerea* hauling out are investigated on a range of temporal scales, including finer scale daily cycles to broader scale breeding cycles; as well as in relation to local environmental conditions. Suggestions are given on the development of survey designs for abundance and trend estimation (from count data) based on the results of this study to support continued monitoring and management of this subpopulation.

Due to the high level of human visitation and the potential detrimental impacts disturbance can cause, the fifth chapter in this thesis investigates exposure levels and behavioural responses of *N. cinerea* to anthropogenic activities. The study is focussed on the the two main haul-out islands in the Perth metropolitan area, which are managed differently; i.e. one is a sanctuary zone without landing permission whereas the other allows people to access the beach. Recommendations to improve management in these areas to limit human disturbance are included in this chapter based on the results of the study.

The final (sixth) chapter provides a general discussion and interpretation of the overall findings of this study, and concluding recommendations for future research, management and conservation of *N. cinerea* in the Perth metropolitan area.

Chapter 2

Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (*Neophoca cinerea*)

Sylvia K. Osterrieder, Chandra Salgado Kent, Carlos J. R. Anderson,
Iain M. Parnum, and Randall W. Robinson

GRADUATE RESEARCH CENTRE

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book:	Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (<i>Neophoca cinerea</i>) Journal of Mammalogy		
Surname:	Osterrieder	First name:	Sylvia
College:	College of Engineering & Science	Candidate's Contribution (%):	50
Status:			
Accepted and in press:	<input type="checkbox"/>	Date:	
Published:	<input checked="" type="checkbox"/>	Date:	25th June 2015

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

	8.8.2015
Signature	Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and

5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

<p>Curtin University, Bentley, Western Australia Palmyra, Western Australia</p>

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Sylvia Osterrieder	50	Planning project, data collection, testing method, writing and editing manuscript		8/8/15
Chandra Salgado Kent	20	Supervision on experimental design, project planning, & manuscript preparation; and code for analyses.		10/8/15
Carlos Anderson	15	Adapting software for matching, and provided suggested edits for improvement to manuscript drafts		6/8/15
Iain Parnum	10	Prepared Matlab code for initial comparisons		8/8/15
Randall Robinson	5	Supervision and input to experiment design, provided suggested edits for improvement to final manuscript		5/8/15

Updated: June 2015

2.1 ABSTRACT

Reliable methods for identification of individual animals are advantageous for ecological studies of population demographics and movement patterns. Photographic identification, based on distinguishable patterns, unique shapes, or scars, is an effective technique already used for many species. We tested whether photographs of whisker spot patterns could be used to discriminate among individual Australian sea lions (*Neophoca cinerea*). Based on images of 53 *N. cinerea*, we simulated 5,000 patterns before calculating the probability of duplication in a study population. A total of 99% (± 1.5 SD) of patterns were considered reliable for a population of 50, 98% (± 1.7 SD) for 100, 92% (± 4.7 SD) for 500, and 88% (± 5.7 SD) for 1,000. We tested a semi-automatic approach by matching 16 known individuals at 3 different angles (70°, 90°, and 110°), 2 distances (1 and 2 m), and 6 separate times over a 1-year period. A point-pattern matching algorithm for pairwise comparisons produced 90% correct matches of photographs taken on the same day at 90°. Images of individuals at 1 and 2 m resulted in 89% correct matches, those photographed at different angles and different times (at 90°) resulted in 48% and 73% correct matches, respectively. Our results show that the Chamfer distance transform can effectively be used for individual identification, but only if there is very little variation in photograph angle. This point-pattern recognition application may also work for other otariid species.

2.2 KEYWORDS

Australian sea lion, individual identification, pattern recognition, pinnipeds, whisker spots

2.3 INTRODUCTION

Many aspects of ecological studies are significantly enhanced by the persistent identification of individuals, achieved for example by capture–recapture models in population-based studies (Nichols, 1992). Behavioural studies focusing on individual differences rely on the recognition of individuals and the ability to follow them through time. Microchips, tags, or artificial marks (e.g., through branding) can be applied to aid in distinguishing among individuals (Summers and Witthames, 1987; Walker *et al.*, 2012). Such methods involve capturing and handling animals, in many cases causing significant stress, can have adverse effects on the animals (Troy *et al.*, 1997; Walker *et al.*, 2012), and increase risk to the researchers themselves. In several species, methods use natural marks for non-invasive individual identification, often through photographic comparison. Identification is based on recognizing unique marks, patterns, shapes of certain body parts, or scars. This is possible with unique fur patterns, such as stripes or spots in tigers (*Panthera tigris*), cheetahs (*Acinonyx jubatus*), or zebras (*Equus quagga*; Peterson, 1972; Ullas Karanth and Nichols, 1998; Kelly, 2001; Hiby *et al.*, 2009). In some phocids, spot patterns in fur have been used to recognise individuals (Hiby and Lovell, 1990; Karlsson *et al.*, 2005). Shapes or outlines of distinctive appendages have successfully been used for individual identification, for example dolphin dorsal fins, whale flukes, badger (*Meles meles*) tails, and sea lion flippers (Würsig and Würsig, 1977; Whitehead, 1990; McConkey, 1999; Dixon, 2003). Scars may also be useful to assist identification in pinnipeds (Forcada and Aguilar, 2000; Vincent *et al.*, 2001), but often change over time, for example when animals moult (McConkey, 1999). On occasion, identification of whisker spot patterns has assisted in identification of individuals (Beentjes, 1989; Miththapala *et al.*, 1989). Australian sea lions (*Neophoca cinerea*) are an endangered species, lacking information on population estimates and demographics for many of their colonies (Goldsworthy *et al.*, 2008). Photo-identification would therefore be a useful tool to gain more knowledge on their population demographics and beneficial for appropriate management and their conservation. *N. cinerea*, however, do not have distinctive patterns in coloration, and readily visible long-term scars are absent for the majority of individuals. Hence, it is highly advantageous to establish a non-invasive and replicable technique for individual identification of *N. cinerea*. (Pennycuick and Rudnai, 1970) first developed and described a method using whisker spot patterns to identify individual lions (*Panthera leo*) successfully. (Anderson *et al.*, 2007a) then tested a similar method for polar bears (*Ursus maritimus*), finding that of 50 individual (*U. maritimus*) whisker spot patterns analysed, 98% contained enough information to reliably identify individuals. For pinnipeds,

no such feasible method has been developed yet which allows effective identification of individuals in the long-term.

Computer-aided photo-identification can increase the efficiency and accuracy of individual recognition and is particularly advantageous for studies on larger populations (e.g. Mizroch *et al.*, 1990). A practical tool may also reduce the costs of a manual-matching research program significantly. The specific objectives of this project were therefore to: 1) establish whether the variability of whisker spot patterns in *N. cinerea* is large enough to reliably use them for individual identification and 2) develop and test the accuracy of pattern recognition on *N. cinerea* whisker spot patterns. The development of a non-invasive photo-identification method for *N. cinerea* would also provide greater confidence in its potential for non-invasive identification in similar species.

2.4 METHODS

2.4.1 Study areas and collection of photographs

Method testing was based on photographs of known individual *N. cinerea* in captivity and in the wild. Images of captive *N. cinerea* comprised 3,036 photographs of 16 individuals, taken by zoos and aquaria including Adelaide Zoo, Pet Porpoise Pool in Coffs Harbour, SEALIFE (previously UnderWater World) in Mooloolaba, and Taronga Zoo in Sydney. Lateral photographs were taken between 1st March 2013 and 25th November 2014 of each *N. cinerea*'s right muzzle at estimated angles of 70°, 90°, and 110° from its anterior, at ranges of 1 and 2 m. An angle of 90° means that the profile view of the animal is perpendicular to the camera. Photo sessions were repeated at approximately 10, 30, 60, 180, and 360 days after the 1st photo session to test the method against any ontogenic changes in whisker spot patterns in *N. cinerea* (Table 2.1). There was minor variability in the timing of photo sessions with some missed due to shortage of zoo staff, busy schedules, or failure of *N. cinerea* to follow trainer instructions when taking photographs.

Table 2.1: Number of individuals and number of photographs taken of the right muzzle of captive *N. cinerea* on different days throughout 1 year.

	Day 1	Day 10	Day 30	Day 60	Day 180	Day 360	Total
Individuals	15	11	15	10	9	5	16
Photographs	396	430	515	580	565	550	3,036

Field-based photographs of wild *N. cinerea* were obtained to increase the sample size of unique individuals. Images from 15 breeding and haul-out islands were included. Selecting a wide variety of locations allowed individuals of both sexes and various age classes to be sampled. Haul-out islands were located in the Perth Metropolitan area in Western Australia and included Seal, Carnac, Penguin, Little, and Dyer Islands, and Burns Rocks. Breeding islands included Haul-off Rock, Red Islet, Middle Doubtful, Glennie, Wickham, Houtman Abrolhos Islands, as well as Anvil and Ford Islands in the eastern group of islands of the Recherche Archipelago off the southwest coast off Albany and Esperance, and Beagle Island off Jurien Bay, Western Australia (Figure 2.1; Table 2.2; Gales *et al.*, 1992). From these locations, a total of 5,766 whisker photographs of *N. cinerea* were taken during 127 field trips between the 8th June 2012 and 15th February 2014 using a Canon EOS 550D with a 100–400 mm zoom lens (Canon, Tokyo, Japan; Table 2.2). We approached focal animals slowly and carefully, up to a minimum distance of 5 m to minimise disturbance. Photographs of *N. cinerea* muzzles in the field were taken from the closest range possible- approximately 5-50 m (5-10 m is minimum distance the public is recommended to maintain from a *N. cinerea*). A maximum range of 50 m was selected as beyond this, photographs were found to be less reliable and blurred in a study on (*U. maritimus*) identification using whisker spot patterns (Anderson *et al.*, 2007a).

Table 2.2: Number of field days and photographs, which were taken of the right side of wild *N. cinerea* muzzles on various islands in Western Australia.

Location	Field days	Photographs
Seal Island	54	2,360
Penguin Island	4	28
Carnac Island	22	1,264
Dyer Island	13	192
Little Island	9	266
Burns Rocks	13	122
Haul-off Rocks	2	100
Middle Doubtful Island	1	190
Red Islet	1	108
Glennie Island	1	90
Wickahm Island	1	96
Recherche Archipelago	1	70
Beagle Island	3	640
Abrohlos Islands	2	2,883
Total	127	5,766

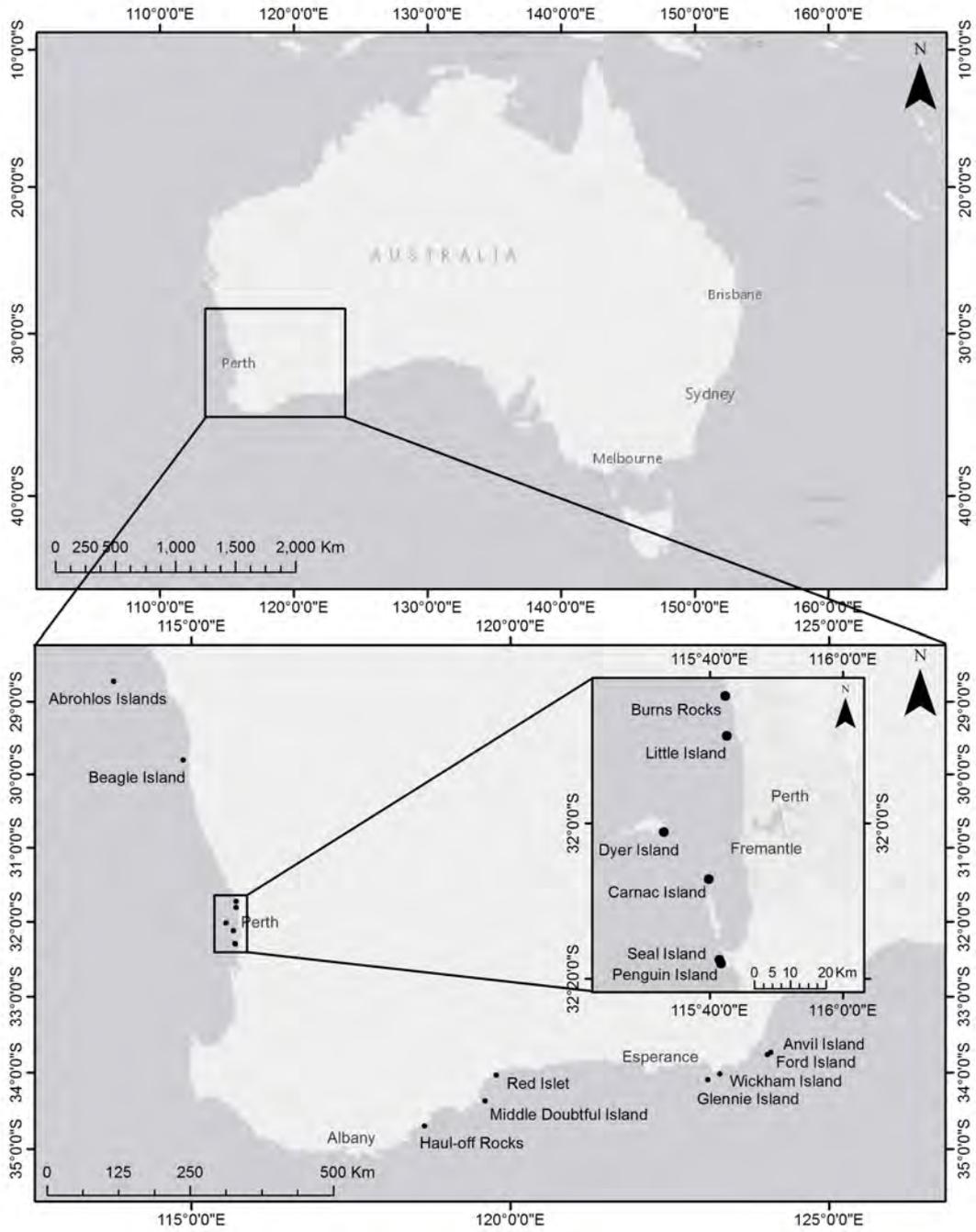


Figure 2.1: Locations of islands where photographs of *N. cinerea* in the wild were obtained.

During each field trip, lateral (90°) photographs of *N. cinerea* muzzles were taken, if possible from its left and right side. Photographed *N. cinerea* were either sitting in the water, swimming with their head raised above the waterline, or hauled out on land. Individuals could be distinguished from each other during a single field day (based on their haul-out locations and movements), and the total number of individuals photographed calculated. Due to unconfirmed movement patterns, the total number of individuals over all field days is unknown. To ensure that unique individuals were tested, a selection of photographs was made from the 5,766 wild *N. cinerea* images. The photographs selected were either taken from multiple locations within a region on a single field day, with the assumption that animals did not have time to move between field sites during the window of field work, or at breeding islands with very large distances between them (i.e., an island near Albany versus an island near Jurien, Western Australia) where there is evidence of site fidelity (Campbell *et al.*, 2008b).

After the selection process, photographs remained from 37 unique wild *N. cinerea*, for many of which, multiple photographs existed. Not all photographs from the original catalogue of 8,802 images (3,036 and 5,766 images from captive and wild *N. cinerea*, respectively) were of sufficient quality to be used in testing, therefore a further selection was required. In all cases, selection was based on user interpreted quality, i.e., in focus, not tilted and taken at the correct angle (70°, 90°, and 110° for captive individuals and 90° for wild individuals). Photographs of captive individuals were only included if taken at a range of 1 and 2 m and photographs of wild individuals only included between 5 and 50 m. In general, suitable photographs of wild individuals were available for one side of the muzzle, with more high-quality photographs from the *N. cinerea*' right-hand side. Thus, only images of the right-hand side of the *N. cinerea* were used in this study to resemble feasible sampling for usage on wild *N. cinerea*. The net result was a library of photographs for analysis, comprising 608 images of 53 individuals: 515 images of 16 captive individuals (including all three orientations and two ranges) and 93 images of 37 wild individuals (at 90°).

This work was conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth). Research on live animals followed American Society of Mammalogists guidelines (Sikes *et al.*, 2011).

2.4.2 Preparation of photographs for reliability testing and matching

Photographs were cropped in Adobe PhotoShop Elements 11 to eliminate superfluous parts of the photograph. In this study, a semi-automated pattern recognition software, originally developed for identifying (*U. maritimus*) using their whisker spots, was adapted for application on sea lions (Anderson *et al.*, 2010; Figure 2.2). The original program was mostly automated, only requiring the user to manually choose three reference points (Anderson *et al.*, 2010). The region of whisker spot patterns in a photograph was automatically extracted and used to match individuals against a database. Due to low and variable contrast between the fur and whisker spots in *N. cinerea* (*N. cinerea* vary in fur colour between sexes as well as change fur colour when maturing; Walker and Ling, 1981), automated whisker spot extraction was not possible, so individual whisker spots were selected manually in the program (see Figure 2.3) for an example of whisker spot patterns).

Once the three reference points (inner corner of the eye, corner of the nostril, and outer end of the mouth; Figure 2.2) and whisker spot locations were marked on the photograph, the program standardised the location of the chosen whisker spot points by applying an affine transformation, such that the eye is located at spatial coordinate (0, 0), the nose is at (1, 0), and corner of the mouth is at (0.5, 0.5). These coordinate values serve to align the whisker spot patterns from different photographs (Figure 2.3). These reference points were chosen based upon their ease of distinction compared to other potential reference points.

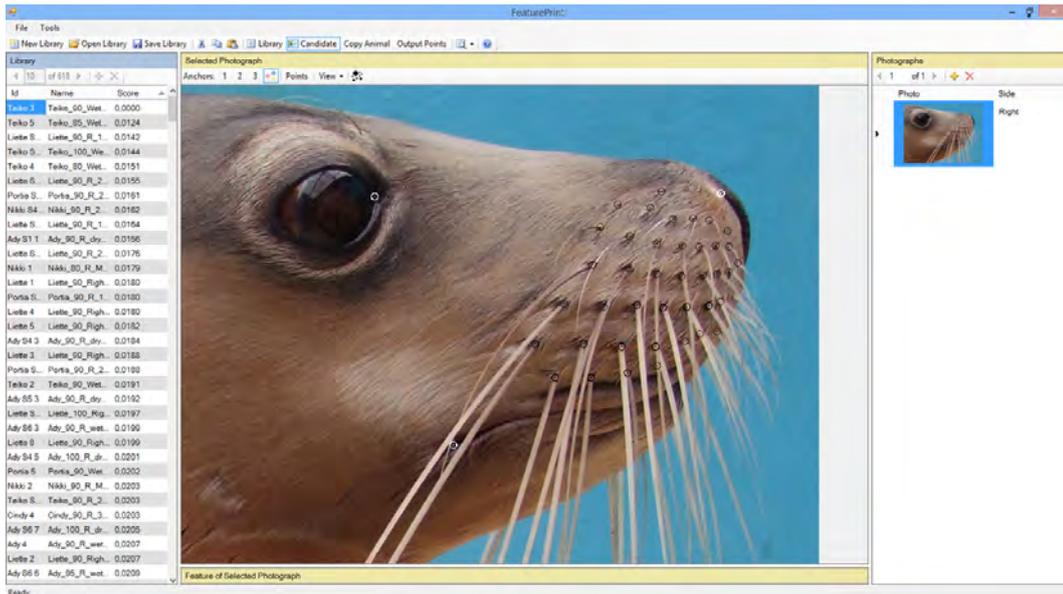


Figure 2.2: Adapted software interface to build a library and match whisker patterns using Chamfer distance transform. Whisker spots in the image are marked with black circles and reference points with white circles. The matching scores with other marked photographs are displayed on the left.

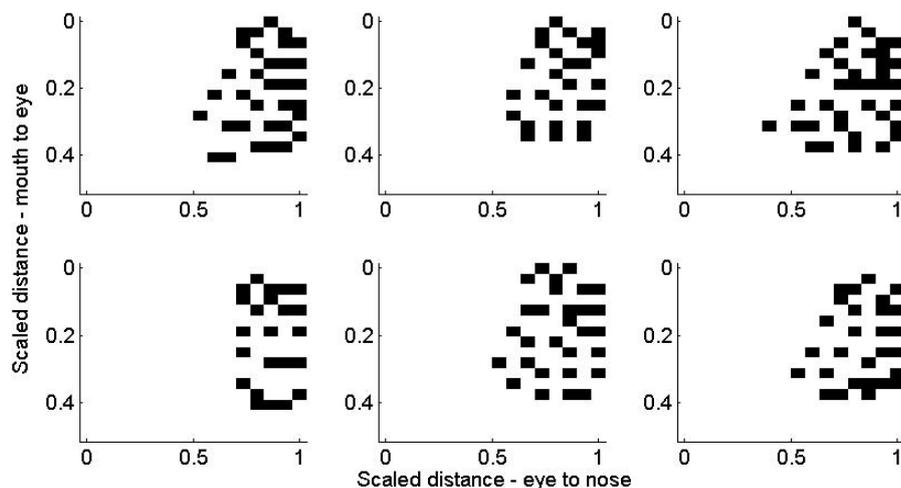


Figure 2.3: Example of marked cells where whiskers are present on grids overlaid over the muzzles of six captive individual *N. cinerea*. Black cells are where whisker spots are present and empty cells where spots are absent. The coordinate [0,0] is the position of the inner corner of the eye, and [1,0] the reference point on the nostril.

The overall methodology required four steps to prepare the data for reliability and matching tests. There were six additional steps for testing reliability of the patterns, and three additional steps for matching the whisker spot patterns (refer to Figure 2.4 for a flow chart). These methods for the additional steps are described below.

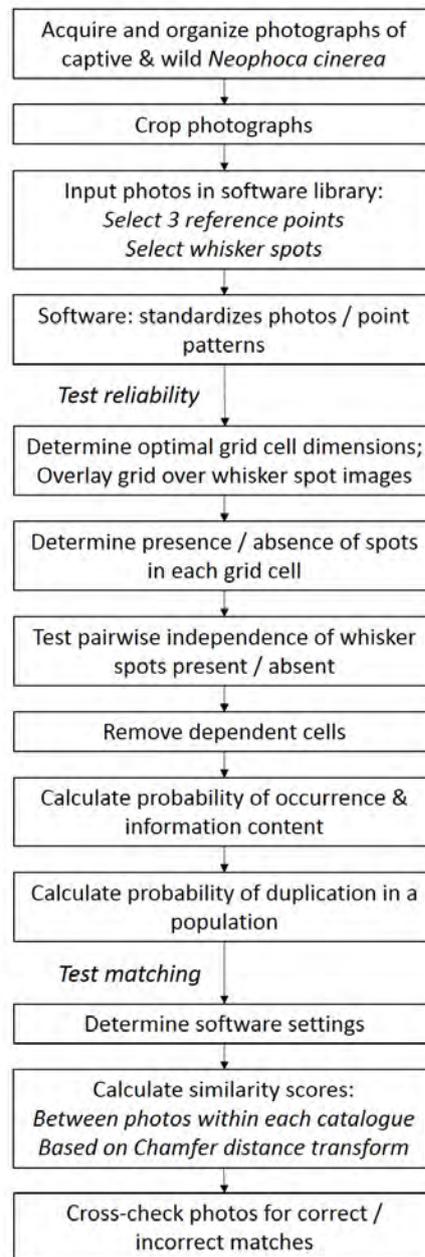


Figure 2.4: Flow chart presenting the entire process of testing the method of using whisker spot patterns for individual *N. cinerea* identification.

2.4.3 Variability of whisker spot patterns in *N. cinerea*

A set of 53 good-quality photographs, one each from 16 captive and 37 wild unique individual *N. cinerea*, were selected to determine whether individual whisker spot patterns were unique enough to reliably identify individual *N. cinerea* in a population. Assessing the variability of whisker spot patterns involved investigating spot locations relative to a normalised grid laid over the standardised photograph of the muzzle and identifying whether spots were “present” or “absent” in each of the cells within that grid. The first step was to select the dimensions of each cell in the grid. The grid cell height and width were chosen using the maximum vertical and horizontal distances, respectively, between the same whisker spots on multiple photographs of the same individuals. The greatest value for each of these two dimensions was taken from 23 photographs of 10 individuals. These individuals were selected because there were 2–3 high-quality photographs available of each.

The grid was applied to one photograph from each of the 53 individuals. The cells were then tested for pairwise independence of whisker spots being present/absent, and one of two dependent cells removed from the analysis (as per Pennycuick, 1978; Anderson *et al.*, 2007a, 2010). To test for mutual independence, the joint probability of two cells having a value of whisker spots “present” was compared to the independent probability of two cells having a value of whisker spots “present.” The probability of a whisker spot present in the cell was tested for each pair of cells. A set of events (such as the presence of whisker spots) is classed as mutually independent if the joint probability for every subset of events (cells) within the set is equal to the product of their individual probabilities (Anderson *et al.*, 2007a). The “joint probability” (called the observed) was calculated as the proportion of each of two adjacent cells having whisker spots present. The individual probability (called the expected) was calculated as the product of the two cell probabilities. Observed and expected probabilities were also calculated for cells having a value of “absent.” To test whether there was a significant difference between observed and expected probabilities, whisker spots for the sample were simulated 5,000 times based on their original probability distribution for the 53 individuals’ patterns. Once dependent cells were removed, the probability of occurrence and information content were calculated for each individual as per (Pennycuick, 1978) and (Anderson *et al.*, 2007a). First the frequency of whisker spot occurrence in each cell was calculated as $f_i = n_i/N$, where n is the number from the sample having a whisker spot in the cell and N is the number of individuals in the sample. The probability of occurrence

was taken as:

$$P = f_a \times f_b \times f_c \times \dots \times (1 - f_q) \times (1 - f_r) \times (1 - f_s) \times \dots, \quad (2.1)$$

where $a, b, c, \text{etc.}$ are cells with spots, and $q, r, s, \text{etc.}$ are cells without spots. The information content was calculated as $I = -\log_2(P)$. As simulations can vary between passes, calculations were conducted 50 times. The mean and standard deviations (SDs) from these calculations are presented.

The probability of duplication, that means that at most one individual has a specific whisker spot pattern, in population sizes of 50, 100, 500, and 1,000 were calculated based on the probability of occurrence of the spot pattern in the study population (as in Pennycuick, 1978 and Anderson *et al.*, 2007a; Table 2.3). This was calculated as:

$$(1 - P)^M + MP(1 - P)^{M-1} \quad (2.2)$$

where M is the number of individuals in a population and P is the probability of a particular pattern occurring in a population.

Table 2.3: The probability (P) of a spot pattern occurring once at most, calculated as: $(1 - P)^M + MP(1 - P)^{M-1}$ and the corresponding information content (I) for a range of population sizes (M).

Population size	Probability of single occurrence	Information content (bits)
50	$\pm 3 \times 10^{-3}$	>8.38
100	$\pm 1.49 \times 10^{-3}$	>9.39
500	$\pm 2.969 \times 10^{-4}$	>11.72
1,000	$\pm 1.4862 \times 10^{-4}$	>12.72

Code written in Matlab R2013a was used to carry out all analyses and produce all figures presented in the results.

2.4.4 Pattern recognition using Chamfer distance transform

Four catalogues of photographs were created from the complete library of 515 photographs of captive individuals to include only those pertinent for the four test scenarios. The ‘‘catalogues’’ consisted of matching photographs of the individuals taken on: 1) the same day at 90° (90 photographs), 2) the same day at different angles (70°, 90°, and 110°; 46 photographs), 3) the same day at 90° at 1- and 2-m

distances (28 photographs), and 4) different days (the 1st photo session, and 10, 30, 60, 180, and 360 days from the 1st photo session) at 90° angle (64 photographs; Table 2.4). An adaptation of the Chamfer distance transform (Borgefors, 1986) was used to compute the similarity score between two images based on the location of their whisker spots (point pattern). The similarity score between two standardised point patterns is calculated as follows: For each point in the 1st pattern, the Euclidian distance to the nearest point in the second pattern is calculated and distances then averaged. The same procedure is carried out in reverse. Both averaged distance scores are averaged together to produce a similarity score between the two point patterns where lower scores indicate higher similarity between two patterns. In addition, the algorithm calculates the similarity score many times, each time shifting one of the patterns by a small distance (chosen by the user), called the step size, and uses the smallest of these scores as the final similarity score. This “shifting” accounts for misalignments of point patterns caused by different facial angles of the animals. The software calculates the similarity score between the “candidate” *N. cinerea* being matched and every *N. cinerea* already in the database (or “library”). Users can cross-check the photographs visually to confirm or reject whether the candidate *N. cinerea* has been matched to one in the library.

2.4.5 Software settings and pairwise matching

Catalog 1 photographs (images from the same day at 90°) were used to determine the best software settings to maximise correct matching results and were then used for all catalogues. Boxplots of Catalog 1 with different settings were displayed to compare the distribution and the overlap of scores for matching and non-matching individuals. An offset (i.e., the “shifting” to account for misalignments of spots in different photographs of the same individual) of 0.07 and step size (i.e., how much a pattern is shifted during the matching process) of 0.005 resulted in the best similarity scores. Best similarity scores in this case mean less variation in score distributions and the least overlap in matching and non-matching scores. Pairwise matching was conducted between all photographs within each catalogue and provided the similarity scores for each scenario based on the Chamfer distance transform. The distributions of scores for correct and incorrect matches for each individual to all other photographs in the catalogues were compared using boxplots for each of the four catalogues.

Table 2.4: Sample sizes of photographs from 16 captive *N. cinerea* used for testing matches for photographs taken: 1) during the same day at 90°; 2) during the same day at 70°, 90°, and 110°; 3) during the same day at 1- and 2-m distances; and 4) during different sessions at 90°.

Name of individual	Same day at 90°	Same day at different angles	Same day at 1 and 2 m	Different days at 90°
Abby	3	3	2	3
Ady	5	2	-	6
April	4	3	2	5
Cindy	3	3	-	3
Lexie	14	3	2	6
Liette	5	3	2	4
Malie	11	3	2	6
Maxine	6	3	2	3
Miri	11	3	2	-
Miya	4	3	2	6
Nala	7	3	2	6
Nikki	2	3	2	3
Orson	6	2	2	4
Portia	5	3	2	3
Rocky	2	3	2	3
Teiko	2	3	2	4
Total	90	46	28	65

2.5 RESULTS

The grid size best suited to discriminating between individuals was found to be 0.0625 cell width and 0.025 cell height, and after testing for pairwise independence of whisker spots being present/absent (Figure 2.5), one of two dependent cells were removed from the analysis. Applying these to test the whisker spot variability and pattern recognition algorithm produced the following results.

2.5.1 Variability of whisker spot patterns in *N. cinerea*

“Dependent” cells were mostly located close to the nose. The cells with the highest probability of whisker spots being present were those close to the nose (between coordinates $x = 0.9$, $y = 0.1$, and $x = 1$, $y = 0.4$; Figure 2.6). Cells with the highest information content were those with lower frequencies of occurrence (Figure 2.6; Pennycuick, 1978). Once dependent cells were removed, 99.0% (± 1.5 SD) of spot patterns were considered reliable for a population size of 50 and 98.2% (± 1.7) for a population size of 100 (Figure 2.7). Reliability estimates dropped to 92.2% (± 4.7)

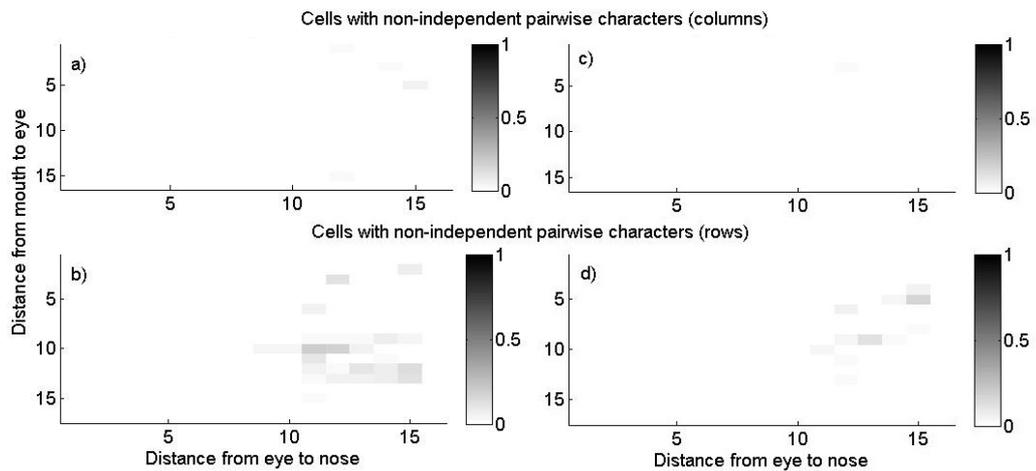


Figure 2.5: Pairwise probabilities of cells having whiskers present a) within columns (cells above and below each other) and b) within rows (cells right and left of each other). Pairwise probabilities of cells having whiskers absent c) within columns and d) within rows.

for a population size of 500, and 88.2% (± 5.7) for a population size of 1,000 (Figure 2.7).

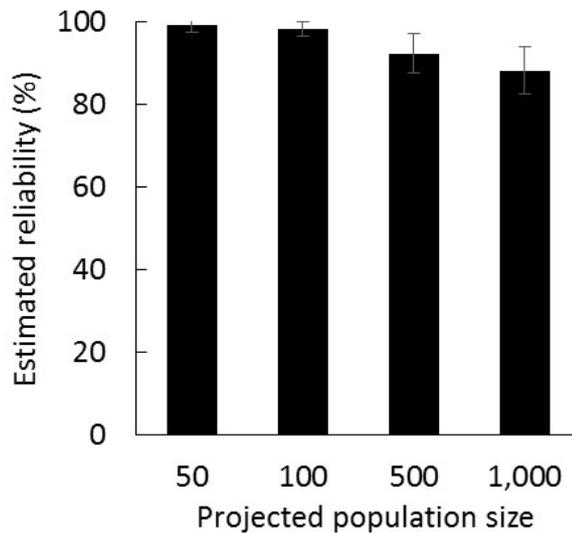


Figure 2.7: Percentage of reliable whisker spot patterns estimated from 50 repeated simulations for a population of 50, 100, 500, and 1,000 individuals, with SD (whiskers).

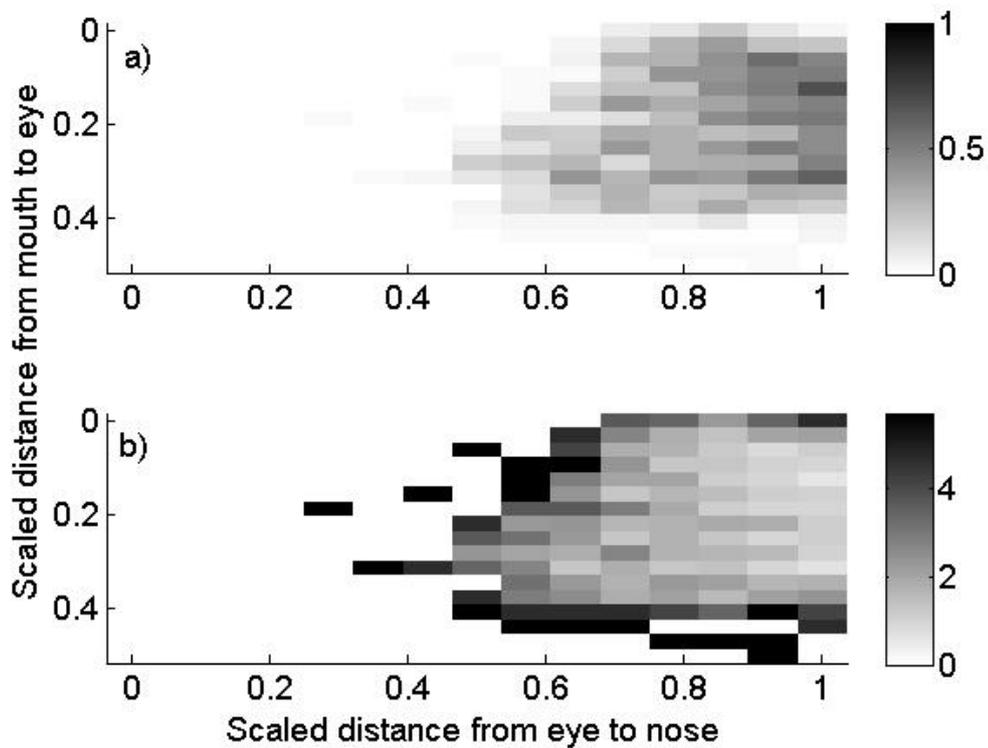


Figure 2.6: a) Frequency of occurrence and information content of whisker spots in grid cells after the removal of dependant cells, b) without removal of dependent cells, based on 53 individual *N. cinerea*, visually illustrating to the reader the locations on the muzzle where dependent cells were predominantly present.

2.5.2 Pattern recognition algorithm and application

Overall, most similarity scores calculated in the adapted software using the Chamfer distance transform were lower for photographs matched correctly than those matched incorrectly (Figure 2.8), where a lower score denotes a better match of two images (Figure 2.9). Similarity scores of pairwise comparisons of photographs of 16 captive animals (in zoos) taken on the same day at a 90° angle (scenario 1, Figure 2.8a) resulted in 90% correct matches. Eighty nine percent of photographs taken at 1- and 2-m distances at 90° were correctly matched (Figure 2.8c), whereas photographs taken from different angles had 48% correct matches (Figure 2.8b). Comparisons of photographs that were taken at different times over a year (at 90°) yielded 73% correct matches by the adapted software (Figure 2.8d). The percentage of correct matches over time did not appear to be related to the time period between photographs.

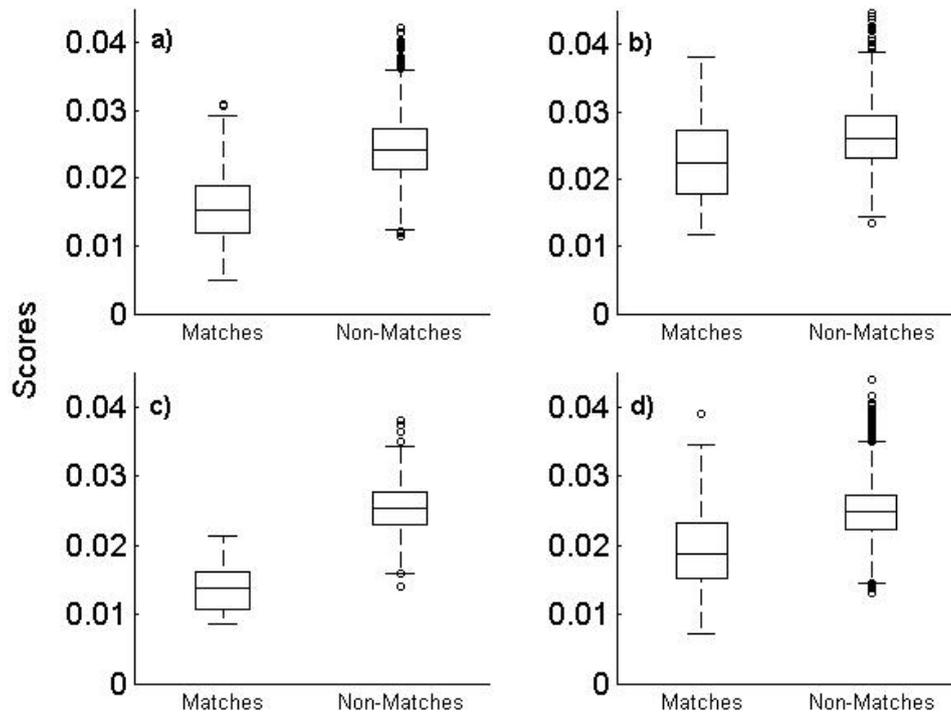


Figure 2.8: Box and whisker plots of averaged similarity scores of “matches” and “non-matches” of whisker spots of 16 individual captive *N. cinerea* for a) 90° angle, b) 70°, 90°, and 110° angles, c) 1- and 2-m distance at 90° angle, and d) 10, 30, 60, 180, and 360 days from the first session at a 90° angle. “Matches” include comparisons of different photographs of the same individuals, whereas “non-matches” are comparisons of photographs from an individual to those from all other individuals. The median is displayed as a black line, 25th and 75th percentiles as vertical boxes, and 90th percentiles as range bars, and outliers as black crosses.

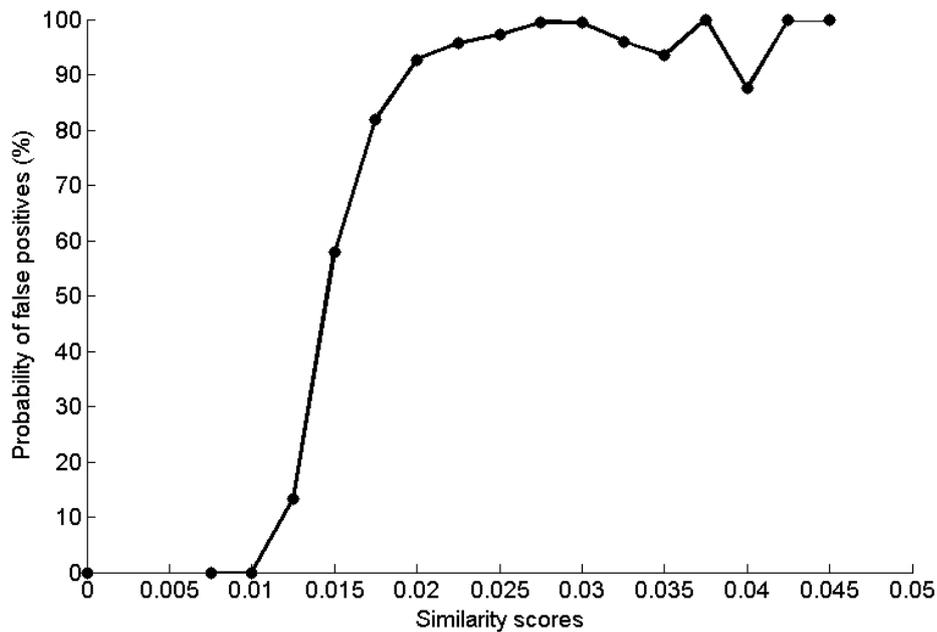


Figure 2.9: Probability of false positive matches based on the similarity scores of whisker spot patterns of 16 captive *N. cinerea* at a 90° angle ($n = 90$).

2.6 DISCUSSION

2.6.1 Variability of whisker spot patterns in *N. cinerea*

Based on the information content of whisker spot patterns calculated here, there is sufficient variability in *N. cinerea* for reliable matching in a relatively small population of 50 individuals. For populations of 1,000 individuals, the reliability estimates decrease and probability of duplication of a whisker spot pattern increases. In *U. maritimus*, whisker spot patterns were estimated to contain more information than in *N. cinerea* and populations of 1,000 individuals were estimated to be able to be matched with 99% reliability (Anderson *et al.*, 2007a). Our results are similar to the results estimated for variations in whisker spot patterns in *P. leo*, which were 92% reliable for a population size of 50 and 64% for a population of 1,000 (Pennycuik and Rudnai, 1970). Similarly, whisker spot patterns in leopards (*Panthera pardus kotiya*) were reliable for smaller populations. Out of 21, 19 had enough information at 95% reliability level, whereas only 15 out of 21 were reliably identifiable at 99% (Miththapala *et al.*, 1989). The main variable that can affect the estimated percentage of individuals considered to be reliable (having an information content above the minimum required for the study population size) is the cell size. For smaller

cell sizes, the information content increases, and so does the percentage of individuals considered to be reliable, since small differences in whisker spot positions can be detected (Pennycuick, 1978). However, if the angle at which the photograph is taken shifts significantly, error in correct whisker spot cell allocation increases significantly. We therefore used a cell size that was equivalent to the maximum distance between the same whisker spots photographed multiple times on the same individuals. Having done this, the authors recognise that there is an untestable assumption that the largest value of maximum distances between the same whisker spots on multiple photographs of the same individuals reflects the maximum shift in angle of photographs taken among different individuals.

2.6.2 Pattern recognition algorithm and application

Overall, from the photographs taken in a controlled environment (captive animals photographed by zoo keepers) on the same day, the Chamfer distance transform performed relatively well with 90% correct matches. The factor most affecting correct matching was the angle at which the photographs were taken, in agreement with Anderson et al.'s (2010) study which found that similarity scores increased (i.e., had poorer matches) with increasing deviance from an angle of 90°. A spot pattern technique to identify *A. jubatus* also performed significantly poorer when photographs were taken from different angles (Kelly, 2001). We suspect that the poorer performance (73% correct matches) of photographs taken during sessions 10, 30, 60, 180, and 360 days after the 1st session is likely due to slight variation in angles from which the photographs were taken. This result highlights the need for very good-quality photographs, taken at the same angle regardless of individual or location, when using this approach. As wild *N. cinerea* are difficult to identify without the use of invasive methods, in the wild, it was impossible to ground truth whether multiple photographs of the same individuals were taken over time. Thus, comparing photographs of an individual separated in time would be difficult and effects of growth could not be tested. *N. cinerea* in captivity for this study were already mature, thus testing changes in growth stage has not been possible. *N. cinerea* in controlled environments were photographed at 1- and 2-m distances to test this method with the highest quality photographs. Distance did not alter matching success compared to 90° photographs at the same distance. We believe that photographs taken at greater distances will not alter matching success when high-quality photographs focused on the muzzle are used. Wild individuals are not permitted to be approached closer than 5 m for safety reasons and to minimise human disturbance. Furthermore, photographs of captive *N. cinerea* were taken with cameras available to the respective

institute, whereas wild individuals were photographed with a 100–400 mm zoom lens, with greater performance over increased distances. The manual selection process of marking all whisker spots means that the matching process is slower than using the original design of the software on *U. maritimus* or *P. leo* (Anderson *et al.*, 2010). In matching through visual inspection, biases and error can be introduced by a person’s perception and level of experience (Oliveira-Santos *et al.*, 2010). Matching through visual inspection is also labour-intensive, can be expensive, and may be exposed to human error. The positive performance of the semi-automated processing illustrates that the software can decrease labour and improve cost efficiency. Verification of the semi-automated matching process could be conducted by laying a grid over whisker spot positions in matched photographs and comparing the grid locations of the whisker spots manually to confirm positive matches.

2.6.3 Application and recommendations

The approach using an adapted Chamfer distance transform has sufficient reliability to be applied to a small population size, when photographs are taken at 90°, without tilt, and are of high contrast and quality. However, we believe that keeping photographs taken at other angles and suboptimal quality photographs on record in the library may improve the chance of re-identifying an individual (Kelly, 2001; Hillman *et al.*, 2003; Arzoumanian *et al.*, 2005). Information content for pattern matching can be increased by adding other features to improve identification, such as forehead spot patterns in *P. pardus kotiya* (Miththapala *et al.*, 1989). Pinniped flipper shape and nicks can be individually specific and offer an additional feature for discriminating individuals. This was previously found to be the most useful feature in identifying Hooker’s sea lions (*Phocarcetos hookeri*; (McConkey, 1999)). However, a limiting factor in photographing all flippers of *N. cinerea* is their tendency to tuck them under the body or cover them with sand, thus this information was not collected. As photograph angle was the greatest cause of reduced matching success, we recommend exploring the effectiveness of the Groth algorithm for pattern matching as an alternative technique as for whale sharks (*Rhincodon typus*; (Arzoumanian *et al.*, 2005)). This approach compensates for distortion in patterns using geometric relationships between spots, similar to how astronomers identify star constellations and the position of stars in relation to other stars (Groth, 1986). In summary, this new technique for identifying *N. cinerea* can be used for small populations or resident communities. *N. cinerea* often occur in small colonies that are distant from each other (Goldsworthy *et al.*, 2008). In conjunction with capture–recapture models to estimate colony size, this method can be used for assess-

ment of localised habitat use and residency in localised areas. Determining the population or resident community size and their areas of use can then be fed into management and conservation of the species, in particular in allocating and defining management zones for high human use areas. The method also provides a way of monitoring these animals over long time periods without the need for capturing and invasively marking the animals. Finally, this point-pattern recognition application may also work for other otariid species.

2.7 ACKNOWLEDGMENTS

This project was made possible with the significant support provided by Adelaide Zoo, Pet Porpoise Pool in Coffs Harbour, SEALIFE (previously UnderWater World) in Mooloolaba, and Taronga Zoo in Sydney through the contribution of all captive *N. cinerea* photographs. Thanks goes to M. Perry for maintenance of the research vessel. The Department of Parks and Wildlife (Western Australia) provided substantial logistical support to and from islands as well as photographs of wild *N. cinerea*. P. Collins based at Department of Parks and Wildlife, Albany, organised several field trips off Albany and Esperance and kindly provided accommodation. The Department of Fisheries and Cockburn Volunteer Sea Search and Rescue contributed through transport to and from islands. The Fremantle Sailing Club provided support through boat ramp access. Many volunteers have allocated their time for considerable assistance in the field. A. Camacho assisted in project management. Wild Encounters Rockingham has contributed by disseminating information regarding the project to the public and has assisted with public outreach. Finally, this project was supported by funding from the Australian Marine Mammal Centre (2012/19) and the Western Australian Government's State NRM Program.

Chapter 3

Difficulties identifying Australian sea lions (*Neophoca cinerea*) in the wild using whisker spot patterns

Sylvia K. Osterrieder, Iain M. Parnum, Chandra Salgado Kent,
Randall W. Robinson

GRADUATE RESEARCH CENTRE

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book:	Difficulties identifying Australian sea lions (<i>Neophoca cinerea</i>) in the wild using whisker spot patterns Australian Mammalogy		
Surname:	Osterrieder	First name:	Sylvia
College:	College of Engineering & Science	Candidate's Contribution (%):	70
Status:			
Accepted and in press:	<input checked="" type="checkbox"/>	Date:	July 2016
Published:	<input type="checkbox"/>	Date:	

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

[Redacted Signature]	13.7.2016
Signature	Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and

5. The original data will be held for at least five years from the date indicated below and is stored at the following **location(s)**:

<p>Curtin University, Bentley, Western Australia Palmyra, Western Australia</p>

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Sylvia Osterrieder	70	Planning project, data collection, data analysis, writing and editing manuscript		13.7.2016
Iain Parnum	15	Supporting data analysis, code writing, review of draft		14.7.2016
Chandra Salgado Kent	10	Planning project, supervision on data analysis and manuscript preparation		14.7.2016
Randall Robinson	5	Supervision		14.7.2016

3.1 ABSTRACT

Individual identification is a beneficial tool in behavioural and ecological research. In mark-recapture studies, for example, it can improve abundance, residency and site fidelity estimates. Two non-invasive, photo-identification approaches, using whisker spot patterns, were tested to identify wild individual Australian sea lions (*Neophoca cinerea*) around Perth metropolitan waters. The Chamfer distance transform algorithm has shown promising results when applied to captive individuals. An alternative matching method using row/column locations of whisker spots, previously applied to lions (*Panthera leo*) was also tested. Resighting wild *N. cinerea* in this study proved unfeasible with both methods. Excessive variation between photographs of the same individual was found when applying the Chamfer distance transform, and similarity between photograph-pairs decreased with increasing time between photographs. Insufficient variation among *N. cinerea* row/column pattern was detected to successfully discriminate among individuals, averaging 39 mystacial spots (range 30-46, n=20) in seven rows and 9-10 columns. Additionally, different observers marking the same photographs introduced considerable variation. Colour difference (red, green and blue colour levels) between the whisker spots and surrounding fur affected marking spot locations significantly, increasing uncertainty when contrast decreased. While other pattern matching algorithms may improve performance, accurate identification of spot locations was the current limitation.

3.2 KEYWORDS

Pattern matching, photo-identification

3.3 INTRODUCTION

Ecological studies of animal residency, movement patterns, population estimates and demographics significantly benefit from identification of individuals within a population. For instance, re-identifying individuals allows animal behaviours and movements to be tracked over time. By obtaining a robust sample of these re-identifications (or resights) from a population, accurate population and demographic parameter estimates can be obtained using mark-recapture statistical approaches (Seber, 1982).

In species that have unique morphology or markings, such as scars or fur patterns, photo-identification has been a useful, non-invasive tool for identifying individuals (e.g. Ardovini *et al.*, 2008; Dixon, 2003; Gowans and Whitehead, 2001; Hiby and Lovell, 1990; Parra and Corkeron, 2001; Whitehead, 1990; Würsig and Jefferson, 1990; Würsig and Würsig, 1977). In species lacking such readily recognizable features, unique marks have been applied more invasively. These methods, including tagging or branding individuals, usually require capturing and handling animals to apply the marks (Troy *et al.*, 1997; Walker *et al.*, 2012) and can cause stress and a risk of infection of the marked area. In some species, however, less prominent features have been found applicable to use as alternative non-invasive marks to identify individuals. For instance, in lions (*Panthera leo*) and polar bears (*Ursus maritimus*), whisker spot patterns have successfully been used to identify individuals over long-term periods (Pennycuick and Rudnai, 1970; Anderson *et al.*, 2007a, 2010). Whisker spot patterns have also assisted in identifying individual leopards (*Panthera pardus kotiya*; Miththapala *et al.*, 1989 and Hooker's sea lions (*Phocarctus hookeri*; Beentjes, 1989).

Whisker spot patterns have been considered 'unique', having sufficient information to identify 92% of *P. leo* and 98% of *U. maritimus* of the populations examined (Pennycuick and Rudnai, 1970; Anderson *et al.*, 2007a). In *P. pardus kotiya*, 91% of examined individuals were distinguishable by their whisker spot patterns. However, using additional patterns in fur was suggested to increase the probability of distinguishing individuals (Miththapala *et al.*, 1989).

Two different approaches have been applied for species in which whisker spot patterns have been used for individual identification. In *P. leo*, the locations of whisker spots in the top row relative to those in the second row (on both sides of the muzzle) have been used (Pennycuick and Rudnai, 1970). The second row has

been used as a reference row to determine the location of the top whisker spots in its quadrants, which has been achieved by drawing the top two rows on gridded paper (Pennycuick and Rudnai, 1970; Kays and Patterson, 2001; Ogutu *et al.*, 2006). Similarly, the location of whisker spots in three rows in relation to a reference row have been used in *P. pardus kotiya* (Miththapala *et al.*, 1989). In contrast, no such consistent reference row is available in *U. maritimus*, and instead, all whisker spots available have been used for pattern matching. In fact, using all available whisker spots increased the information content available for identifying an individual (i.e. including all whiskers made the pattern more distinctive). The method used in *U. maritimus* is based on comparing whisker spot patterns, by standardising them using three reference points. Differences between two whisker spot patterns are then determined using a Chamfer distance transform algorithm, comparing the distances between standardised whisker spots within one pattern to those in another pattern. A distance score indicates how similar two patterns are to each other. The lower the distance score, the higher the similarity between two patterns, i.e. the higher the probability of a resighting (Anderson *et al.*, 2007a). Following this method, a recent study showed that in controlled conditions, using captive Australian sea lions (*Neophoca cinerea*), whisker spot patterns contain sufficient information for individual identification in small populations, and matching success was relatively high (Osterrieder *et al.*, 2015a). This method has, however, not been tested for re-identifying individuals in the wild.

N. cinerea is an endangered species with several populations decreasing, and is endemic to South and Western Australia. For many *N. cinerea* colonies, there is limited information on population trends and demographics (Goldsworthy, 2015). This is often insufficient for their effective long-term conservation. There are six haul-out islands in the Perth metropolitan area that are used only by male *N. cinerea*, predominantly outside the breeding season (Gales *et al.*, 1992). Little is known about the abundance, residency and movement patterns of *N. cinerea* residing in this area. The high variability of *N. cinerea* hauling out at a given time means that careful planning is required for count data to be used as abundance indices (Gales *et al.*, 1992; Osterrieder *et al.*, 2015b). Identifying individual *N. cinerea* would therefore be a valuable tool with which a better understanding of the subpopulation's movement and residency patterns can be gleaned to inform management and conservation strategies and improve their effectiveness.

The overall aim of this study was to apply and test the performance of the Chamfer distance transform method to identify and resight individual *N. cinerea* in the wild. This study also explored the potential of applying a method similar to that used in *P. leo* as an alternative, potentially simpler approach, based on row/column locations of whisker spots. The aims were achieved by: 1) mapping out the composition of whisker spot patterns from a sample of wild *N. cinerea*, 2) testing whisker spot pattern matching using the Chamfer distance transform and row/column mapping as a photo-identification technique for wild *N. cinerea* in the Perth metropolitan area, 3) identifying whisker spot occurrence of row/column locations and uncertainty in detection by a single observer, 4) quantifying the variation in detecting whisker spots among multiple observers, and 5) identify whether consistency in identifying the presence of spots among observers was associated with colour differences between whisker spots and surrounding fur.

3.4 MATERIAL AND METHODS

3.4.1 Study sites

Male *N. cinerea* are known to haul out on six islands in the Perth metropolitan area, Western Australia, between breeding seasons (Figure 1 in Osterrieder *et al.*, 2015a). Most islands are small and have less than 10 *N. cinerea* hauling out at one time (Department of Parks and Wildlife [DPaW], unpublished data). However, over 30 *N. cinerea* have been documented to haul out during peak periods on the two most used haul-out islands in this area: Seal (-32.29° S, 115.69° E) and Carnac (-32.12° S, 115.66° E) Islands (Gales *et al.*, 1992; Osterrieder *et al.*, 2015b). The relatively large numbers of *N. cinerea* on Seal and Carnac Islands provide a greater opportunity to obtain a relatively large photo-ID sample size (per unit effort) than at other islands. Therefore, Seal and Carnac Islands were selected as the study sites. The breeding islands in the Houtman Abrolhos Islands (ca. -28.7° S, 113.7° E) are approximately 60 km off Geraldton, Western Australia and approximately 400 km north-west of the haul-out islands in the Perth metropolitan area. The Houtman Abrolhos Islands were added as study sites to increase the sample size of whisker spot photographs of wild individual *N. cinerea*.

3.4.2 Collection and selection of photographs

Whisker spot photographs were taken during 35 field days in the Perth metropolitan area (16 days at Carnac Island and 19 at Seal Island, between 20th November 2013 and 6th June 2014), and during two field days at the Houtman Abrolhos Islands (the 9th and 10th April 2013). *N. cinerea* were approached with care to limit disturbance and were not approached closer than 5-10 m, which is the required minimum distance the public is to maintain according to guidelines (DEC, 2007; DEC and Fisheries, 2011). The lateral sides of the muzzles (90° to the camera's direction of view) were photographed as described in Osterrieder *et al.* (2015a). Lateral photographs of *N. cinerea* muzzles were taken at a range between five and approximately 50 m using a Canon EOS 550D with a 100-400 mm zoom lens (Canon, Tokyo, Japan). Beyond 50 m, the quality of photographs has been shown to be too poor to identify *U. maritimus* and used as a guide in this study (Anderson *et al.*, 2007a). Photographs were taken as close to 90° as possible, without tilt and of the left and right sides of the muzzles when possible. To aid in correctly identifying two photographs of an individual as a match (a resighting), photographs of the whole body of an individual were taken when possible in addition to the whisker spot photographs. These supplementary photographs may contain additional information for the individual, such as scarring, age and overall size.

To map out the general composition of whisker spot patterns, photographs of *N. cinerea*'s muzzles were chosen from a single field day, 15th February 2014, in the Perth metropolitan area to ensure that the same individuals were not re-sampled (unknowingly on different days). It was assumed unlikely that hauled-out (i.e. typically resting) *N. cinerea* would haul-out and travel between Seal and Carnac Islands, which are ≈19 km apart, within the 7 h timeframe that the photographs were taken. The 15th of February was selected because the largest number of individuals were photographed on Carnac and Seal Islands on that day. Similarly, photographs of *N. cinerea* at the Houtman Abrolhos Islands were added from only one day, 10th April 2013, to increase the sample size without resampling from the same location. It is not known whether *N. cinerea* move between Houtman Abrolhos Islands and the Perth metropolitan area. The distance of ≈400 km, between these study sites is relatively large. In addition, the Perth region consists only of what could possibly be a group (the males) of the population at the Houtman Abrolhos Islands. While the time between sampling dates of ≈10 months is relatively large (allowing time to travel between locations), the likelihood of the same *N. cinerea* being photographed twice in this study was assumed to be small.

Photographs were selected for analysis by removing poor quality photographs. Photographs that were poor quality were defined as those that were out of focus, taken at a different angle than the approximate 90° to the animal's muzzle, or were tilted. Whisker spots were not always clearly visible to be marked and confidence in the accuracy of marking was reduced accordingly. In extreme cases, such as a muzzle completely covered by sand, the photograph was discarded. In other studies, poor quality photographs have been shown to result in comparatively poor matching results due to low visibility of natural marks (Gowans and Whitehead, 2001; Kelly, 2001; Stevick *et al.*, 2001; Hastings *et al.*, 2008) - whisker spots in this case. For most *N. cinerea*, it was possible to photograph only one side of their muzzle, and therefore photographs of the side having the largest sample size were used.

The total number of individuals photographed was recorded on each field day, based on their haul-out locations and movements during the period of photographing. However, individual *N. cinerea*, were not distinguishable from each other on different field days by field personnel, and the total number of individuals over all field days was unknown. 'Individuals' referred to in the section below describing pairwise matching were the individuals photographed on any single day (which could be the same individual on a different day, but is considered a 'different individual' for describing methods for matching).

3.4.3 Application of the Chamfer distance transform method for identification

3.4.3.1 Preparation of photographs for matching using the Chamfer distance transform method

All procedures to prepare selected photographs for the matching process followed the description in Osterrieder *et al.* (2015a). Photographs were cropped in Adobe PhotoShop Elements 11 to include the required section of each image. Photographs were then added to a database ('library') in software previously used for whisker spot identification in *U. maritimus* (Anderson *et al.* 2010), and adapted for use for *N. cinerea* (Osterrieder *et al.*, 2015a). Three reference points, consisting of the inner corner of the eye, the corner of the nostril and the edge of the mouth, and all visible whisker spots were marked manually (Osterrieder *et al.*, 2015a). Where an exact spot location was difficult to determine, the corresponding whisker was traced back as close to the origin as possible to identify the whisker spots.

The software standardised all photographs to each other using the selected reference points, so that the eye was at a spatial coordinate of (0, 0), the nose at (1, 0) and the mouth at (0.5, 0.5) as in (Osterrieder et al. 2015a)

3.4.3.2 *Pairwise matching of photographs method*

The software calculates distance scores between pairwise matched individual photographs, based on a Chamfer distance transform (Anderson *et al.*, 2010; Osterrieder *et al.*, 2015a). The lower the distance score, the greater the similarity between two matched patterns. In this study, it was often possible to take a number of photographs of an individual in a single approach. Multiple photographs were taken to maximise the likelihood that one or more photographs met the required conditions of angle and tilt (Osterrieder *et al.*, 2015a). These photographs were all placed in a software library and all photographs taken that could be confirmed as being of the same individual (e.g. multiple approaches of an individual that had not moved throughout the day, or multiple photographs from a single approach to one individual) placed into an individual's folder. Each individual's photographs in the final library were pairwise matched with other individuals, producing a distance score for each comparison. The software tests two groups of photographs (all those of Individual A with all those of Individual B) and provides the best matching score (i.e. that with the lowest distance score) for the pair.

The same software settings used by Osterrieder *et al.* (2015a) were used for this study, which included an offset of 0.07 and step size of 0.005. The offset accounts for misalignments of whisker spots in different photographs by shifting spots from a pattern to align with spots of a pattern being matched to improve the match. The step size determines the amount a pattern can be shifted during the matching process.

3.4.3.3 *Thresholds to identify matching pairs of photographs using the Chamfer distance transform method*

Distance scores were calculated for photographs of individuals taken on a single day to obtain the range of scores for confirmed 'matches'. Distance scores from comparisons of photographs of different individuals on the same day were calculated to obtain the range of scores for confirmed 'non-matches'. Due to the non-normal distribution of the data according to a Shapiro-Wilk normality test, a Mann-Whitney U-test was used to test for differences of distance scores of confirmed 'non-matches' and confirmed 'matches'. These distance scores were also used to identify a threshold of scores that would likely represent correct positive matches. On the basis

of these scores, the probability of pairwise matches being correct matches or non-matches was calculated and plotted, and the distance score for 50% probability of a match estimated. Pairs with distance scores below the 50% probability (of not being a match; i.e. scores indicating more similar patterns) were considered ‘more likely’ to be true matches, and were selected for manual (visual) inspection to confirm or reject the match. The 50% probability threshold was selected to allow for a thorough manual verification of potential pairs that exhibited a higher probability of being a match. As a preliminary stop-go test, the manual checks were conducted only on pairs more likely to be a match. It was deemed that if the method was not successful in matching individuals with scores $\geq 50\%$ probability of being a match, it would be less likely to be successful using the remaining photographs. Scores larger than the threshold were discarded as they were more likely to be false matches.

3.4.3.4 Variability between photographs of the same individual using the Chamfer distance transform method

To assess variability among sequences of photographs of the same individual, the distance scores of pairwise matched photographs of a single individual taken throughout the day were compared. This allowed the investigation of variability between photographs taken within short (within seconds) succession, to assess the impact of re-composition (altering position or orientation of either camera or subject and potentially light conditions) on the matching performance.

3.4.3.5 Manual verification of photographs to identify correct matches

Two steps were used to manually compare individual *N. cinerea* and their patterns, which resulted in distance scores below the 50% probability threshold (described above). For both steps selected photographs of individuals were compared by manual inspection by an observer, and identified as the same or different individual based on qualitative evaluation. If identified as the same individuals, then this match was considered a resight.

1. Firstly, photographs were searched for little marks, spots and scars (other than whisker spot patterns), and identifying features compared across photographs. Age variation of individuals was also compared between photographs, and if the age variation was too large for the time difference between the photographs, these were considered as different individuals (i.e. an individual photographed as an adult cannot be photographed as a juvenile a few months later). Male *N. cinerea* change their fur colour when maturing which therefore

gives an indication of the individual's age. The age category adult, sub-adult or juvenile was determined based on fur colour and size of an animal (Gales *et al.*, 1992; Jefferson *et al.*, 2011; Osterrieder *et al.*, 2015b) and the date and location of the sighting was documented.

2. Whisker spot patterns were compared through manual inspection, by plotting the coordinates of the whisker spots of matched photographs on top of each other in different colours on a gridded space. In addition to this, whisker spot patterns were compared by comparing cells used for estimating information content in whisker spot patterns by Osterrieder *et al.* (2015a). Grids with 0.0625 cell width and 0.025 cell height were previously determined to be the best fitting grid size for this method and species (Osterrieder *et al.*, 2015a).

Following manual verification of 'Matches' (confirmed resighting), 'Non-matches' (confirmed rejections) and 'Unconfirmed matches' (no confirmed resightings or rejections), a Mann-Whitney U-test was used to test for differences of distance scores between these categories. Additionally, comparisons of distance scores were conducted between individuals with wet and dry muzzles. Mann-Whitney U-tests (exact distribution method in cases of small sample sizes) were applied to test for variations in distance scores of 'Matches', 'Non-matches', and 'Unconfirmed matches' in pairs of wet-wet, wet-dry and dry-dry pairs of photographs. Mann-Whitney U-tests were applied due to the non-normal distribution of the data according to a Shapiro-Wilk normality tests.

3.4.4 Identification using row-column locations

3.4.4.1 Processing of photographs, analysing occurrence of whisker spots

All visible whisker spots on the selected photographs were manually marked in Adobe PhotoShop Elements 11. Lines tracing the rows and columns of whiskers were added to facilitate allocation of whisker spots to rows and columns (Figure 3.1). This was carried out by manually fitting a line through the points in which the spots most closely aligned. The average and standard deviation, as well as minimum and maximum numbers of whiskers occurring in each row and column was then calculated, and a general schematic of the composition of whisker spot patterns (i.e. in rows and columns) was drawn based on these results.

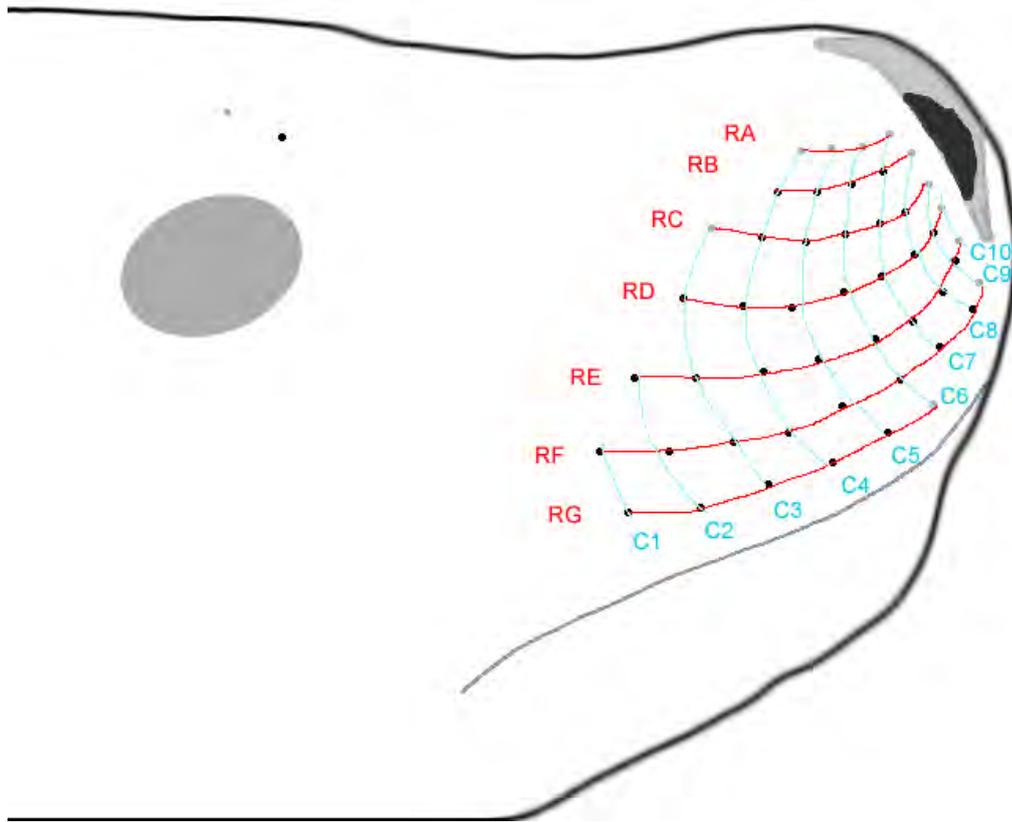


Figure 3.1: General whisker spot sketch based on 20 individual wild *N. cinerea*, ordered from row A (RA) to G (RG) and columns 1 (C1) to 10 (C10). Black spots indicate those present in >80% of photographs, grey spots indicate those in 30-80% of photographs. Rows A to G are illustrated by red lines and Columns 1 to 10 by light blue lines. Whiskers may also occur above the eye.

In addition to documenting the presence and absence of whisker spots at each row/column location (e.g. presence/absence of a spot in row A, Column 2; Figure 3.1), a qualitative score was allocated based on whether there was uncertainty as to whether a row/column location contained a spot. Uncertainty in a spot's presence occurred as a result of spots being hidden by fur and the corresponding emerging whiskers being thin and short, or whiskers overlapping and masking the spots and whiskers being evaluated (Osterrieder *et al.*, 2015a). In cases where presence or absence of a spot or whisker could not be definitively determined, it was marked as an uncertain.

The frequency of individuals having spots at each row/column locations was quantified to identify areas of consistency and variation among individuals. The frequency was mapped as the percentage of whisker spots occurring at each row/column location of the total photographs evaluated. The frequency in the uncertainty of

determining the presence of whisker spots at each row/column location was also mapped to identify areas of high certainty. A cross-correlation was conducted to pair-wise compare all different patterns to determine differences and matches between all whisker spot patterns. Cross-correlation was also carried out after removing all locations showing an arbitrary chosen value of >10% uncertainty of a row/column location showing a spot.

3.4.5 Observer variability

Observer bias has been shown to affect the results of photo-identifying individuals (Oliveira-Santos *et al.*, 2010). It is reasonable to assume, however, that multiple observers in various studies over time would apply this photo-identification method if this approach of whisker spot pattern matching proved useful. Therefore, different operators were asked to select all whisker spots in selected photographs (selection criteria described below) to examine the consistency in marking the spots. The lead observer in the overall study, plus five experienced marine mammal observers marked all visible whisker spots on each of 10 selected photographs (independently from each other). Photographs included varying whisker spots and fur colouration; animals at different stages of maturation; wet and dry muzzles, and two images of a single individual taken at different times (the photographs were marked randomly, and those of the same individual over time were not marked immediately after each other). As training, the observers were supplied one additional photograph, prior to marking the following images. All observers had a minimum of three years' experience working in marine biology projects and three had been involved in photo-identification of other marine mammals previously.

3.4.6 Analysing uncertainties of marking whisker spots

N. cinerea possess different fur colours between sexes, and while both sexes change fur colour as they mature from pups to juveniles, males also change fur colour as they progress from juveniles to adults. As a result, the difference between the colour of the whiskers, whisker spots, and fur vary depending upon the age and sex of the animals. In comparison, *U. maritimus* and *P. leo* exhibit light fur colours and dark whisker spots throughout their lives and between sexes.

To determine the impact of fur colour in selecting whisker spots, the colours (Red, Green and Blue) of whisker spots were compared to surrounding fur. There are various metrics that could be calculated to quantify the contrast. The authors chose to do this by calculating the Root-Mean-Squared (RMS) difference of the

Red (R), Green (G) and Blue (B) levels between the ‘spot’ and surrounding ‘fur’. Calculating the RMS of the RGB levels to quantify contrast has been used successfully in various applications (such as in Pallottino *et al.* (2010) to determine hazelnut peeling). This study used the following implementation, Equation (3.1):

$$RMS = \sqrt{\left(\frac{1}{3}\right) \sum (R_{spot} - R_{fur})^2 + (G_{spot} - G_{fur})^2 + (B_{spot} - B_{fur})^2} \quad (3.1)$$

Where R_{spot} is the mean value of the red component (0-255) of the spot and R_{fur} is the mean value of the red component of the fur, with the same principle applied to green (G_{spot} and G_{fur}) and blue (B_{spot} and B_{fur}) components. The mean levels were calculated from user defined areas of the spot and surrounding fur. The user attempted to define a similar ratio of spot area to surrounding fur area throughout the markings to limit bias. The spot itself was used as a mask to exclude those pixels in the calculation of the RGB mean levels of the fur. To calculate the RGB mean levels, six whisker spots were chosen in a stratified approach from each of the 10 photographs marked by six observers. The rationale of the stratified approach was to choose three of these whisker spots marked by five to six observers and three spots marked by one to four observers. To ensure that pooling of whisker spots marked by one to two and three to four observers was validated, a Mann-Whitney U-test was performed, and groups not significantly different from each other were subsequently pooled. The RMSs of the RGB mean levels were then compared by applying a Mann-Whitney U-test between whisker spots marked by most or all observers with those marked by low number of observers. The Mann-Whitney U-test was used due to the non-normal distribution of most groups according to a Shapiro-Wilk normality test applied beforehand. Where possible, spots for colour extraction were chosen if no other whisker was overlaying the area and the whisker spot was clearly visible. Where these spots were not available the pixels comprising overlaying whiskers were included in the analysis as this contributed to the variation in RGB levels that the observer used to discriminate between to identify the whisker spot.

All analyses were carried out and figures produced in Matlab R2014a. This work was conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth).

3.5 RESULTS

3.5.1 Composition of whisker spot patterns

Photographs of a total of 76 *N. cinerea* were selected to determine the general composition of whisker spot patterns; including 44 *N. cinerea* from the Houtman Abrolhos Islands, 17 from Seal Island and 14 from Carnac Island. A larger number of good quality photographs were available of the right side of *N. cinerea*, and therefore were selected for analyses. Of the photographs of the right side of the animals, good quality photographs of whisker spot patterns were available from a total of 20 individuals: eight from Houtman Abrolhos, four from Seal Island, and eight from Carnac Island.

The general whisker spot pattern composition of *N. cinerea* consisted of seven distinct rows of mystacial whisker spots (rows A to G in Figure 3.1), ordered in nine to 10 columns (columns 1 to 10, Figure 3.1). Some individuals also had whiskers above the eye. Based on 20 individuals, the mode of whisker spot numbers in *N. cinerea* was 40, ranging from 30 to 46, and on average 38.8 (± 3.4 SD). There were between zero and two whiskers above the eye, called superciliary whiskers.

3.5.2 Application of the Chamfer distance transform method for identification

A total of 2884 photographs of *N. cinerea* were taken for individual identification between November 2013 and June 2014; 1171 on Carnac Island and 1713 photographs on Sea Island, to test the application of spot pattern matching in wild *N. cinerea* based on the Chamfer distance transform algorithm (Osterrieder *et al.*, 2015a). As before, a greater number of individuals were photographed from the right hand side. Therefore, only photographs taken from this side were considered for matching. A rigorous selection due to the stringent criteria for photographs, to be at 90° without any tilt, for successfully matching captive individuals (Osterrieder *et al.*, 2015a), resulted in a library with 135 photographs of 66 *N. cinerea*. Of these, 25 individuals were photographed on Carnac Island and 41 on Seal Island. Multiple photographs existed for 45 individuals, with up to five photographs for each of these.

3.5.2.1 Identifying similarity thresholds for matches

Distance scores varied significantly ($Z = 21.8697$, $p < 0.001$) between multiple photographs of the same (confirmed match, number of scores = 332) and different (confirmed non-match, number of scores = 1080) individuals taken on the same day (Figure 3.2). Comparison of these distance scores resulted in an approximate 50% probability of a false positive match (a score at which there is an approximately equal probability of the two whisker spot patterns coming from the same individual as from two different individuals) at a score of 0.02 (Figure 3.3). Therefore, all compared photographs with distance scores ≤ 0.02 were manually checked to identify whether they were a correct match and considered to be of a resighted individual.

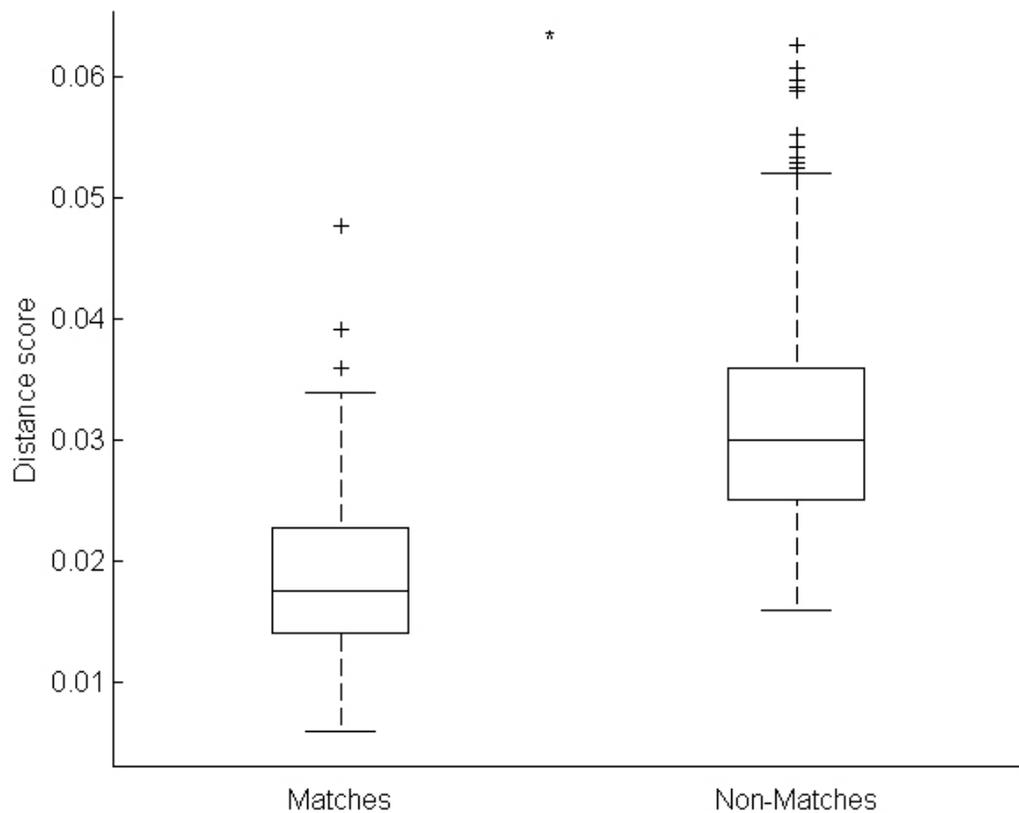


Figure 3.2: Box-whisker plots of the distance scores of confirmed ‘matches’ (same individual, number of scores = 332) and confirmed ‘non-matches’ (different individuals, number of scores = 1080) of whisker spot patterns photographed on the same day. The median is shown as a black line, 25th and 75th percentiles as vertical boxes, 90th percentiles as range bars, and outliers as black crosses.

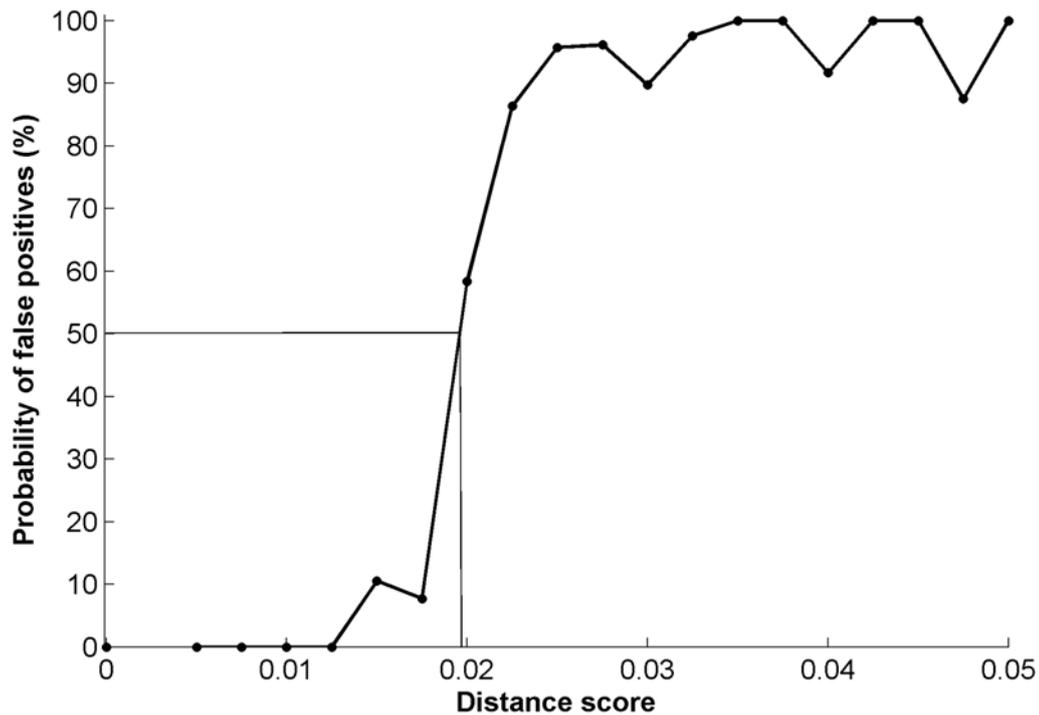


Figure 3.3: Probability of confirmed matches and confirmed non-matches based on the distance scores of whisker spot patterns calculated using the Chamfer distance transform algorithm. Distance scores of pattern comparisons are based on multiple photographs of an individual taken on a single day and of known different individuals on the same day ($n = 135$ photographs of 66 individual sea lions).

3.5.2.2 *Variability between photographs of the same individual*

Distance scores ($n = 105$) of multiple photographs of the same individual (49 individuals) resulted in an average score of 0.01747 ($SD \pm 0.006434$). These distance scores varied considerably, also when two matched photographs of the same individual were taken within a few seconds of each other (Figure 3.4; higher scores mean they were less similar). The high variability in scores, with several scores exceeding the threshold of 0.02, occurred up to a time difference of 50 s between tested photographs, after which the sample size was very small and therefore not presented ($n = 10$ after 70 s, max. duration between photographs 23.48 min). Although taken within short succession of each other, distance scores of >0.02 between photographs occurred throughout the entire period of 70 s, showing that an individual cannot be identified reliably in the wild (Figure 3.4).

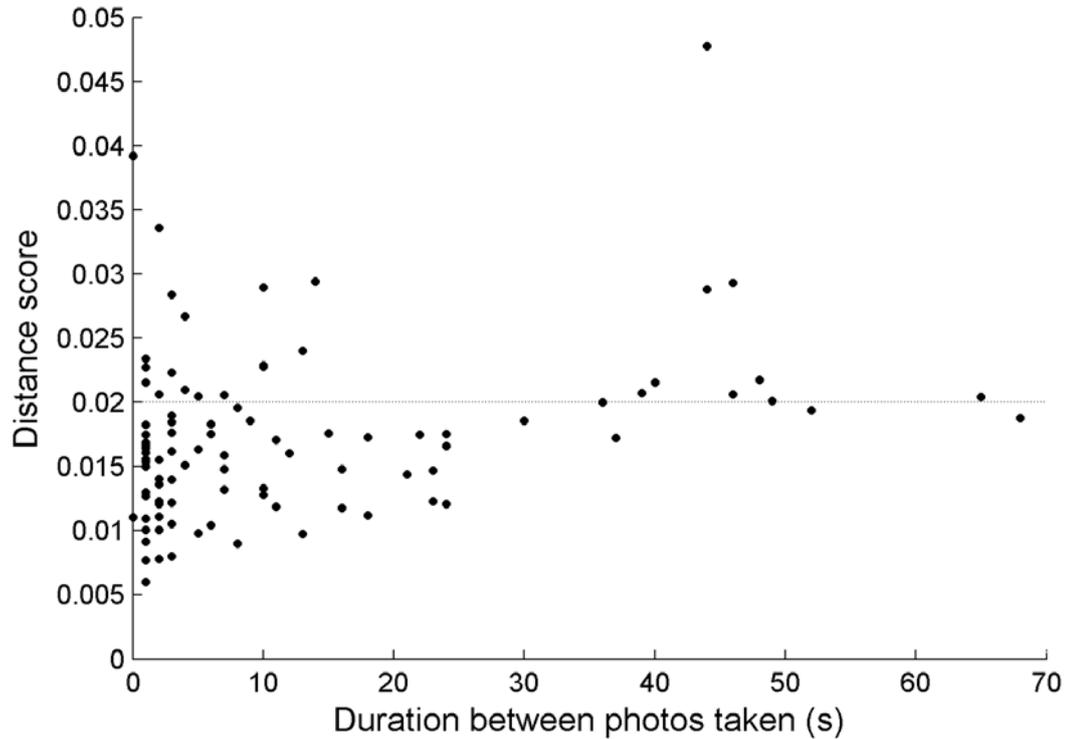


Figure 3.4: Distance scores of whisker spot patterns between pairs of photographs of the same individuals taken within 70 s of each other. 10 scores beyond 70 s were excluded from the figure for display purposes (four data points around 150-250 s, four data points around 800 s, and two data points around 1400 s).

3.5.2.3 *Manual verification*

Out of the total 66 tested individuals, 128 comparisons involving 38 individuals (unconfirmed matches or unconfirmed non-matches) had distance scores ≤ 0.02 , with up to 19 potential matches (i.e. resightings) per individual. After manually (visually) inspecting these 128 comparisons to verify or reject the match (example in Figure 3.5), 82 of the matches with a distance score of ≤ 0.02 were rejected and classed as confirmed ‘non-matches’. Rejections were mainly based on the age gap of the individuals matched being impossible (e.g. a bull being resighted as a sub-adult). There were also a few individuals photographed on the same day and documented as a different individual. Scars large enough to be easily noticed were rarely seen and scars were therefore not helpful for manual verification of potential matches. None of the potential matches, based on their whisker spot patterns, from different days that were visually inspected could be confirmed as a ‘match’ (i.e. a resighting). Therefore the remaining 46 matches could neither be confirmed nor rejected as resightings, and were classed as ‘unconfirmed’ matches (example in

Figure 3.6). The 10 lowest distance scores ranged from 0.0147 to 0.0162, and resulted in five confirmed non-matches and five unconfirmed matches. The plotted patterns, neither as filled cells in a grid nor as dots on top of each other, supported a clear decision as to whether a *N. cinerea* had been sighted previously (Figure 3.6, example of a 'match' and 'non-match' in Figures 3.7 and 3.8). The distance scores of rejected matches, i.e. confirmed non-matches, were similar to the scores assigned to unconfirmed matches (Figure 3.9, $Z = 0.3358$, $p > 0.05$). The confirmed non-matches and unconfirmed matches plotted as cells or spots on a scaled grid proved too variable to decipher the pattern visually to be able to confirm a resighted individual.

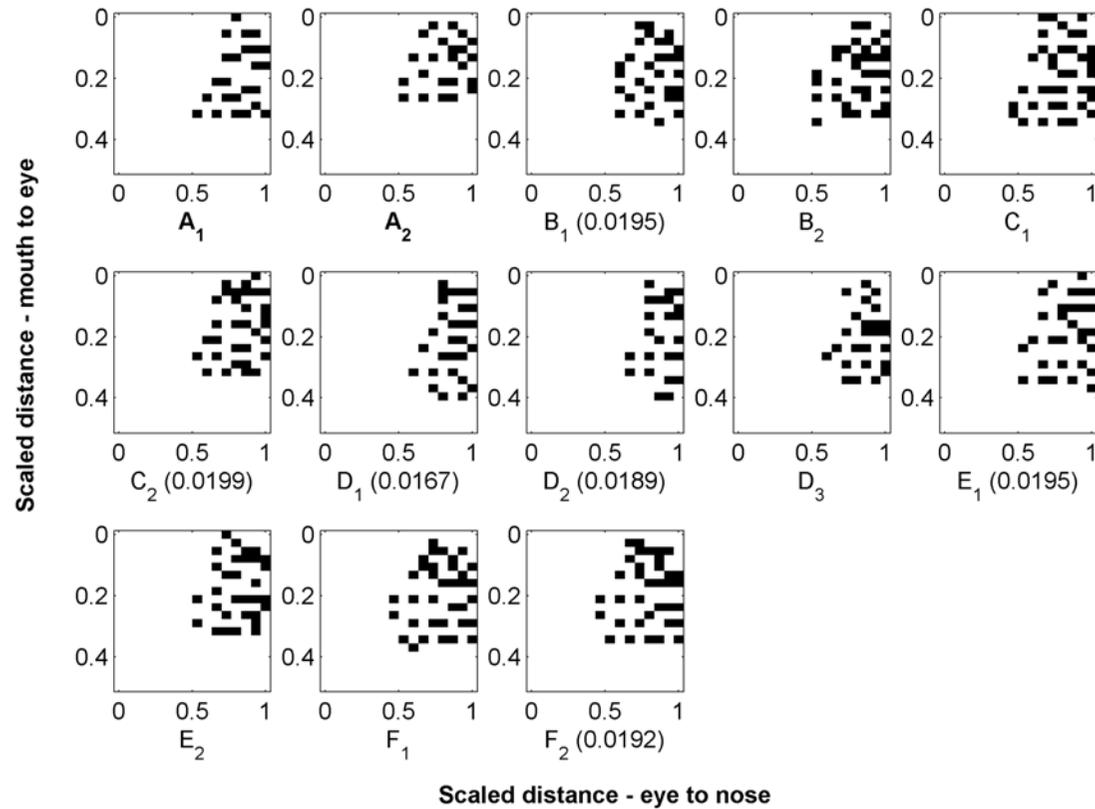


Figure 3.5: Standardised whisker spot patterns of individuals A – F, index numbering the good photographs available for each individual. Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Example of individual A (bold) pairwise matched with all individuals in the final library. B – F are individuals, each returning at least one spot pattern matched with a distance score ≤ 0.02 (numbers in parentheses show distance scores to A₁, the lowest score resulted for the combination of individuals). Panels without distance scores show spot patterns of other photographs available to aid the matching process. Individuals C and E are unconfirmed matches and individuals B, D, and F confirmed non-matches with individual A (based on age classes). Only spots between reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth 0.5,0.5] are shown.

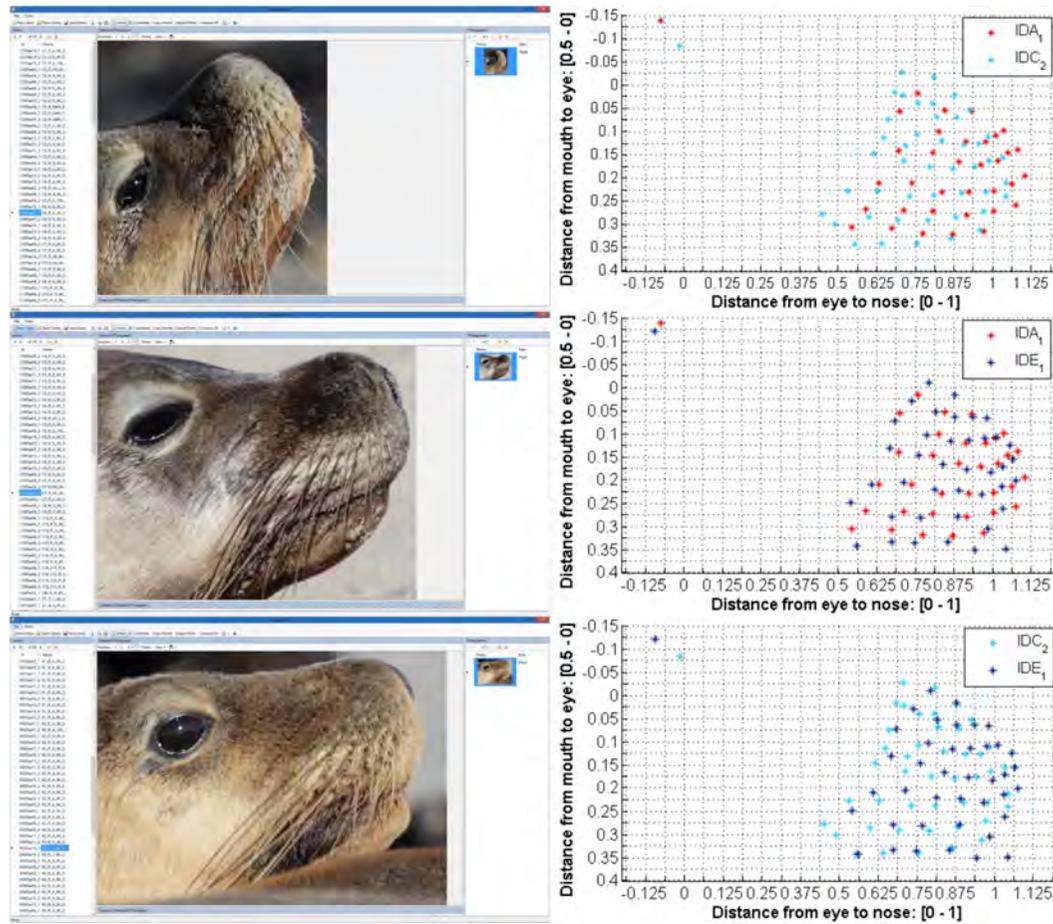


Figure 3.6: ‘Unconfirmed’ matches of A_1 (top photograph) with C_2 (middle photograph) and E_1 (bottom photograph) after visual inspection of all potential matches (distance score ≤ 0.02) in the library (Figure 3.4). Standardised spot patterns were plotted on top of each other to aid visual comparison of A_1 and C_2 (top grid) and A_1 and E_1 (bottom grid), including spots beyond the reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5].

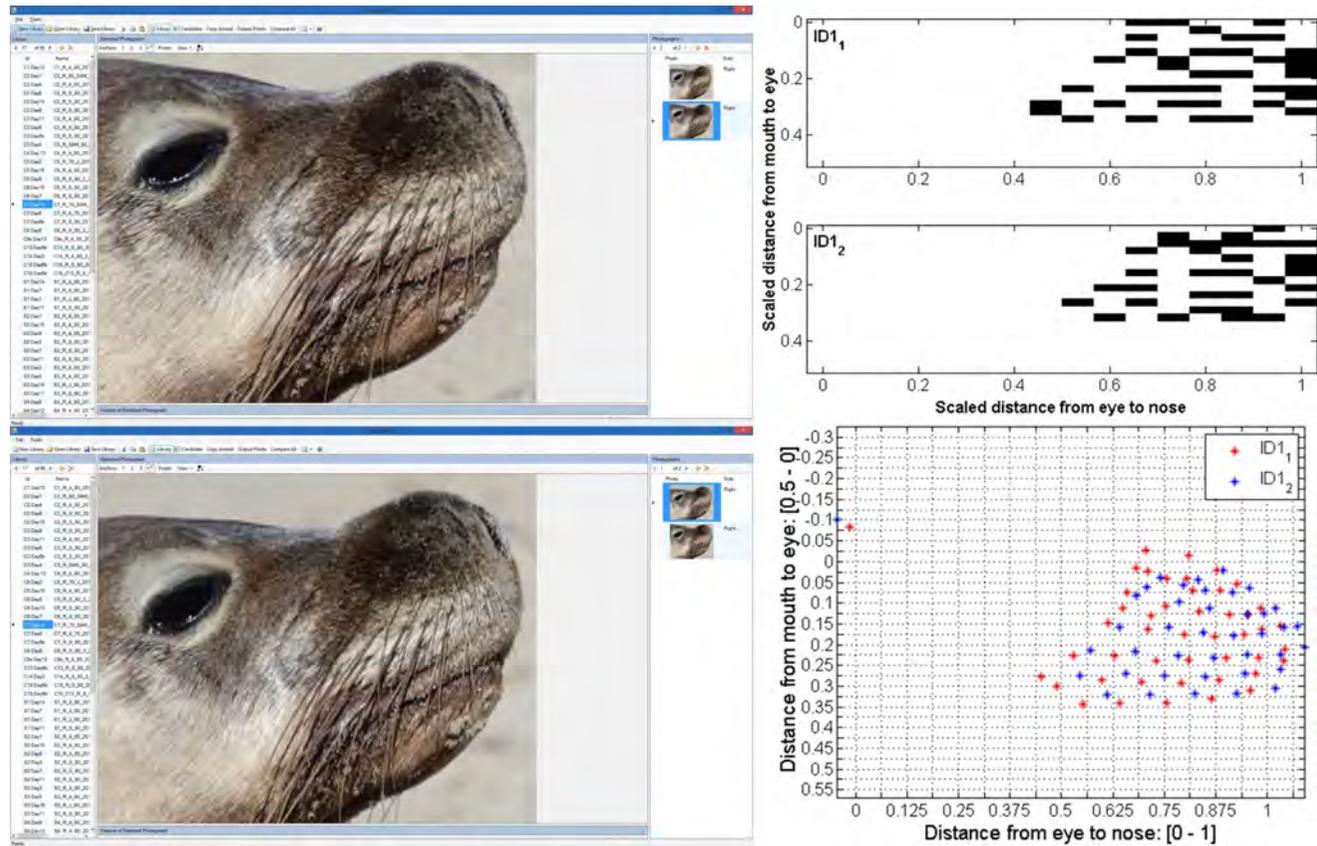


Figure 3.7: Example of a confirmed match of two photographs of the same individual taken 1 s apart with a distance score of 0.0182 (top, ID₁ and bottom, ID₂, photographs). The standardised marked cells of ID₁ and ID₂ indicate where whiskers are present on grids overlaid over the muzzle (top right). Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Only spots between the reference points are displayed. The bottom right grid shows the standardised spot patterns of ID₁ and ID₂ plotted on top of each other, including spots beyond the reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5].

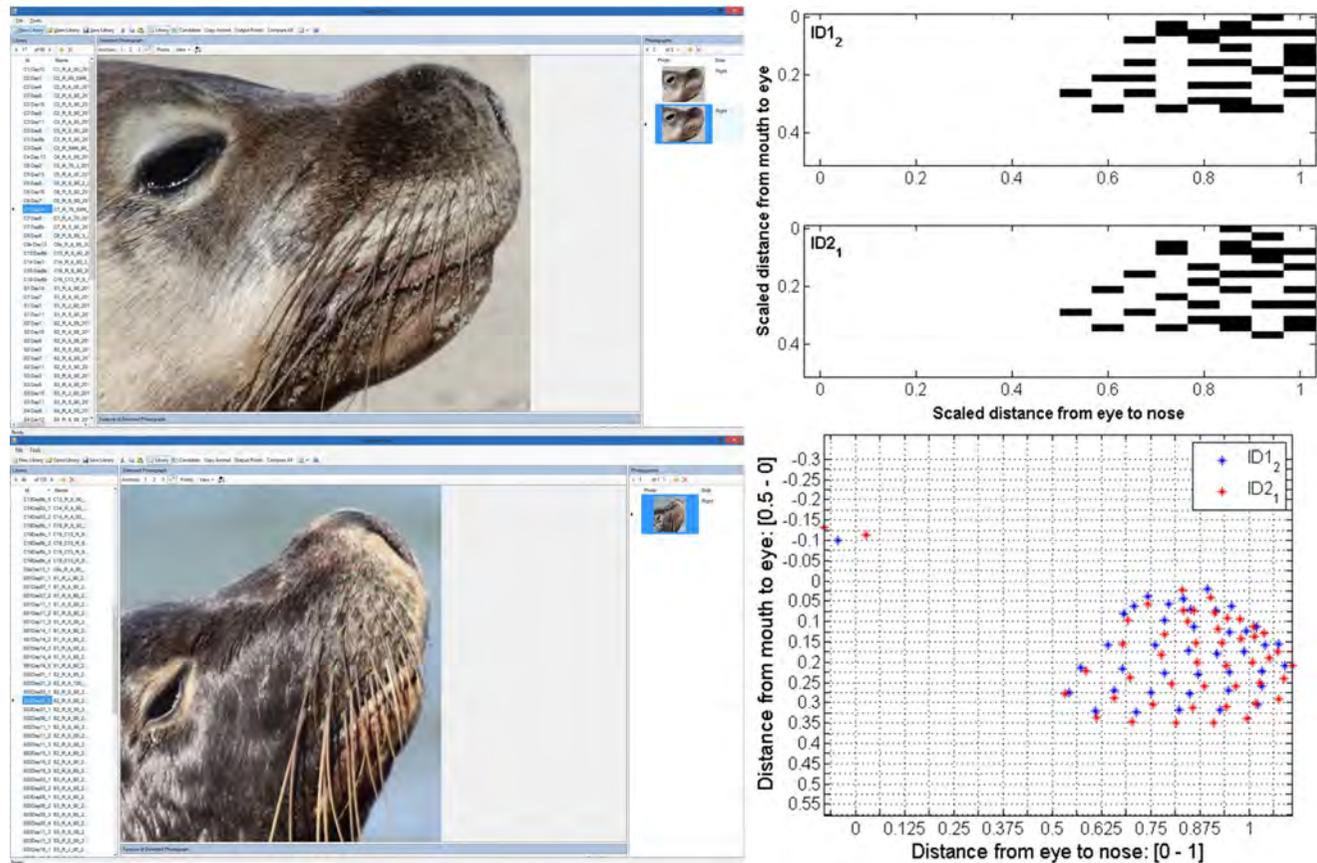


Figure 3.8: Example of a confirmed non-match of two photographs of different individuals with a distance score of 0.0188 (top ID₁₂ and bottom ID₂₁ photographs). The standardised marked cells of ID₁₂ and ID₂₁ indicate where whiskers are present on grids overlaid over the muzzle (top right). Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Only spots between the reference points are shown. The bottom right grid shows the standardised spot patterns of ID₁₂ and ID₂₁ plotted on top of each other, including spots beyond reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5].

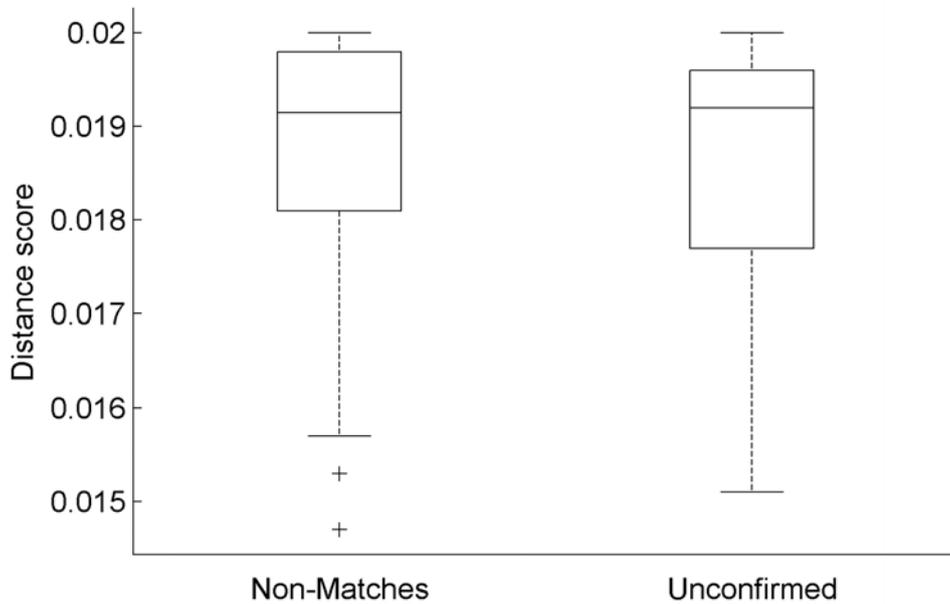


Figure 3.9: Box-whisker plots of the distance scores of ‘Non-matches’ (confirmed rejections) and ‘Unconfirmed’ (no confirmed resightings or rejections) of whisker spot patterns with distance scores of ≤ 0.02 . The median is shown as a black line, 25th and 75th percentiles as vertical boxes, and 90th percentiles as range bars, and outliers as black crosses.

3.5.2.4 Variability between photographs of wet and dry individuals

The library to test the effects of whether the individual had wet or dry fur included photographs of 18 wet individuals of which nine individuals resulted in 66 distance scores of ≤ 0.02 when compared in pairs. These matches included 14 pairs of both wet individuals and 52 pairs of which one individual was wet and the other dry. 10 of the wet-wet matches could be confirmed as non-matches and four resulted in unconfirmed matches, whereas of the wet-dry comparisons, 38 pairs were confirmed non-matches and 14 pairs unconfirmed matches (Figure 3.10). Pairwise comparison of dry individuals resulted in 62 matched pairs with distance scores ≤ 0.02 , of which 36 were non-matches and 26 pairs remained unconfirmed matches. The variation of the distance scores was similar amongst each group of comparison of wet-wet ($W = 34.0$, $p > 0.05$, exact distribution method due to small sample size), wet-dry ($Z = 0.1654$, $p > 0.05$) and dry-dry ($Z = 0.5434$, $p > 0.05$) individuals (Figure 3.10).

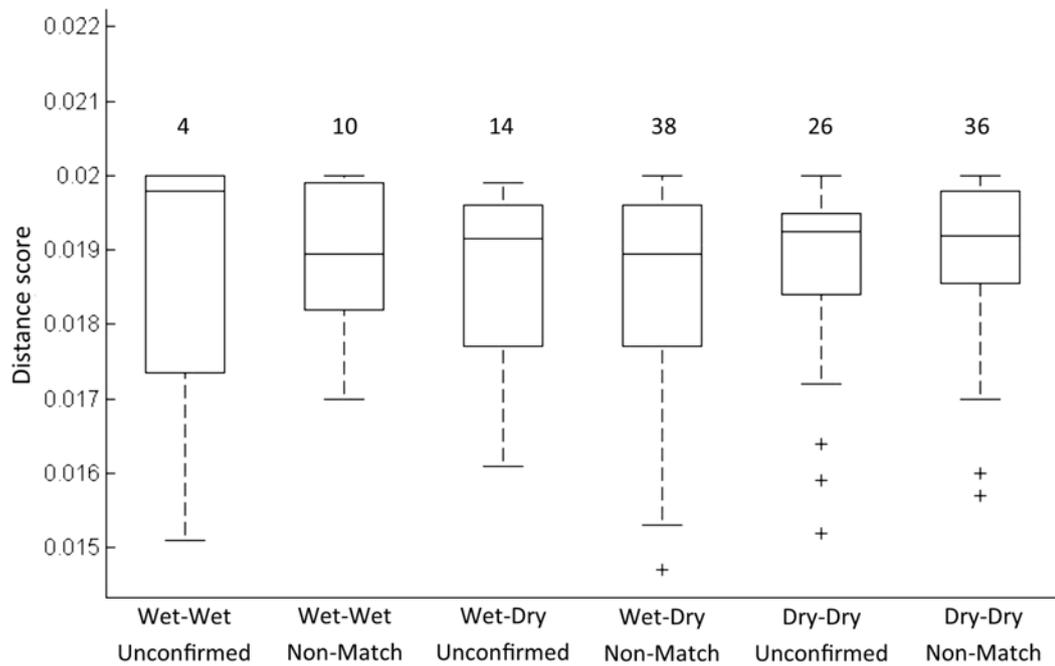


Figure 3.10: Box-whisker plots of the distance scores of ≤ 0.02 between whisker spot patterns of wet and dry individuals and their combinations, which resulted in ‘Unconfirmed’ (no confirmed resightings or rejections) and ‘Non-matches’ (confirmed rejections). The median is shown as a black line, 25th and 75th percentiles as vertical boxes, 90th percentiles as range bars, and outliers as black crosses. Numbers on top of each box show the number of distance scores for each category.

3.5.3 Identification using row-column locations

Of 57 confirmed mystacial whisker spot locations (i.e. at least one of the 20 individuals had a whisker spot present at these locations), 32 locations occurred in 18 to 20 individuals (90-100%; Figure 3.11a). The remaining whisker spots were located in the lower and upper column ranges of each row (Figure 3.11a). Marking the locations of visual uncertainties of whisker spots’ presence, indicated highest uncertainties of whisker spots occurring towards the lower and upper column ranges of each row, particularly columns 9 and 10 and rows A, B and F (Figure 3.11b). Of the cells in the mystacial rows and columns, 35% had more than 20% uncertainty, and 40% had more than 10% uncertainty (Figure 3.11). Whisker spots occurring in ≥ 5 individuals showed a decreasing trend in uncertainty (i.e. whisker spots occurring in most individuals had least uncertainty). Whisker spots occurring in < 5 individuals had variable uncertainty of a whisker spot’s presence (Figure 3.11c). A cross-correlation between all pair-wise matched individuals resulted in 20 out of 20 individuals having different whisker spot compositions of marked spots from each

other. After removing all whisker spot locations with a nominally chosen uncertainty of $>10\%$ across all individuals, 12 out of the 20 individuals showed distinct whisker spot compositions based on gridded locations.

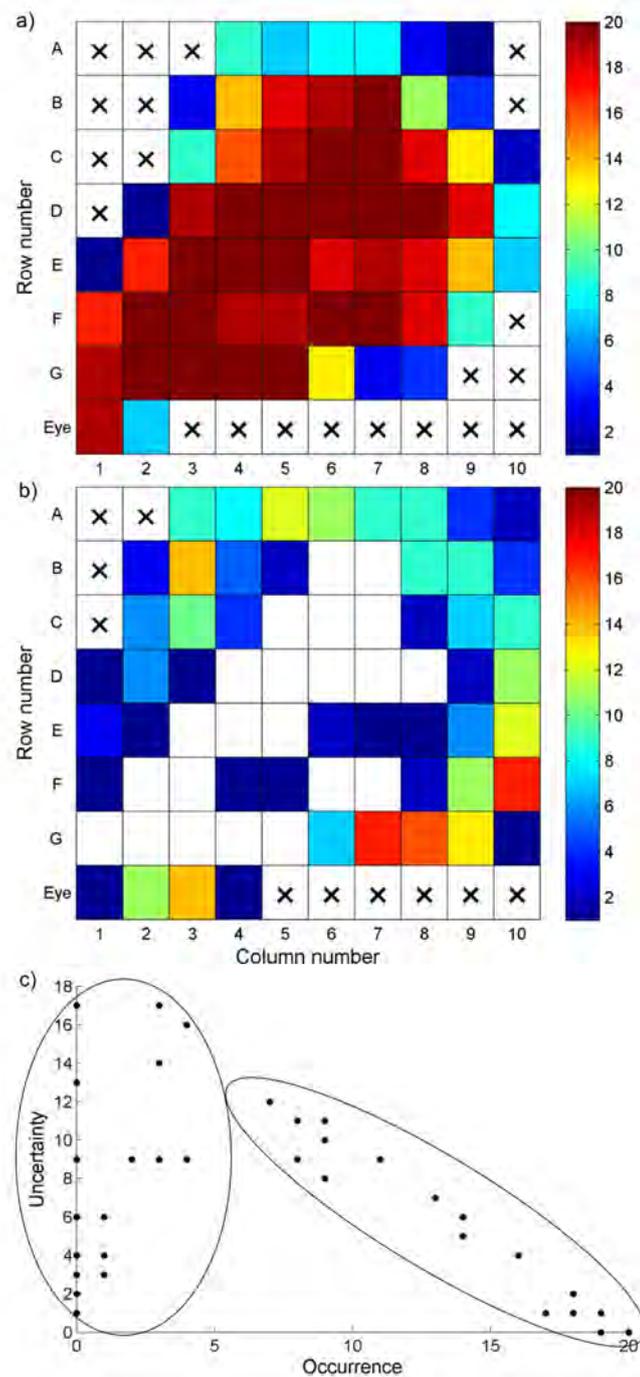


Figure 3.11: Top and middle panels: a) number of whisker spots' presence based on 20 wild individuals ('x' indicates locations in which whiskers are absent), and b) uncertainty of whisker spots' presence (white indicates 0 uncertainty, and 'x' indicates locations in which whiskers were absent with 0 uncertainty). The bottom row (Eye) represents the number of whiskers occurring above the eye. Bottom panel: c) uncertainty of a whisker spot's presence Frequency of mystacial whisker spots occurring (e.g. uncertainty of 10 means that in 10 individuals it was uncertain if a whisker spot was present) as a function of mystacial whisker spots occurring (e.g. an occurrence of 15 means that 15 individuals had this whisker spot) plotted for 20 individuals (excluding superciliary whiskers).

3.5.4 Observer variability

There was considerable variation in the number of whisker spots marked by the first author and five additional observers for 10 photographs of nine individual *N. cinerea* when comparing the different observers' marking of the same individual photographs (Figures 3.12 and 3.13, Table 3.1). Re-matching of the same individual appears unlikely if the two photographs are analysed by different observers (Figure 3.13, Table 3.1). Although ID 8 showed the highest variation in terms of the number of whiskers selected, ID 8 also showed the most consistent marking amongst all observers according to the distance scores, followed by ID 2 (relatively low distance scores; Figure 3.13). IDs 8 and 2 are both wet individuals, ID 8 with black fur colour and light coloured whiskers and ID 2 with light fur colour and dark whisker spots (Table 3.1). ID 1 (another wet individual), followed by IDs 5 and 6 (both dry individuals), individuals with similar brown coloured fur, whiskers and whisker spots, showed the highest inconsistency in marking between the observers according to the distance scores (Figure 3.13, Table 3.1). IDs 4, 5 and 6 displayed the greatest range of whisker spot numbers between observers and high SDs in the average number of whiskers marked (Figure 3.13, Table 3.1). Row A, followed by rows B and G, were the most inconsistently marked rows as were columns 9 and 10 (Figure 3.12), indicating that visibility of the whisker spots was lower than in rows C to F in the first eight columns.

Up to four eye whiskers were marked on most individuals by at least one observer (except for on one photograph where none were marked by all observers). On one photograph, ID 8, each observer marked two to three whiskers above the eye (Table 3.1). Up to five additionally selected mystacial whisker spots were marked outside of the row/column locations. However, only one of these additional whisker spots was marked by all observers. All other additional whisker spots varied considerably between observers, and usually only one to two observers selected the same additional whisker spot. At times, the shadows of overlapping whiskers were marked which is why these additional whisker spots were analysed separately.

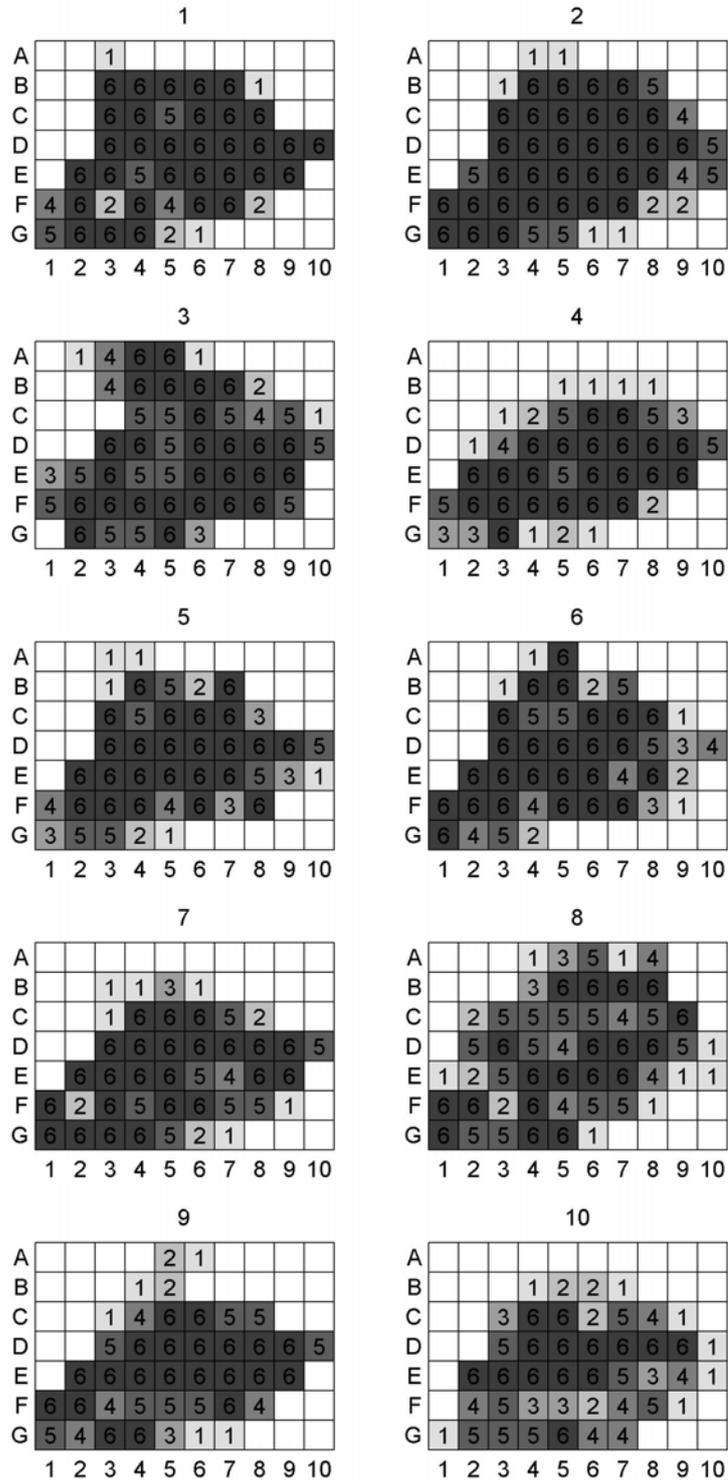


Figure 3.12: Number of observers (n = 6) that marked whisker spots in rows A to G in columns 1 to 10; where 10 whisker spot patterns (IDs 1 to 10) were marked by the first author and five observers experienced in marine science, and the numbers of selected whisker spot locations summed (i.e. 1: one of the observers marked a whisker spot at this location, 6: all of the observers marked a whisker spot at this location). Note that panels 4 and 9 were patterns from two different photographs of the same individual.

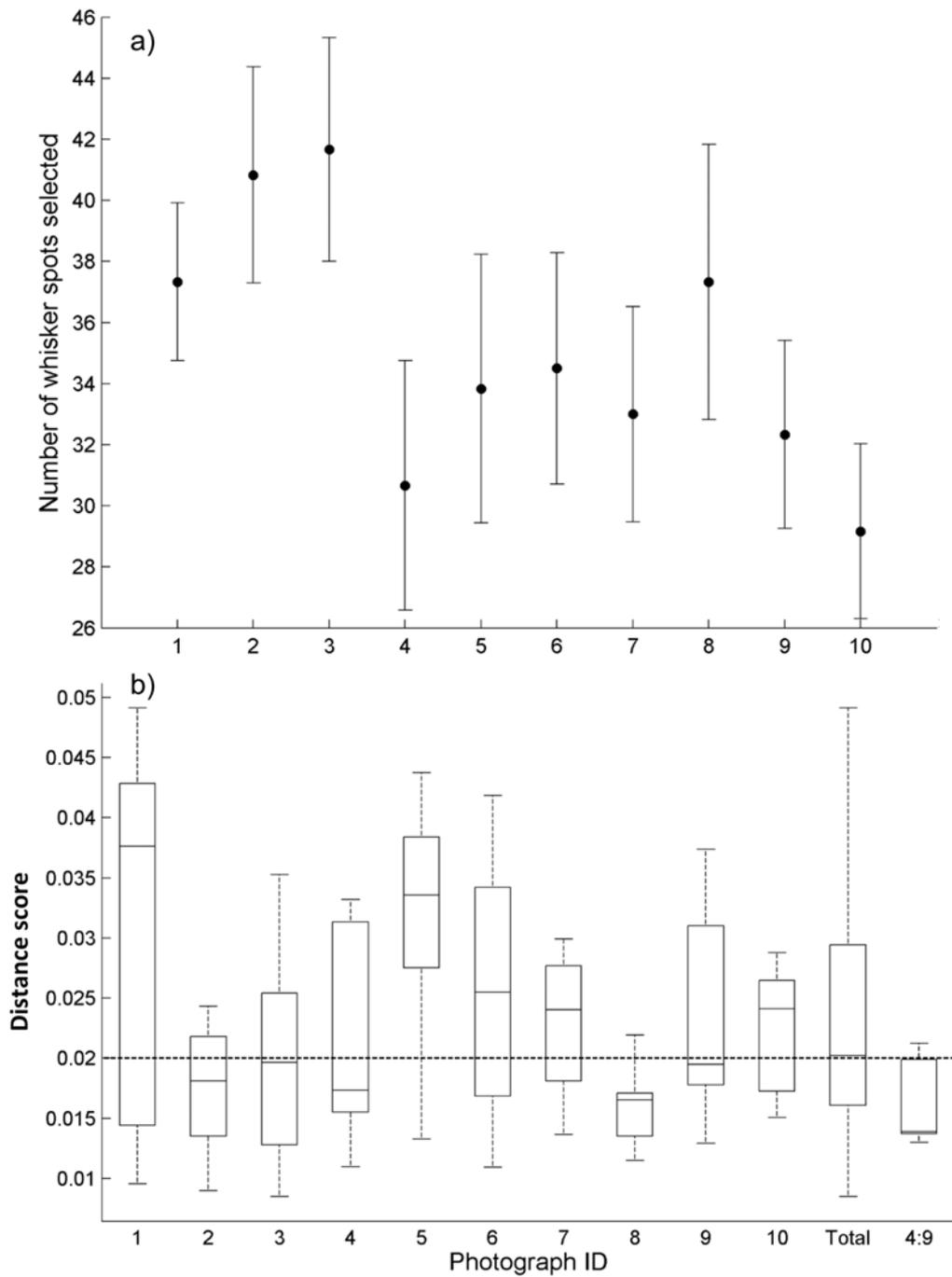


Figure 3.13: a) Average (dot), and standard deviation (lower and upper error bars) of whisker spots marked by six observers on 10 different photographs (IDs 4 and 9 are different photographs from the same individual). b) Box-whisker plot of distance scores for selected whisker spot patterns by six different observers of the same photographs (IDs 1 to 10). ‘Total’ shows the distribution of distance scores from all pairs (IDs 1 to 10). IDs 4 and 9 are photographs of the same individual, taken on the same day, and ‘4:9’ shows the distribution of distance scores from each observer matching the two photographs of the same individual.

Table 3.1: Number of whiskers marked by six different observers on 10 photographs (IDs 1 to 10): range (min - max), mode, mean (\pm SD) as well as the number of whiskers marked above the eye, and additional whiskers marked outside of the row/column arrangement. Characteristics for each *N. cinerea* are given as the fur and whisker spot colour as well as wet or dry condition of the fur and any other notable feature (and whisker colours generally light to dark brown unless stated otherwise). Note that IDs 4 and 9 were two photographs of the same individual.

ID	Number whiskers (range)	Number whiskers (mode)	Number whiskers (average (\pm SD))	Number whiskers eye (range)	Number additional whiskers (range)	Characteristics: Fur, whisker spot colour, wet/dry, other
ID 1	35 - 41	35	37.3 (\pm 2.6)	0 - 4	0 - 5	Dark brown, dark brown/black, wet
ID 2	37 - 45	38	40.8 (\pm 3.5)	0 - 1	0 - 1	Light brown, dark brown/black, brown, wet
ID 3	37 - 46	41	41.6 (\pm 3.7)	0 - 2	0 - 3	Dark brown, dark brown/black, dry
ID 4	27 - 38	27	30.7 (\pm 4.1)	0 - 2	0 - 3	Light to dark brown, dark brown/black, dry
ID 5	28 - 40	32	33.8 (\pm 4.4)	0 - 3	0 - 2	Brown, light to dark brown, dry, little bit sand
ID 6	30 - 41	34	34.5 (\pm 3.8)	0 - 3	1 - 5	Brown, light to dark brown, dry
ID 7	31 - 40	31	33.0 (\pm 3.5)	0	0 - 2	Light brown, light to dark brown, dry
ID 8	33 - 43	43	37.3 (\pm 4.5)	2 - 3	0 - 2	Black, black, wet, white whiskers
ID 9	29 - 38	31	32.3 (\pm 3.1)	0 - 2	0 - 3	Light to dark brown, dark brown/black, dry
ID 10	26 - 34	30	29.2 (\pm 2.9)	0 - 1	0 - 2	Light to dark brown, dark brown/black, dry

3.5.5 RGB levels of fur and whisker spots

The mean difference in Root-Mean-Squared (RMS) of RGB levels between whisker spots and surrounding fur was significantly greater for spots that were consistently marked by most or all observers (5-6 of the six observers, number of spots = 30) compared to spots identified less consistently, by 1-4 observers (which averaged 26.61 (± 19.8 SD, number of spots = 30; $Z = 3.1417$, $p < 0.002$).

3.6 DISCUSSION

Identification of individuals from their whisker spot patterns was non-trivial. The variation between photographs of potential resightings was similar to the variation between photographs of different individuals. Two techniques were tested to assess performance in correctly resighting individuals, however, under the conditions of this survey, neither exhibited satisfactory confidence in resightings.

Variation between photographs of the same individual, which were taken in short succession, increased with time between photographs. This suggests that movements of *N. cinerea* and the photographer alter the relative orientation between them and the resulting identified spot pattern, such that re-matching was not possible with the Chamfer distance transform method. The reduction in matching performance with time implies that as time passes it becomes more difficult to return to the original relative orientation, possibly also changing light conditions between photographs. Here, time (i.e. duration between photographs) was effectively acting as a proxy for differences in orientation between animal and photographer and the potential differences in lighting conditions between photographs taken in succession. While a direct relationship between these effects and time has not been established, the premise is that a time between photographs increases, so does the likelihood of difference in e.g. orientation, position, muzzle lighting and, in the extreme, subject state (for example, a dry subject photographed entering the water and getting wet before the second photograph, or rolling around covering itself in sand before the second photograph). A reduction in performance was also observed in tests with captive animals, although with a reduction from 90% to 73%, it was not as pronounced as in this study of wild animals (Osterrieder *et al.*, 2015a).

Although individual *N. cinerea* showed variation in their whisker spot pattern composition, the numbers of whiskers in each row and column were less variable than for *P. leo* (Pennycuik and Rudnai, 1970). In *P. leo*, the number of whiskers

occurring in the top row varied between zero and five, with 13 possible positions compared to the reference row below and thus this variation could be used to identify individuals. Additionally, the use of both sides of the muzzle, provided sufficient information for a reliable photo-identification method (Pennycuick and Rudnai, 1970). In *U. maritimus*, there was no consistent row for this type of testing (Anderson *et al.*, 2007a). In this study, there was a subtle difference from findings in *U. maritimus* in that there was no row in *N. cinerea* that exhibited a relatively high variability in occurrence in whisker spot location that could also be consistently marked. Therefore, the reason was similar to *U. maritimus* in that applying the pattern matching method used in *P. leo* was inapplicable to *N. cinerea*, under the conditions in this study.

When applying the Chamfer distance transform approach to wild individuals, the probability of 50% of the matches being false positives was determined to be 0.02, 33% higher than the 0.015 determined for captive individuals by (Osterrieder *et al.*, 2015a). Photographs taken in controlled environments therefore showed less variation than when taken in the wild. However, as mentioned above, success rates decreased when matching photographs taken over time of captive *N. cinerea*, showing that repositioning caused differences in spot patterns even in controlled environments (Osterrieder *et al.*, 2015a). Orientation of photographer and *N. cinerea* to each other appeared to be too difficult to duplicate with reliable accuracy for matching purposes, both in the wild and in controlled surroundings.

In controlled environments and with trained individuals, 70% of photographs taken on different days were matched correctly using the Chamfer distance transform approach. In comparison, unconfirmed matches and confirmed non-matches of wild individuals were not significantly different in their distance scores. Although only good quality photographs were used, small changes between photographs in *N. cinerea* position (how they are holding their heads or lying on the sand), different light conditions or slight variations in photograph angle appear to introduce too much variation to enable resightings in the wild.

Observer bias was significant in this study resulting in re-matching even the same photographs unlikely if analysed by different observers. The selection of the exact locations of the reference points, particularly at the end of the mouth, varied considerably among observers, contributing to variation in detected whisker spot patterns when marked by multiple observers. Three of the observers commented

that marking spots and determining exact locations of reference points was difficult. Photographs in which *N. cinerea* had closed eyes made the first reference point (the corner of the eye) more difficult to distinguish, and best guesses in these cases may have resulted in inaccuracy in standardisations of the images. Applying the row/column approach, however, showed that the areas of highest uncertainties detected by the main observer were the same areas where inconsistencies were found between multiple observers. Thus, the difficulty in marking the whisker spots was independent of those found in marking the reference points. Whisker spots located on the periphery of the populated area of the muzzle presented the highest uncertainty of all spots when marked by the main observer. At the same locations, inconsistencies in detections of whisker spots occurred among observers. Several studies have shown variations in photo-identifying individual animals when different observers analysed the photographs (e.g. Anderson *et al.*, 2007a; Friday *et al.*, 2000; Oliveira-Santos *et al.*, 2010). Differences may occur when observers are less experienced (Friday *et al.*, 2000) or when photographs are poor in quality. In this study all observers were trained scientists and were given detailed instructions for marking, and only good quality photographs were used. This supports the premise that variation between different individual animals may be too small and even the best photographs too variable to identify *N. cinerea* in the wild in conditions similar to those of this study.

Variability in marking photographs can also occur when the unique mark or part of it is inconspicuous or too similar to other marks present (Anderson *et al.*, 2007a; Friday *et al.*, 2000; Oliveira-Santos *et al.*, 2010). Comparing the distinctiveness of a whisker spot within the surrounding fur showed that whisker spots selected by most or all observers were more visible on an RGB scale than whisker spots selected by few observers. This association suggests that whisker spots may not have been clearly discernible at certain locations, particularly, the mystacial whiskers in the top row, above the top row, and in the columns close to the nostril. Due to the inconsistency in spot detection among observers, Anderson *et al.* (2007a) suggested identifying unambiguous spots to ensure consistent selection of whisker spots, in addition to using only high quality photographs. For methods that use the relative location of these whisker spots, this method could be effective (if distortion from photographs taken at slightly different angles does not affect accuracy). However, if the method relies on the presence and absence of spots within rows and columns, removal of inconsistent and uncertain columns and rows for individual identification would reduce the probability of a whisker spot pattern being unique.

The highest individual variability in whisker spot patterns occurred in the areas of highest inconsistency in whisker spot selection (Osterrieder *et al.*, 2015a). However, some of this variation among individuals in Osterrieder *et al.* (2015a) may have been caused by inconsistent selection of whisker spots. Nonetheless, most individuals in this study, still showed unique patterns based on row/column locations after removing areas of high uncertainties, suggesting that differences in whisker spot patterns do occur in wild *N. cinerea*. There was high variability in uncertainty of spots with low number of occurrences, with some whisker spots showing low uncertainty when occurring, and adding to the variation of spot patterns detected. The rate of unique pattern composition, 60% in 20 individuals, however, was lower than found in photo-identification methods in other species, e.g. 98% in 50 individuals in *U. maritimus* and 92% in 25 *P. leo* (Anderson *et al.*, 2007a; Pennycuik and Rudnai, 1970) and while the latter methods differed slightly from this study, the difference in performance is tangible.

The contrast between whisker spots and fur is one of the most obvious differences between *N. cinerea* and *U. maritimus*, *P. leo* and *P. pardus kotiya* (Anderson *et al.*, 2007a; Miththapala *et al.*, 1989; Pennycuik and Rudnai, 1970). In *P. leo* and *P. pardus kotiya*, the black whisker spots are highly visible against the contrasting pale fur colour, and the authors of the studies indicated that there was little ambiguity in identifying their presence and absence. With the greater accuracy of selecting presence and location of whisker spots, smaller differences between individual patterns are also easier to detect and greater information can be gleaned from smaller areas. Among individual *N. cinerea* in this study, there was a high variation in fur and whisker spot colours. This study showed that the colouration in *N. cinerea* impacted the consistency of marking whisker spots significantly, with whisker spots with higher colour variability to the surrounding fur being marked by more observers.

The difference in RGB levels between the whisker spots and surrounding fur was calculated conservatively in that only spots that were perceived by the main observer were used. However, whisker spots in *N. cinerea* can also be covered or partly covered by fur, decreasing the area with colour differences available to be detected, especially in those areas with smaller and thinner whiskers (Ling, 1977). Different light conditions can obscure whisker spot marking as well by introducing glare or by darkening sections of the muzzle on the photograph. Sand on the muzzle

of *N. cinerea* complicated marking as well, but muzzles predominantly covered in sand were excluded from analysis. Due to these difficulties, some spots may have been missed on some photographs and manual marking of spot patterns may have also introduced some variation. Some colour combinations were particularly difficult, specifically in cases where whiskers and whisker spots blended in with the fur colour. The whisker spot to fur colour difference appeared to be more pronounced in wet than in dry individuals, with the whisker spot selection in two out of three wet individuals showing consistency between the observers. The photograph of the third wet individual showed some sun glare in the top rows, contributing to the difficulties of selecting the whisker spots. Although wet individuals were marked with greater consistency between observers, there was still some marking variation in the columns towards the nostril as well as in the lower rows. However, distance scores of wet and dry individuals did not differentiate between these more easily marked matches, which could not be ground truthed either.

In harbour seals (*Phoca vitulina*), the use of multiple photographs for each individual in an automated matching process using spot patterns in their fur increased matching success significantly (Hastings *et al.*, 2008). It is possible that the use of multiple photographs improves matching for photographs with small variations (for example, minor changes in lighting or shade, environmental conditions or animal - photographer composition). However, in this study, while many individuals had more than one good quality photograph available, the matching did not appear to be improved with greater number of photographs. Furthermore, a larger number of photographs had to be discarded due to the stringent selection required. The low success rate of suitable photographs obtained also presents limitations in this method in the field.

Pinnipeds have well-developed whiskers in regards to their length and thickness, and are generally ordered in rows and columns as is typical of mammals (Ahl, 1986; Ling, 1966, 1977; Miller, 1975). The numbers of mystacial whiskers per individual recorded in this study was between values reported from two previous studies, which reported 26 and 44-48 whiskers for *N. cinerea*, respectively (data obtained from unknown sample sizes; Ling, 1977, 1992). Only one of the individuals marked by six observers resulted in a mode as low as 27 mystacial whiskers, and 45-46 whiskers were recorded as maximum values in this study (Ling 1977; Ling 1992). Compared with those of *U. maritimus* and *P. leo*, the whiskers of *N. cinerea* are more pronounced and frequently overlap spots in rows below (Ling,

1977; Pennycuik and Rudnai, 1970). This further explains the difficulty in selecting some whisker spots in the lower rows, particularly row G. Two and three superciliary whiskers have previously been recorded, which is consistent with this study although they were detected by only a few observers (Ling, 1977, 1992). Similar to the infrequently selected mystacial whiskers, superciliary whiskers often appeared inconspicuous within the surrounding fur and were often missed.

Whisker spots outside the row/column locations add information to patterns, making them more distinctive. However, additional mystacial whisker spots, apart from one on a single individual, were not usually selected by most observers and increased the inconsistency in whisker spot detection. For additional whisker spots to be useful, they need to be distinctive and readily detectable by observers. On some occasion, the shadows of whiskers gave the impression of additional whiskers occurring; and on other occasions, very small and short whiskers growing adjacent to the main whisker identified for that row/column were marked. These detections added to the inconsistency of marking whisker spots. More experience in whisker spot selection with varying colour combinations of fur, whisker spots and whiskers and light conditions could reduce inconsistencies due to colour contrast. However, difficulties in selecting whisker spots on photographs taken under environmental conditions such as obscuring sand, light and glare would still affect consistency, which could perhaps be removed by considering these as poor quality photographs. This would, however, considerably limit the number of photographs available, or extend the time required to acquire photographs of sufficient quality.

The use of the Chamfer distance transform approach on spots that can be consistently identified and are of high colour contrast may overcome some limitations experienced in the previous test of its application to wild *N. cinerea*. However, the variability in patterns resulting from a smaller number of spots may potentially limit the method to small colonies. If only consistent spots were used and there was sufficient uniqueness in the relative distances between them, approaches that correct for distortion could have some success. The Groth algorithm has successfully been used for pattern matching in whale sharks (*Rhincodon typus*). It is based on using triangles, matching the dimensions of all possible triangles between selected spots, and may improve matching results between photographs taken with slight angle variations (Groth, 1986; Arzoumanian *et al.*, 2005). The visual confirmation of potential resightings by either comparing plotted spot patterns alone or by including the whole body was unsuccessful in this study. An alternative method would need

to overcome uncertainties of confirming matches or be sufficiently robust so that visual checks would be unnecessary. Other possible discrimination methods could include using other features such as flippers (McConkey, 1999; McNally, 2001). However, the hind flippers are rarely observed spread out and were deemed unfeasible to be used for photo-identification (by the author). Hence, this method, previously used in Hooker's sea lions (*Phocarctos hookeri*), was not tested here. It may be possible to test whether a single front flipper contains enough information for photo-identification. In general, the authors have observed that animals spend long periods with their flippers tucked under the body, or buried in sand. Hence, a considerable amount of effort (and time) may be required for *N. cinerea* to move and stretch or lift one of its front flippers into an appropriate position for photographing.

An automated software developed for using the outline of sea lion flippers to match sea lions by their flipper shapes provides a list of the most likely matches (Gope *et al.*, 2005). In testing the software, Gope *et al.* (2005) found that 27 incorrect matches were suggested before the correct image on the list was suggested as a match. Thus, the software was reported to support individual identification with required visual double-checking. If confirmations of potential matches by eye were robust and feasible, and there was sufficient information in flipper shapes to apply to large populations, this method may then be more viable than using whisker spot patterns. The whisker spot patterns showed large variation between photographs of the same individual under the conditions and with animals tested in this study.

3.7 ACKNOWLEDGMENTS

This project was made possible by significant field support by many volunteers. In addition, this project would not have been possible without the substantial logistical support to and from the islands provided by the Department of Parks and Wildlife (Western Australia). The Department of Fisheries and Cockburn Volunteer Sea Search and Rescue also contributed by providing transport to and from islands. Malcom Perry maintained the research vessel, and boat ramp access was provided by the Fremantle Sailing Club. Thanks to Miles Parsons for editing and improving first drafts of the manuscript. Finally, this project was supported by funding from the Australian Marine Mammal Centre (2012/19) and the Western Australian Government's State NRM Program.

Chapter 4

Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia

Sylvia K. Osterrieder, Chandra Salgado Kent, and Randall W. Robinson

GRADUATE RESEARCH CENTRE

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book:	Variability in haul-out behaviour by male Australian sea lions <i>Neophoca cinerea</i> in the Perth metropolitan area, Western Australia Endangered Species Research		
Surname:	Osterrieder	First name:	Sylvia
College:	College of Engineering & Science	Candidate's Contribution (%):	70
Status:			
Accepted and in press:	<input checked="" type="checkbox"/>	Date:	June 2015
Published:	<input type="checkbox"/>	Date:	

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

	8.8.2015
Signature	Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and

5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

Curtin University, Bentley, Western Australia
Palmyra, Western Australia

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Sylvia Osterrieder	70	Planning project, data collection, data analysis, writing and editing manuscript		8/8/15
Chandra Salgado Kent	20	Planning project, supervision on data analysis and manuscript preparation		10/8/15
Randall W. Robinson	10	Advice on experimental design and comments on manuscript drafts		3/8/15

4.1 ABSTRACT

Pinnipeds spend significant time hauled out, and their haul-out behaviour can be dependent on environment and life stage. In Western Australia, male Australian sea lions *Neophoca cinerea* haul out on Perth metropolitan islands, with numbers peaking during aseasonal (~17.4 months in duration), non-breeding periods. Little is known about daily haul-out patterns and their association with environmental conditions. Such detail is necessary to accurately monitor behavioural patterns and local abundance, ultimately improving long-term conservation management, particularly where, due to lack of availability, typical pup counts are infeasible. Hourly counts of *N. cinerea* were conducted from 08:00 to 16:00 h on Seal and Carnac Islands for 166 days over two years, including two peak periods. Generalised additive models were used to determine effects of temporal and environmental factors on *N. cinerea* haul-out numbers. On Seal Island, numbers increased significantly throughout the day during both peak periods, but only did so in the second peak on Carnac. During non-peak periods there were no significant daytime changes. Despite high day-to-day variation, a greater and more stable number of *N. cinerea* hauled out on the significantly smaller beach of Seal Island than at Carnac Island during one peak. Overall, numbers hauled out were associated with temperature and tidal height, but not wind speed. Relative percentages of age classes (juveniles, sub-adults and adults) hauled out also varied with time of breeding cycle. Due to high variability in haul-out behaviour in space and time, and its association with environmental conditions, we conclude that counts for monitoring relative abundance to inform management objectives should be conducted systematically, using robust survey designs with relatively large sample sizes.

4.2 KEYWORDS

Keywords: Haul-out pattern, Australian sea lion, *Neophoca cinerea*

4.3 INTRODUCTION

Hauling out is a behaviour displayed by pinnipeds where animals temporarily leave the water to spend time on land between periods of activity. Hauling out onto land is important for pinnipeds during specific periods of their life cycle, such as breeding and moulting, but most pinniped species also haul out for other reasons. For example, they may travel considerable distances to foraging grounds and may forage at their aerobic dive limits (e.g. Arnould and Hindell, 2001; Costa and Gales, 2003; Chilvers *et al.*, 2005), thus hauling out may help conserve energy and contribute to recuperation (Riedman, 1990). Pinnipeds may also haul out to avoid predation (LeBoeuf *et al.*, 1982), as shown by an inverse relationship between successful great white shark *Carcharodon carcharius* attacks and distance from a haul-out island (Hammerschlag *et al.*, 2006) or increased instances of haul-out behaviour in the presence of killer whales *Orcinus orca* (London *et al.*, 2012). Haul-out sites are also commonly used for social interactions and thermoregulation (e.g. Ling *et al.*, 1974; Marlow, 1975; Kriebler and Barrette, 1984; Riedman, 1990).

The duration and frequency of hauling out can be specific to species, population or individual. Duration of haul out may be longer during the breeding and pupping season of some pinniped species (e.g. Lake *et al.*, 1997; Southwell, 2005), which may be related to variations between female and male haul-out duration and pattern (Thompson *et al.*, 1989; Reder *et al.*, 2003; Southwell, 2003). Accordingly, within a population of the same species, age and sex, as well as timing within the breeding, pupping or moulting cycles, can influence haul-out patterns (Thompson *et al.*, 1989; Reder *et al.*, 2003; Bengtson and Cameron, 2004).

Previous studies of seals and sea lions have shown considerable differences between species in diurnal and seasonal fluctuations in haul-out numbers (Thompson *et al.*, 1989; Sepúlveda *et al.*, 2001; Reder *et al.*, 2003; Sepúlveda *et al.*, 2012). Some phocids and otariids have shown lower numbers in the morning, with peaks in the afternoon (Stirling, 1968; Lake *et al.*, 1997; Carlens *et al.*, 2006), though the timing of the peaks can vary between seasons (Lake *et al.*, 1997). Some seals, for example, hauled out more over midday in some seasons, but less at the same time of day during others, such as harbour seals *Phoca vitulina* in Scotland, where there was a greater probability of hauling out around midday in summer (Cunningham *et al.*, 2009). Diurnal haul-out patterns can also vary between locations (Cunningham *et al.*, 2009), a trait observed in South American sea lions *Otaria flavescens* (Rosas *et al.*, 1994; Sepúlveda *et al.*, 2001, 2012).

Several studies have shown that variations in numbers of pinnipeds hauled out can be affected by weather conditions, such as temperature, cloud cover or wind speeds (Schneider and Payne, 1983; Watts, 1992; Carlens *et al.*, 2006; Andrews-Goff *et al.*, 2010). Additionally, oceanographic factors, such as tidal heights, times or currents may play a significant role in haul-out patterns (Stirling, 1968; Pauli and Terhune, 1987b; Thompson *et al.*, 1989; Watts, 1992). There may also be a combined effect of several environmental conditions combined with time of day and season (e.g. Schneider and Payne, 1983; Pauli and Terhune, 1987a,b; Thompson *et al.*, 1989; Lake *et al.*, 1997; Reder *et al.*, 2003; Carlens *et al.*, 2006; Mogren *et al.*, 2010).

Australian sea lions *Neophoca cinerea* have been listed as Endangered on the IUCN Red List and are endemic to South and Western Australia (Goldsworthy, 2015). *N. cinerea* have an unusual breeding cycle, unique among pinnipeds. Breeding takes place aseasonally on average every 17.4 months (range: 16.0-19.9 months) and asynchronously between breeding locations, during a period of five to nine months (Ling and Walker, 1978; Gales *et al.*, 1992; Higgins, 1993; Gales *et al.*, 1994; Goldsworthy *et al.*, 2008). Haul-out sites in the Perth metropolitan area (Western Australia), approximately 250 km south of the closest breeding islands at Jurien Bay, are known to be used exclusively by males (Gales *et al.*, 1992). Male *N. cinerea* travel significant distances away from breeding colonies, presumably to maximise foraging success and efficiency (Gales *et al.*, 1992). Thus, access to these non-breeding haul-out sites is critical to the health of male sea lions and successful reproduction of the species. Management to reduce human impacts at key haul-out sites where animals rest is often implemented (e.g. Gales, 1995; Lovasz *et al.*, 2008), especially in areas of high human visitation such as the Perth metropolitan area. To determine the effectiveness of management guidelines, monitoring of animals using the islands is often undertaken by conducting counts over time (Cassini *et al.*, 2004; Salgado Kent and Crabtree, 2008). These counts can be used as estimates of relative abundance on which to base trends. Male numbers at the Perth haul-out islands peak aseasonally, aligned with the non-breeding season (Ling and Walker, 1978; Gales *et al.*, 1992; Higgins, 1993; Gales *et al.*, 1994; Goldsworthy *et al.*, 2008). While these peaks can be predicted, little is known about the extent and variability in daily use of these islands, or how they vary with environmental conditions. Variation in the proportion of age classes on these islands is also unknown.

Counts are often used for management purposes to monitor numbers of animals hauled-out on islands over time. Based on the resulting counts, measures, such as establishing sanctuary zones in areas used by large number of animals, may be implemented to minimise human disturbance (e.g. Gales, 1995; Cassini *et al.*, 2004). However, single counts used as an indicator of numbers of animals using a haul-out site can be subject to significant inaccuracies when there is large day-to-day variability (Southwell, 2005). Consequently, information on the extent of use of these islands and its associated variability with environmental conditions is critical for accurately monitoring changes in behaviour and relative abundance and is essential for the long-term conservation management of *N. cinerea* in the region.

To enhance current knowledge and thereby improve future monitoring practices and conservation management of *N. cinerea*, we aimed to identify whether *N. cinerea* had a consistent pattern in daily haul-out behaviour. We recorded haul-out numbers over a two year period on two main haul-out islands in the Perth metropolitan area, and assessed whether hauling out was associated with local environmental conditions.

4.4 METHODS

4.4.1 Study sites

Male *N. cinerea* are known to regularly haul out on six islands off the Perth metropolitan coast in Western Australia (WA; Figure 4.1). On these six male-only haul-out islands, Seal (-32.29° S, 115.69° E) and Carnac (-32.12° S, 115.66° E) Islands are used by the largest number of *N. cinerea*, with up to 28 and 45 individuals hauled out during the Perth peak season, respectively (Gales *et al.*, 1992), and were therefore chosen as the field sites for this study. The Perth peak season refers to the season in which the number of hauled out *N. cinerea* around Perth peaks, which shall now be referred to as the peak season, unless stated otherwise. Approximately 15 *N. cinerea* can be found hauled out at Burns Rocks and Little Island combined, mostly less than 10 at Dyer Island during the Perth peak season, and one or two can be found infrequently on Penguin Island (Department of Parks and Wildlife [DPaW], unpublished data). It is highly likely that sea lions move between the haul-out islands given their proximity to each other; however, this has rarely been documented (Gales *et al.*, 1992).

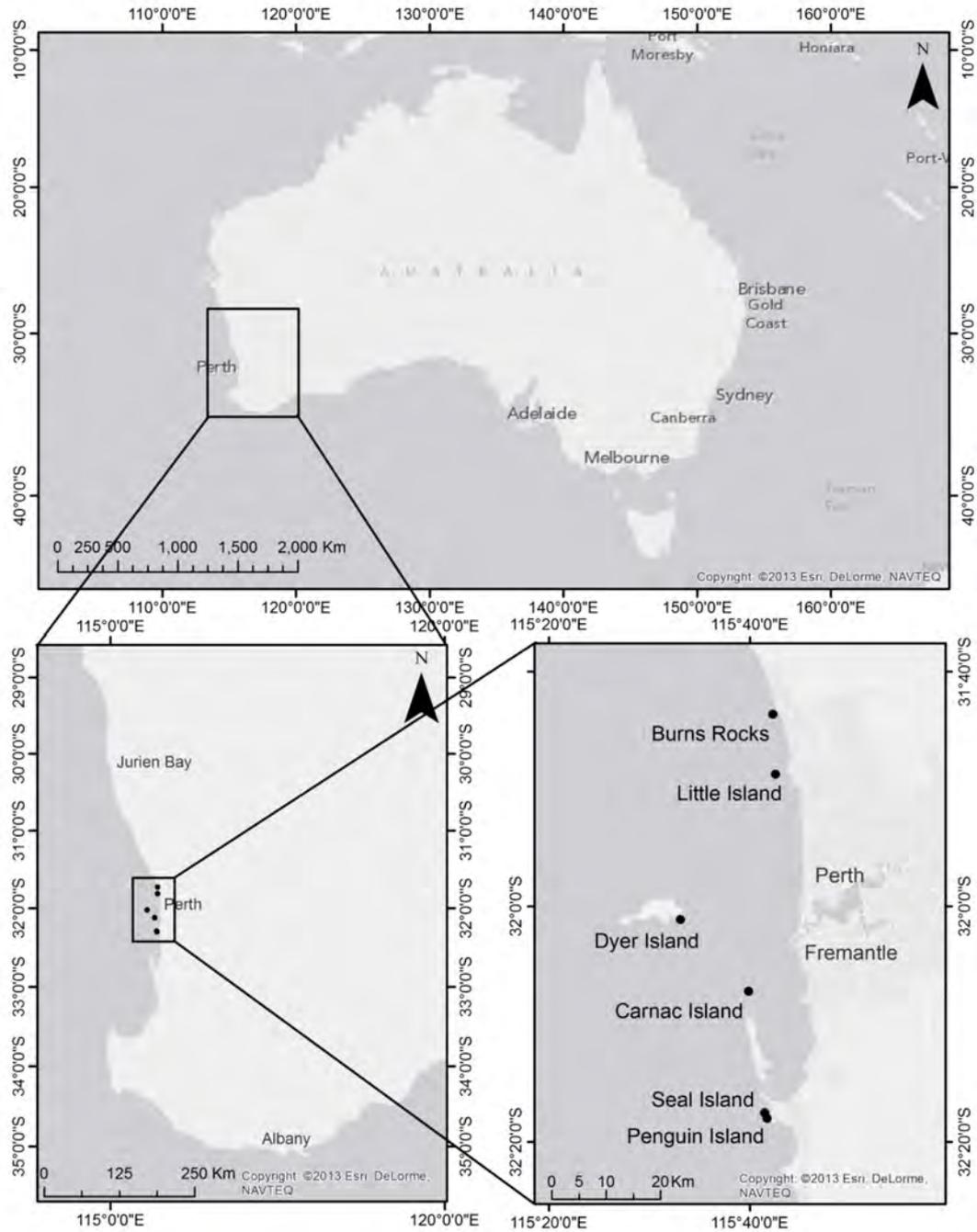


Figure 4.1: Haul-out sites of *N. cinerea* in the Perth metropolitan area.

Seal Island is located in the Shoalwater Islands Marine Park, approximately 0.9 km offshore from Shoalwater (ca. 45 km south of Perth, capital city of WA, Figure 4.1). Seal Island is a sanctuary zone, managed as a ‘look, but don’t take’ area and, as such, landing on the island is prohibited (DEC, 2007; DEC and Fisheries, 2011). The island provides a sandy beach of approximately 0.27 ha on the eastern side of the island (estimated from a Google Earth, 2014 image from 1st January 2014) with shrubs at the edges, small rocky overhangs and caves north and south of the beach, as well as along the southern bay. Apart from the beach area, the shores of the island are rocky. *N. cinerea* mainly use the beach and occasionally use adjacent shrubs or caves to haul out.

Carnac Island, approximately 10 km south-west of Fremantle (ca. 15 km south of Perth, Figure 4.1) is classified as an A class nature reserve. Access to most of the island is prohibited, but the southern part of the eastern beach is available for public access during the day (CALM, 2003). *N. cinerea* mainly haul out on the sandy beach of ca. 0.78 ha (estimated from a Google Earth, 2014 image from 1st January 2014) located on the east side of the island. The beach is fringed with shrubs and small bushes. There are some rocks, small rocky overhangs and caves to the north and south of the main beach where *N. cinerea* have been observed occasionally.

4.4.2 Data collection

4.4.2.1 *N. cinerea* counts

Counts of *N. cinerea* on the main haul-out beaches of Seal and Carnac Islands were undertaken over 166 days between June 2012 and April 2014. The study was designed so that days on which counts were undertaken occurred approximately once or twice a week over most of the study period; however, on occasion, sampling was less frequent. During each day of counts, all *N. cinerea* within view of the observer were counted hourly, primarily between 08:00 and 16:00 h. *N. cinerea* not in view at the time of counting, but known to be present during the count (i.e. seen when entering a cave as well as when exiting) were also included in the counts. Counts were made every hour, except during inclement weather or when there were technical difficulties that caused some counts to be missed during the day or some days cut short. Counts on Seal Island were conducted over 78 days and on Carnac Island over 88 days.

On Seal Island, the observer was located strategically, where the entire beach was within view, and counts were conducted with the aid of binoculars (Nikon Eagleview 8-24 × 25). At Carnac Island, a live camera (AVT284 IP Camera with remote Pan, Tilt, and Zoom capability and 22× optical zoom), owned and serviced by DPaW, with a radio link to the local office (using a Proxim 8150 PTP microwave radio link), was used. This radio link allowed remote control over the camera's viewing direction and zoom (Salgado Kent and Crabtree, 2008). The camera is located on a vantage point overlooking the eastern beach and was panned from north to south along the beach to count the *N. cinerea*. The zoom was used to aid counts when necessary, particularly to distinguish *N. cinerea* from some rocks on the far, southern part of the beach. This method was considered to accurately reflect counts that would have been made at a strategic vantage point at the site, based on the results of a study comparing simultaneous *in situ* beach counts and counts made with a previous model of this camera on the island (Salgado Kent and Crabtree, 2008).

There were several cases when these methods were not used, due to logistical constraints (for example, in instances when a vessel was not available to travel to Seal Island or the live camera was not working at Carnac Island). On Seal Island, between July and August 2012 as well as on the 9th October 2012, a vantage point on the mainland (on the coast of Shoalwater, WA) with the entire beach area on Seal Island in view was used. Counts from this location were conducted using either a telescope (115 mm Tasco reflecting with either a 25, 20 or 10 mm eye piece with 36 ×, 45 × or 90 × magnification, respectively) or a spotting scope (Televid 77 with 20 × to 60 × zoom). The vantage point on the mainland (at 32.2855° S, 115.7035° E) was approximately 1.4 km away from Seal Island, and the telescope and spotting scopes were considered to give sufficient magnification to count *N. cinerea* easily and accurately. On Carnac Island, between the end of November 2013 and the end of April 2014 (with the exception of 8th and 13th January 2014), counts were conducted directly from the vantage point where the live camera was located.

During all counts (with the exception of the first six months of the study; i.e. counts before 22nd October 2012), animals were visually classified into age groups by S. K. O., either as juvenile, sub-adult, adult or otherwise unknown, following the description of Jefferson *et al.* (2011). Juvenile males were identified based on their silvery grey backs and light brown or fawn lower fronts. They are also lighter in colour than sub-adults and adults. Sub-adult males are larger and darker than juveniles and most show a white ring around their eyes which diminishes as they grow

into bulls. Bulls are usually dark brown with a light creamy coloured crown. A number of *N. cinerea* present in the Perth metropolitan area are in intermediate stages of becoming either a sub-adult or adult and it can be difficult to distinguish between the age classes. For classification purposes here, younger *N. cinerea*, with undersides darker in colouration than juveniles and showing darker spots, were classed as sub-adults. Distinguishing between sub-adults and adults was based on size and the light coloured crown. If the individual showed a pronounced white eye ring and the light coloured crown was at the initial stages of showing through, it was classified as a sub-adult; however, if a full white crown was visible it was classified as an adult. For most *N. cinerea*, the age classification was straightforward. However, if no decision could be made or an individual *N. cinerea* was obscured sufficiently so that no age class could be determined, the class was recorded as unknown. Counts and age classifications were made by the same observer to avoid observer variability (Udevitz *et al.*, 2005).

4.4.2.2 Environmental data

Beaufort condition and cloud cover (in percentage) were recorded qualitatively at the time each count was made. Quantitative measures of air temperature, wind speed, wind direction, precipitation and atmospheric pressure at sea level were accessed through the Bureau of Meteorology (BOM) half-hourly weather station measurements made on Garden Island. This station was the closest to the study sites; located between Seal and Carnac Islands at a range of approximately 5.5 and 13.8 km from the islands, respectively. Weather station readings within 30 min from the start time of *N. cinerea* counts were taken to represent those at the time counts were made. Tide levels (from the lowest astronomical tide) at the start of counts were interpolated based on half-hourly tidal measurements from the tidal gauge at Fremantle, WA (provided by BOM). The times in which counts were conducted were recorded using a GPS set to local time (UTC +8) unless the remote controlled camera was used, in which the current local time was accessed from www.timeanddate.com (which uses Australian Western Standard Time). The times were also expressed as decimal hours since sunrise in the final database by subtracting the time of sunrise (accessed from www.timeanddate.com) from the local start time of counts (with minutes being divided by 60 to express decimal hours).

4.4.3 Statistical analysis

4.4.3.1 Modelling

Generalised additive models (GAM) with Poisson distribution and log link functions for count data were used to determine the relationship between temporal and environmental factors and numbers of *N. cinerea* hauled out at the two islands. GAMs were chosen to allow the inclusion of smoothers in the model as well as an autocorrelation structure to account for the dependency of subsequent counts in a short time period. Explanatory variables included survey interval (as a factor) with eight levels (1 to 8), location as a factor with two levels (Seal and Carnac Islands), hours since sunrise (in decimal hours) in which the counts were made (as a continuous variable), and temperature, tide level and wind speed (as continuous variables). The entire survey time was split into eight survey intervals, hereafter called ‘periods’. Each period consisted of an average of 83.6 days (varying by a maximum of three days), with Period 1 starting on Day 1 of the study and Period 8 starting on Day 586 (corresponding to 6th July 2012 and 12th February 2014, respectively). Periods 1 and 7 were found to be the peak periods for *N. cinerea* counts, and Period 4 fell on the minimum of the cycle. As not all intervals within the 17.4 months breeding cycle of *N. cinerea* could be sampled multiple times, ‘period’ is treated as a sequential survey interval from 1 to 8 rather than a period relative to the aseasonal cycle. Thus, Periods 1 and 2 and Periods 7 and 8 represented intervals during sequential peak and post-peak periods in the aseasonal cycles, respectively. Each period included between 20 and 26 survey days (across both islands), except Period 6 (October 2013) which had nine survey days. The autocorrelation structure used decimal days rescaled so that values were relative to the time since sunrise, reported as hours since sunrise from Day 1.

Interaction terms included time since sunrise by location and location by period. Correlation structures tested to account for temporal correlation among counts included autoregressive correlation of order 1 (AR-1), continuous AR(1), and exponential and spherical correlations (available in the R package ‘mgcv’ used for the GAMs; Wood, 2006).

Variance inflation factors (VIF) were used to test for collinearity. All covariates showed VIFs <3, indicating no collinearity between covariates (Zuur *et al.*, 2007, 2009). Sea level pressure, however, showed high nonlinear correlation with air temperature and was excluded from the analysis to prevent model misfit. Air tem-

perature was chosen as biologically more significant than sea level pressure because air temperature has shown significant influence on haul-out behaviour in previous studies (e.g. Carlens *et al.*, 2006). Precipitation was excluded from the models because of the lack of rainy days and, therefore, the poorly balanced data set. Cloud cover was recorded as a percentage of the whole sky. This was removed from the analysis (to simplify an already complex model) as it was considered of low biological significance, since recorded clouds could have been far away and not impacting *N. cinerea* locally. Wind speed influences the sea conditions, the Beaufort scale measures, and therefore, we included wind speed as the more direct covariate in the model, excluding Beaufort from the analysis. Wind direction was excluded from the analysis to keep the model as simple as possible (and to avoid problems in convergence), since it was considered biologically of less significance than the other variables. The full model thus included air temperature, tide level and wind speed as environmental covariates. Temperature, wind speed and tide level showed some non-linear patterns with period, hence the validity and interpretation of models including these environmental factors were assessed carefully. Three counts lacked temperature and wind speed measurements and were thus removed from the analysis.

4.4.3.2 Data exploration and model validation

Data exploration was undertaken to identify and remove any outliers or any single exceptionally large or small values that would overly influence the model results, and to check general assumptions of GAMs. Counts from two days, Days 38 and 550 (within Periods 1 and 7, respectively) from Carnac Island were excluded from the model to avoid influential data in the analysis. Counts on Day 38 were exceptionally low and were made two days after an unusually large storm event. Day 550 had exceptionally high numbers of *N. cinerea*.

The general approach to model construction and validation was to begin with the most complex model, with all effects that were considered to be of relevance to the numbers of *N. cinerea* hauling out based on biological knowledge (Flom and Cassell, 2007; Zuur *et al.*, 2009). The model was restricted a priori to an acceptable level of complexity, based on a general rule of thumb of at least 20 samples per covariate level (Harrell, 2001), with the exception of Period 6 with nine sampling days. Period 6 was included in the analysis to avoid a large data gap between Periods 5 and 7, and was interpreted carefully. To validate the model, residuals were plotted against each individual explanatory variable to ensure there were no obvious

patterns. To test that the inclusion of a correlation structure accounted for dependency sufficiently with no persisting autocorrelated residuals, normalised residuals were inspected for remaining pattern using variograms (Zuur *et al.*, 2009). Fewer counts were made before 08:00 h and after 16:30 h which appeared to influence the autocorrelation left in the normalised residuals. Therefore, only counts between 08:00 and 16:30 h (which included 0.7 until 10.9 h after sunrise) were included in the models.

Following this first complex model, submodels were created by removing insignificant explanatory terms ($p > 0.05$) with very small estimated variances one by one from the model, starting with the least significant term for model simplification (Wood, 2006; Zuur *et al.*, 2009). Each time, the resulting submodel was refitted and re-validated. Submodels were compared, and of these the final model was selected by finding the simplest validated model using Akaike's information criterion (AIC) as a guide. While identifying the simplest model that reduced the AIC by >2 units (Burnham and Anderson, 2002) was the initial aim, because environmental variables were related (non-linearly) to period, selection of the lowest AIC reduced by >2 units would have meant removing significant terms from the model. We minimised AIC for model selection, while including all significant terms based on p values for which to explain phenomena (Valpine, 2014). Smoothers fit to the variable 'time since sunrise' were straight lines, indicating linear relationships between response through the link function and covariates, and did not improve the model from one using a nonsmoothed 'time since sunrise' term according to the AIC. When adding a smoother to tide level in the final model selected the model could not converge; however, comparisons in previous submodels leading up to the final model fit better with tide as a nonsmoothed function. Time since sunrise and tide level were therefore added without smoothing functions.

Finally, there were some submodels that could not be tested, for the simple reason that they did not converge. All submodels, regardless of which insignificant terms had been removed, showed the same explanatory variables as significant, providing confidence in the final model selected.

As a large number of *N. cinerea* used the islands during peak periods (i.e. Periods 1 and 7 in this study), maximum counts were also compared between islands. Finally, models were not generated for identifying patterns associated with age class since data sets split by age class did not contain sufficient samples. These patterns

were, however, investigated using exploratory analyses. The maximum numbers of juveniles, sub-adults, adults and unknowns counted for each day were averaged over each period. Percentages of each class were then calculated from the averages. All analyses and figures were produced using R version 3.1.1 (R Development Core Team, 2014) run through RStudio Version 0.98.983 - © 2009-2013 RStudio.

4.5 RESULTS

Neophoca cinerea was observed on 163 of 166 survey days. There were 620 hourly counts made over 78 days on Seal Island and 712 counts over 88 days on Carnac Island, totalling 1332 hourly counts. Models were produced using 1227 counts, which covered all survey intervals (603 from Seal Island and 624 from Carnac Island).

The final GAM selected which produced the most parsimonious model followed the form:

$$\begin{aligned} \log(\mu_i) = & \alpha + \beta_1 \times TimeSinceSunrise_i + \beta_2(Period_i \times Location_i) + \beta_3 \times Tide_i \\ & + s(Temperature_i) + e_i, \end{aligned} \quad (4.1)$$

where

$$e_i = \sim Time + \varepsilon_i \quad (4.2)$$

with μ being the number of *N. cinerea* observed at count i , α being the intercept, β the corrections of the slope for each covariate at count i , s the smoothing function, and e_i consisting of the correlation structure indicated by the Time + noise ε (with noise normally distributed with mean 0 and variance σ^2). Time here was expressed as decimal days, rescaled so that values were relative to the time the sun rises (rather than relative to GMT). A spherical correlation structure resulted in the best fit, and was given by the equation in Pinheiro and Bates (2000), their Table 5.2:

$$\gamma(s, \rho) = 1 - [1 - 1.5(\frac{s}{\rho}) + 0.5(\frac{s}{\rho})^3] I(s < \rho) \quad (4.3)$$

with $\gamma(s, \rho)$ as the correlogram with correlation parameters ρ as the range and s as the time, and where function ' $I(s < \rho)$ denotes a binary variable taking value 1 when $s < \rho$ and 0 otherwise' Pinheiro and Bates (2000, p. 231). The greatest variation in numbers of *N. cinerea* on Seal and Carnac Islands was from the 17.4 month aseasonal cycle in arrival at and departure of animals from breeding grounds. Peak numbers at both islands occurred between July and August 2012 and December 2013 and January 2014 (Period 1 and 7, respectively, Figure 4.2). The trough

in numbers was between April and May 2013 (Period 4). This was reflected in the model by 'Period' being the most influential variable ($p < 0.001$, see Table A1 in the Supplement). There was a significant difference in numbers of *N. cinerea* hauling out on Seal and Carnac Islands ($p < 0.001$, Table A1), with overall numbers greater at Seal than at Carnac Island (Figure 4.2). The interaction between period and location was significant ($p < 0.001$, Table A1), showing that the greater numbers at Seal Island than at Carnac occurred during Periods 1, 2, 5 and 6 (Figure 4.2). Numbers of *N. cinerea* present did not show any significant difference between the two islands in Periods 3, 4 and 7 but numbers on Carnac Island were greater than on Seal Island in Period 8 (Figure 4.2). A small increase with time since sunrise was observed for Carnac Island in the second peak season, but not in the first peak season (Figure 4.3). The time since sunrise and its interaction with period significantly influenced the number of *N. cinerea* ($p < 0.001$, Table A1), with increasing numbers of *N. cinerea* hauled out over the course of a day during certain periods (Figure 4.3). On Seal Island, this trend occurred during Periods 1, 6 and 7 with a slight increase during Period 2. On Carnac Island the trend occurred during Period 7 and to a lesser extent during Periods 2 and 8. No increase over the course of a day was observed at either location during Periods 3, 4 and 5, which were the periods with the lowest numbers of *N. cinerea* present. Additionally, no trend was observed during Periods 1 and 6 on Carnac Island (Figure 4.3). The interaction term between location and time since sunrise did not show any significant effect on the number of *N. cinerea* hauled out and was thus removed from the final model.

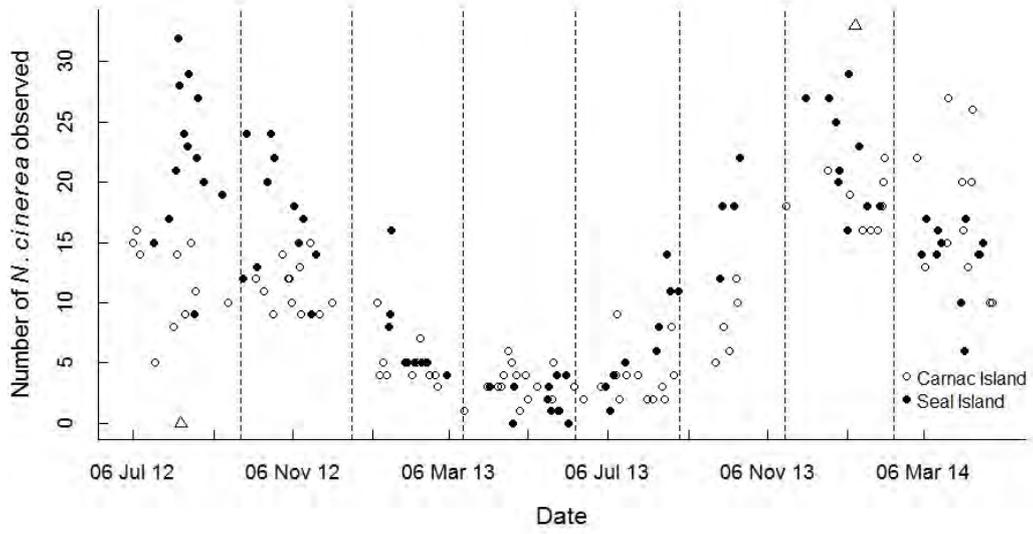


Figure 4.2: Maximum number of *N. cinerea* observed on Carnac (○) and Seal Islands (●) during 166 survey days between June 2012 and April 2014, including the exceptionally high and low observations (△) on Carnac Island. Dashed lines mark the survey periods (survey intervals 1 to 8).

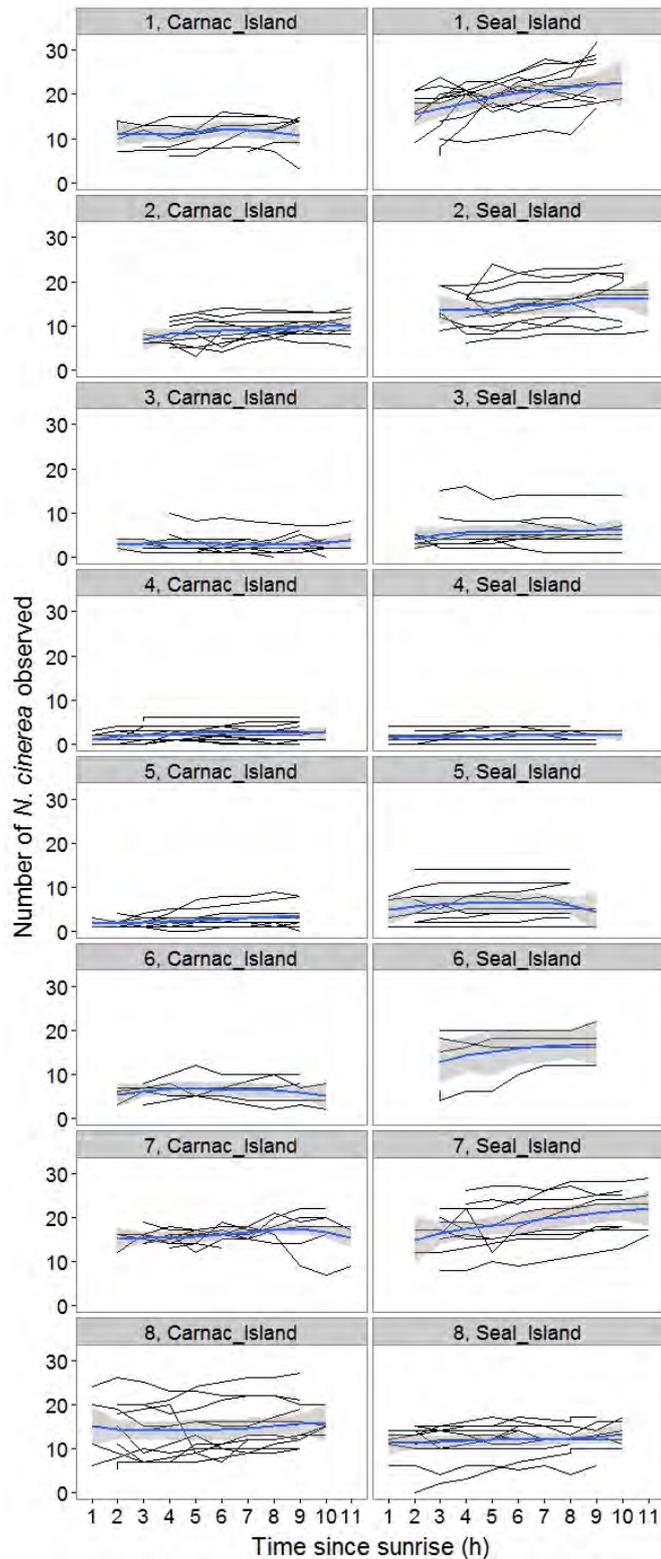


Figure 4.3: Number of *N. cinerea* observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represent counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) was added as visual aid. (The two exceptionally high and low observation days on Carnac Island were excluded.)

Air temperature and tide level had significant effects on the number of *N. cinerea* hauled out on Seal and Carnac Islands (Figure 4.4 and 4.5). The numbers of *N. cinerea* on the islands decreased with increasing tide level ($p = 0.003$, Table A1, Figure 4.5). The pattern was more pronounced on Seal Island than on Carnac Island (Figure 4.5). On Carnac Island, a steeper decrease in *N. cinerea* numbers hauled out was only observed when the tide had reached 1.0 m above the lowest astronomical tide (Figure 4.5). The numbers of *N. cinerea* increased with air temperature up to approximately 21°C ($p < 0.001$, Table A1) at which point they became comparatively stable. Smoothing function confidence intervals (95%) at temperatures below 15°C and above 27°C were large, thus interpretation at these temperatures is unreliable (Figure 4.4). Wind speed did not have a significant effect on numbers of *N. cinerea* hauled out ($p > 0.1$).

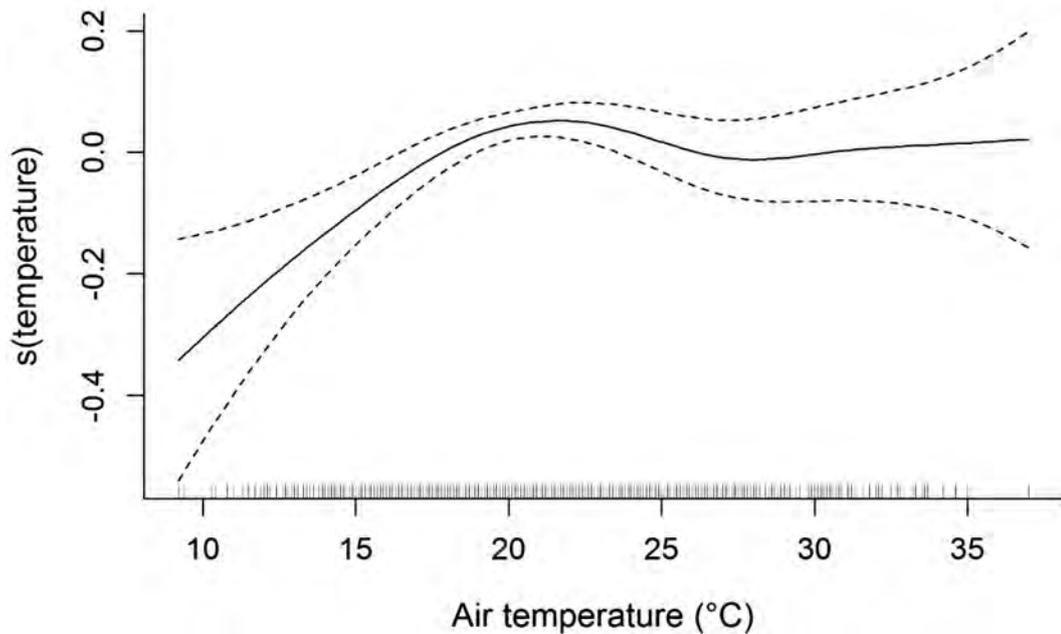


Figure 4.4: Smoothing function (solid line) with 95% confidence intervals (dashed lines) fitted to air temperature (°C) estimated in the final generalised additive model (GAM) model to show the influence of air temperature on the number of *N. cinerea* hauling out. The small ticks above the x-axis represent air temperature values of the observations.

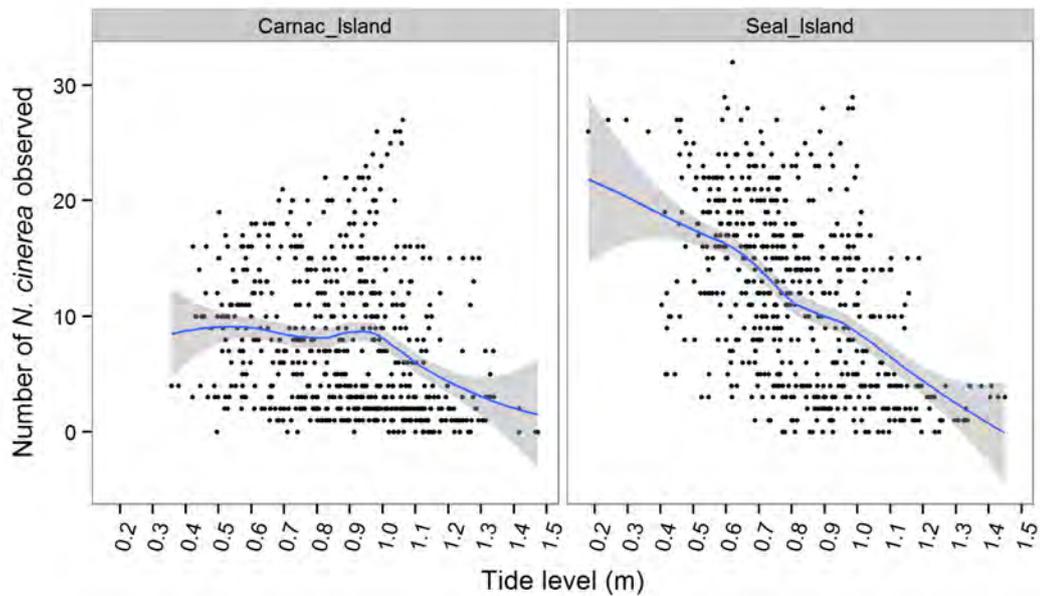


Figure 4.5: Relationship between number of *N. cinerea* and tide level (m) observed on Seal and Carnac Islands. A LOESS smoother (blue line) with 95% confidence intervals (grey) was added to aid visual interpretation.

4.5.1 Peak periods

There were 308 counts conducted in the peak periods, 174 on Seal Island and 134 on Carnac Island over 21 and 23 days, respectively. More *N. cinerea* were observed on Seal than on Carnac Island during this period. The maximum numbers counted were 32 and 29 on Seal Island and 16 and 33 on Carnac Island in the first and second peaks, respectively. The maximum numbers of animals hauling out in each peak season were counted on Days 35 and 550 on Seal and Days 3 and 556 on Carnac Island, and were 515 (16.9 months) and 553 days (18.3 months) apart, respectively. However, excluding the highest and most influential count, the highest count was conducted on Day 578 (19.0 months after Day 3) with 22 individuals counted on Carnac Island. More *N. cinerea* hauled out on Carnac Island in the second than in the first peak season (Figure 4.2). There was a high variation in numbers counted on different days in the peak seasons, more so on Seal than on Carnac Island (Figures 4.2 and 4.3).

4.5.2 Age classes

The majority of animals on Seal and Carnac Islands consisted of sub-adults and adults, with a maximum of 18 sub-adults and 24 adults hauled out at any one time (Figures 4.6 and 4.7). Juveniles were present on both Seal and Carnac Islands, but were low relative to overall numbers, not exceeding eight individuals at any time. Juveniles did not show any visible variation in hauled out numbers throughout the day. Sub-adults increased throughout the day during Periods 6 and 7, and slightly increased during Period 8 on Seal Island. A similar increasing pattern was observed during Periods 7 and 8 on Carnac Island. An increase in numbers of *N. cinerea* was evident in Period 7 between 2 h and 9 h after sunrise, but numbers decreased between 10 h and 12 h after sunrise. However, the variability in these last three hours of observations was visibly greater. Sub-adults on Carnac Island during Period 8 showed a slight decrease in numbers until 6 h after sunrise, but numbers thereafter increased. With few *N. cinerea* remaining in the area during the non-peak period, a small variation in *N. cinerea* numbers had a large influence in their percentages (indicated by the large standard deviations). Thus, patterns in haul-out behaviour of different age classes could only be detected in plots when *N. cinerea* numbers were high. Adult *N. cinerea* came ashore throughout the day during Periods 2 and 7 on both Seal and Carnac Islands and also during Periods 5 and 8 on Carnac Island. The sample size in Period 6 on Seal Island was too small and variability of adult counts too high to identify a pattern.

The ratio of adult to sub-adult *N. cinerea* increased at both islands during the breeding season (i.e. the period with few individuals occurring around Perth). During the breeding season (Periods 2 to 6), the period-averaged, maximum daily percentage (\pm SD) of sub-adults on the islands ranged from 2% (\pm 8 SD) to 32% (\pm 34 SD), compared with the percentage of adult males which ranged from 47% (\pm 23 SD) to 92% (\pm 49 SD; Table A2 in the Appendix). When numbers of *N. cinerea* in the area increased, however, the ratio of adults to sub-adults was closer to parity, with adult males ranging from 43% (\pm 26 SD) to 47% (\pm 24 SD) and sub-adults ranging from 49% (\pm 17 SD) to 52% (\pm 20 SD) (Table A2, Figure 4.8).

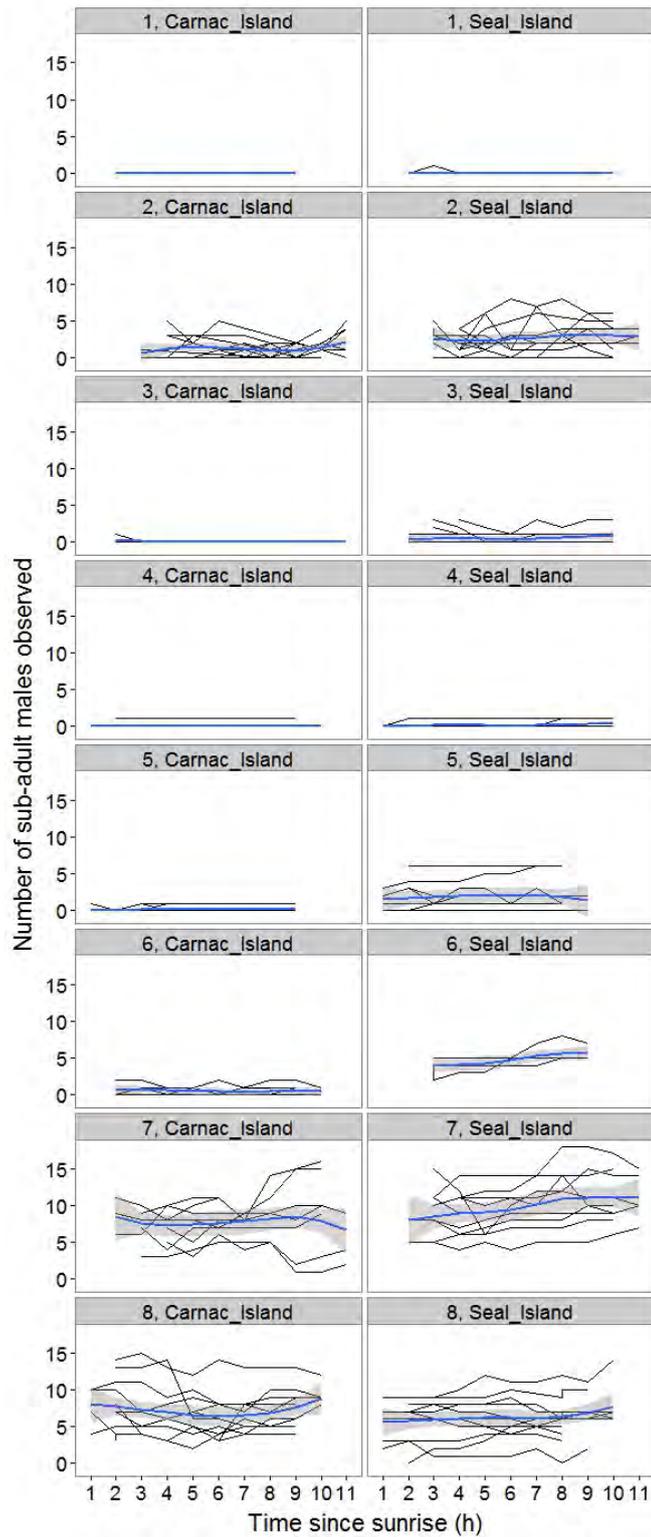


Figure 4.6: Number of sub-adult male *N. cinerea* observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represent counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The two exceptionally high and low observation days on Carnac Island were excluded.)

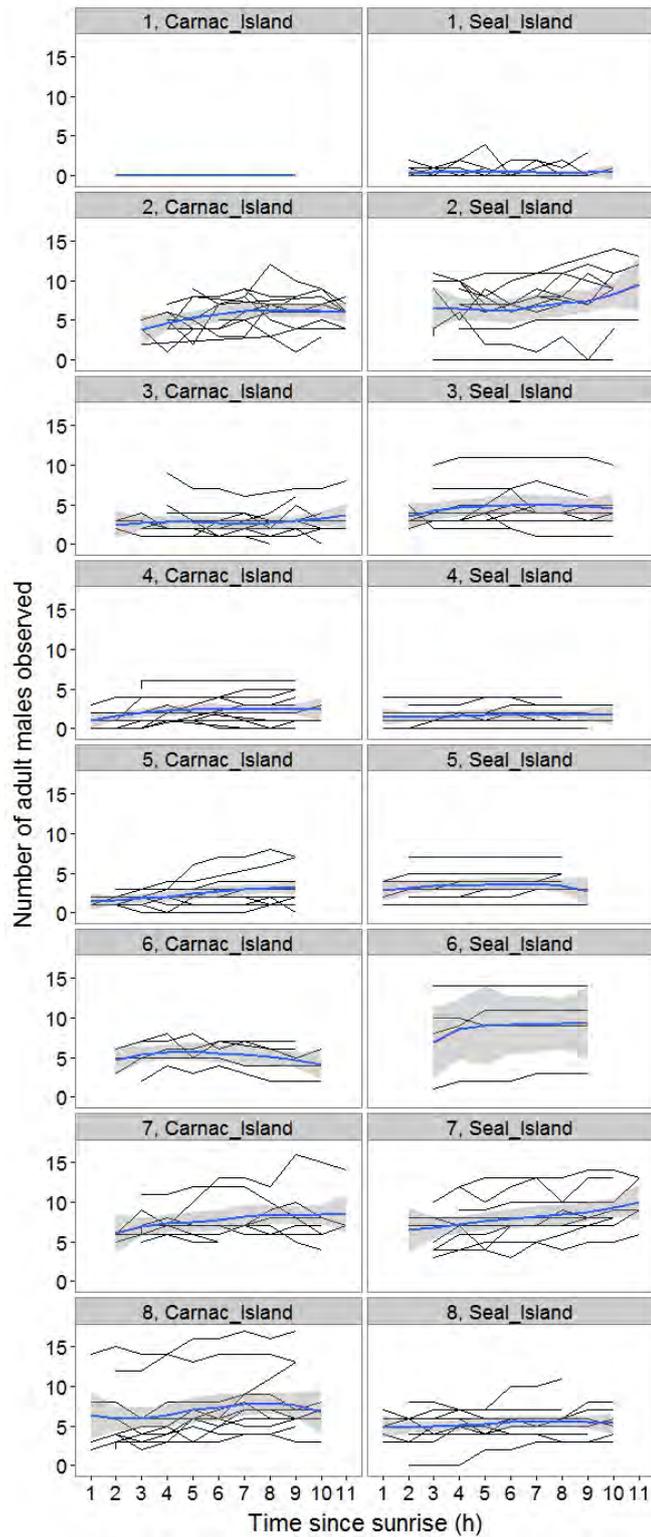


Figure 4.7: Number of adult male *N. cinerea* observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represent counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The two exceptionally high and low observation days on Carnac Island were excluded.)

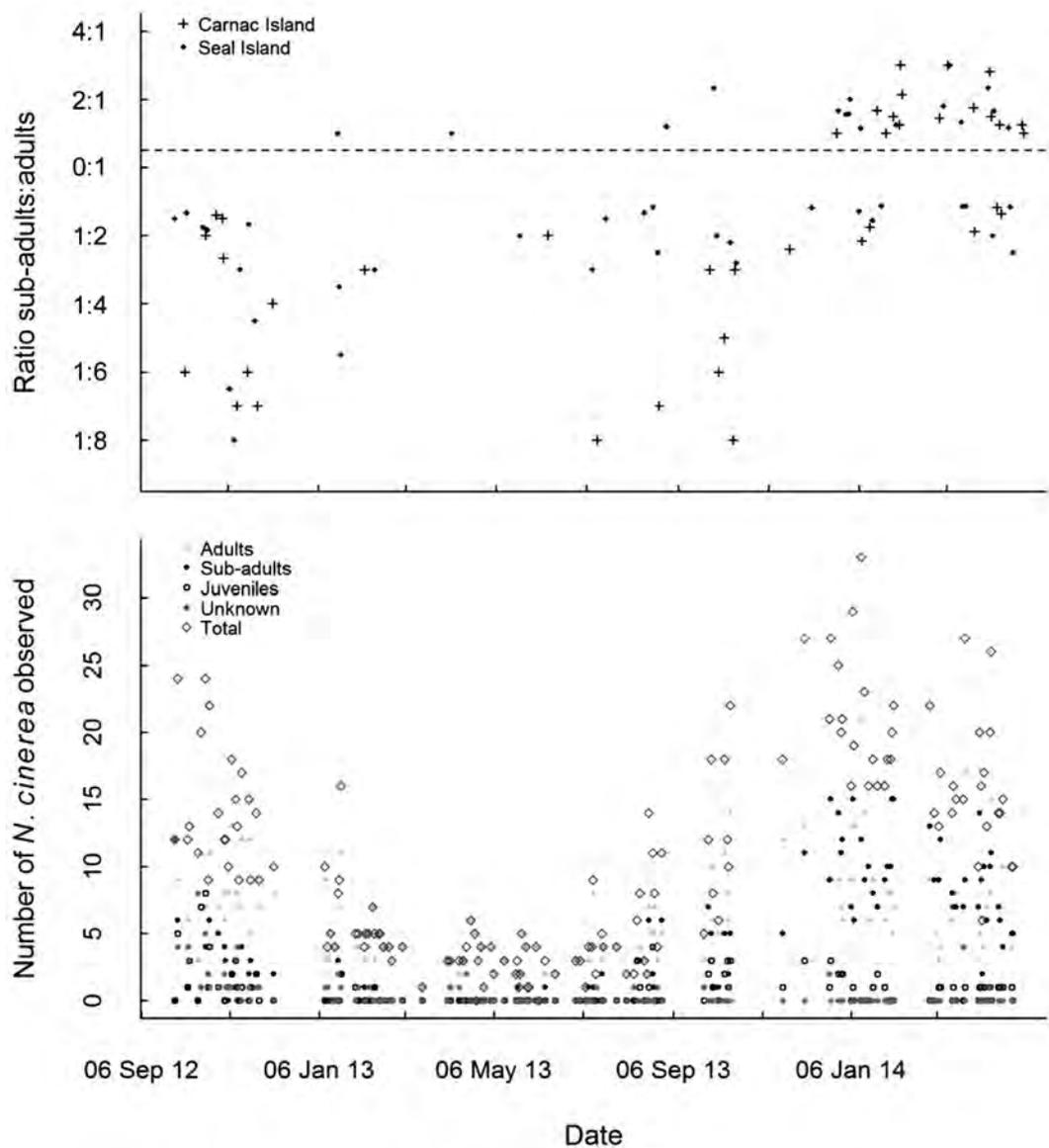


Figure 4.8: Age classes of *N. cinerea* observed at time of maximum count on 166 days on Carnac and Seal between September 2012 and April 2014 (after the first non-breeding season, i.e. high numbers in Perth). Top: Ratio of sub-adults to adults on Carnac (black crosses) and Seal (diamonds) Islands. The dashed horizontal line marks the ratio of sub-adults to adults at 1:1. Three data points are missing due to zero sub-adults or adults present and no ratio could be calculated. Bottom: Number of *N. cinerea* observed in each age classes (adults: grey triangles; sub-adults: black, solid circles; juveniles: white-filled circles; unknown: black asterisks) (bottom graph).

4.6 DISCUSSION

Numbers of male *N. cinerea* hauling out in Perth metropolitan waters display aseasonal cycles in abundance, varying according to the approximately ~17.4 month breeding cycle of the species. Sampling period, in relation to the breeding season, was the most influential variable on numbers of *N. cinerea* hauled out, similar to other sea lion species (Sepúlveda *et al.*, 2001; Marcotte, 2006). Results here were similar to those of Gales *et al.* (1992) in that overall *N. cinerea* numbers on Perth metropolitan haul-out islands followed a 17 to 18 months cycle, with troughs in Perth aligning with the peaks on breeding islands to the north (Jurien Bay).

At their peaks, numbers of hauled out *N. cinerea* varied between peaks and islands; with greater numbers at Seal than Carnac Island throughout all of the first peak and part of the second. There are many possible reasons for these differences. While a larger beach, potentially allowing more sea lions to haul out, has been suggested (Kriebler and Barrette, 1984), beach area and hauled out numbers appeared to be unrelated in this study. The beach area on Carnac Island was estimated to be approximately three times larger than that at Seal Island. The intertidal region where animals hauled out at Carnac Island appeared to be equally as large as that on Seal Island. We suggest that other drivers, such as proximity to preferred foraging locations, influenced haul-out site choice. If there are shifting prey locations, we suggest that this may be reflected by changes in haul-out site selection. There is evidence of this behaviour in other pinnipeds, such as Steller's sea lions *Eumetopias jubatus* in Alaska, suggested to depart to follow herring spawn and eulachon runs (Marcotte, 2006), and California sea lions *Zalophus californianus* in California, responding to prey-abundance (Ainley *et al.*, 1982). Since breeding, and the following peak numbers in the Perth metropolitan area, follow a 17 to 18 months cycle, successive peaks in the Perth area occur in different seasons. In this study, the first peak occurred in winter, and the second in summer. A seasonal change in targeted prey location is possible, and therefore a change in foraging location for the same prey or a change in target prey species in different seasons could be expected (Lowry *et al.*, 1991; Sinclair and Zeppelin, 2002; Sigler *et al.*, 2004; Womble *et al.*, 2005; Winter *et al.*, 2009; Womble *et al.*, 2009). Such instances could have resulted in Carnac Island being relatively less favourable during the second peak than the first peak. A second plausible explanation is that the numbers using the islands are directly related to human use reflected by the differing management regulations at the two islands. Carnac Island has direct access for recreational use on most of the beach, and despite the presence of a sanctuary zone on a section of the beach, the

entire beach is used for recreation. At Seal Island, recreational users are completely and effectively restricted from landing on any part of the island, including the beach.

In the present study, the percentage of adults to sub-adults increased at both islands from close to parity to above 90%, as the abundance of *N. cinerea* dropped from peak to trough in the cycle. A range of age class distributions have been observed among pinniped species at haul-out and breeding sites elsewhere. At a *E. jubatus* breeding island in Southeast Alaska, for example, more bulls than sub-adults hauled out consistently across the survey period (Marcotte, 2006). In contrast, sub-adult Subantarctic fur seals *Arctocephalus tropicalis* dominate during most of the year at a haul-out site where no breeding occurs as well as at a breeding site on the same island during the non-breeding season (at Marion Island, close to Antarctic Convergence; Kerley, 1983). Results that are more similar to those in this study were observed in *A. tropicalis*, with a sub-adult to adult ratio of approximately five to three at a haul-out site with occasional breeding on Amsterdam Island (6% females, Roux and Hes, 1984). In the current study, the changes in the composition of different age classes were consistent between the two islands and appeared to follow the timing of the breeding season. It is not known if age classes depart at different times from the breeding sites in this species; however, staggered departures have been observed between females and pups in *E. jubatus* (Marcotte, 2006). We suggest that observations here are likely a result of the following premise: as young males grow older, they increasingly travel longer distances and stay away for longer periods (Goldsworthy *et al.*, 2009b).

Day-to-day haul-out numbers of *N. cinerea* on Seal and Carnac Islands often fluctuated considerably, similar to South American *Otaria flavescens*, *E. jubatus* and Hooker's sea lions *Phocarctos hookeri* (Beentjes, 1989; Rosas *et al.*, 1994; Kucey, 2005). The positive relationship between numbers of hauled out *N. cinerea* and time since sunrise each day, observed during periods of high numbers of *N. cinerea* in the study area, is not uncommon. *Phocarctos hookeri* on the Otago Peninsula in New Zealand were found to increasingly arrive ashore mid-morning, with numbers reaching a plateau at mid-day before decreasing again around 18:00 h (Beentjes, 1989). Similarly, studies of other otariids, of varying sex and age class, have also shown a pattern of increasing numbers hauling out throughout the day, until mid-afternoon or early evening, when these numbers began to decrease (Stirling, 1968; Harestad, 1978; Ainley *et al.*, 1982; Sepúlveda *et al.*, 2001, 2012). On Carnac Island, this pattern was less prominent, and only occurred significantly dur-

ing the second non-breeding season, where there were overall greater numbers of animals hauling out.

During a study of *N. cinerea* at Dangerous Reef in South Australia, animals mostly arrived and hauled out between 05:00 and 07:00 h, and departed between 18:00 and 20:00 h to forage mainly at night (Goldsworthy *et al.*, 2009b). The study, however, was on lactating adult females, rather than males. While there are variations in behaviour among species, there are also variations within different populations of the same species. This has been shown to be true for *E. jubatus* which displayed no evidence of a diurnal pattern in hauling out at one site (Kucey, 2005; Marcotte, 2006); while Harestad (1978) indicated a clear diurnal pattern, dissimilar from the Perth findings, at another. Furthermore, *N. cinerea* in this study represent a unique group of juvenile, sub-adult, and adult males, reflecting the unique haul-out patterns observed. Adult male *N. cinerea* are known to forage further offshore, spend longer periods at sea, and have higher variations among individuals in distances travelled than other age and sex classes (Goldsworthy *et al.*, 2009b). The large number of adult males at Carnac and Seal Islands likely influenced the arrival times, as *N. cinerea* travelling longer distances may return later in the day. Conversely, juvenile foraging behaviour is reportedly similar to the more restricted ranges of adult females, compared to the more distant and longer duration foraging by adult males (Goldsworthy *et al.*, 2009b). The low numbers of juveniles counted on Seal and Carnac Islands suggest that daily patterns in behaviour would be mostly due to sub-adults and bulls using the islands.

While this study showed similarities and contrasts to findings in studies elsewhere, studies referred to here have reported haul-out timing in relation to local time rather than relative to the time of sunrise (e.g. Stirling, 1968; Sepúlveda *et al.*, 2001; Carlens *et al.*, 2006). We suggest that by reporting haul-out patterns relative to sunrise and sunset rather than relative to GMT, studies will be more comparable and meaningful in terms of their biological significance. Circadian rhythms of wild animals are more closely related to daily solar patterns and seasonal changes than our clocks (Reebs, 2002; McCauley, 2012). Also, we note, that in this study, logistical constraints limited observations before 07:00 h and after 17:00 h. It is possible that numbers at Seal and Carnac Island decrease at dusk or later, as was observed of *N. cinerea* in South Australia (Goldsworthy *et al.*, 2009b). *Phocarctos hookeri* and *O. flavescens* have also been reported to depart haul-out islands in the evening, likely to forage (Beentjes, 1989; Sepúlveda *et al.*, 2001, 2012). Alterna-

tively, numbers present at Seal and Carnal Islands later in the day after observations had been made could have remained constant if diurnal foraging patterns are absent, such as reported by Costa and Gales (2003) for female *N. cinerea* on Kangaroo Island, South Australia. The few counts conducted in this study before 07:00 h did not indicate a spike in numbers of *N. cinerea* returning ashore just after sunrise as reported by Goldsworthy *et al.* (2009b).

During the breeding season, when overall numbers of *N. cinerea* were low, there was no distinguishable diurnal pattern. This was likely due to either too few numbers of *N. cinerea* present to detect a relatively small effect, or highly variable foraging patterns (and thus arrival times) of adult males.

In this study, numbers of *N. cinerea* hauled out increased with increasing air temperature up to 21°C. This association has also been observed in harbour seals (*Phoca vitulina*) and Weddell seals (*Leptonychotes weddellii*; Watts, 1992, 1996; Andrews-Goff *et al.*, 2010). In winter months, the local waters around Perth can drop to below 16°C (BOM). Immersion in water below 17°C is energetically costly and may stop cell growth in *E. jubatus* (Feltz and Fay, 1966). Thus, for winter months, temperature could be expected to be a contributing factor to haul-out patterns as it affects cell growth and recovery. Hauling out also conserves energy, reducing heat loss by spending less time in a high temperature-conducting medium, such as water (Riedman, 1990). When temperature on land increases, however, pinnipeds may return to water to support thermoregulation, which may explain why there were no further increases in association beyond 21°C. However, the effects of temperature on seals' diurnal haul-out pattern has also been observed to vary at different times of year in *P. vitulina* in Scotland and in captive harp seals *Pagophilus groenlandicus* (Grellier *et al.*, 1996; Watts, 1996; Moulton *et al.*, 2000).

N. cinerea decreased in numbers in the present study when tidal height increased. While this pattern was similar to that reported for *E. jubatus* on islands off of Alaska, tide level did not have a significant influence at other locations in Alaska (Kastelein and Weltz, 1991; Kucey, 2005). Tidal heights have been reported to have variable effects in different seasons in *P. vitulina*; (Reder *et al.*, 2003), and other pinnipeds, regardless of season (Thompson *et al.*, 1989). In *E. jubatus*, more adult males entered the water during low tides, despite the tide level having little to no impact on their preferred haul-out location (Kastelein and Weltz, 1991). The variation observed in other studies is comparable to that seen in *N. cinerea* in the present

study, where tide level was more influential on Seal Island than on Carnac Island. While Seal Island was, overall, a preferred site over the larger beach of Carnac Island, the decrease in the number of *N. cinerea* hauling out became more prominent when tidal heights were above 1.0 m. This increase in tide would have greater impact on available intertidal area and overall beach on Seal Island than Carnac Island because of the wider beach on Carnac Island. Variation in tidal heights in this study was small compared to the 4 m tidal heights that impact some *P. vitulina* haul-out sites. Where higher proportions of *N. cinerea* haul out and tides above 4 m constrain the available size of the haul-out sites, numbers hauled out reduce (Watts, 1993). Extremely high tides, combined with strong winds, can push the water over the whole beach on Seal Island; however, a large proportion of the beach on Seal Island does not appear to be impacted during typical high tides, suggesting that the association between tidal height and numbers hauled out may not be simplistic. It is not only beach availability that is affected by lunar variation, however. Localised movement of fish species in relation to tides and lunar phase have been documented in the Perth metropolitan area (Wakefield, 2010), which may also be true for prey species of *N. cinerea*. Some prey species may be influenced by tide and may become easier to target during low tide (Morrison *et al.*, 2002; Ribeiro *et al.*, 2006), suggesting that *N. cinerea* might follow their food source during a time when it is easier to catch, leaving lower numbers of *N. cinerea* on the haul-out islands during lower tidal heights.

Wind speed did not have a significant effect on the numbers of *N. cinerea* hauling out in this study, similar to the case for *P. vitulina* in Scotland and Norway (Grellier *et al.*, 1996; Reder *et al.*, 2003) although it has been observed to affect diurnal haul-out patterns of other pinniped species (e.g. Lake *et al.*, 1997; Sato *et al.*, 2003). One sampling day on Carnac Island was removed of the analysis because of the unusual low number (zero) of *N. cinerea* hauling out during the peak season. Within three days prior to this count, a storm including time-averaged winds of up to 54 km/h and heavy rains with up to 10.2 mm/day precipitation passed Carnac Island and may have affected the *N. cinerea* haul-out pattern. Extreme environmental conditions have been shown to alter sea lion behaviour, for example in *E. jubatus* during stormy weather (Kenyon and Rice, 1961) or *O. flavescens* after an earthquake and tsunami (Sepúlveda *et al.*, 2012).

Finally, different methods of sampling can often bias count data. It is unlikely that the different sampling methods used here would have caused significant variations in count numbers in this type of study (Balouin *et al.*, 2014). Salgado Kent and Crabtree (2008) have previously shown that the remotely controlled camera on Carnac Island does not produce significantly different counts to those made by an observer on the island. Though considered infrequent, individuals may have remained undetected during the few surveys when counts were conducted from the vantage point at Shoalwater. On one occasion, rangers aboard a DPaW vessel, near Seal Island, conducted a count on Seal Island at the same time as counts were conducted from the Shoalwater vantage point. The authors observed 19 of the 21 observed from the vessel. Two were hidden from view from the vantage point.

While sub-models resulting in the same significant explanatory variables provided confidence in the final model selected, none of the sub-models accounted for absolutely all of the autocorrelation in the residuals. A smoother through numbers of *N. cinerea* observed versus normalized residuals still explained approximately 7.6% of the variation remaining in the residuals. Nevertheless, despite modelling constraints and convergence problems experienced in modelling these complex, longitudinal data, the models provided an improvement in our current knowledge of *N. cinerea*, which is needed for management and conservation.

The variability in numbers of *N. cinerea* hauling out at Carnac and Seal Islands within a day can affect the accuracy of trend in relative abundance over time if counts are undertaken at different times each day. For monitoring trends in relative abundance, counts would be best conducted between 9 and 11 h after sunrise if this is logistically possible. If this is not possible, a similar time of day across all survey days (in relation to the time of sunrise) should be targeted, so that they are comparable between sites and years. Conducting surveys only during periods of comparable temperature and tidal conditions, in addition to comparable times, would be logistically highly restrictive, resulting in a very small sample size. We have therefore suggested maintaining consistency in the most influential variable, which is the time of day. However, temperature and tidal heights can be recorded so that relative abundance can be adjusted using a correction factor to improve comparability over time (Seber, 1986; Huber *et al.*, 2001). Through the systematic collection of count data during periods when haul-out behaviour is expected to be comparable, more accurate trend estimations can be obtained to improve management outcomes.

4.7 ACKNOWLEDGEMENTS

This project was made possible with the significant support provided by the Department of Parks and Wildlife (Western Australia) who made the spotting scope available to use, provided substantial logistical support to and from islands, as well as local and scientific knowledge. The Department of Fisheries and Cockburn Volunteer Sea Search and Rescue also contributed transports to and from islands. The project was also supported by Malcom Perry for maintenance of the research vessel and the Fremantle Sailing Club for access to their boat ramp. Mike Todd lent us his telescope. Many volunteers have allocated substantial time in assistance in the field and data entry. We are grateful to Sven Gestauer for helping with the R coding and to Alain Zuur who gave useful advice concerning initial models. Miles Parsons provided comments on multiple drafts of the manuscript, which significantly improved its quality. Finally, this project was supported by funding from the Holsworth Grant, and was conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth).

Chapter 5

Responses of Australian sea lions, *Neophoca cinerea*, to anthropogenic activities in the Perth metropolitan area, Western Australia

Sylvia K. Osterrieder, Chandra Salgado Kent, and Randall W. Robinson

GRADUATE RESEARCH CENTRE

DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS BY PUBLICATION

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book:	Responses of Australian sea lions, Neophoca cinerea, to anthropogenic activities in the Perth metropolitan area, Western Australia Aquatic Conservation: Marine and Freshwater Ecosystems		
Surname:	Osterrieder	First name:	Sylvia
College:	College	Candidate's Contribution (%):	75
Status:			
Accepted and in press:	<input checked="" type="checkbox"/>	Date:	March 2016
Published:	<input type="checkbox"/>	Date:	

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

	4.5.2016
Signature	Date

3. CO-AUTHOR(S) DECLARATION

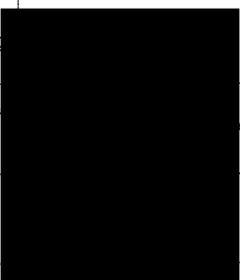
In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and

5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

<p>Curtin University, Bentley, Western Australia Palmyra, Western Australia</p>

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Sylvia Osterrieder	75	Planning project, data collection, data analysis, writing and editing manuscript		4/5/16
Chandra Salgado Kent	20	Planning project, supervision on data analysis and manuscript preparation		5/5/16
Randall W. Robinson	5	Supervision and review of draft		5/5/16

5.1 ABSTRACT

1. Tourist-based activities, partly due to their rapid increase, have raised concerns regarding the impacts of anthropogenic activity on marine fauna. Documented effects on pinnipeds in proximity to humans include changes in behaviour, site use and potentially higher aggression levels towards people. Effects vary considerably between populations and sites, thus requiring separate assessment of human impacts on activity and energy budgets.
2. Responses of the endangered Australian sea lion, *Neophoca cinerea*, to human visitation were recorded from November 2013 through April 2014. Exposure levels and response types to anthropogenic activities were assessed at two easily accessible locations with different management schemes, Seal (landing prohibited) and Carnac (landing permitted) Islands, Western Australia. Exposure levels were measured as both stimulus type (i.e. 'People', 'Paddlers', 'Small', 'Medium', and 'Large vessels', 'Tour vessels', and 'Jet skis'), and people ('Direct', 'Attract', 'Interact', 'View', 'Incidental', 'Water', 'Low-level') and vessel activities ('Interact', 'Approach/Follow', 'Anchor noise', 'Engine noise', 'Close to beach', 'Moderate/Fast travel', 'Slow travel', 'Transit', 'Drift/At anchor', 'Aircraft noise').
3. Exposure levels varied significantly between the islands in numbers, stimuli type, duration and minimum approach distances. The instantaneous behaviours of 'Lift head', 'Interact' and 'Sit' were the most frequent responses. 'Aggressive' and 'Retreat' responses, the highest disturbance levels measured, occurred on Carnac approximately once per day, but rarely on Seal Island. 'Aggressive' behaviour towards 'People' was observed only on Carnac Island and elicited only by 'People'. 'People', 'Tour vessels', and scenic 'Aircrafts' on both islands as well as 'Jet skis' on Carnac Island had the highest probability of triggering responses. Owing to their relatively high visitation at Seal Island, 'Paddle powered vessels', followed by 'Tour vessels' elicited the highest number of responses, compared with 'People', 'Small', and 'Medium vessels' at Carnac Island. The majority of responses occurred when any stimulus type was at short-range (≤ 10 m), and 'People' 'Viewing' *N. cinerea* elicited most. Vessels triggered more responses at larger ranges than 'People'.

4. To limit close-range access to *N. cinerea*, one possibility is to close the beach at Carnac Island to human visitation and increase the minimum approach distance by vessels and 'People' by installing marker buoys at least 15 m from the shore.

5.2 KEYWORDS

Keywords: coastal; island; disturbance; endangered species; habitat management; mammals; pinnipeds; anthropogenic activities

5.3 INTRODUCTION

Conservation of animal populations requires accurate knowledge of the potential impacts that anthropogenic activities may have on their health and survival. Impacts from anthropogenic activities are wide-ranging, and the level of severity depends upon factors including the type of activity, duration and their proximity to the animals. Marine ecotourism such as whale and dolphin watching is increasing in popularity and often includes direct (swimming with) and indirect (observational) interaction. More recently, excursions to observe other fauna such as seals and sea lions at haul-out locations, have also increased. In the Southern Hemisphere alone, 1.3 million people visit pinnipeds every year (estimated average from 1995 to 2000), with an annual value of US \$12.6 million (Kirkwood *et al.*, 2003). Activities range from swimming with seals and sea lions to watching them from boats, planes or land (Boren *et al.*, 2002; Kirkwood *et al.*, 2003; Lovasz *et al.*, 2008; Cowling *et al.*, 2014).

The Australian sea lion, *Neophoca cinerea*, endemic to Western and South Australia (Gales *et al.*, 1994; Goldsworthy *et al.*, 2008), is classified as Vulnerable by the Environment Protection and Biodiversity Conservation (EPBC) Act 1999 and by the two states in which it occurs (National Parks and Wildlife Act 1972, South Australia; Wildlife Conservation Act 1950, Western Australia), and is listed as Endangered on the IUCN Red List of Threatened Species (Goldsworthy, 2015). In Australia, *N. cinerea* is increasingly targeted by marine tourism. Seal Bay at Kangaroo Island in South Australia is the most popular location to watch *N. cinerea* in the wild, receiving up to 110,000 visitors in any one year since the 1950s (Kirkwood *et al.*, 2003). Haul-out islands off Port Lincoln, South Australia and Jurien Bay, north of Perth in Western Australia (WA) also receive large numbers of visitors (Kirkwood *et al.*, 2003).

Pinniped tourism is very popular and economically beneficial (Kirkwood *et al.*, 2003). However, there is public and scientific concern that these activities may have detrimental effects on the health of marine wildlife populations (Gerrodette and Gilmartin, 1990). Various studies have endeavoured to document behavioural changes, for example aggressive displays, avoidance or habituation, physiological responses, and direct threats to the survival of animals, such as entanglement and increased risk of boat strikes (Gerrodette and Gilmartin, 1990; Constantine, 1999; Stevens and Boness, 2003; Newsome and Rodger, 2008; French *et al.*, 2011). There is evidence that animals may reduce time spent resting or hauling out, possibly af-

fecting their energy budget; or may leave pups unattended, which, while currently untested, could potentially increase pup mortality (Kovacs and Innes, 1990; Jansen *et al.*, 2010). Assessing the magnitude of effects is complex as age, sex, degree of exposure, and stage in the breeding cycle may influence responses to disturbance and level of impact (Boren *et al.*, 2002; Cowling *et al.*, 2015). Furthermore, most studies have been limited to assessing short-term (over the course of a day) and immediate responses of individuals, rather than long-term impacts (over months and years) on the population. This is probably a result of the challenges involved in long-term monitoring requiring long-term funding, and the non-trivial nature of disentangling the effects of human disturbance from changes in a complex environment.

To reduce the impact of human/pinniped interactions, various regulations and guidelines have been initiated (e.g. spatial and temporal restrictions or limitations in vessel speed and visitor numbers) to both maintain the health of the marine environment and to protect animals and tourists during interactions (Orams, 1999). In Australia, several patrolled marine parks and sanctuary zones, with limited access for visitors to view and interact with animals, have been established (Gales, 1995; Kirkwood *et al.*, 2003; Cassini *et al.*, 2004; Salgado Kent and Crabtree, 2008; Young *et al.*, 2014). Where close approaches are allowed, there are guidelines recommending safe distances for viewing and for reducing disturbance to pinnipeds. Although regulations and guidelines are in place for management, the scientific basis for these management decisions in relation to *N. cinerea* is limited (DEC, 2007; Lovasz *et al.*, 2008; Salgado Kent and Crabtree, 2008; Young *et al.*, 2014).

In metropolitan waters around Perth, for example, moving/approaching *N. cinerea* slowly and keeping a minimum distance of 5-10 m are recommended (DEC and Fisheries, 2011). At this location, the largest numbers of *N. cinerea* haul out on Seal and Carnac Islands, which are two of the six main local haul-out sites (Gales *et al.*, 1992). Owing to the close proximity of the islands to Perth (~2 million people) and their ease of access, both islands are heavily used for tourism and recreational activities, including viewing *N. cinerea* in the wild (Orsini and Newsome, 2005). On Seal Island, located within a marine park, landing by either vessels or people is prohibited. In comparison, the beach on Carnac Island is divided into two different zones with only the sanctuary zone off limits to the public. While the designation of the zone was based on the area used most often by *N. cinerea* in a study in 2005 (Orsini and Newsome, 2005), a follow-up study, two years later, showed that *N.*

cinerea used the beach outside the sanctuary zone just as frequently (Salgado Kent and Crabtree, 2008). It was determined that the most effective approach for reducing disturbance on Carnac Island was to expand the sanctuary zone over the entire beach (Salgado Kent and Crabtree, 2008).

Beyond the study at Carnac Island, the effectiveness of small sanctuaries, or no-go zones, in reducing disturbance in the Perth metropolitan area, and other areas, is not accurately known (Gormley *et al.*, 2012; Hartel *et al.*, 2015). Owing to the competing interests in use and access to the islands by conservationists, recreational users, and commercial operators, as well as the underlying need for conservation of the species, the effectiveness of sanctuary zone size and applied management strategies must be assessed (Salgado Kent and Crabtree, 2008). Establishing baseline data and determining impacts of various types of use is critical for improving the design of reserves and management outcomes (Kelleher, 1999).

This study investigates the responses of *N. cinerea* to anthropogenic activity at two sites (Seal Island and Carnac Island) with different management strategies. Various activity types were documented as pinnipeds have been shown to respond differently to varying stimuli (Cassini, 2001; Boren *et al.*, 2002; Jansen *et al.*, 2010). Specifically, stimulus types (i.e. vessel types and people), their activities, and *N. cinerea*'s response behaviours were categorized and recorded. Distances between the stimuli and responding *N. cinerea* were also recorded. Thus, the influence of stimulus types, their activities, and distances on *N. cinerea* behaviour were investigated.

The specific objectives of this study were to: 1) compare the numbers of vessels and/or people present, and to quantify their activities at Seal and Carnac Islands, two islands with contrasting management types, to provide context to the response of *N. cinerea* for wider application; 2) compare the frequency and level of disturbance to *N. cinerea* at the two islands, in relatively close proximity to urban areas (0.9 and 10 km); and 3) assess the influence of anthropogenic activity types and their proximity to the animals through measurements of the frequency and level of *N. cinerea* responses. Understanding the key impacts of tourism on *N. cinerea* behaviour is necessary for improved, scientific-based, long-term management, and where necessary, recovery plans for endangered species, such as *N. cinerea*, on both a local, regional and national scale. To do this requires an understanding of the context within which the animals have been observed (Objectives 1 and 2).

5.4 METHODS

5.4.1 Study sites

Six islands are known to be used as haul-out sites by male *N. cinerea* off the Perth metropolitan coast, Western Australia (Figure 5.1). Of the six islands, the islands included in this study - Seal Island (-32.29° S, 115.69° E) and Carnac Island (-32.12° S, 115.66° E; Figure 5.1) - have the largest proportions of *N. cinerea* hauling out; more than 30 during the peak season (Osterrieder *et al.*, 2015b). Usually less than 10 *N. cinerea* haul out at the other metropolitan haul-out islands (Department of Parks and Wildlife, unpublished data).

Seal Island is a sanctuary zone where landing is not permitted, located in the Shoalwater Islands Marine Park, ≈ 0.9 km from the coast and ≈ 45 km south of Perth (Figure 5.1). Here, *N. cinerea* can be viewed on a kayaking or boat tour and the sanctuary zone's 'look, but don't take' area offers the highest level of protection allowing boating, snorkelling and nature appreciation activities, but prohibits fishing (DEC and Fisheries, 2011). *N. cinerea* predominantly haul out on the beach of ≈ 0.27 ha (estimated from a Google Earth, 2014 image from 1st January 2014) on the eastern side of the island. They also haul out adjacent to the shrubs or caves at the southern bay on occasion, but have not been seen to haul out on the other sides of the island that comprise mostly rocky outcrops.

Carnac Island (≈ 10 km south-west of the Fremantle coast and ≈ 15 km south of Perth; Figure 5.1) is an A class nature reserve, with part of the island designated as a sanctuary zone. Access to most of the island is prohibited, but the southern part of the eastern beach is available for public access during the day (CALM, 2003). The eastern beach is ≈ 0.78 ha (estimated from a Google Earth, 2014 image from 1st January 2014) and *N. cinerea* mainly haul out on this sandy beach. Charter and tour vessels travel to Carnac Island, though less frequently than to Seal Island. Both, Seal and Carnac Islands, can also be easily accessed by private recreational vessels.

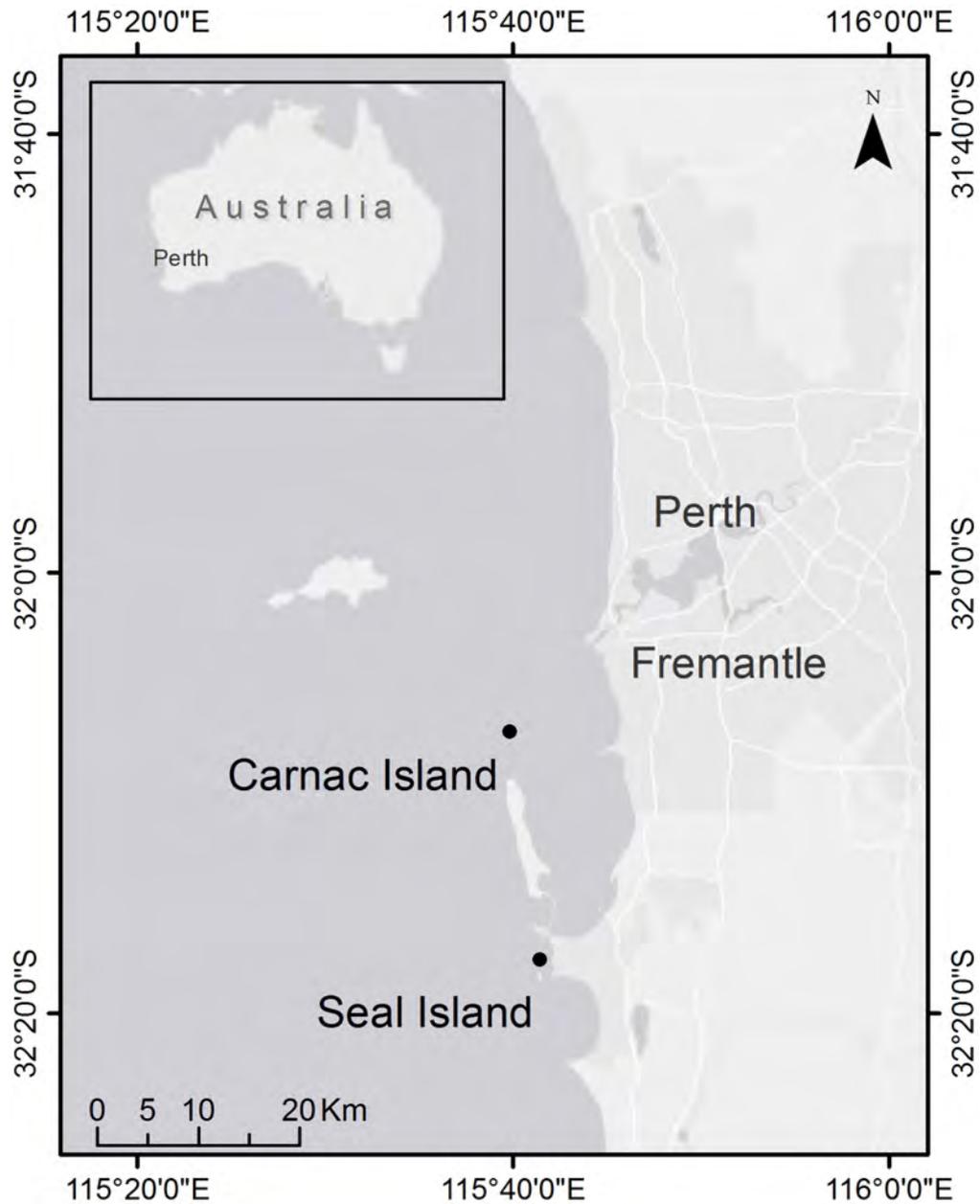


Figure 5.1: Seal Island and Carnac Island, largest *N. cinerea* haul-out sites in the Perth metropolitan area, Western Australia.

5.4.2 Experimental design

Count data for Seal and Carnac Islands, either conducted by an observer located on the islands, or remotely using a locally installed, live video camera, were collected over a period of two years, between June 2012 and April 2014 (Figure 5.2a)). Within this period, individual responses to anthropogenic activities were recorded over five months, from the end of November 2013 until the end of April 2014.

Observations at Seal Island were predominantly made from a vantage point located on the island with a view of the entire beach, using either the naked eye or binoculars (Nikon Eagleview 8-24 × 25). Transfer issues restricted travel to Seal Island between July and August 2012 and on 9th October 2012 (Figure 5.2a and b)). As a result, observations during those times were made from a vantage point on the mainland (32.2855° S, 115.7035° E), with the entire beach area in view, using either a telescope (115 mm Tasco reflecting with either a 25 mm, 20 mm or 10 mm eye piece 36×, 45× or 90× magnification, respectively) or a spotting scope (Televid 77 with 20× to 60× zoom).

Data for Carnac Island were predominantly collected remotely, via an at the time of operation live, remote controlled camera (AVT284 IP Camera with remote Pan, Tilt, and Zoom capability and 22× optical zoom) with a radio link to a local Department of Parks and Wildlife office (using a Proxim 8150 PTP microwave radio link). The camera was located overlooking the eastern beach. Direct observations made on Carnac Island from the same vantage point as the camera were conducted between the end of November 2013 and the end of April 2014, with the exceptions of the 8th and 13th January 2014 (Figure 5.2a) and b)). To minimize disturbance caused by the researchers upon arrival, the vantage points on Seal and Carnac Islands were approached from a small bay at the back of the beach (Seal Island), or by landing in gaps between *N. cinerea* (Carnac Island), always remaining as far away from *N. cinerea* as possible. While on-island, observations were always conducted from a range >20 m, movement minimized (e.g. no sudden standing up) and conversation kept to a level thought to be inaudible at the ranges where *N. cinerea* had hauled out.

Remote observations were limited to counts of vessels and ‘People’ (i.e. people in the water or on the beach, not attached to any floatation device, and herein classified as ‘People’) to ensure comparable and accurate data were collected. More detailed behavioural data were collected only when observers were on the islands. The telescope and spotting scope were considered to give sufficient magnification for easy and accurate counts, and the remotely operated camera has previously been shown to reflect counts accurately (Salgado Kent and Crabtree, 2008).

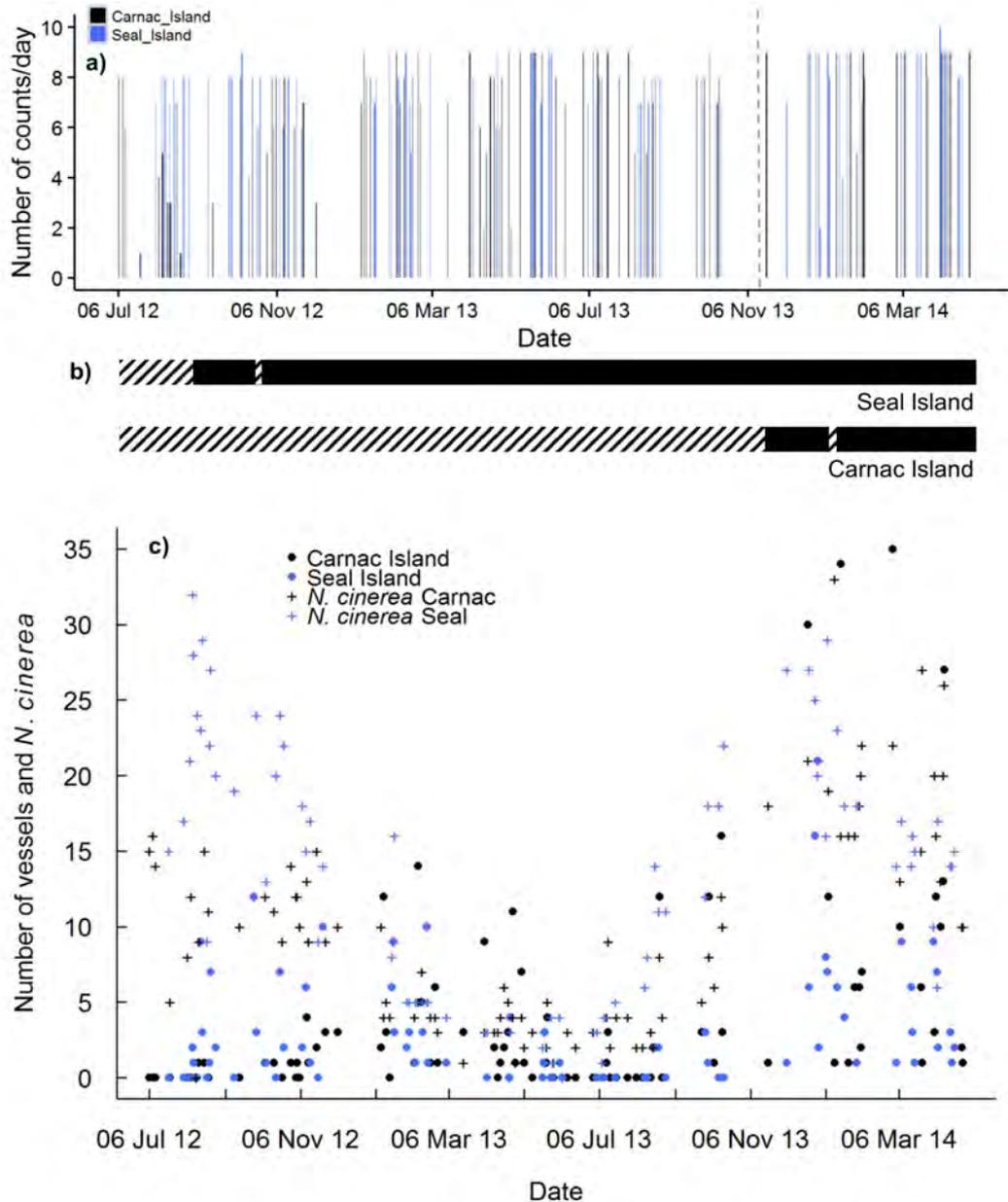


Figure 5.2: a) Sampling frequency with number of counts conducted per sampling day (Carnac Island is demarcated in black, Seal Island in blue, and dashed line at end of November 2013 indicates the start of the collection of disturbance data). b) Sampling method used throughout the sampling period displayed in Figure 2a (black stripes = remote, including Seal Island observations from the vantage point in Shoalwater using the telescope or spotting scope, and Carnac Island observations with the remote controlled, live camera). c) Maximum number of vessels (○) and *N. cinerea* (+) observed on Carnac Island (black) and Seal Island (blue) each day during 166 survey days between June 2012 and April 2014.

5.4.2.1 Counts of vessels, 'People' and *N. cinerea*

All vessels approaching or passing within approximately 400 m of the beach were counted by one to three observers during island-based monitoring. In addition, counts were made of all 'People' and *N. cinerea* within view, either on land or in the water. Counts were generally conducted during 5 to 10 min scans, and were made every hour primarily between 08:00 and 16:00 h, with the exceptions of inclement weather or when technical difficulties cut some days short. *N. cinerea* known to be present during the count but temporarily out of view (i.e. sighted when going behind rocks and again when coming back into view) were also recorded.

Hourly counts conducted remotely were carried out by panning from north to south, from one side of the beach to the other, to count vessels, 'People' and *N. cinerea* (Salgado Kent and Crabtree, 2008). The zoom on the live camera was used to aid counts when necessary, particularly to distinguish *N. cinerea* from some rocks on the far, southern part of the beach.

5.4.2.2 Behavioural responses to human activities

An observer recorded arrival and departure times of anthropogenic stimulus types (e.g. vessels, and 'People', Table 5.1), including the time 'People' entered or left the water or the beach, on a dictaphone. These arrival and departure times were used to calculate the total number of 'People' and vessels, except on five days when high activity and numbers of vessels at Carnac Island (up to a maximum of 36 vessels and 20 'People' at any one time) made this unfeasible. During these periods, counts were conducted every 5 to 15 min instead to determine totals and numbers of *N. cinerea* present during each behavioural response taken from the nearest count. At all other times on Carnac Island and at all times on Seal Island vessel numbers were accounted for at each response. During these 'busy' periods, particular attention was paid to those closest to *N. cinerea* and vessels involved in activities anticipated to have greater impacts (e.g. varying the engine throttle or playing music) to capture detailed behavioural response information. Overall documented responses of interactions anticipated to have 'lower' impacts were not affected. Rather, the more detailed information was used separately - for focal behavioural response analysis.

Table 5.1: Definition of stimulus categories.

Stimulus type	Description
People	People in the water or on the beach, not attached to a floatation device or vessel. On Carnac ‘People’ occurred in the water and on the beach. On Seal Island people were restricted (legally) to the water (except for on 10 occasions when people accessed the island illegally)
Paddle powered/(Paddlers)	Small vessels ≤ 3 m in length with no engine (e.g. kayak, paddleboard, canoe, row boat, body board)
Small vessels	Vessels up to 6 m in length (e.g. recreational/fishing vessels, dinghies)
Medium vessels	Vessels >6 m and ≤ 15 m in length with a single deck (including government department vessels and power/speed boats)
Large vessels	Vessels >15 m in length or fitted with multiple decks (e.g. charter boats, catamarans, party boats, commercial dive vessels, and sailing boats)
Tour vessels	Vessels visiting the islands with the aim of observing <i>N. cinerea</i> (these were usually medium sized vessels on Seal and large vessels on Carnac Island)
Jet ski	Jet propelled personnel water craft
Aircraft	Planes (usually scenic and military) and helicopters

*only encountered once and therefore excluded from analysis and figures

‘People’s and vessels’ activities (Tables 5.2 and 5.3), including the times the activities were undertaken, were also recorded. Groups of ‘People’ were defined as one or more closely-spaced humans displaying similar or associated behaviour. Groups of vessels (such as several kayaks travelling in close proximity) were considered in the same way.

Table 5.2: List of categories of activities associated with vessels ordered from highest to lowest anticipated impact.

Activity Classification	Description
Interact	Vessels interacting with <i>N. cinerea</i> , including: animals following a vessel, swimming or porpoising around a vessel
Approach/Follow <i>N. cinerea</i>	Vessels which are seeking to interact with <i>N. cinerea</i> by approaching for a better view, driving in circles around <i>N. cinerea</i> or following/chasing <i>N. cinerea</i>
Anchor noise	Setting or retrieving the anchor with associated rattling noise of the anchor chain and splashing when dropping the anchor
Engine noise	Activities producing higher level of engine noise than when travelling, including revving engine, reversing, travelling with particularly noisy engines
Close to Beach	Activities within the vicinity of the beach, including approaching, staying close to or leaving the beach, and landing on the beach
Moderate/Fast Travel	Travelling at moderate to fast speeds (including rapid circles)
Slow travel	Travelling at a slow speeds (such as paddling)
Transit	Approaching, passing, leaving or returning to the vicinity of the island, including paddle powered vessels placed in the water from a vessel anchored off Carnac Island
Drift/At anchor	Activities with no or low movement and/or noise levels associated with them, including drifting, vessels anchored, or no activity
Aircraft noise	Planes or helicopters flying overhead

Table 5.3: List of categories used for recording anthropogenic activities in the order of the highest to lowest anticipated impact levels (if different activities were performed at the same time, the highest activity was recorded). Abbreviated activity names used in text and figures are marked in bold.

Activity	Description
Direct / Invasive Interaction	Invasive activities in direct contact or attempting direct contact with <i>N. cinerea</i> , including touching <i>N. cinerea</i> directly or with a tool (e.g. stick), feeding <i>N. cinerea</i> (including throwing fish towards <i>N. cinerea</i>), throwing objects towards <i>N. cinerea</i> , and splashing water at <i>N. cinerea</i>
Deliberately Attracting Attention	Activities seeking <i>N. cinerea</i> 's attention and provoking responses, without <i>N. cinerea</i> 's engagement, including splashing water (to attract <i>N. cinerea</i> , but not splashing directly at them), imitating <i>N. cinerea</i> noises (barking), clapping, honking, and banging vessel, hitting paddles on the water's surface, following <i>N. cinerea</i> (usually swimming), circling <i>N. cinerea</i> (e.g. standing/crowding around <i>N. cinerea</i> in a circle), yelling, screaming, whistling, loud talking, laughing, loud speaker systems on vessels, playing music, barking dog, jumping into the water
Mutual Interaction	Interacting, people and <i>N. cinerea</i> engaged with each other, i.e. people playing with <i>N. cinerea</i> (in the water), such as mimicking <i>N. cinerea</i> behaviour and achieving a similar response from the <i>N. cinerea</i>
Viewing	Activities involved in viewing <i>N. cinerea</i> such as standing and observing <i>N. cinerea</i> , taking photos, being in close proximity to <i>N. cinerea</i> , approaching, passing or leaving <i>N. cinerea</i> , retreating from aggressive <i>N. cinerea</i>
Incidental Activities	Activities not aimed to provoke <i>N. cinerea</i> responses, typically occurring on the beach or in shallow (knee-deep) water, including playing on the beach, dragging a boat onto the beach or into the water, picnicking, talking at a conversation level (including humans on a vessel), walking on the beach, in the wash zone or in shallow water (but not passing <i>N. cinerea</i>)
Water-related Activities	Activities undertaken in the water (more than knee-deep) including swimming, snorkelling, diving, playing in water (but not interacting with <i>N. cinerea</i>), entering and leaving water from the boat, fishing
Low-level Recreational Activities	Activities, not fitting in previous categories and only when of low level noise, and without rapid movements such as 'quiet', i.e. barely audible talking humans not moving or moving little on the boat or beach

Vessel categories included 'Paddle powered', 'Small', 'Medium', 'Large' and 'Tour vessels' as well as 'Jet skis', and 'Aircrafts' (Table 5.1). Vessel activities included 10 categories ranging from activities anticipated to have a low impact, such as 'Drifting' and 'At anchor', to those anticipated to have a high impact such as 'Interactions with *N. cinerea*' (Table 5.2). Activities undertaken by 'People' fell into one of seven categories ranging from 'Low-level' recreational activities to 'Direct', invasive interactions (Table 5.3). *N. cinerea*'s behavioural responses to the activities were classed as 'Aggressive', 'Retreat', 'Enter water', 'Interact', 'Travel', 'Sit', 'Lift head', 'Move head', 'Look', and 'No response' (Table 5.4). If multiple activities occurred at the same time (e.g. standing close to and watching *N. cinerea* - 'Viewing' activity) and clapping hands or screaming ('Attract' activity), the activity with the highest anticipated impact was recorded ('Attract' in this example; Table 5.3). Similarly, if a *N. cinerea* responded with multiple behavioural responses (e.g. 'Moving its head' to look towards the stimulus and 'Sitting' up at the same time) the highest response level was recorded ('Sit' in this case; Table 5.4). Ethograms were compiled based on proven techniques from previous studies (Beentjes, 1989; Cassini *et al.*, 2004; Salgado Kent and Crabtree, 2008; Bowles and Anderson, 2012), and adjusted to capture those relevant to this study. For each interaction, numbers of *N. cinerea* responding, frequency of responses and *N. cinerea*'s behavioural response types were recorded. *N. cinerea* do not have readily identifiable patterns and do not often have scars which aid discrimination among individuals. Therefore, on some rare occasions, during periods when greater numbers were hauling out and multiple individuals responded to the different stimuli, it was not always possible to assign responses to particular individuals.

Table 5.4: Definitions of response types of *N. cinerea* responses to vessel and human activities, in order from highest to lowest level anticipated responses (if different responses occurred in combination with each other, the most severe was recorded).

<i>N. cinerea</i> response	Description
Aggression	Aggressive behaviour displayed towards a stimulus (e.g. gaping or lunging at ‘People’)
Retreat	<i>N. cinerea</i> walking or swimming away from vessels or ‘People’ to deliberately increase the distance between vessel/‘People’
Enter water	<i>N. cinerea</i> entering water, including running into water
Interact	Socializing with vessels or ‘People’, includes behaviours such as porpoising, spy hopping, following and swimming in circles around vessels or ‘People’
Sit	<i>N. cinerea</i> sitting upright, including when near or facing a stimulus
Travel	<i>N. cinerea</i> swimming or walking in a specific direction (e.g. swimming or walking past ‘People’)
Lift head	<i>N. cinerea</i> lifting its head off the sand, such as when looking at a stimulus
Move head	<i>N. cinerea</i> moving its head by turning its head and looking around when sitting up or after lifting its head, such as when looking at vessels or ‘People’
Look	Opening or moving eyes to look at a stimulus (i.e. vessels or ‘People’)

Whenever possible, distances and angles from the observer to the stimuli (vessel or ‘People’), from the observer to the *N. cinerea* closest to the stimulus, and from the observer to any *N. cinerea* responding to anthropogenic activities (regardless of the distance) were measured using laser rangefinder and compass (TruPulse 360 R with accuracies of ± 0.5 m in distance to high quality targets such as *N. cinerea* and stimuli types, and ± 1 deg azimuth). The distance between the stimulus and the nearest *N. cinerea* (unless another *N. cinerea* was responding to the stimulus which was then measured) was calculated using basic trigonometry. Distances were not measured on 18th January 2014 at Carnac Island, on 28th December 2013 and 3rd April 2014 at Seal Island, nor after 10:05 h on 8th March 2014 on Seal Island because of the unavailability of the rangefinder or the lack of functioning replacement batteries. *N. cinerea* in the water did not typically present a sufficiently reflective target for the rangefinder and could not be measured. When appropriate, distance from the closest vessel or ‘People’ to the closest *N. cinerea* was estimated in *N. cinerea* body lengths (≈ 2 m) and was used for estimating distances up to 10 m. ‘People’

within arm's reach of a *N. cinerea* were recorded as at 1 m and those touching a *N. cinerea*, as 0 m. Distances were measured when *N. cinerea* responded to groups of vessels or 'People' (in the water or on beaches) and when groups were seen to approach *N. cinerea*.

5.4.3 Analytical approach

Overall numbers of each vessel type and 'People', and numbers of *N. cinerea* were based on data collected over the entire study period. However, for comparing responses of *N. cinerea* with anthropogenic disturbance at Carnac and Seal Islands, a subset of data was used from the same period at both islands (from 20th November 2013 to 27th April 2014), to ensure observations had comparable seasonal conditions. Observation effort at the two islands differed by 8 h (equivalent to approximately one survey day of 20), thus effort was accounted for by normalizing the frequency of activities and responses at each of the islands to an hourly rate. 'Aircrafts' were considered in analyses of the total numbers of groups of stimulus types visiting and in the total number of responses elicited by anthropogenic activities. However, owing to their relatively low overall numbers and different types of behaviours, they were excluded from all other analysis. All analyses and figures were produced using R version 3.2.0 (R2014) run through RStudio Version 0.98.1103 - © 2009-2014 RStudio, Inc.

5.4.3.1 Number of vessels and 'People'

The number and composition of different stimulus types at Carnac and Seal Islands were compared using Pearson's Chi² tests with Yates' continuity correction (Yates, 1934). While sampling effort was approximately 6% greater on Seal Island than on Carnac Island, Chi² tests are robust with unequal sample sizes (McHugh, 2013). Furthermore, the difference in sampling effort between the islands was small.

The duration of visits and minimum approach distances of stimulus types at Carnac and Seal Islands were compared using Kruskal-Wallis tests (Kruskal and Wallis, 1952). Comparisons of duration and minimum approach distances between Carnac Island and Seal Island for the different vessel types and 'People' were also analysed using Kruskal-Wallis tests. Kruskal-Wallis tests were used due to the non-normal distribution of the data.

For all analyses on numbers of each stimulus type, duration of their visits, and their minimum approach distances, multiple tests were conducted using the entire dataset and several subsets of the data. Therefore, the family-wise error rate (the probability of rejecting at least one null hypothesis erroneously) could be expected to increase since the tests are no longer independent. A sequential Bonferroni correction on the p -values considered as significant was therefore applied (Rice, 1989). Eight Chi² tests were performed on the exposure of *N. cinerea* to the number of stimuli and were considered significant when $p < 0.006$. Duration and minimum approach distance of different vessel types and 'People' were considered significant when $p < 0.006$ and $p < 0.005$ to account for the eight and nine Chi² tests conducted, respectively.

5.4.3.2 Behavioural responses to anthropogenic activities

On occasion, individual *N. cinerea* responded several times to a single stimulus, sometimes in quick succession, such as 'Lifting their head' to 'Look' at the stimulus and then 'Sitting up' within a few seconds or minutes. If the same individual (*N. cinerea* A, for example) responded to the same stimulus within a 5 min period only one response, the behaviour considered to represent the greatest response, was used in analyses. If, however, *N. cinerea* A responded to a different stimulus at a different location, or a different individual (*N. cinerea* B) responded to the same stimulus as *N. cinerea* A, these were counted as separate responses. Once the 5 min period was completed, a response to the original stimulus by *N. cinerea* A was counted as a new response. During a subsample of 310 responses, the number of repeat responses (i.e. responses to the same stimulus by the same individual) occurring more than 5 min after the initial responses and prior to the stimulus departing the area occurred <3% of the time. Ongoing 'Interactions' between humans and *N. cinerea* can feasibly extend over 5 min (for example a *N. cinerea* may follow a vessel or play with a person for a prolonged period). Continued 'Interactions' of this sort (also exceeding 5 min) with one stimulus group were analysed as a single 'Interaction' response.

The number of *N. cinerea* responses to each stimulus type were compared among each other and between islands. In addition, a comparison of number of responses for each behaviour type was made among the stimulus types and the two islands. Either the Chi² test, or in cases with small sample sizes Fisher's exact test (Fisher, 1922; Yates, 1934), were used. A sequential Bonferroni correction was carried out and p -values of $p < 0.005$, $p < 0.005$, $p < 0.008$ and $p < 0.007$ were considered as significant for analyses of the number of responses for each behavioural type level

to: groups of vessels vs. groups of ‘People’ regardless of the location, a stimulus regardless of the type (vessels and ‘People’ combined) at Carnac Island vs. Seal Island, groups of ‘People’ at Carnac Island vs. Seal Island, and groups of vessels at Carnac Island vs. Seal Island, respectively (Rice, 1989). To assess whether the percentage of *N. cinerea* responding (of those hauled out at any one time) was related to the number of vessels and ‘People’ visiting the island at that time, a linear regression was applied to the data and the corresponding R^2 value was calculated.

To investigate the influence of stimulus activities (regardless of whether they were on a vessel, swimming, or on land), *N. cinerea* behavioural responses to each activity level were calculated per hour of sampling effort and plotted. Response behaviours per hour of sampling effort were also calculated for each stimulus type and plots were used for comparisons.

5.4.3.3 Response distances

The relationship between stimulus range and frequency of occurrence of a response was investigated through histograms. To ensure all possible errors were accounted for across all ranges between stimulus and *N. cinerea* (maximum error ranges over all measured distances averaged 1.77 m (± 0.96 SD) due to triangulation error), and for ease of viewing, the distances were analysed in 5 m bins. This was also plotted for the stimulus groups and activity types.

5.5 RESULTS

5.5.1 Numbers of vessels, ‘People’ and *N. cinerea*

Vessels, ‘People’ in the water or on the beach, and *N. cinerea* were observed on 127, 57 and 163 days, respectively, during a total of 166 survey days (Figure 5.2). On Seal Island, 619 hourly counts were made during 78 days, and on Carnac Island 709 hourly counts were conducted on 88 days.

Between 20th November 2013 and 27th April 2014, when behavioural responses to anthropogenic activities were recorded from observation points on the islands, 134 h of observations were conducted over 19 days on Carnac Island and 142 h during 20 days on Seal Island. Eight of the days spent on each island were weekend days or public holidays. Over the six-month period, a maximum of 35 and 21 vessels and a maximum of 19 and six ‘People’ were recorded at any one time on Carnac and Seal Islands, respectively (Figure 5.2). During this period, 402 and 521

groups of vessels and 164 and 38 groups of 'People' were observed on Carnac and Seal Islands, respectively. Owing to the high number of vessels visiting during five days at Carnac Island, several vessels were unaccounted for and the total number of groups of vessels on Carnac Island is therefore probably an underestimate by an order of tens of vessels (cf. Orsini, 2004).

Significantly more vessels visited the islands than 'People' ($p < 0.0001$, Table 5.5), and both varied significantly. The total number of groups of vessels was greater on Seal Island than on Carnac Island; however, the number of groups of vessels on Carnac Island was underestimated on five days. The composition of vessel types during these days was similar to the overall composition of vessel types on the remaining days. It is likely that the sample accurately represents the data, hence, the total number of vessels is reported with the inclusion of the five days. The exposure of *N. cinerea* to different vessel types differed between Seal and Carnac Islands (Table 5.5, Figure 5.3). While 'Small vessels', 'Paddle powered', and 'Tour vessels' visited Seal Island most frequently, Carnac Island was mostly visited by 'Medium' and 'Large vessels' ($p < 0.0001$ for each vessel type except for 'Large vessels', Table 5.5, Figure 5.3). 'Large vessels' were only observed on Seal Island on one occasion. Carnac Island was visited by more than four times as many groups of 'People' as Seal Island ($p < 0.0001$, Table 5.5).

Table 5.5: Results of Chi² and Kruskal-Wallis tests comparing the number of groups of vessels of different types and ‘People’ visiting, the duration of visits, and the minimum approach distances to *N. cinerea* at Carnac and Seal Islands. Numbers in bold represent significant values, type of test added as + Chi² test or * Kruskal-Wallis rank sum test, and X² or KW-X², respectively, in brackets following the *p*-value. (Sample size for ‘Large vessels’ were too small for calculations and not included.)

	<i>P</i> -value: Number stimuli Carnac vs. Seal	<i>P</i> -value: Duration stimuli Carnac vs. Seal	<i>P</i> -value: Min approach distance stimuli Carnac vs. Seal
Overall	<2.2x10⁻¹⁶ (91.895) ⁺	3.608x10⁻⁷ (25.893)*	<2.2x10⁻¹⁶ (179.29)*
People	<2.2x10⁻¹⁶ (78.594) ⁺	0.4378 (0.602)*	0.687 (0.162)*
Paddle powered	<2.2x10⁻¹⁶ (147.63) ⁺	0.6561 (0.198)*	0.310 (1.034)*
Jet ski	0.00343 (8.565) ⁺	0.9746 (0.00101)*	0.371 (0.801)*
Small vessel	<2.2x10⁻¹⁶ (105.98) ⁺	0.000933 (10.956)*	3.065x10⁻⁶ (21.775)*
Medium vessel	5.971x10⁻¹⁶ (65.447) ⁺	0.0319 (4.606)*	0.434 (0.609)*
Tour vessel	<2.2x10⁻¹⁶ (94.308) ⁺	NA	<0.005 (7.881)*
Carnac only, stimuli overall	NA	0.000203 (26.219)*	<2.2x10⁻¹⁶ (17.349)*
Seal only, stimuli overall	NA	0.0487 (11.141)*	9.111x10⁻¹⁰ (50.89)*
Bonferroni corrected	0.006	0.006	0.005
<i>p</i> -value for significance			

⁺ Chi² test

*Kruskal-Wallis rank sum test

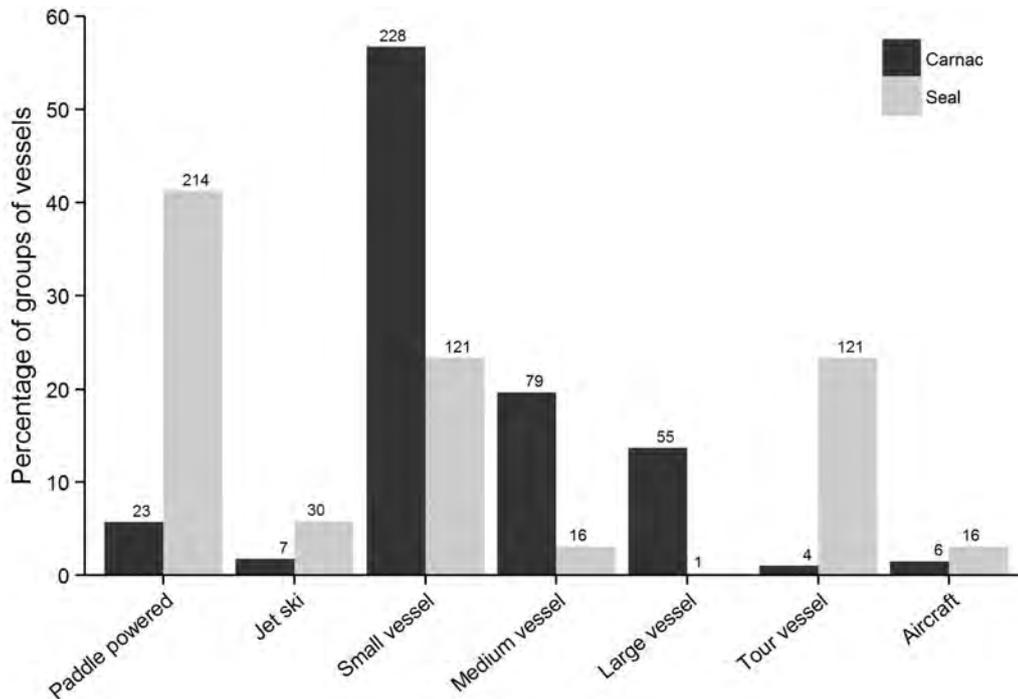


Figure 5.3: Percentage of groups of vessels observed visiting Carnac Island and Seal Island. Percentages are of the total at each island rather than the total combined at both islands. Values on top of each bar display the number of times each vessel type was observed (with Carnac Island having 134 h, and Seal Island 142 h of sampling effort between November 2013 and end April 2014).

The duration of time stimulus source spent in proximity to *N. cinerea* varied significantly among stimulus types at Carnac Island ($p = 0.0002$, Table 5.5, Figure 5.4), but not at Seal Island ($p = 0.05$) spending on average 0.56 h (± 0.79 SD) at Carnac Island and 0.23 h (± 0.30 SD; $p < 0.0001$) at Seal Island. At Carnac Island, the variation among vessel types was greater with ‘Jet skis’ staying the shortest periods (on average 6 min), and ‘Tour vessels’ and ‘Large vessels’ staying up to several hours at Carnac Island; longer than any vessel type at Seal Island. The sample size, however, was too small to test for differences (Figure 5.4).

Minimum approach distances varied significantly among vessel types and ‘People’ on each island ($p < 0.0001$, Table 5.5, Figure 5.4) as well as between Seal and Carnac Islands ($p < 0.0001$). The average distance to which groups of vessels approached *N. cinerea* was 57.5 m (± 64.1 SD) and 34.4 m (± 33.5 SD) on Carnac Island and Seal Island, respectively. ‘People’ approached *N. cinerea* more closely than any other stimuli type at both islands, on average 10.8 m at Carnac Island and 15.7 m at Seal Island.

5.5.2 Number of responses

In total, 1348 responses were recorded. Of those, 1300 responses were associated with vessels and 'People', and 48 with 'Aircrafts'. Of the responses to 'Aircrafts', nine occurred on Carnac Island and 39 on Seal Island (Figure 5.3). 'Aircrafts' were not included in statistical analysis owing to their combination of relatively small sample size, lack of determined range, and non-distinct noise source direction. Of the responses to vessels, 250 occurred at Carnac Island and 568 on Seal Island. The total numbers of responses to 'People' were 373 on Carnac Island and 109 on Seal Island, significantly more than to vessels in relation to the total number of stimuli ($p < 0.0001$, Table 5.6, Figure A1).

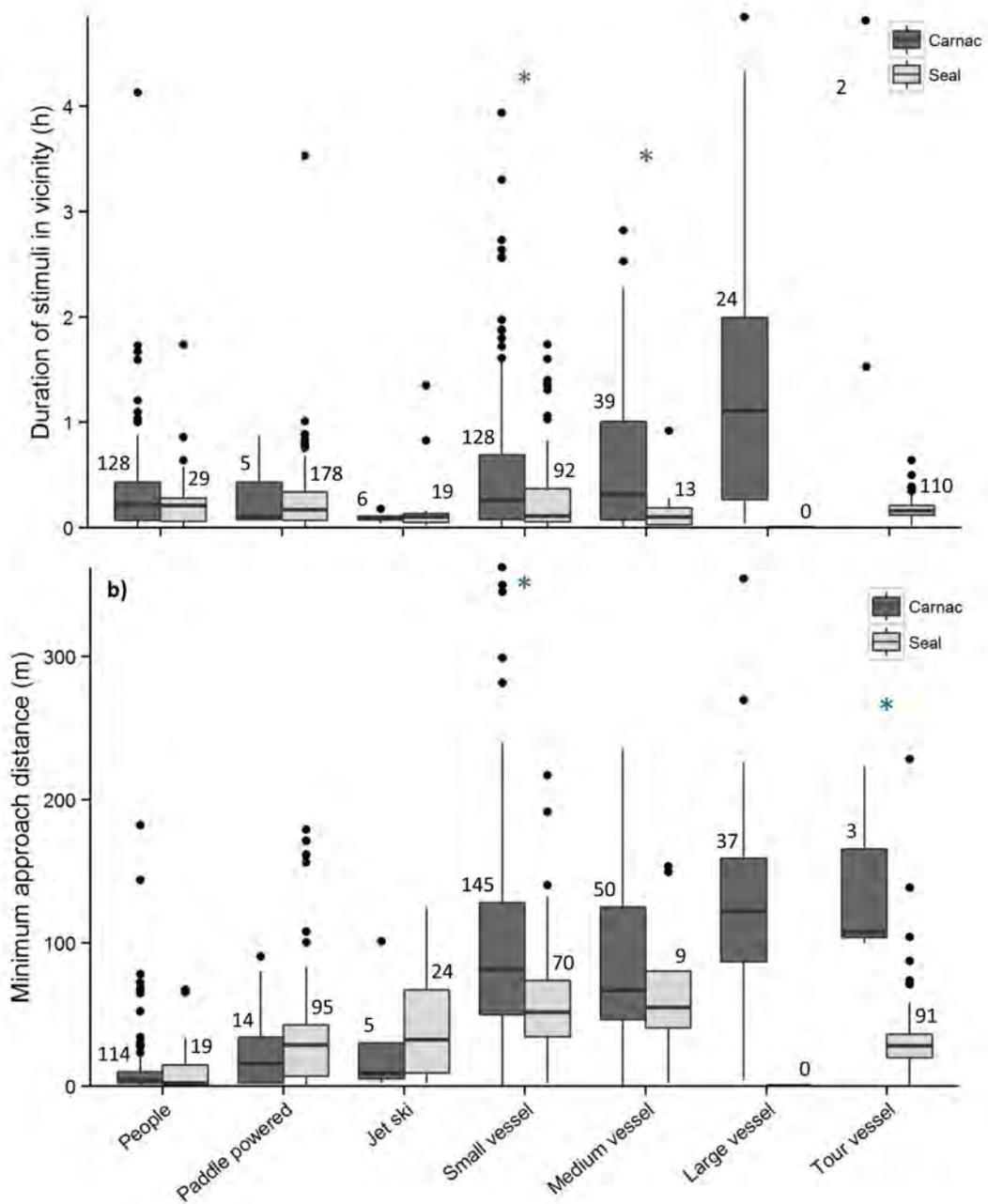


Figure 5.4: a) Duration (h) and b) minimum approach distance (m) of ‘People’ and vessels staying in the vicinity of Carnac Island and Seal Island. Values next to each bar display sample size of recorded approach and departure times for groups of vessels and ‘People’ observed (with Carnac Island having 134 h and Seal Island having 142 h sampling effort between November 2013 and end April 2014). * = significant differences between the islands.

Table 5.6: Results of Chi² and Fisher's exact tests comparing the number of *N. cinerea* responses elicited by groups of vessels and 'People' at Carnac and Seal Islands. Numbers in bold represent significant values.

	<i>P</i> -value: Number responses vessels vs. 'People'	<i>P</i> -value: Number responses Carnac vs. Seal	<i>P</i> -value: Number responses to 'People' Carnac vs. Seal	<i>P</i> -value: Number responses to vessels Carnac vs. Seal
Overall	<2.2x10⁻¹⁶ (74.755) ⁺	0.317 (0.999) ⁺	0.491 (0.474) ⁺	2.116x10⁻⁵ (13.725) ⁺
Retreat	3.516x10⁻⁸ (0.0696)*	4.234x10⁻³ (8.181) ⁺	NA	NA
Enter water	3.01x10⁻¹¹ (44.17) ⁺	0.353 (0.862) ⁺	1.225x10⁻⁴ (0.1589)*	0.561 (0.337) ⁺
Interact	2.511x10⁻¹⁴ (58.085) ⁺	0.994 (5.106x10 ⁻⁵) ⁺	0.581 (0.305) ⁺	0.0305 (4.68) ⁺
Travel	2.372x10⁻³ (0.274)*	1 (2.672x10 ⁻²⁸) ⁺	0.439 (0.604)*	0.718 (0.131) ⁺
Sit	6.926x10⁻⁸ (29.085) ⁺	0.625 (0.239) ⁺	0.818 (0.0530) ⁺	3.451x10⁻³ (8.552) ⁺
Lift head	3.78x10⁻³ (8.386) ⁺	0.983 (4.524x10 ⁻⁴) ⁺	0.61 (0.260) ⁺	0.274 (1.199) ⁺
Move head	1.314x10⁻³ (14.622) ⁺	0.63 (0.232) ⁺	NA	0.273 (1.201) ⁺
Look	3.257x10⁻³ (0.240)*	0.714 (0.135) ⁺	NA	NA
Bonferroni corrected <i>P</i> -value for significance	0.005	0.005	0.008	0.007

⁺ Chi² test

*Kruskal-Wallis rank sum test

NA: sample size too small for calculation

Overall, 40% and 39% of all groups of vessels on Carnac Island and Seal Island, respectively, elicited one or more responses from one or more *N. cinerea* (Figure 5.5). ‘Aircrafts’ flying over or past the islands, triggered responses in 67% and 81% of their passes from one or more *N. cinerea* at Carnac Island and Seal Island, respectively. *N. cinerea* responded to 66% and 74% of all groups of ‘People’ at Carnac Island and Seal Island, respectively. The percentage of different vessel types that triggered responses in one or more *N. cinerea* varied little between the islands apart from ‘Jet skis’ and ‘Large vessels’. A linear regression was applied to assess whether the percentage of *N. cinerea* responding (of those hauled out at any one time) was related to the number of vessels and ‘People’ visiting the island at the time, but did not reveal a linear relationship (Seal Island: adjusted $R^2_{\text{‘People’}}$ = 0.01, adjusted R^2_{vessels} = 0.0007; Carnac Island: adjusted $R^2_{\text{‘People’}}$ = 0.006, adjusted R^2_{vessels} = 0.001). Additionally, here was also no obvious non-linear pattern associated with the total number of vessels or ‘People’ at either island.

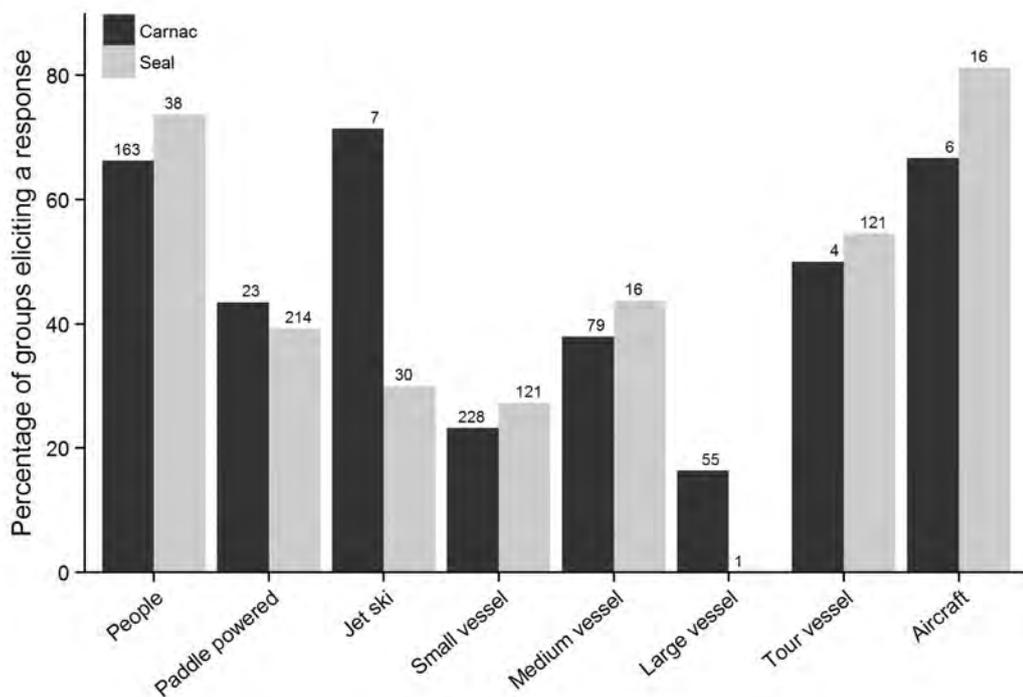


Figure 5.5: Percentage of groups for the different stimulus types (different vessels and ‘People’) that elicited one or more responses from one or more *N. cinerea* (with Carnac Island having 134 h and Seal Island having 142 h observation effort between November 2013 and end April 2014). Values on top of each bar display the sample size of groups of vessels or ‘People’.

5.5.3 Response distances

Out of the total 1300 responses (623 and 677 on Carnac Island and Seal Island, respectively, excluding aircrafts), the distance between a stimulus and a responding *N. cinerea* was measured for 482 responses; 280 on Carnac Island and 202 on Seal Island. For the remaining 973 responses, a number of factors inhibited measuring the response distance; including lack of available range finder on survey, multiple *N. cinerea* responding either simultaneously or in short succession, multiple active stimuli, or a fast-moving stimulus. Responses were triggered between 0 and 345 m, with a mean of 29.6 m (± 39.18 SD). The majority of responses of *N. cinerea* elicited by vessels or 'People' were observed from the shortest ranges (≤ 10 m) to *N. cinerea*, and decreased with increasing distance, most prominently at Carnac Island (Figure 5.6). The number of responses measured between 0 and 5 m at Carnac Island was double that at Seal Island, and *N. cinerea* appeared to respond to vessels and 'People' at greater distances at Carnac Island (Figure 6). Responses triggered from medium ranges (15-50 m), were predominantly due to 'Tour vessels' and to a lesser extent to 'Paddle powered vessels' at Seal Island, and 'Small vessels' at Carnac Island (Figure 5.7).

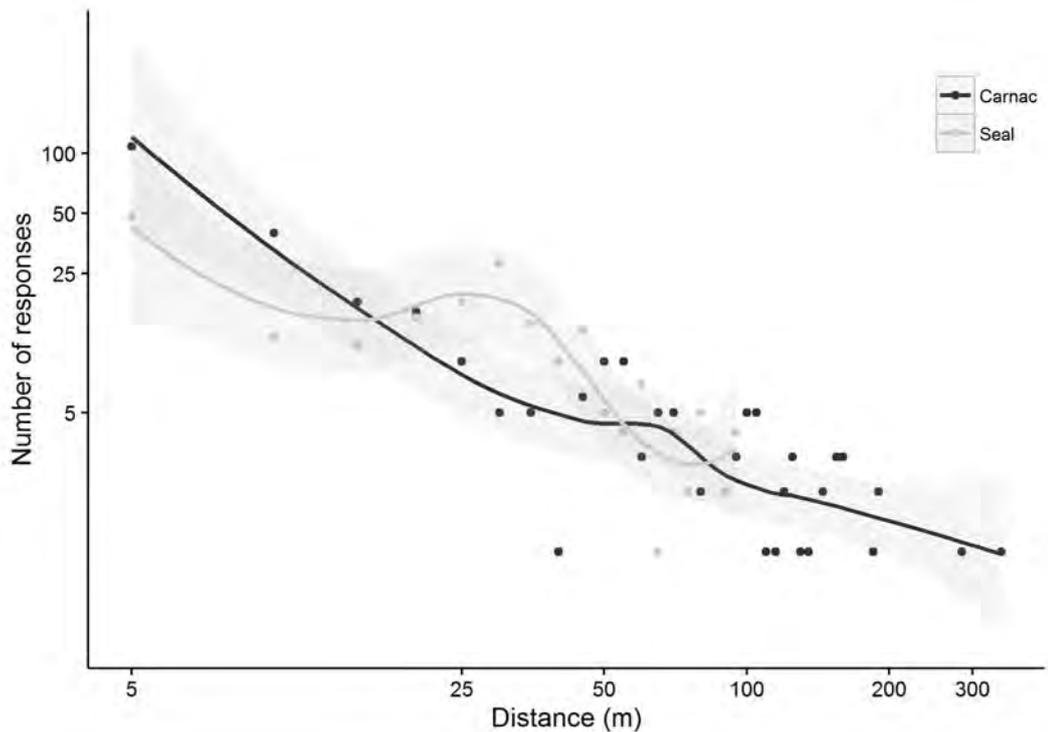


Figure 5.6: Number of *N. cinerea* responses occurring at 5 m binned distances at Carnac Island (n = 280) and Seal Island (n = 202), displayed on log 10 transformed axis. Loess smoothers for Carnac Island (black) and Seal Island (gray) with 95% confidence intervals were added to aid visual interpretation.

All activities carried out by ‘People’ (regardless of whether on board, in the water or on the beach) potentially induced a response within 10 m, and the probability of a response increased within 5 m range (Figure 5.8). Overall, the shortest ranges causing the highest number of responses were from ‘Viewing’ ($11.9 \text{ m} \pm 11.27 \text{ SD}$) and ‘Low-level’ activities ($41.4 \text{ m} \pm 43.61 \text{ SD}$), followed by ‘Interacting’ ($5.73 \text{ m} \pm 1.77 \text{ SD}$). The number of responses decreased with increasing distances for most activities (Figure 5.8). Distance had less effect than activity when humans were involved in ‘Attracting’ greater numbers of response occurrences at longer distances. Although the frequency of responses to ‘Low-level’ activities decreased with increasing distance, many responses were still triggered beyond 30 m.

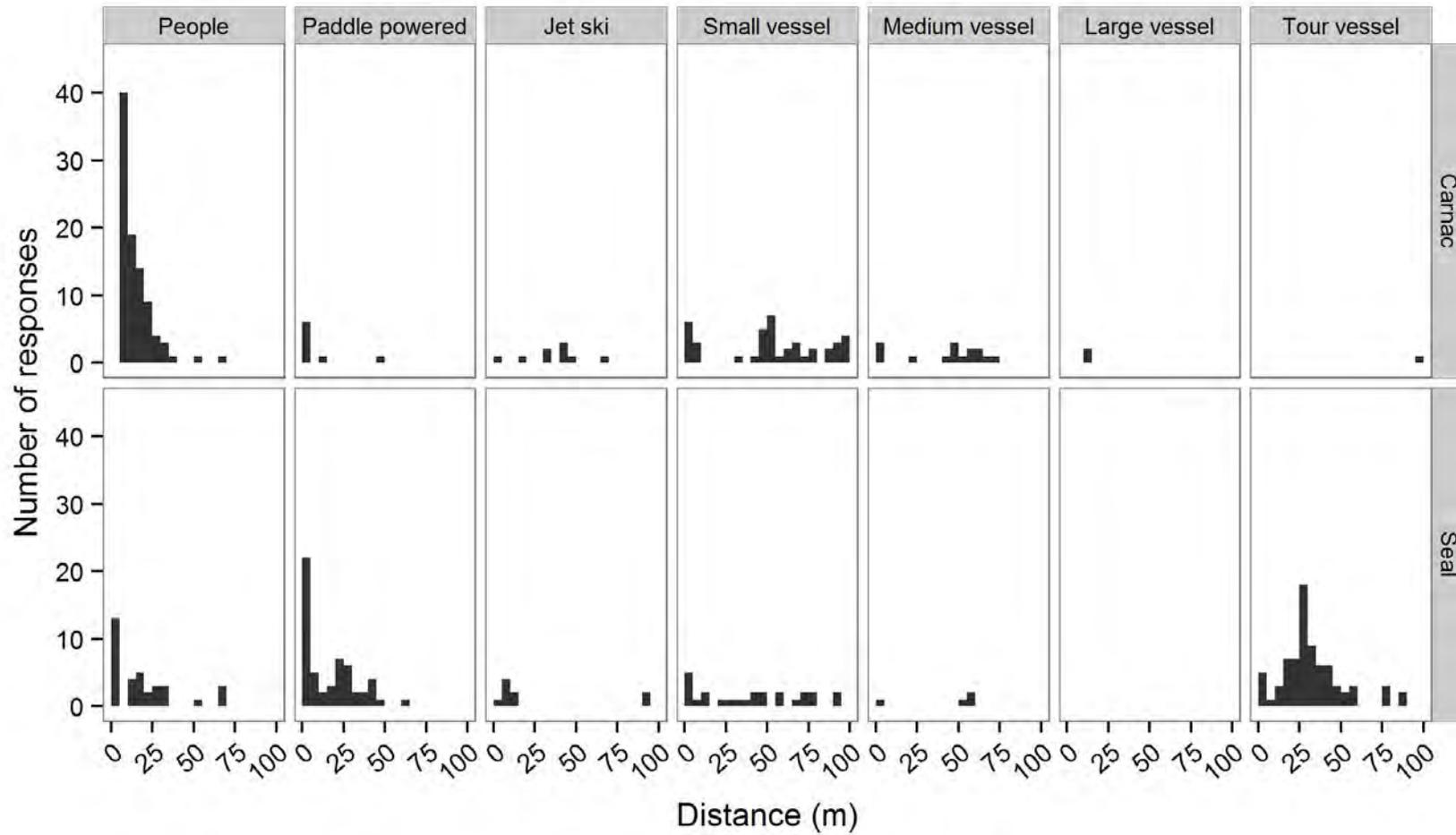


Figure 5.7: Number of *N. cinerea* responses elicited by groups of ‘People’ and vessels at Carnac Island and Seal Island, in 5 m bins and truncated at 100 m.

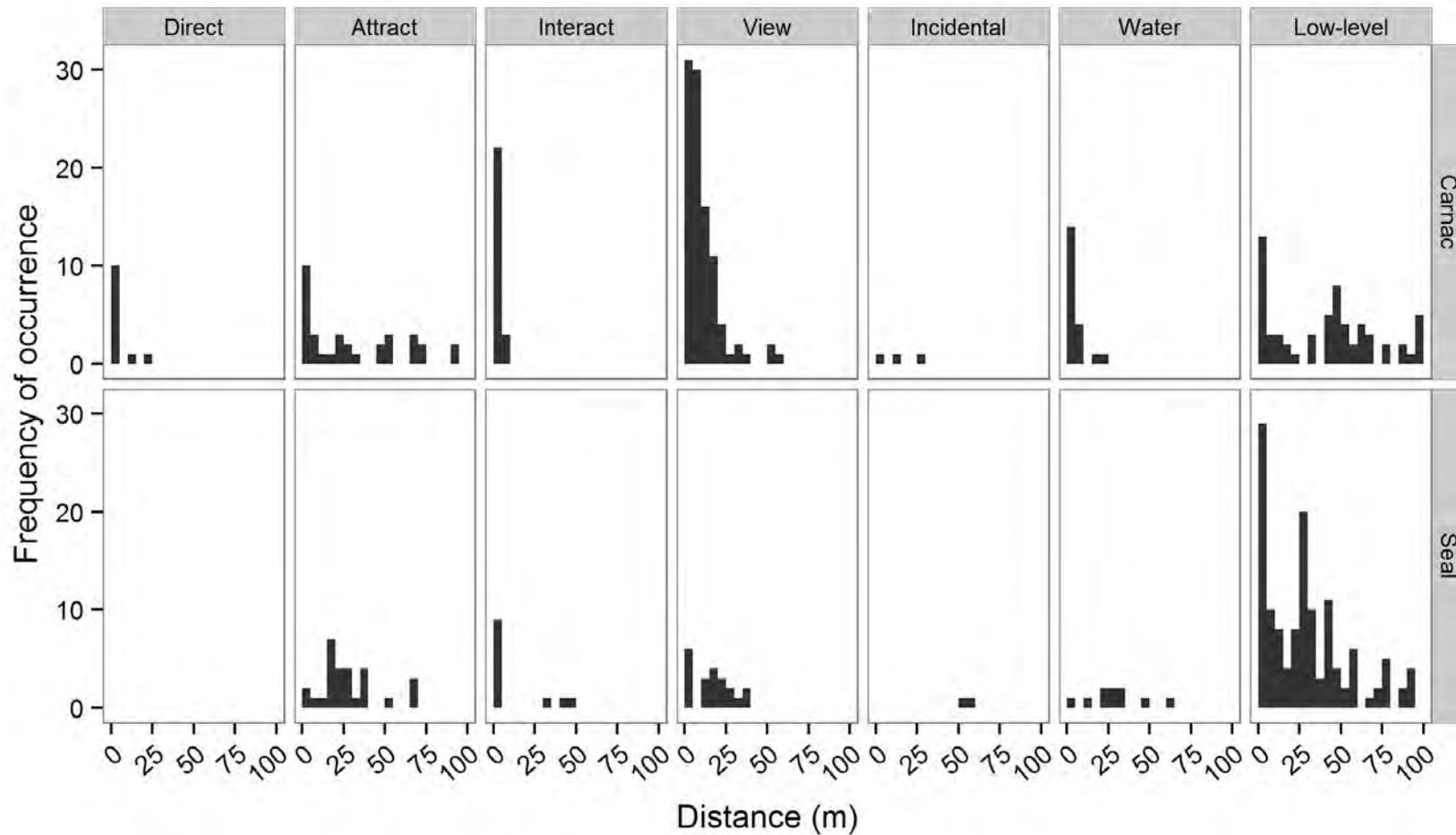


Figure 5.8: Frequency of a variety of *N. cinerea* responses elicited by ‘People’s’ activities on board, in the water and on the beach at Carnac Island and Seal Island, in 5 min bins and truncated at 100 m.

5.5.4 Response behaviours

'Lift head', 'Interaction' and 'Sit' were the most frequent behavioural responses triggered by both vessels and 'People' (Figure 5.9). Most behavioural responses were more likely caused by 'People' than vessels on a per visit basis ($p < 0.005$ for each response level; Table 5.6, Figure 5.9). The number of responses provoked by 'People' did not vary significantly between Carnac and Seal Islands ($p = 0.5$, Table 5.6). 'Aggressive' behaviours, however, occurred only at Carnac Island, in response to 'People'. 'Retreat' behaviours occurred mainly at Carnac Island, also mostly in response to 'People' ($p = 0.004$ Carnac/Seal for 'Retreat' behaviour; Figure 5.9). At Carnac Island, 'Viewing' elicited the most responses, however, on Seal Island 'People' involved in 'Interact', 'Attract', 'Viewing' or 'Water' activities all elicited responses (Figure 5.10). 'Lift head' accounted for half of the total number of responses at Seal Island provoked by vessels and was triggered at a rate of about one per hour at Carnac Island and over two per hour at Seal Island (Figure 5.9). The relationships between the different types of response to each stimulus group are shown in Table 5.6 and displayed in Figures 5.10 and 5.11. 'Small' and 'Medium vessels' elicited most responses at Carnac Island. Of the vessel activities and anthropogenic activities on vessels at Carnac Island, 'Anchor' and 'Engine' noises elicited most responses in *N. cinerea* (Figure A2). At Seal Island, most vessel related responses were triggered by 'Low-level' activities (Figure A2).

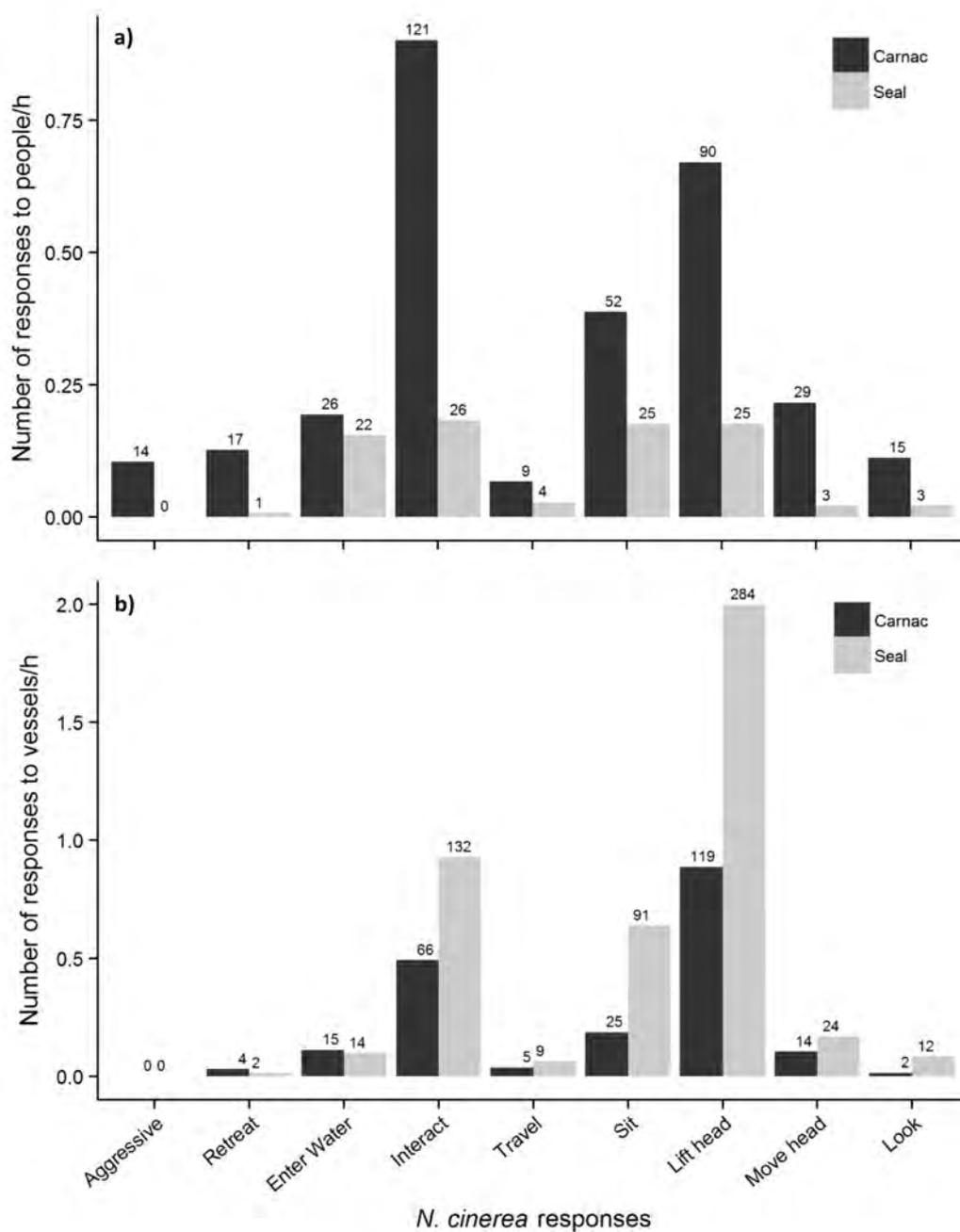


Figure 5.9: Number of *N. cinerea* responses per hour of sampling elicited by a) 'People' and b) vessels at Carnac Island and Seal Island (excluding 'Aircrafts'). Numbers above each bar indicate the total number for each behaviour observed (Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).

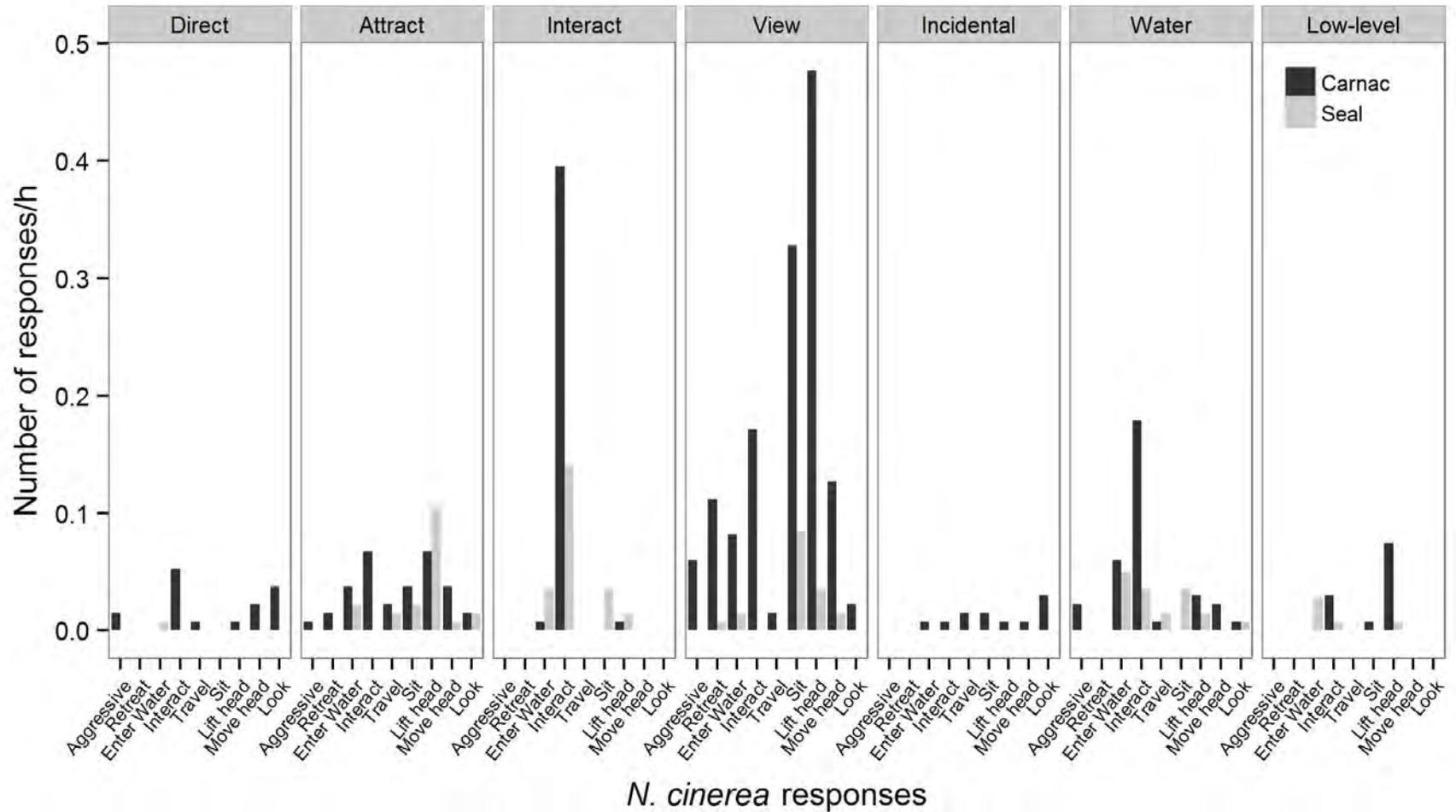


Figure 5.10: Number of *N. cinerea* responses elicited per hour as a result of groups of ‘People’ undertaking different activities at Carnac Island and Seal Island (Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).

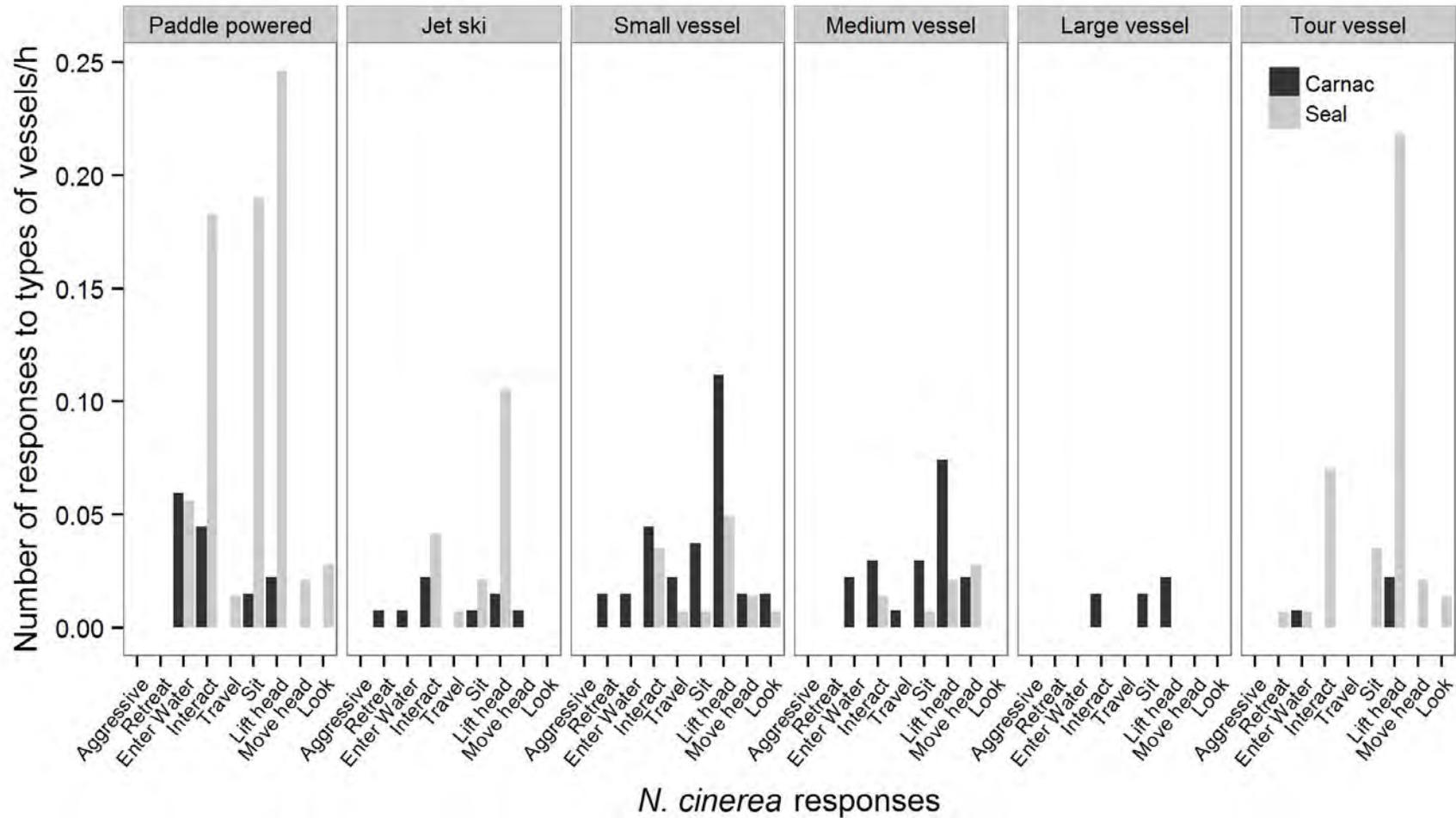


Figure 5.11: Number of *N. cinerea* responses per hour of sampling elicited by different vessel types at Carnac Island and Seal Island (excluding 'Aircrafts'; Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).

5.6 DISCUSSION

N. cinerea frequently respond to anthropogenic activities and the response type and frequency can be dependent on the stimulus itself, its range and the activity. In this study, anthropogenic stimulus and activity types varied at two differently managed islands. While response levels were, in general, similar at both locations, the most severe behavioural response levels, ‘Aggressive’ and ‘Retreat’, occurred mostly at Carnac Island, predominantly elicited from approaches by ‘People’ and probably because of their proximity (≤ 10 m) to *N. cinerea*. The majority of responses were generated from stimuli that achieved the closest range and decreased with increasing range. Responses elicited at greater ranges were more likely to occur when stimuli were undertaking activities associated with elevated noise levels or actions directed at attracting *N. cinerea*’s attention.

Distance has been identified in many studies as the main factor in altering pinniped behaviour, eliciting stronger responses when disturbance occurred within closer ranges (Cassini, 2001; Boren *et al.*, 2002; Labrada-Martagón *et al.*, 2005; Szaniszló, 2005; Shaughnessy *et al.*, 2008; Strong and Morris, 2010; Pavez *et al.*, 2014; Young *et al.*, 2014). Here, ‘Viewing’ activities were associated with low levels of noise, as any discernible sound (e.g. screaming, banging objects or splashing water) reclassified the activity to a higher level. ‘Viewing’ elicited one of the highest rates of response (apart from ‘Low-level’ activities) and were mostly conducted at relatively short ranges where animals could perceive them without auditory cue (Schusterman and Balliet, 1971; Schusterman, 1972). In phocids, such close range approaches of various stimulus types caused Saimaa ringed seals (*Phoca hispida saimensis*) (Niemi *et al.*, 2013) and harbour seals (*Phoca vitulina*) at various locations to exhibit avoidance behaviour and enter the water ((Allen *et al.*, 1984; Suryan and Harvey, 1999; Henry and Hammill, 2001; Jansen *et al.*, 2010; Anderson *et al.*, 2012; Osinga *et al.*, 2012). In this study, more than 40% of all responses elicited by ‘People’ were attributed to ‘Viewing’ activities with most of these approaches being classified as breaches of the required 5 m minimum distance. These findings are consistent with a study of *N. cinerea* at Seal Bay, South Australia, which exhibited elevated response rates, including aggressive and avoidance behaviours, when approached within 10 m and even more so when approached within 5 m (Lovasz *et al.*, 2008).

While distance has a significant effect on responses elicited, human behaviour also has been noted to significantly contribute to disturbance of otariids (South

American fur seal, *Arctocephalus australis*; Cassini, 2001; Labrada-Martagón *et al.*, 2005; Pavez *et al.*, 2014), and phocids. Vessel activities that involve higher in-air noise levels have been shown to have similar effects to direct, i.e. interactive human disturbance. *N. cinerea* in this study ‘Lifted their heads’ to ‘Engine’ and ‘Anchor’ noise, similar to Australian fur seals (*Arctocephalus pusillus*) that exhibited increased response rates at higher noise levels of vessels (Tripovich *et al.*, 2012). It should be noted that the presence of noise in this study was based on its perceived presence by researchers located within proximity of the animals, and was not based on measurements of in-air noise levels. However, otariid’s hearing sensitivity includes the frequency band in which much of the energy from an engine, human speech, and anchor noise occurs (Gramming *et al.*, 1988; Richardson *et al.*, 1995; Badinoa *et al.*, 2012; Muslow *et al.*, 2014). During visits to Seal Island, the ‘Tour vessel’s’ amplified guides were regularly audible to the researchers on the island, and probably the cause of frequent *N. cinerea* responses. This probably also contributes to the peak of responses occurring at 25-30 m at Seal Island, reflecting the most common shortest range to which the ‘Tour vessel’ approached. Similarly, anthropogenic activity in association with noise was identified as likely to cause disturbance in harbour seals, during a nonbreeding season in Iceland (Granquist and Sigurjonsdottir, 2014). Elevated noise levels of passing ‘Aircrafts’, such as scenic flights resulted in responses in *N. cinerea*, similar to responses of Steller sea lions (*Eumetopias jubatus*) and *P. vitulina* to low-flying aircraft (Osborn, 1985; Henry and Hammill, 2001; Kucey, 2005; Szaniszlo, 2005).

‘Aggressive’ gaping and launching behaviours in *N. cinerea* towards ‘People’ were primarily evoked by close proximity ‘Viewing’ and occasionally ‘Direct’ invasive activities. Proximity of ‘People’ to *N. cinerea* occurred mainly at Carnac Island owing to easy (and non-restricted) beach access at a limited number of specific points, and was probably the main trigger of ‘Aggressive’ behaviours and higher numbers of ‘Retreat’ responses here compared with Seal Island. Approaches from land are potentially perceived as a more immediate and greater threat than approaches by vessels, and the resulting behaviours have been observed in other pinnipeds (Stirling, 1972; Boren *et al.*, 2002; Osinga *et al.*, 2012).

While stimulus type had a significant influence on the level of *N. cinerea* responses, stimulus types varied in exposure level, minimum approach distance and duration between the two islands. Although not directly studied here, draft associated with vessel type may limit a vessel’s approach range to a beach, and thus the

distance at which different vessel types may trigger responses from hauled out *N. cinerea*; simply put, larger vessels did not approach as closely as smaller vessels at either island. Furthermore, the relative proximity of the island to the mainland coast also affected the type of vessel that visited the islands. The close proximity of Seal Island to shore and its location within the sheltered waters of Shoalwater Bay, allowed 'Paddlers' to access the island easily and 'Tour vessels' to offer multiple trips per day to view *N. cinerea*. Thus, the high number of 'Paddle powered' vessels able to approach the island to within a few metres may explain the high number of responses at Seal Island. In addition, 'Paddlers' elicited responses mostly during 'Low'-level activities, i.e. mostly by their presence alone, indicating range, rather than activity per se, was the driving factor. Such 'surprise' appearances (i.e. no engine noise) at close range and higher mobility have been thought to have similar impacts on pinnipeds elsewhere (Allen *et al.*, 1984; Osborn, 1985; Suryan and Harvey, 1999; Henry and Hammill, 2001). In comparison, there were fewer responses to other vessel types, particularly large vessels associated with large drafts, which on average were at greater distances from *N. cinerea*.

Carnac Island's longer distance from shore is probably the reason for the greater number of large vessels visiting compared with Seal Island. Moreover, the draft associated with larger vessels meant that they remained further from the beach than smaller vessels. Furthermore, Carnac Island has a relatively large area of sandy beach with non-restricted access by people during the day, resulting in greater numbers of people on the beach than 'no access' Seal Island. In general, 'People' approached *N. cinerea* more closely than 'Paddlers', possibly explaining their greater probability of eliciting behavioural responses. The number of 'Paddlers' visiting Carnac Island was approximately 10 times lower than at Seal Island, maybe due to the increased distance from the mainland coast.

Apart from Carnac Island's greater distance from shore, the intention to visit Carnac Island as a 'day trip', rather than the 'stop-off' that Seal Island represents, may also explain the longer times vessels stayed at Carnac Island than at Seal Island. 'People' visit Carnac Island mainly for other recreational purposes and 'Viewing' *N. cinerea* is a secondary activity (Orsini and Newsome, 2005). Conversely, as landing on Seal Island is prohibited, viewing *N. cinerea* is the primary reason for visitation which most groups carried out for relatively short times resulting in *N. cinerea* being exposed to human activity for shorter individual periods.

The total number of vessels and ‘People’ can have variable influences on pinned reactions (Jansen *et al.*, 2010). Here, the proportion of responding *N. cinerea* did not appear to vary with increasing or decreasing numbers of vessels or ‘People’, which is similar to some studies where response behaviours remained comparatively consistent (Kovacs and Innes, 1990; Strong and Morris, 2010). However, in other studies varying behavioural responses occurred with differing numbers of people in the vicinity, such as adult male *N. cinerea*, during the breeding season reportedly responding to individual people at greater distances than to groups of people (Lovasz *et al.*, 2008). In contrast, females and other age groups observed in the same study did not show variation when approached by people on their own or in groups. Lovasz *et al.* (2008) speculated that the breeding season may play a role in responses, but was not able to ascertain what that might be. Quite the opposite, however, has been observed in *A. australis* (Cassini *et al.*, 2004).

Long-lasting interactions between *N. cinerea* and vessels or ‘People’ in the water were a common occurrence in this study (26.5% of all responses), similar to *A. forsteri* approaching kayaks or interacting with swimmers from a swim tour (Boren *et al.*, 2002; Cowling *et al.*, 2014). In contrast, at Seal Bay, South Australia, *N. cinerea* have been recorded to only rarely respond to interacting behaviours (Lovasz *et al.*, 2008). In this study, one example of long-lasting duration occurred at Carnac Island when no other vessel or ‘People’ were in the vicinity. One adult and three sub-adult *N. cinerea* ‘Entered the water’ immediately when one of the marine park rangers removed star pickets from the beach (always remaining at >10 m range). The first sub-adult to haul out after leaving the beach did so more than one hour after the incident, and more than 40 min after the rangers’ vessel had left. This is comparable with *Z. californianus* mostly re-hauling out within 10 min after disturbance ceased, though they could take up to 3.5 h (Labrada-Martagón *et al.*, 2005). Anthropogenic impacts may, therefore, have altered *N. cinerea*’s natural behaviour in this study considerably, especially when *N. cinerea* ‘Entered the water’ or began ‘Interactions’, although ‘Interactions’ may have occurred voluntarily.

A. pusillus have shown increased levels of aggression among themselves when exposed to higher sound levels (Tripovich *et al.*, 2012). In contrast, similar behavioural changes as a response to noise were not observed in this study, and aggressive behaviours towards stimuli were comparatively rare. This difference may be explained by age and sex composition of the study populations, as well as timing within the breeding cycle (Boren *et al.*, 2002; Labrada-Martagón *et al.*, 2005;

Tripovich *et al.*, 2012; Cowling *et al.*, 2014; Pavez *et al.*, 2014). How human impacts affect different age and sex classes is known to vary between different species of pinnipeds. Females were more sensitive to anthropogenic activities in *P. vitulina* (Selvaggi *et al.*, 2001), whereas sub-adult males were more responsive to anthropogenic activities in South American sea lions (*Otaria bryonia*), and adult male *N. cinerea* elsewhere reacted at slightly greater distances than females and other age classes (Lovasz *et al.*, 2008). In (*O. bryonia*), more frequent disturbance was elicited at a breeding site compared with a haul-out site, whereas female *P. vitulina* displayed less pronounced responses, appearing reluctant to leave their pups (Anderson *et al.*, 2012; Pavez *et al.*, 2014).

The high frequency of anthropogenic activities, the resulting disturbance, and the time to return to previous behaviours may have an important effect on *N. cinerea* activity and energy budgets of individual animals. The accumulation over time of these may lead to long-term effects. *N. cinerea* have a ~2.3 times higher field metabolic rate and a ~6.2 times higher basal metabolic rate than terrestrial animals of comparable size (Costa and Gales, 2003). Based on this knowledge, the energy demands on individual *N. cinerea* are relatively high. *N. cinerea* are benthic foragers and their foraging trips are highly demanding and energy intensive (Costa and Gales, 2003). Hauling out may help conserve energy and contribute to recuperation between foraging trips (Riedman, 1990). Interrupting *N. cinerea*'s recovery time from strenuous foraging trips may, therefore, alter their activity budgets and increase energetic requirements. This could mean that *N. cinerea* frequently responding to anthropogenic activity while resting, must increase time spent foraging to gain sufficient energy to offset the time spent at higher activity levels, which, consequently, could result in less time spent resting. If *N. cinerea* spend less time resting between foraging trips, they may be more susceptible to disease and other threats if their fitness is reduced (Taillier, 2014; Shaughnessy *et al.*, 2007). This study did not attempt to track movements of identified individuals over time or investigate impacts on overall numbers of animals hauled out. However, pinnipeds may face displacement from preferred sites and move to less suitable habitat as a result of ongoing disturbance (Allen *et al.*, 1984; Stevens and Boness, 2003; Kucey, 2005). The impact of anthropogenic activities on overall numbers of *N. cinerea* hauling out at Carnac and Seal Islands is unknown, but recommended to be investigated in future studies.

Habituation to people has been suspected in *N. cinerea* at Seal Bay, South Australia. *N. cinerea* show more disturbance at a rarely disturbed site compared with a long-term, frequently visited site where people are able to approach within close range (Stirling, 1972; Lovasz *et al.*, 2008). Carnac and Seal Islands are both visited frequently and *N. cinerea* may show some level of habituation, especially considering the high number of interactions with vessels and 'People'. However, quantifying habituation in animals so commonly visited over such a prolonged period as occurs at the islands studied would not be trivial.

Some biases may have been introduced by the inability to equally measure all distances between responding animals and stimuli. However, the difference between the distribution of total minimum approach distances and that of response distances illustrate that the sample size across ranges was sufficient to detect the inverse relationship of response with range. Furthermore, while behavioural changes of *N. cinerea* were excluded when there was uncertainty as to whether the response was to anthropogenic activities, some responses might have been misclassified as a response to humans, when they were not. The authors, however, believe that these cases were rare and that responses were more likely underestimated. In particular, while measurements were taken of vessels, 'People', and closest *N. cinerea* during heavy visitation periods (although priority was placed on 'People', vessels in close proximity to *N. cinerea* and vessels involved in conspicuous activities), some *N. cinerea* responses or measured distances may have been missed. 'Look', for example, was often an inconspicuous behaviour, particularly if *N. cinerea* faced away from the researchers, and was therefore possibly underestimated. A previous study, conducted at Carnac Island during summer months, approximately six months prior to the *N. cinerea* peak season, investigated responses to people. This documented relatively high numbers of responses in the three response categories measured (lift head, sit and look) and include repeated responses to the same stimuli (Orsini, 2004; Orsini *et al.*, 2006). Hourly sampling periods, observing these responses were conducted on one *N. cinerea* at a time, totalling 240 *N. cinerea* sampling periods. The sampling method and measurements, however, differ from that of this study, in particular that the observer was positioned within close proximity to the animals and thus while detecting a greater number of low level responses, may also have contributed to them (Orsini, 2004; Orsini *et al.*, 2006).

5.6.1 Suggestions for management

This study showed that not only did distance play a major role in eliciting responses in *N. cinerea*, but human and vessel activity types were also contributors. These factors should be included as primary considerations for programmes aiming at reducing disturbance. The impact of disturbance on individual energetics has not been investigated here and, similar to response levels, are likely to vary between species and location. However, it is feasible that many of the following suggestions, and indeed the current management protocols put in place by the Department of Parks and Wildlife, Western Australia, would reduce responses of *N. cinerea* if applied to haul-out locations of pinniped species elsewhere. Thus by increasing the minimum approach distance for vessels and people to 30 m, disturbance would be expected to decrease significantly as high rates in this study were observed at the current minimum approach distance restrictions of 5-10 m (DEC and Fisheries, 2011). The frequent breaches of the current limit was a notable feature in this study, thus enforced minimum distances may improve the effectiveness of the regulations.

In a separate study in South America, fencing limited the distance people were able to access, approach, and view *A. australis* from land, and significantly decreased human disturbance, including attacks on people which were reduced from four in a month to zero (Cassini *et al.*, 2004). ‘Aggressive’ behaviour towards ‘People’ and ‘Retreat’ behaviours, were observed more than once a day on Carnac Island in 74% of all *N. cinerea* observation days in this study. Limiting the approach distance and/or beach access may reduce the highest response levels and lower the chances of danger to both humans and pinnipeds. Designating all of Carnac Island (rather than a section of the beach) as a sanctuary zone, as presently exists on Seal Island, may assist in reducing disturbance. It would perhaps also provide visitors with a stronger awareness of their responsibilities when interacting with wild animals. As a control measure, marker buoys installed 15 m off the waterline at low tide at Carnac and Seal Islands may reduce the disturbance of *N. cinerea* thermoregulating in the wash zone during periods of higher air temperatures (Marlow, 1975; Riedman, 1990). Creating a demarcation of a boundary with buoys where vessels and ‘People’ should not pass may help reduce ‘People’ accidentally beaching their kayaks (as occurred during 50% of the field days at Seal Island) and also increase awareness of the sanctuary zone.

In addition, standardizing control measures across *N. cinerea* haul-out locations may assist in generating more consistent behaviour from the public to limit disturbance. This study has not investigated the impacts of disturbance on the energetics of *N. cinerea*. The following suggestions are therefore made based on a precautionary approach, given that the level of effects of disturbance on *N. cinerea* energetics has yet to be quantified.

In general, it is probable that most visitors are not aware they are causing a disturbance to *N. cinerea* or what effects these disturbances may have on colonies and the overall population (Orsini and Newsome, 2005). Clear signage and other forms of information and educational material, including increased direct communication from patrol officers, may improve awareness of the importance of haul-out and resting behaviours to *N. cinerea* health and body condition. Furthermore awareness of the potential impacts of noise may alter peoples' behaviours so that noise levels and overall disturbance are reduced when in close proximity to animals (Newsome and Rodger, 2008). In a previous study, the combination of approaching slower, maintaining greater ranges, and having quieter passengers reduced disturbance of *P. vitulina* by 60-80% (Hoover-Miller et al., 2013).

In conclusion, this study has shown that a considerable number of responses and behavioural changes were elicited by anthropogenic activities. Significant differences occurred between Seal and Carnac Islands in levels of exposure, including the exposure duration and types of stimuli, as well as in the elicited response levels. However, most responses occurred in close ranges to *N. cinerea*. If minimum approach distances in guidelines are increased, and the public is made aware that calm and quiet behaviour around Seal and Carnac Islands would significantly reduce the potential impacts of anthropogenic activity, the number of responses due to disturbance may be reduced. Longer-term studies measuring the cumulative duration of interactions, assessing the effects of anthropogenic activities on *N. cinerea*'s energy budgets, and determining the impacts of fitness and habitat displacement at an individual and population level are recommended. However, it should also be noted that pinniped responses to humans varies widely between species and that context is an important factor in the application of protocols to mitigate disturbance.

5.7 ACKNOWLEDGMENTS

The Department of Parks and Wildlife (Western Australia) made this project possible with substantial logistical support provided including transport to and from the islands and loan of a spotting scope, as well as provision of local and scientific knowledge. Transports to and from islands was also provided by the Department of Fisheries and Cockburn Volunteer Sea Search and Rescue. The Fremantle Sailing Club provided access to their boat ramp. The project was also supported by Malcom Perry through maintenance of the research vessel and Mick Todd through loan of his telescope. Many volunteers contributed substantial time assisting in the field and entering data into spreadsheets in the office. The authors are grateful to Miles Parsons for providing editorial and analytical advice which significantly improved the quality of this manuscript, Sven Gastauer for providing advice on R code, and Julie Sims who gave useful advice on data analysis. The authors appreciate the constructive feedback and helpful advice from both reviewers and the editor to improve this manuscript. Finally, this project was supported by funding from the Holsworth Wildlife Research Endowment. This project was also conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth). The authors have no conflicting interests to declare.

Chapter 6

Discussion

Natural and anthropogenic fragmentation of Australian sea lions, *Neophoca cinerea*, into widely dispersed colonies coupled with historical localised extinctions due to various forms of human activity has led to suggestions that management of the species may be required on a colony level rather than on the population as a whole (Campbell, 2003). Whilst population estimates are based on pups, these are not applicable for local abundance estimates of animals in the Perth metropolitan area as these colonies represent a cohort of the population comprised of male animals. When animals can be reliably identified, re-sighted individuals can be used for estimating local abundance sizes, monitoring trends, and conducting mark-recapture estimates (e.g. Buckland, 1980; Seber, 1986; Lebreton *et al.*, 1992).

In animal species lacking natural marks or other identifiable features for use in non-invasive photo-identification, painted marks have been suggested as a means to more readily support identification of individuals over a relatively short period of time (e.g. Gales *et al.*, 1992; Robertson *et al.*, 2006). However, for long-term identification, short-lasting marks are not effective, and therefore more invasive methods such as tagging or branding have been applied successfully in other pinniped species (e.g. Merrick *et al.*, 1996; McMahon *et al.*, 2006). Invasive methods involve capturing and handling individuals, and may pose significantly greater risk to animals (e.g. Mellish *et al.*, 2007; Walker *et al.*, 2009, 2012). This is of particular concern in species such as *N. cinerea*, where disturbance might have detrimental effects on animals occurring in small colonies of an endangered population (Campbell, 2005). Due to the need and desire for minimally invasive survey techniques, this study investigated alternative possibilities for abundance estimates and for investigating the behaviour of *N. cinerea* in an area where human/animal interactions are already at a high level. Through progress made here, it is anticipated that the findings of this study will contribute to the development of standards for improved non-invasive monitoring that is more appropriate for this and other populations whether impacted by humans or not.

Consistent to other studies of large mammals, the use of whisker spot patterns to identify individual *N. cinerea* showed high reliability in small populations, under controlled conditions. Additionally, the photographs were obtained from particular distances and at a perpendicular angle. However, in contrast to photographs taken under controlled conditions, the variation of whisker spot patterns obtained by marking the positions in photographs of wild *N. cinerea* was found to be too large. The degree of difference between photographs of the same individual in

comparison with photographs of different individuals to confirm matches (i.e. re-sightings) manually proved problematic. Although success rate of correct matches was high in captive individuals with good quality photographs taken at 90° (lateral) without tilt, photographing wild *N. cinerea* appeared to introduce too much variation into the determination of whisker spot location for identification. This is in contrast to whisker spot patterns of lions (*Panthera leo*) and polar bears (*Ursus maritimus*) which have shown greater variation between individuals which allows easier identification (Pennycuik and Rudnai, 1970; Anderson *et al.*, 2007a). In *P. leo* identification is even possible by using whisker spot positions manually marked in a grid, during visual observation by eye (Kays and Patterson, 2001; Ogutu *et al.*, 2006). In *P. leo* the position of the top row of whisker spots, in relation a reference row below, contain most of the information needed for identification (Pennycuik and Rudnai, 1970). By contrast, this study suggests that *N. cinerea* have a similar level of information spread across all whisker spots. Moreover, their top row of whisker spots is the most inconsistent row for marking or determining whisker spots (see Chapter 2). The whiskers in the top row in *N. cinerea* are very thin and the spots difficult to see, partly due to blending in with and becoming camouflaged by fur. Light conditions and spot colours may also affect the visibility of these spots. Using the top row as the main indicator for identification was, therefore, unreliable and could not be used for further ecological studies. Whisker spots in *U. maritimus* appear to be less abundant and less ordered than in *N. cinerea* and *P. leo*, potentially increasing the variation between individuals (Anderson *et al.*, 2005, 2007a). In both controlled conditions and in the wild, similarity scores for two photographs of the same individual, while variable, increased as the time between photographs increased, i.e. matching performance was inversely related to the time interval between photographs. This highlighted the sensitivity of the technique to movement by either photographer or target (i.e. re-composition of photographs), and its effect in significantly reducing performance.

Work by the author of this thesis and other co-authors has commenced on testing alternatives, such as the Groth algorithm, which has been used in other applications to improve matching at different angles of incidence (Groth, 1986). The Groth algorithm has been successfully employed to match spot patterns in whale sharks (*Rhincodon typus*), and is based on comparing triangles between all spots of one pattern to those of another (Groth, 1986; Arzoumanian *et al.*, 2005). The preliminary work, however, is still underway and the results are not yet available.

Nevertheless, accurate and consistent identification of the whisker spot locations on *N. cinerea* in the wild has proved to be the factor that leads to excessive variation in the patterns, which resulted in low matching performance of tests carried out so far. The low accuracy and constancy of performance appears to be the result of a combination of environmental conditions (sunlight, wet/dry fur) and user error (photographer/subject movement) that impact the ease with which spots can be correctly located. When marking of the same individual on the same photographs was independently carried out by different observers, the software matching probability often resulted in identification as two different individuals. Given that similarity scores (using the Chamfer transform) did not result in accurate identification of individuals in the wild in this study, the application of an alternative algorithm on the same photographs taken in the wild may be met with limited success. One inference from the differing results between photos taken of animals in the wild and those in captivity is that environmental conditions add extra ‘noise’ (measurement error) to whisker spot patterns in wild animals. These factors would need to be able to be sufficiently controlled for the method to have (at least) the same level of success as in captive environments. However, when identifying resightings for behavioural or abundance monitoring a recognition algorithm should be as close to 100% accurate as possible to prevent mismatches. Whether this is possible with *N. cinerea* is unknown.

Based on the results presented here, alternative methods of identification should be tested, such as the comparison of photographs of front flippers of wild *N. cinerea*. Flippers have been shown to contain sufficient information for identification in Hooker’s sea lions (*Phocarcos hookeri*), although in that study all four flippers were photographed from close ranges (McConkey, 1999). In 75% of matching trials (whether only one flipper was photographed is unspecified), preliminary testing of computer-assisted curve matching of sea lion flippers from 92 photographs of 37 individuals (2-4 photographs each) resulted in the first correct match only after matching at least 28 photographs incorrectly (Gope *et al.*, 2005). In *N. cinerea*, hind flippers are barely seen spread apart, and it was rare that both front flippers were available for photographs. As a result, this method (using all four flippers) was originally deemed unfeasible in a preliminary study by the author. However, the reliability of using a single flipper for photo-identification in a larger population could be tested and is suggested here. In addition, to account for variations in angle and potentially allow for easier manual matching, testing of the use of 3D stereo

photographs is recommended (Heike *et al.*, 2010). Nevertheless, whether one front flipper would provide sufficient information for identification is not known.

While scarring is often used to identify marine animals such as cetaceans (e.g. Würsig and Jefferson, 1990), *N. cinerea* in Perth metropolitan areas with scars of sufficient size and visible position that could be used for re-identification were rarely observed (see Figure A1 and Table A1 in the Appendix for images of the four individuals that were resighted by using scars, for which no applicable photo on their whisker spot pattern was available). Marine mammals generally exhibit extensive and rapid healing capacities and in pinnipeds, regrowth of fur obscures or hides smaller scars. Furthermore, *N. cinerea* moult every ~17 months, a process that may also alter a scar's appearance (e.g. Bruce-Allen and Geraci, 1985; Lockyer and Morris, 1990). The low number of *N. cinerea* with large scars suitable for re-identification, together with the healing and changing of scars over time, limits the extent in which this method can be used reliably for long-term studies. However, in this study, resightings of four individuals based on the distinct patterns of their scars were possible. Two of these individuals showed evidence of movement between Seal and Carnac Islands, while the other two were observed twice on either Seal or on Carnac Islands. Although the sample size of resightings was small, the results showed *N. cinerea* moved between these two islands as well as returned to the same islands. Although *N. cinerea* have previously been documented to move freely between the Perth haul-out islands in a period of four months (Gales *et al.*, 1992), in this study, movement between islands was confirmed over a period of approximately eight months. Resightings in this study were, through any method, insufficient to interpret information beyond these observations (frequency of returning and movement between islands, abundance estimates, etc.).

Despite whisker spot identification of individuals not proving to be a feasible method of resighting in this study, more general knowledge on haul-out patterns in *N. cinerea* in the Perth metropolitan area provided alternative information to support the design of more robust surveys and abundance estimates based on repeated count data (e.g. Kéry *et al.*, 2005; Dail and Madsen, 2011). The overall long-term fluctuation of numbers of *N. cinerea* hauling out in the Perth metropolitan area generally followed a 17-18 month cycle that aligned with the population's known breeding cycle. It is likely that male *N. cinerea* migrate to the Perth metropolitan area during the non-breeding season to reduce competition for food resources around breeding sites or to seek out more readily available food sources (Gales *et al.*, 1992). Due

to the non-annual breeding cycle, successive peak haul-out seasons in the Perth metropolitan area are out of synchrony with the regular seasonal pattern and consecutive peaks never occur in the same annual seasons. Numbers of *N. cinerea* hauling out on Seal and Carnac Islands during winter and summer peaks have been reported to be comparable, according to count data collected from 1982 until 1991 (Gales *et al.*, 1992). However, while the peak numbers on Seal Island in this study were similar between consecutive peak periods (the first occurring in the winter and the second 17-18 months later in the summer), significantly fewer *N. cinerea* hauled out during the first peak (the winter peak) than during the second peak (in the summer) on Carnac Island. Whether the earlier count data, comprising six consecutive peaks (three peaks during summer and three during winter), did not capture maximum numbers during the peak season, and so did not detect such a phenomenon is unknown. The exact number of surveys conducted in a month in the previous study is unknown (Gales *et al.*, 1992). The variation in peak numbers on Carnac Island is thought to be due to environmental drivers, or the lower winter peak in this study being a lower peak in general.

Count data during the summer peak (second peak in the study) indicated a potential lag in the increase in numbers on Carnac Island compared to Seal Island. Differences in the timing of the peak season on haul-out islands in the Perth metropolitan area were unexpected, given the proximity of the islands and movement between islands (Gales *et al.*, 1992). Similar lags in breeding behaviour have been observed of the same population of *N. cinerea*. Asynchronous breeding seasons occur in Jurien Bay, Western Australia, where *N. cinerea* hauling out in the Perth region are expected primarily to migrate for breeding. Also, the breeding season at Buller Island's lags approximately one month behind that of Fisherman and Beagle Islands (breeding islands around Jurien Bay; Gales *et al.*, 1992; Campbell, 2003). If individuals from the island where breeding occurred earlier, migrated to one haul-out island whilst the remaining breeding animals migrated at a later time to a different haul-out island, this may explain some of the observed delay in peak numbers from one island to another. Perhaps the habitat is better on Seal than Carnac Island for nonbreeding male sea lions. Alternatively, the earlier returnees may simply display a preference for one island over another for some temporary, as yet unknown, environmental condition.

This lag between peak numbers at the two main haul-out islands near Perth appears to be only recently documented. Data collected from 1982 until 1991 does not reveal the same lag between the peak seasons on Carnac Island, including from 1987 onwards when data were collected more often (Gales *et al.*, 1992). However, as only maximum values were often reported, the actual sample size and survey intervals for this data set are unknown and final monthly counts most likely do not show changes in numbers at sufficient resolution to detect a time lag in hauled-out numbers between the islands. Difficulty in identifying short-term trends or differences could be exacerbated by the high variation in day-to-day numbers of individuals, as was found in this study, highlighting the importance of a high sample size when monitoring *N. cinerea* numbers based on haul-out data alone.

While the data collected here had a much higher sample size and smaller intervals between surveys, consistent data collection on both Carnac and Seal Islands during a greater number of peak seasons would need to be conducted to determine if lags of the peak season are typical in this area, and if there are differences in haul-out numbers between winter and summer peak seasons. It is also unknown if males hauling out in the Perth metropolitan area show breeding preferences for one or more of the breeding islands in Jurien Bay. Females have been shown to exhibit a high level of natal site fidelity (Campbell *et al.*, 2008b). Therefore, if males exhibited similar behaviour and were not locally migrating between breeding islands, gene flow and genetic variation between colonies could likely be more segregated (Campbell, 2003; Campbell *et al.*, 2008b; Lowther *et al.*, 2013) and more susceptible to collapse.

The highest numbers of males on the breeding islands off Jurien Bay, approximately aligned with the lowest numbers of *N. cinerea* hauling out in the Perth metropolitan area (Gales *et al.*, 1992; DPaW unpublished data). The difference in time between peak numbers of *N. cinerea* hauling out around Perth and the breeding season in Jurien Bay is approximately six months (Gales *et al.*, 1992). This reflects a relatively long migration time between the two locations, in both directions. Where *N. cinerea* spend their time in transition from haul-out to breeding islands and back is currently unknown. Prior to breeding, male *N. cinerea* are thought to spend most of their time foraging to build energy and fat reserves in preparation to defend territories, however, no information is available on feeding grounds or haul-out locations along their migratory route from metropolitan Perth to the Jurien Bay region (Marlow, 1975; Ling, 1992).

Very small numbers of male *N. cinerea* remained in the two Perth metropolitan haul-out islands in this study during the breeding season in the Jurien Bay region, and were mostly adults which may be too old or weak to breed or defend a territory (Gales *et al.*, 1992). Juveniles and sub-adults that are not of breeding age, nor able to defend territories, appeared to leave the Perth metropolitan area along with the mature males, although the destination of these individuals is unknown. Some juveniles have previously been shown to migrate to breeding sites, even though they do not set up territories (Gales *et al.*, 1992), though the reason for this migration is also unknown. It has been presumed that the juveniles migrating could be females returning to their natal sites during the breeding season (Gales *et al.*, 1992). However, in this study, male juveniles and sub-adults left the haul-out islands after the peak seasons, presumably to migrate as well.

During the non-breeding season, when sufficient numbers of *N. cinerea* resided in the Perth Metropolitan area to detect more fine-scale patterns, increasing numbers of *N. cinerea* hauled-out with increasing time after sunrise. It is feasible that *N. cinerea* left the islands after dusk, though this could not be tested as surveys ceased prior to sunset. The findings in this study showing numbers doubling from morning to afternoon on some days highlights that the timing of counts used for abundance and trend estimates can significantly influence results (e.g. Southwell, 2005). Either consistent timing is required, or the underlying pattern needs to be able to be accurately predicted to correct for variability in the timing of counts.

N. cinerea's hauling-out behaviour was found to be complex, influenced not only by the breeding cycle, but also a number of environmental drivers. *N. cinerea* preferred to haul out when air temperatures were higher and tidal heights were lower, however they were less influenced by wind speed. The high variability in numbers of hauled-out *N. cinerea* at any given time during the day when coupled with variable weather and tide conditions can affect the accuracy of abundance estimates considerably (Southwell, 2005). Due to the interacting influences that the environmental conditions listed above have on haul-out behaviour, repeated counts over several closely-spaced days during the peak may be a way of normalising data. This will certainly overcome some of the inherent difficulties of single-day counts in the peak season undertaken opportunistically for monitoring and management purposes (as has been common practice over many years in this region). Repeated counts would be best done when they are most comparable. Time since sunrise was the most significant variable influencing haul-out behaviour (following season

and location), indicating that counts should be conducted between 9 and 11 h after sunrise at Carnac and Seal Islands. However, during population monitoring, regional managers conduct standard surveys that include counts across all six islands in the Perth area. It may not be logistically possible for them to consistently conduct counts at particular sites in the afternoon. In these cases, it is recommended that counts be conducted at a similar time of day at particular islands across all survey days (in relation to the time of sunrise). This will help ensure that counts are comparable between sites and years. Additionally, air temperature and tidal heights should be recorded so that relative abundance can be modelled and if possible adjusted using a correction factor to improve comparability over time (Seber, 1986; Huber *et al.*, 2001).

The ~17 month breeding cycle in the Perth region results in peak periods occurring during entirely different seasons in sequential years; thus, environmental conditions during peaks will vary significantly from peak to peak. Peak seasons in alternate years also occur in earlier months (two peaks are less than three years apart). Therefore, to determine whether the variation in *N. cinerea* haul-out behaviour reflects a spatial (preferred haul-out location), temporal (timing and duration of peaks), or abundance (population numbers) change, a multi-cycle dataset is needed. Thus continued monitoring is required to determine whether trends are a direct result of seasonal environmental conditions, or are a function of some alternative longer-term driver such as long-lasting anthropogenic pressures and climate change.

In fact, *N. cinerea* residing in the Perth metropolitan area have been exposed to a variety of anthropogenic activities. Given the proximity of a state capital city and the high rate of vessel ownership per capita, there is a constant and high level of anthropogenic visitation and interaction at the major haul-out islands where *N. cinerea* occurs. The exposure of *N. cinerea* to vessels and people was high in this study at both islands, but the composition of stimuli types (vessels and people) varied significantly between the islands. This variation can be explained by the proximity to the mainland and the topographic features of the islands, as well as the purpose for people's visits to each island (Orsini and Newsome, 2005). Furthermore, approach distances and duration of visitation varied among stimulus types. Hence the frequency and level of *N. cinerea* responses were likely not only a function of the stimulus type, but the type in combination with its approach distance and visitation duration.

Overall, responses of *N. cinerea* to anthropogenic activities were frequently observed, most often at the shortest ranges, and predictably increased with decreasing range. This highlights that the level of disturbance was greatest when humans approached to within close ranges. The most severe responses, i.e. aggressive behaviour towards people, were only observed on Carnac Island. Here, startle responses were also most frequent, and most retreating behaviours were recorded when people approaching along a beach were highly visible to hauled-out animals. In both cases, people often breached the 5-10 m minimum approach distance in guidelines (CALM, 2003; DEC, 2007; DEC and Fisheries, 2011). Even at 10 m distances, responses remained high, thus approach limits may need to be increased and further enforced, e.g. using the already installed buoys as boundary markers for all stimuli, including 'Paddle powered' vessels and 'People'. However, changing people's activities and behaviour around *N. cinerea* can only be achieved if they are aware of the concerns for this population and the impacts of their activities. This may be achieved by continued patrols to enforce guidelines and broadening education programs. The number of higher-level responses elicited could be reduced by restricting Carnac Island's beach access and designating the entire beach area as a sanctuary zone. This would be in place of the current limited area, where boundaries marking the sanctuary zone are not easily discerned. A reduction in aggressive behaviours towards people would also be safer for those visiting the island.

The results here identify impacts from human disturbance as playing a direct role in *N. cinerea*' overall behaviour while hauling out at the two islands, and more importantly indicate that the level of disruption may be significant, potentially having a detrimental impact on the overall energy budget of disturbed animals. Due to the high frequency of disturbance experienced by a potentially significant number of *N. cinerea* in this region, future studies estimating the extent of its impact on energy budget of *N. cinerea* are recommended. These include the behavioural assessment of hauled-out individuals during non-disturbed periods, i.e. during the absence of anthropogenic activities in the vicinity, to determine changes in their activity budgets during times with anthropogenic disturbance. These can then be used to estimate energy budgets of undisturbed versus disturbed individuals. As non-disturbed periods are few during a summer non-breeding cycle, it may be necessary to conduct these comparisons during spring/autumn parts of the cycle, or during a winter non-breeding period. Furthermore, quantifying foraging behaviour of the individuals for which activity budgets of hauled-out periods are available,

would be beneficial to investigate differences between undisturbed and disturbed periods. Tagging *N. cinerea* in Perth's metropolitan waters would also help to reveal the main foraging grounds of this population and potential concurrences with other anthropogenic activities, such as recreational fishing and boating. It is also strongly suggested that management authorities endeavour to reduce disturbance at both non-breeding and breeding colonies of *N. cinerea*.

N. cinerea have previously been shown to be sensitive to anthropogenic activities in their vicinity (Campbell, 2005), and similar to other marine mammals, a consequence of long-term lower impact level activities may be a reduction in population size (e.g. Gerrodette and Gilmartin, 1990; Stevens and Boness, 2003). This type of reduction in numbers is difficult to detect in small populations, such as *N. cinerea* in Perth waters especially when it is difficult to estimate true numbers of animals (e.g. Taylor and Gerrodette, 1993; Taylor *et al.*, 2007). Populations may also significantly decline in numbers before the change is detected (e.g. Taylor and Gerrodette, 1993). The high variability in hauled-out *N. cinerea* around Perth increases the uncertainty in abundance estimates, and long-term trend assessment so far has not been possible. The limited information available for effective management also emphasizes the importance of obtaining sound baseline knowledge, implementing robust survey design and collecting sufficient sample sizes for monitoring such a population. This study has highlighted some environmental conditions and anthropogenic activities these *N. cinerea* respond to, and provides several recommendations for future monitoring and management.

Continued monitoring is recommended not only to assess current abundance and population trends, but also to determine the effect of updated regulations or guidelines. Management of *N. cinerea* in the Perth metropolitan area is important for successful conservation of this iconic species, and to keep them healthy and happily returning ever after.

Bibliography

- Abbott, I. (1979). The past and present distribution and status of sea lions and fur seals in Western Australia. *Records of the Western Australia Museum*, **7**, 375–390.
- Agler, B.A. (1992). Testing the reliability of photographic identification of individual fin whales (*Balaenoptera physalus*). *Report of the International Whaling Commission*, **42**, 731–737.
- Ahl, A.S. (1986). The role of vibrissae in behavior: a status review. *Veterinary research communications*, **10**(1), 245–268.
- Ainley, D.G., Huber, H.R., and Bailey, K.M. (1982). Population fluctuations of California sea lions and the Pacific whiting fishery off central California. *Fishery Bulletin*, **80**(2), 253–258.
- Airoidi, L., Balata, D., and Beck, M.W. (2008). The gray zone: Relationships between habitat loss and marine diversity and their applications in conservation. *Journal of Experimental Marine Biology and Ecology*, **366**(1-2), 8–15.
- Allen, S.G., Ainley, D.G., Page, G.W., and Ribic, C.A. (1984). The effect of disturbance on harbor seal haul out patterns at Bolinas Lagoon, California. *Fishery Bulletin*, **82**(3), 493–500.
- Allison, G.W., Lubchenco, J., and Carr, M.H. (1998). Marine reserves are necessary but not sufficient for marine conservation. *Ecological applications*, **8**(sp1), S79–92.
- Anderson, C.J., Waterman, J.M., and Roth, J.D. (2005). Noninvasive identification of individual polar bears by whisker spot patterns. *Integrative and Comparative Biology*, **45**(6), 1106.
- Anderson, C.J.R., Roth, J.D., and Waterman, J.M. (2007a). Can whisker spot patterns be used to identify individual polar bears? *Journal of Zoology*, **273**, 333–339.

- Anderson, C.J.R., Vitoria Lobo, N.d., Roth, J.D., and Waterman, J.M. (2010). Computer-aided photo-identification system with an application to polar bears based on whisker spot patterns. *Journal of Mammalogy*, **91**(6), 1350–1359.
- Anderson, S.M., Teilmann, J., Dietz, R., Schmidt, N.M., and Miller, L.A. (2012). Behavioural responses of harbour seals to human-induced disturbances. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **22**, 113–121.
- Anderson, S.M., Teilmann, J., Harders, P.B., Hansen, E.H., and Hjøllund, D. (2007b). Diet of harbour seals and great cormorants in Limfjord, Denmark: interspecific competition and interaction with fishery. *ICES Journal of Marine Science*, **64**(6), 1235–1245.
- Andrews-Goff, V., Hindell, M.A., Field, I.C., Wheatley, K.E., and Charassin, J.B. (2010). Factors influencing the winter haulout behaviour of Weddell seals: consequences for satellite telemetry. *Endangered Species Research*, **10**, 83–92.
- Arcangeli, A., Marini, L., and Crosti, R. (2013). Changes in cetacean presence, relative abundance and distribution over 20 years along a trans-regional fixed line transect in the Central Tyrrhenian Sea. *Marine Ecology*, **34**(1), 112–121.
- Ar dovini, A., Cinque, L., and Sangineto, E. (2008). Identifying elephant photos by multi-curve matching. *Pattern Recognition*, **41**, 1867–1877.
- Arnould, J.P.Y. and Hindell, M.A. (2001). Dive behaviour, foraging locations, and maternal attendance patterns of Australian fur seals (*Arctocephalus pusillus doriferus*). *Canadian Journal of Zoology*, **79**, 35–48.
- Arzoumanian, Z., Holmberg, J., and Norman, B. (2005). An astronomical pattern-matching algorithm for computer-aided identification of whale sharks (*Rhincodon typus*). *Journal of Applied Ecology*, **42**, 999–1011.
- Atkinson, S. (1997). Reproductive biology of seals. *Reviews of Reproduction*, **2**, 175–194.
- Aurioles, D. and Trillmich, F.I.S.P.S.G. (2008). *Zalophus californianus*. *The IUCN Red List of Threatened Species*, **Version 2015.2**. www.iucnredlist.org, Downloaded on 05 August 2015.
- Badinoa, A., Borellib, D., Gaggeroc, T., Rizzutod, E., and Schenonee, C. (2012). Normative framework for ship noise: Present situation and future trends. *Noise Control Engineering Journal*, **60**(6), 740–762.

- Balmer, B.C., Schwacke, L.H., Wells, R.S., Adams, J.D., Clay George, R., Lane, S.M., McLellan, W.A., Rosel, P.E., Sparks, K., Speakman, T., Zolman, E.S., and Pabst, D.A. (2013). Comparison of abundance and habitat usage for common bottlenose dolphins between sites exposed to differential anthropogenic stressors within the estuaries of southern Georgia, U.S.A. *Marine Mammal Science*, **29**(2), E114–E135.
- Balouin, Y., Rey-Valette, H., and Picand, P.A. (2014). Automatic assessment and analysis of beach attendance using video images at the Lido of Sète beach, France. *Ocean & Coastal Management*, **102**, 114–122.
- Bannister, J. (2001). Status of southern right whales (*Eubalaena australis*) off Australia. *Journal of Cetacean Research and Management Special Issue*, **2**, 103–110.
- Baylis, A.M.M., Hamer, D.J., and Nichols, P.D. (2009). Assessing the use of milk fatty acids to infer the diet of the Australian sea lion (*Neophoca cinerea*). *Wildlife Research*, **36**, 169–176.
- Beentjes, M.P. (1989). Haul-out patterns, site fidelity and activity budgets of male Hooker's sea lions (*Phocarctos hookeri*) on the New Zealand mainland. *Marine Mammal Science*, **5**(3), 281–297.
- Bejder, L., Samuels, A., Whitehead, H., Finn, H., and Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. *Marine Ecology Progress Series*, **395**, 177–185.
- Bejder, M., Johnston, D.W., Smith, J., Friedlaender, A., and Bejder, L. (2016). Embracing conservation success of recovering humpback whale populations: Evaluating the case for downlisting their conservation status in Australia. *Marine Policy*, **66**, 137–141. doi:10.1016/j.marpol.2015.05.007.
- Bengtson, J.L. and Cameron, M.F. (2004). Seasonal haulout patterns of crabeater seals (*Lobodon carcinophaga*). *Polar Biology*, **27**, 344–349.
- Berggren, P. (1994). Bycatches of the harbour porpoise (*Phocoena phocoena*) in the Swedish Skagerrak, Kattegat and Baltic Seas; 1973-1993. *Report of the International Whaling Commission*, **15**, 211–215.
- Berkson, J.M. and DeMaster, D.P. (1985). Use of pup counts in indexing population changes in pinnipeds. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**(5), 873–879.

- Berry, T. (2013). *Development of molecular tools for dietary analysis: A tail end look at Western Australia's aquatic ecosystems*. Thesis, Murdoch University, Perth, Western Australia.
- Best, P.B. (1990). Trends in the inshore right whale population off South Africa, 1969-1987. *Marine Mammal Science*, **6**(2), 93–108.
- Bester, M. (2014). Marine mammals - natural and anthropogenic influences. In B. Freedman (Ed.), *Global environmental change*, vol. 1 of *Handbook of Global Environmental Pollution*, chap. 21. Springer Netherlands, pp. 167–174.
- Birkun, A.A.J. and Frantzis, A. (2008). *Phocoena phocoena ssp. relicta*. *The IUCN Red List of Threatened Species*, (19 April 2016), Downloaded on 19 April 2016. E.T17030A6737111.
- Birkun, A.J. (2002). Interactions between cetaceans and fisheries in the Black Sea. In G. Notarbartolo di Sciara (Ed.), *Cetaceans of the Mediterranean and Black Seas: state of knowledge and conservation strategies*, chap. 10. ACCOBAMS Secretariat, Monaco, p. 11 p.
- Boehme, L., Thompson, D., Fedak, M., Bowen, D., Hammill, M.O., and Stenson, G.B. (2012). How many seals were there? the global shelf loss during the last glacial maximum and its effect on the size and distribution of grey seal populations. *PloS ONE*, **7**(12), e53000.
- Bohnsack, J.A. (1996). Maintenance and recovery of reef fishery productivity. In N.V.C. Polunin and C.M. Roberts (Eds.), *Reef fisheries*. Springer Netherlands, pp. 283–313.
- Booth, D.J., Bond, N., and Macreadie, P. (2011). Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research*, **62**, 1027–1042.
- Boren, L., Gemmill, N.J., and Barton, K.J. (2002). Tourist disturbance on New Zealand fur seals *Arctocephalus forsteri*. *Australian Mammalogy*, **24**, 85–95.
- Borgefors, G. (1986). Distance transformation in digital images. *Computer Vision, Graphics, and Image Processing*, **34**, 344–371.
- Bowles, A.E. and Anderson, R.C. (2012). Behavioral responses and habituation of pinnipeds and small cetaceans to novel objects and simulated fishing gear with and without a pinger. *Aquatic Mammals*, **38**(2), 161–188.

- Branch, T.A., Stafford, K.M., Palacios, D.M., Allison, C., Bannister, J.L., Burton, C.L.K., Cabrera, E., Carlson, C.A., Galletti Vernazzani, B., Gill, P.C., Huckle-Gaete, R., Jenner, K.C.S., Jenner, M.N.M., Matsuoka, K., Mikhalev, Y.A., Miyashita, T., Morrice, M.G., Nishiwaki, S., Sturrock, V.J., Tormosov, D., Anderson, R.C., Baker, A.N., Best, P.B., Borsa, P., Brownell Jr, R.L., Childerhouse, S., Findlay, K.P., Gerrodette, T., Ilangakoon, A.D., Joergensen, M., Kahn, B., Ljungblad, D.K., Maughan, B., Mccauley, R.D., Mckay, S., Norris, T.F., Group, O.W., Research, D., Warneke, R.M., Rankin, S., Samaran, F., Thiele, D., and Van Waerebeek, K. (2007). Past and present distribution, densities and movements of blue whales *Balaenoptera musculus* in the Southern Hemisphere and northern Indian Ocean. *Mammal Review*, **37**(2), 116–175.
- Bruce-Allen, L.J. and Geraci, J.R. (1985). Wound healing in the bottlenose dolphin (*Tursiops truncatus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **42**(2), 216–228.
- Buckland, S.T. (1980). A modified analysis of the jolly-seber capture-recapture model. *Biometrics*, **36**(3), 419–435.
- Burnham, K.P. and Anderson, D.R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach*. Springer, New York.
- CALM (2003). Carnac Island Nature Reserve - Management Plan 2003. Report, Department of Conservation and Land Management.
- Campbell, R. (2005). Historical distribution and abundance of the Australian sea lion (*Neophoca cinerea*) on the west coast of Western Australia. Report, Fisheries Research Division.
- Campbell, R., Holley, D., Christianopoulos, D., Caputi, N., and Gales, N. (2008a). Mitigation of incidental mortality of Australian sea lions in the west coast rock lobster fishery. *Endangered Species Research*, **5**, 345–358.
- Campbell, R., Holley, D., Collins, P., and Armstrong, S. (2014). Changes in the abundance and distribution of the New Zealand fur seal (*Arctocephalus forsteri*) in Western Australia: are they approaching carrying capacity? *Australian Journal of Zoology*, **62**(4), 261–267.
- Campbell, R.A. (2003). *Demography and genetic population structure of the Australian sea lion (Neophoca cinerea)*. Thesis, University of Western Australia, Perth, Western Australia.

- Campbell, R.A., Gales, N.J., Lento, G.M., and Baker, C.S. (2008b). Islands in the seas: extreme female natal site fidelity in the Australian sea lion, *Neophoca cinerea*. *Biology Letters*, **4**, 139–142.
- Carlens, H., Lydersen, C., Krafft, B.A., and Kovacs, K.M. (2006). Spring haul-out behavior of ringed seals (*Pusa hispida*) in Kongsfjorden, Svalbard. *Marine Mammal Science*, **22**(2), 379–393.
- Cassini, M.H. (2001). Behavioural responses of South American fur seals to approach by tourists - a brief report. *Applied Animal Behaviour Science*, **71**, 341–346.
- Cassini, M.H., Szteren, D., and Fernández-Juricic, E. (2004). Fence effects on the behavioural responses of South American fur seals to tourist approaches. *Journal of Ethology*, **22**, 127–133.
- Childerhouse, S., Dix, B., and Gales, N. (2001). Diet of New Zealand sea lions (*Phocartos hookeri*) at the Auckland Islands. *Wildlife Research*, **28**, 291–298.
- Childerhouse, S., Hamer, D., Maloney, A., Michael, S., Donnelly, D., and Schmitt, N. (2014). Preliminary Report for CSP Project 4522 New Zealand sea lion ground component 2013/14. Report, New Zealand Department of Conservation.
- Chilvers, B.L., Wilkinson, I.S., Duignan, P.J., and Gemmell, N.J. (2005). Summer foraging areas for lactating New Zealand sea lions *Phocartos hookeri*. *Marine Ecology Progress Series*, **304**, 235–247.
- Connor, R.C. and Smolker, R.S. (1985). Habituated dolphins (*Tursiops sp.*) in Western Australia. *Journal of Mammalogy*, **66**(2), 398–400.
- Constantine, R. (1999). Effects of tourism on marine mammals in New Zealand. Report, Department of Conservation.
- Costa, D.P. and Gales, N.J. (2003). Energetics of a benthic diver: Seasonal foraging ecology of the Australian sea lion, *Neophoca cinerea*. *Ecological Monographs*, **73**(1), 27–43.
- Cowling, M., Kirkwood, R., Boren, L., and Scarpaci, C. (2015). The effects of vessel approaches on the New Zealand fur seal (*Arctocephalus forsteri*) in the Bay of Plenty, New Zealand. *Marine Mammal Science*, **31**(2), 501–519.
- Cowling, M., Kirkwood, R., Boren, L.J., and Scarpaci, C. (2014). The effects of seal-swim activities on the New Zealand fur seal (*Arctophoca australis forsteri*)

- in the Bay of Plenty, New Zealand, and recommendations for a sustainable tourism industry. *Marine Policy*, **45**, 39–44.
- Cox, T.M., Read, A.J., Swanner, D., Urian, K., and Waples, D. (2003). Behavioral responses of bottlenose dolphins, *Tursiops truncatus*, to gillnets and acoustic alarms. *Biological Conservation*, **115**, 203–212.
- Cunningham, L., Baxter, J.M., Boyd, I.L., Duck, C.D., Lonergan, M., Boss, S.E., and McConnell, B. (2009). Harbour seal movements and haul-out patterns: implications for monitoring and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **19**, 398–407.
- Dail, D. and Madsen, L. (2011). Models for estimating abundance from repeated counts of an open metapopulation. *Biometrics*, **67**(2), 577–587.
- Dans, S.L., Degradi, M., Pedraza, S.N., and Crespo, E.A. (2012). Effects of tour boats on dolphin activity examined with sensitivity analysis of Markov chains. *Conservation Biology*, **26**(4), 708–716.
- Dawson, S.M. (1991). Incidental catch of Hector's dolphins in inshore gillnets. *Marine Mammal Science*, **7**(3), 283–295.
- Dawson, S.M. and Slooten, E. (1993). Conservation of Hector's dolphins: the case and process which led to establishment of the Banks Peninsula Marine Mammal Sanctuary. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **3**(3), 207–221.
- DEC (2007). Shoalwater Islands Marine Park Management Plan 2007-2017. Report, Department of Parks and Wildlife.
- DEC and Fisheries (2011). Shoalwater Islands Marine Park. Marine parks... WA's submerged wonders. Recreation guide. Perth, Western Australia.
- Demarchi, M.W., Holst, M., Robichaud, D., Waters, M., and MacGillivray, A.O. (2012). Responses of Steller sea lions (*Eumetopias jubatus*) to in-air blast noise from military explosions. *Aquatic Mammals*, **38**(3), 279–289.
- Department of Fisheries (2012). Sea lion exclusion devices - A guide to assist rock lobster fishers install SLEDs. Western Australia.
- Dixon, D.R. (2003). A non-invasive technique for identifying individual badgers *Meles meles*. *Mammal Review*, **33**, 92–94.

- Donaldson, R., Finn, H., and Calver, M. (2010). Illegal feeding increases risk of boat-strike and entanglement in bottlenose dolphins in Perth, Western Australia. *Pacific Conservation Biology*, **16**(3), 157–161.
- Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L., and Sullivan, C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, **81**(02), 163–182.
- Feltz, E.T. and Fay, F.H. (1966). Thermal requirements in vitro of epidermal cells from seals. *Cryobiology*, **3**(3), 261–264.
- Fergusson, I., Compagno, L.J.V., and Marks, M. (2009). *Carcharodon carcharias*. *The IUCN Red List of Threatened Species, Version 2015.2*. www.iucnredlist.org, Downloaded on 06 August 2015. URL www.iucnredlist.org.
- Fisher, R.A. (1922). On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society*, **85**(1), 87–94.
- Flom, P.L. and Cassell, D.L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. *NorthEast SAS Users Group Inc 20th Annual Conference*, 1–6.
- Forcada, J. and Aguilar, A. (2000). Use of photographic identification in capture-recapture studies of Mediterranean monk seals. *Marine Mammal Science*, **16**(4), 767–793.
- French, S.S., Gonzáles-Suárez, M., Young, J.K., Durham, S., and Gerber, L.R. (2011). Human disturbance influences reproductive success and growth rate in California sea lions (*Zalophus californianus*). *PloS ONE*, **6**(3), e17686.
- Frid, A. and Dill, L. (2002). Human-caused disturbance stimuli as a form of predation risk. *Conservation Ecology*, **6**(1), 11.
- Friday, N., Smith, T.D., Stevick, P.T., and Allen, J. (2000). Measurement of photographic quality and individual distinctiveness for the photographic identification of humpback whales, *Megaptera novaeangliae*. *Marine Mammal Science*, **16**(2), 355–374.
- Gailey, G., Würsig, B., and McDonald, T.L. (2007). Abundance, behavior, and movement patterns of western gray whales in relation to a 3-D seismic survey,

- Northeast Sakhalin Island, Russia. *Environmental Monitoring and Assessment*, **134**, 75–91.
- Gales, N.J. (1995). Hooker's sea lion recovery plan (*Phocarctos hookeri*). Report, Threatened Species Unit, Department of Conservation.
- Gales, N.J., Cheal, A.J., Pobar, G.J., and Williamson, P. (1992). Breeding biology and movements of Australian sea lions, *Neophoca cinerea*, off the west coast of Western Australia. *Wildlife Research*, **19**, 405–416.
- Gales, N.J. and Costa, D.P. (1997). The Australian sea lion: a review of an unusual life history. In M. Hindell and C. Kemper (Eds.), *Marine Mammal Research in the Southern Hemisphere*, vol. 1: Status, Ecology and Medicine. Surrey Beatty and Sons, Devon, UK, pp. 78–87.
- Gales, N.J. and Fletcher, D.J. (1999). Abundance, distribution and status of the New Zealand sea lion, *Phocarctos hookeri*. *Wildlife Research*, **26**, 35–52.
- Gales, N.J., Haberley, B., and Collins, P. (2000). Changes in the abundance of New Zealand fur seals, *Arctocephalus forsteri*, in Western Australia. *Wildlife Research*, **27**(2), 165–168.
- Gales, N.J., Shaughnessy, P.D., and Dennis, T.E. (1994). Distribution, abundance and breeding cycle of the Australian sea lion *Neophoca cinerea* (Mammalia: Pinnipedia). *Journal of Zoology*, **234**, 353–379.
- Gerrodette, T. and Gilmartin, W.G. (1990). Demographic consequences of changed pupping and hauling sites of the Hawaiian monk seal. *Conservation Biology*, **4**(4), 423–430.
- Goldstein, T., Johnson, S.P., Phillips, A.V., Hanni, K.D., Fauquier, D.A., and Gulland, F.M.D. (1999). Human-related injuries observed in live stranded pinnipeds along the central California coast 1986-1998. *Aquatic Mammals*, **25**(1), 43–51.
- Goldsworthy, S.D. (2015). *Neophoca cinerea*. *The IUCN Red List of Threatened Species*, **Version 2015.4**. www.iucnredlist.org, Downloaded on 19 January 2016. (IUCN SSC Pinniped Specialist Group).
- Goldsworthy, S.D., Lowther, A., Shaughnessy, P.D., McIntosh, R.R., and Page, B. (2007a). Assessment of pup production and the maternal investment strategies of the Australian sea lion (*Neophoca cinerea*) at Dangerous Reef in the 2006-07 breeding season. Report, South Australian Research and Development Institute.

- Goldsworthy, S.D., Mackay, A.I., Shaughnessy, P.D., Bailleul, F., and McMahon, C.R. (2014). Maintaining the monitoring of pup production at key Australian sea lion colonies in South Australia (2013/14). Report, South Australian Research and Development Institute.
- Goldsworthy, S.D., McKenzie, J., Shaughnessy, P.D., McIntosh, R.R., Page, B., and Campbell, R. (2009a). An update of the report: understanding the impediments to the growth of Australian sea lion populations. Report, South Australian Research and Development Institute.
- Goldsworthy, S.D. and Page, B. (2007). A risk-assessment approach to evaluating the significance of seal bycatch in two Australian fisheries. *Biological Conservation*, **139**, 269–285.
- Goldsworthy, S.D., Page, B., Shaughnessy, P.D., Hamer, D., Peters, K.D., McIntosh, R.R., Baylis, A.M.M., and McKenzie, J. (2009b). Innovative solutions for aquaculture planning and management: addressing seal interactions in the fin-fish aquaculture industry. Report, South Australian Research and Development Institute.
- Goldsworthy, S.D., Shaughnessy, P.D., McIntosh, R.R., Kennedy, C., Simpson, J., and Page, B. (2008). Australian sea lion populations at Seal Bay and the Seal Slide (Kangaroo Island): continuation of the monitoring program. Report, South Australian Research and Development Institute.
- Goldsworthy, S.D., Shaughnessy, P.D., Page, B., Dennis, T.E., McIntosh, R.R., Hamer, D., Peters, K.J., Baylis, A.M.M., Lowther, A., and Bradshaw, C.J.A. (2007b). Developing population monitoring protocols for Australian sea lions. Report, South Australian Research and Development Institute.
- Gope, C., Kehtarnavaz, N., Hillman, G., and Würsig, B. (2005). An affine invariant curve matching method for photo-identification of marine mammals. *Pattern Recognition*, **38**, 125–132.
- Gormley, A.M., Slooten, E., Dawson, S., Barker, R.J., Rayment, W., du Fresne, S., and Bräger, S. (2012). First evidence that marine protected areas can work for marine mammals. *Journal of Applied Ecology*, **49**(2), 474–480.
- Gowans, S. and Whitehead, H. (2001). Photographic identification of Northern bottlenose whales (*Hyperoodon ampullatus*): Sources of heterogeneity from natural marks. *Marine Mammal Science*, **17**(1), 76–93.

- Gramming, P., Sundberg, J., Ternström, S., Leanderson, R., and H., P.W. (1988). Relationship between changes in voice pitch and loudness. *Journal of Voice*, **2**(2), 118–126.
- Granquist, S.M. and Sigurjonsdottir, H. (2014). The effect of land based seal watching tourism on the haul-out behaviour of harbour seals (*Phoca vitulina*) in Iceland. *Applied Animal Behaviour Science*, **156**, 85–93.
- Gray, J.S. (1997). Marine biodiversity: patterns, threats and conservation needs. *Biodiversity and Conservation*, **6**, 153–175.
- Grellier, K., Thompson, P.M., and Corpe, H.M. (1996). The effect of weather conditions on harbour seal (*Phoca vitulina*) haulout behaviour in the Moray Firth, northeast Scotland. *Canadian Journal of Zoology*, **74**, 1806–1811.
- Groth, E.J. (1986). A pattern-matching algorithm for two-dimensional coordinate lists. *Astronomical Journal*, **91**, 1244–1248.
- Halpern, B.S. and Warner, R.R. (2002). Marine reserves have rapid and lasting effects. *Ecology Letters*, **5**, 361–366.
- Hamer, D.J., Goldsworthy, S.D., Costa, D.P., Fowler, S.L., Page, B., and Sumner, M.D. (2013). The endangered Australian sea lion extensively overlaps with and regularly becomes by-catch in demersal shark gill-nets in South Australian shelf waters. *Biological Conservation*, **157**, 386–400.
- Hamer, D.J., Page, B., and Goldsworthy, S.D. (2007). The spatial distribution of foraging effort in Australian sea lion populations in close proximity to areas of high catch and effort in the SA rock lobster and shark gill-net fisheries. Report, South Australian Research and Development Institute.
- Hamer, D.J., Ward, T.M., Shaughnessy, P., and Clark, S.R. (2011). Assessing the effectiveness of the Great Australian Bight Marine Park in protecting the endangered Australian sea lion (*Neophoca cinerea*) from by-catch mortality in shark gill-nets. *Endangered Species Research*, **14**, 203–2016.
- Hammerschlag, N., Martin, R.A., and Fallows, C. (2006). Effects of environmental conditions on predator-prey interactions between white sharks (*Carcharodon carcharias*) and Cape fur seals (*Arctocephalus pusillus pusillus*) at Seal Island, South Africa. *Environmental Biology of Fishes*, **76**, 341–350.

- Hammond, P., Berggren, P., Benke, H., Borchers, D., Collet, A., Heide-Jørgensen, M., Heimlich, S., Hiby, A., Leopold, M., and Øien, N. (2002). Abundance of harbour porpoise and other cetaceans in the North Sea and adjacent waters. *Journal of Applied Ecology*.
- Harestad, A.S. (1978). Diurnal activity of Northern sea lions, *Eumetopias jubatus* (Schreber). *Syesis*, **11**, 279–280.
- Harrell, F.E. (2001). *Regression modeling strategies*. Springer Science & Business Media.
- Harris, R.E., Miller, G.W., and Richardson, W.J. (2001). Seal responses to airgun sounds during summer seismic surveys in the Alaskan Beaufort Sea. *Marine Mammal Science*, **17**(4), 795–812.
- Hartel, E.F., Constantine, R., and Torres, L.G. (2015). Changes in habitat use patterns by bottlenose dolphins over a 10-year period render static management boundaries ineffective. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **25**, 701–711.
- Hastings, K.K., Hiby, L.A., and Small, R.J. (2008). Evaluation of a computer-assisted photograph-matching system to monitor naturally marked harbor seals at Tugidak Island, Alaska. *Journal of Mammalogy*, **89**(5), 1201–1211.
- Hastings, K.K., Small, R.J., and Hiby, L. (2001). Use of computer-aided matching of photographs to examine population parameters of Alaskan harbor seals. Harbor seal investigations in Alaska. annual report for NOAA award NA87FX0300, Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK.
- Heike, C.L., Upson, K., Stuhaug, E., and Weinberg, S.M. (2010). 3d digital stereophotogrammetry: a practical guide to facial image acquisition. *Head Face Med*, **6**(1), 18.
- Henry, E. and Hammill, M.O. (2001). Impact of small boats on the haulout activity of harbour seals (*Phoca vitulina*) in Métis Bay, Saint Lawrence Estuary, Québec, Canada. *Aquatic Mammals*, **27**(2), 140–148.
- Hiby, L. and Lovell, P. (1990). Computer aided matching of natural markings: a prototype system for grey seals. *Report of the International Whaling Commission*, **12**, 57–61.

- Hiby, L., Lovell, P., Patil, N., Samba Kumar, N., Gopaldaswamy, A.M., and Ullas Karanth, K. (2009). A tiger cannot change its stripes: using a three-dimensional model to match images of living tigers and tiger skins. *Biology Letters*, **5**, 383–386.
- Higgins, L.V. (1990). *Reproductive behavior and maternal investment of Australian sea lions*. Thesis, California Sea Grant College Program.
- Higgins, L.V. (1993). The nonannual, nonseasonal breeding cycle of the Australian sea lion, *Neophoca cinerea*. *Journal of Mammalogy*, **74**(2), 270–274.
- Higgins, L.V. and Gass, L. (1993). Birth to weaning: parturition, duration of lactation, and attendance cycles of Australian sea lions (*Neophoca cinerea*). *Canadian Journal of Zoology*, **71**, 2047–2055.
- Higham, J.E.S. and Shelton, E.S. (2011). Tourism and wildlife habituation: Reduced population fitness or cessation of impact? *Tourism Management*, **32**(6), 1290–1298.
- Hillman, G.R., Würsig, B., Gailey, G.A., Kehtarnavaz, N., Drobyshevsky, A., Araabi, B.N., Tagare, H.D., and Weller, D.W. (2003). Computer-assisted photo-identification of individual marine vertebrates: a multi-species system. *Aquatic Mammals*, **29**(1), 117–123.
- Hooper, D.U., Chapin III, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., and Schmid, B. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological monographs*, **5**(1), 3–35.
- Hoyt, E. (2011). *Marine Protected Areas for Whales, Dolphins and Porpoises: A World Handbook for Cetacean Habitat Conservation and Planning*. Earthscan, London and New York, 2 ed.
- Huber, H.R., Jeffries, S.J., Brown, R.F., DeLong, R.L., and Vanblaricom, G. (2001). Correcting aerial survey counts of harbor seals (*Phoca vitulina richardsi*) in Washington and Oregon. *Marine Mammal Science*, **17**(2), 276–293.
- Jansen, J.K., Boveng, P.L., Dahle, S.P., and Bengtson, J.L. (2010). Reaction of harbor seals to cruise ships. *The Journal of Wildlife Management*, **74**(6), 1186–1194.
- Jefferson, T.A. and Curry, B.E. (1994). A global review of porpoise (*Cetacea: Phocoenidae*) mortality in gillnets. *Biological Conservation*, **67**(2), 167–183.

- Jefferson, T.A., Webber, M.A., and Pitman, R.L. (2011). *Marine mammals of the world: A comprehensive guide to their identification*. Academic Press.
- Jones, G.P., Srinivasan, M., and Almany, G.R. (2007). Population connectivity and conservation of marine biodiversity. *Oceanography*, **20**(3), 100–111.
- Karlsson, O., Hiby, L., Lundberg, T., Jüssi, M., Jüssi, I., and Helander, B. (2005). Photo-identification, site fidelity, and movement of female gray seals (*Hali-choerus grypus*) between haul-outs in the Baltic Sea. *Ambio*, **34**(8), 628–634.
- Kastelein, R.A. and Weltz, F.C. (1991). Distribution and behaviour of Steller sea lions (*Eumetopias jubatus*) in Prince William Sound, Alaska, June 1989. *Aquatic Mammals*, **17**(2), 91–97.
- Kays, R.W. and Patterson, B.D. (2001). Mane variation in African lions and its social correlates. *Canadian Journal of Zoology*, **80**, 471–478.
- Kelleher, G. (1999). *Guidelines for Marine Protected Areas*. World Commission on Protected Areas of IUCN - The World Conservation Union: Gland, Switzerland, and Cambridge, UK.
- Kelly, B.P., Burns, J.J., and Quakenbush, L.T. (1988). Responses of ringed seals (*Phoca hispida*) to noise disturbance. *Port and Ocean Engineering under Arctic Conditions*, **2**, 27–38.
- Kelly, M.J. (2001). Computer-aided photograph matching in studies using individual identification: an example from Serengeti cheetahs. *Journal of Mammalogy*, **82**(2), 440–449.
- Kemper, C.M., Permberton, D., Cawthorn, M., Heinrich, S., Mann, J., Würsig, B., Shaughnessy, P., and Gales, R. (2003). Aquaculture and marine mammals: co-existence or conflict? In N. Gales, M. Hindell, and R. Kirkwood (Eds.), *Marine mammals: fisheries, tourism and management issues*, chap. 11. CSIRO, Melbourne, Australia, pp. 208–225.
- Kenyon, K.W. and Rice, D.W. (1961). Abundance and distribution of the Steller sea lion. *Journal of Mammalogy*, **42**(2), 223–234.
- Kerley, G.I.H. (1983). Comparison of seasonal haul-out patterns of fur seals *Arctocephalus tropicalis* and *A. gazella* on Subantarctic Marion Island. *South African Journal of Wildlife Research*, **13**(3), 71–77.

- Kéry, M., Royle, J.A., and Schmid, H. (2005). Modeling avian abundance from replicated counts using binomial mixture models. *Ecological Applications*, **15**(4), 1450–1461.
- Kirkman, S.P., Oosthuizen, W.H., Mejer, M.A., Kotze, P.G.H., Roux, J.P., and Underhill, L.G. (2007). Making sense of censuses and dealing with missing data: trends in pup counts of Cape fur seal *Arctocephalus pusillus pusillus* for the period 1972-2004. *African Journal of Marine Science*, **29**(2), 161–176.
- Kirkwood, R., Boren, L., Shaughnessy, P., Szteren, D., Mawson, P., Huckstadt, L., Hofmeyr, G., Oosthuizen, H., Schiavini, A., Campagna, C., and Berris, M. (2003). Pinniped-focused tourism in the southern hemisphere: a review of the industry. In N. Gales, M. Hindell, and R. Kirkwood (Eds.), *Marine mammals: fisheries, tourism and management issues*. Csiro Publishing, Collingwood, pp. 257–272.
- Koschinski, S. (2001). Current knowledge on harbour porpoises (*Phocoena phocoena*) in the Baltic Sea. *Ophelia*, **55**(3), 167–197.
- Kovacs, K.M., Aguilar, A., Aurioles, D., Burkanov, V., Campagna, C., Gales, N., Gelatt, T., Goldsworthy, S.D., Goodman, S.J., Hofmeyr, G.J.G., Härkönen, T., Lowry, L., Lydersen, C., Schipper, J., Sipilä, T., Southwell, C., Stuart, S., Thompson, D., and Trillmich, F. (2012). Global threats to pinnipeds. *Marine Mammal Science*, **2**, 414–436.
- Kovacs, K.M. and Innes, S. (1990). The impact of tourism on harp seals (*Phoca groenlandica*) in the Gulf of St. Lawrence, Canada. *Applied Animal Behaviour Science*, **26**, 15–26.
- Kovacs, K.M. and Lavigne, D.M. (1992). Maternal investment in otariid seals and walruses. *Canadian Journal of Zoology*, **70**, 1953–1964.
- Kraus, S., Read, A., Anderson, E., Baldwin, K., Solow, A., Spradlin, T., and Williamson, J. (1997). Acoustic alarms reduce porpoise mortality. *Nature*, **388**, 525.
- Kriebler, M. and Barrette, C. (1984). Aggregation behaviour of harbour seals at Forillon National Park, Canada. *Journal of Animal Ecology*, **53**(3), 913–928.
- Kruskal, W.H. and Wallis, W.A. (1952). Haulout patterns of grey seals *Halichoerus grypus* in the Baltic Sea. *Journal of the American Statistical Association*, **47**(260), 583–621.

- Kucey, L. (2005). *Human disturbance and the hauling out behaviour of Steller sea lions (Eumetopias jubatus)*. Thesis, University of British Columbia, Vancouver, Canada.
- Kyhn, L.A., Jørgensen, P.B., Carstensen, J., Bech, N.I., Tougaard, J., Dabelsteen, T., and Teilmann, J. (2015). Pingers cause temporary habitat displacement in the harbour porpoise *Phocoena phocoena*. *Marine Ecology Progress Series*, **526**, 253–265.
- Labrada-Martagón, V., Aurióles-Gamboa, D., and Martínez-Díaz, S.F. (2005). Natural and human disturbance in a rookery of the California sea lion (*Zalophus californianus californianus*) in the Gulf of California, Mexico. *Latin American Journal of Aquatic Mammals*, **4**(2), 175–185.
- Lake, S.E., Burton, H.R., and Hindell, M.A. (1997). Influence of time of day and month on Weddell seal haul-out patterns at the Vestfold Hills, Antarctica. *Polar Biology*, **18**, 319–324.
- Lalas, C. and Bradshaw, C.J. (2001). Folklore and chimerical numbers: review of a millennium of interaction between fur seals and humans in the New Zealand region. *New Zealand Journal of Marine and Freshwater Research*, **35**(3), 477–497.
- LeBoeuf, B.J., Riedman, M., and Keyes, R.S. (1982). White shark predation on pinnipeds in California coastal waters. *Fishery Bulletin*, **80**(4), 891–895.
- Lebreton, J.D., Burnham, K., Clobert, J., and Anderson, D.R.R. (1992). Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological monographs*, **62**(1), 67–118.
- Ling, J.G. and Walker, G.E. (1978). An 18-month breeding cycle in the Australian sea lion? *Search*, **9**(12), 464–465.
- Ling, J.K. (1966). The skin and hair of the southern elephant seal, *Mirounga leonina* (Linn.) I. The facial vibrissae. *Australian Journal of Zoology*, **14**, 855–866.
- Ling, J.K. (1977). *Functional Anatomy of Marine Mammals*, vol. 3, chap. Vibrissae of marine mammals. London: Academic, pp. 387–415.
- Ling, J.K. (1992). *Neophoca cinerea*. *Mammalian Species*, **392**, 1–7.
- Ling, J.K., Button, C.E., and Ebsary, B.A. (1974). A preliminary account of grey seals and harbor seals at Saint-Pierre and Miquelon. *Canadian Field Journalist*, **88**, 461–468.

- Linnane, A., James, C., Middleton, J., Hawthorne, P., and Hoare, M. (2010). Impact of wind stress anomalies on the seasonal pattern of southern rock lobster (*Jasus edwardsii*) settlement in South Australia. *Fisheries Oceanography*, **19**(4), 290–300.
- Lockyer, C.H. and Morris, R.J. (1990). Some observations on wound healing and persistence of scars in *Tursiops truncatus*. *Report of the International Whaling Commission*, **12**, 113–118.
- London, J.M., Ver Hoef, J.M., Jeffries, S.J., Lance, M.M., and Boveng, P.L. (2012). Haul-out behavior of harbor seals (*Phoca vitulina*) in Hood Canal, Washington. *PLoS ONE*, **7**(6), e38180.
- Lovasz, T., Croft, D., and Banks, P. (2008). Establishing tourism guidelines for viewing Australian sea lions *Neophoca cinerea* at Seal Bay Conservation Park, South Australia. In D. Lunney, A. Munn, and M. Meikle (Eds.), *Too close for comfort*. Royal Zoological Society of New South Wales, Mosman, NSW, Australia, pp. 225–232. doi:doi:10.7882/FS.2008.026.
- Lowry, M.S., Stewart, B.S., Heath, C.B., Yochem, P.K., and Francis, J.M. (1991). Seasonal and annual variability in the diet of California sea lions *Zalophus californianus* at San Nicolas Island, California, 1981–86. *Fishery Bulletin*, **89**, 331–336.
- Lowther, A.D., Harcourt, R.G., and Goldsworthy, S.D. (2013). Regional variation in trophic ecology of adult female Australian sea lions inferred from stable isotopes in whiskers. *Wildlife Research*, **40**(4), 303–311.
- Lowther, A.D., Harcourt, R.G., Hamer, D.J., and Goldsworthy, S.D. (2011). Creatures of habit: foraging habitat fidelity of adult female Australian sea lions. *Marine Ecology Progress Series*, **443**, 249–263.
- Marcotte, M. (2006). *Steller watch: Timing of weaning and seasonal patterns in numbers and activities of Steller sea lions (Eumetopias jubatus) at a year-round haulout site in southeast Alaska*. Thesis, University of British Columbia, Vancouver, Canada.
- Marcus, A.D., Higgins, D.P., and Gray, R. (2014). Epidemiology of hookworm (*Uncinaria stenocephala*) infection in free-ranging Australian sea lion (*Neophoca cinerea*) pups. *Parasitology research*, **113**(9), 3341–3353.

- Marlow, B.J. (1975). The comparative behaviour of the Australasian sea lions, *Neophoca cinerea* and *Phocarcos hookeri* (Pinnipedia: Otariidae). *Mammalia*, **39**(2), 159–230.
- McCauley, R.D. (2012). Fish choruses from the Kimberley, seasonal and lunar links as determined by long term sea noise monitoring. *Proceedings of Acoustics 2012 Fremantle*, 21–23.
- McConkey, S.D. (1999). Photographic identification of the New Zealand sea lion: A new technique. *Journal of Marine and Freshwater Research*, **33**(1), 63–66.
- McDonald, R.I., Kareiva, P., and Forman, R.T.T. (2008). The implications of current and future urbanization for global protected areas and biodiversity conservation. *Biological Conservation*, **141**(6), 1695–1703.
- McHugh, M.L. (2013). The Chi-square test of independence. *Biochemia Medica*, **23**(2), 143–149.
- McIntosh, R.R., Page, B., and Goldsworthy, S.D. (2006). Dietary analysis of regurgitates and stomach samples from free-living Australian sea lions. *Wildlife Research*, **33**, 661–669.
- McMahon, C.R., Burton, H.R., van den Hoff, J., Woods, R., and Bradshaw, C.J. (2006). Assessing hot-iron and cryo-branding for permanently marking southern elephant seals. *Journal of Wildlife Management*, **70**(5), 1484–1489.
- McNally, N.C.W. (2001). *New Zealand sea lion abundance, demographics and movements in southern New Zealand*. Thesis, University of Otago, Dunedin, New Zealand.
- Mellish, J.A., Hennen, D., Thomson, J., Petrauskas, L., Atkinson, S., and Calkins, D. (2007). Permanent marking in an endangered species: physiological response to hot branding in Steller sea lions (*Eumetopias jubatus*). *Wildlife Research*, **34**, 43–47.
- Merrick, R.L., Loughlin, T.R., and Calkin, D.G. (1996). Hot branding: a technique for long-term marking of pinnipeds. Report, U.S. Department of Commerce.
- Meÿer, M.A., Best, P.B., Anderson-Reade, M.D., Cliff, G., Dudley, S.F.J., and Kirkman, S.P. (2011). Trends and interventions in large whale entanglement along the South African coast. *African Journal of Marine Science*, **33**(3), 429–439.

- Miller, E.H. (1975). A comparative study of facial expressions of two species of pinnipeds. *Behaviour*, **53**(3/4), 268–284.
- Miththapala, S., Seidensticker, J., Phillips, L.G., Fernando, S.B.U., and Smallwood, J.A. (1989). Identification of individual leopards (*Panthera pardus kotiya*) using spot pattern variation. *Journal of Zoology*, **218**, 527–536.
- Mizroch, S.A., Beard, J.A., and Lynde, M. (1990). Computer assisted photo-identification of humpback whales. *Report of the International Whaling Commission*, **12**, 63–70.
- Mogren, H.G., Lindstrøm, U., Nilssen, K.T., and Haug, T. (2010). Haulout behaviour of harbour seals (*Phoca vitulina*) during breeding and moult in Vesterålen, Norway. *NAMMCO Scientific Publications*, **8**, 302–312.
- Morrison, M.A., Francis, M.P., Hartill, B.W., and Parkinson, D.M. (2002). Diurnal and tidal variation in the abundance of the fish fauna of a temperate tidal mudflat. *Estuarine, Coastal and Shelf Science*, **54**, 793–807.
- Morton, A.B. and Symonds, H.K. (2002). Displacement of *Orcinus orca* (L.) by high amplitude sound in British Columbia, Canada. *ICES Journal of Marine Science*, **59**, 71–80.
- Moulton, V.D., Miller, E.H., and Ochoa-Acuña, H. (2000). Haulout behaviour of captive harp seals (*Pagophilus groenlandicus*): Incidence, seasonality, and relationships to weather. *Applied Animal Behaviour Science*, **65**, 367–378.
- Muslow, J., Houser, D.S., and Finneran, J.J. (2014). Aerial hearing thresholds and detection of hearing loss in male California sea lions (*Zalophus californianus*) using auditory evoked potentials. *Marine Mammal Science*, **30**(4), 1383–1400.
- Nemeth, R.S. (2005). Population characteristics of a recovering us virgin islands red hind spawning aggregation following protection. *Marine Ecology Progress Series*, **286**, 81–97.
- Newsome, D. and Rodger, K. (2008). Impacts of tourism on pinnipeds and implications for tourism management. In J.E.S. Higham and M. Lück (Eds.), *Marine wildlife and tourism management: insights from the natural social sciences*, chap. 10. CABI, Oxfordshire, pp. 182–205.
- Nichols, J.D. (1992). Capture-recapture models. *BioScience*, **42**(2), 94–102.

- Niemi, M., Auttila, M., Valtonen, A., Viljanen, M., and Kunnasranta, M. (2013). Haulout patterns of Saimaa ringed seals and their response to boat traffic during the moulting season. *Endangered Species Research*, **22**, 115–124.
- Notarbartolo-di Sciara, G. (2014). Sperm whales, *Physeter macrocephalus*, in the Mediterranean Sea: a summary of status, threats, and conservation recommendations. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **24**(S1), 4–10.
- Nowacek, S.M., Wells, R.S., and Solow, A.R. (2001). Short-term effects of boat traffic on bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay, Florida. *Marine Mammal Science*, **17**(4), 673–688.
- Ogutu, J.O., Piepho, H.P., Dublin, H.T., Reid, R.S., and Bhola, N. (2006). Application of mark-recapture methods to lions: satisfying assumptions by using covariates to explain heterogeneity. *Journal of Zoology*, **269**, 161–174.
- Oliveira-Santos, L.G.R., Zucco, C.A., Antunes, P.C., and Crawshaw Jr., P.G. (2010). Is it possible to individually identify mammals with no natural markings using camera-traps? A controlled case-study with lowland tapirs. *Mammalian Biology-Zeitschrift für Säugetierkunde*, **75**(4), 375–378.
- Orams, M. (1999). *Marine tourism: development, impacts and management*. Routledge.
- Orphanides, C.D. and Palka, D.L. (2013). Analysis of harbor porpoise gillnet by-catch, compliance, and enforcement trends in the US northwestern Atlantic, January 1999 to May 2010. *Endangered Species Research*, **20**, 251–269.
- Orsini, J.P. (2004). *Human impacts on Australian sea lions, Neophoca cinerea, hauled out on Carnac Island (Perth, Western Australia): implications for wildlife and tourism management*. Thesis, Murdoch University, Perth, Western Australia.
- Orsini, J.P. and Newsome, D. (2005). Human perceptions of hauled out sea lions (*Neophoca cinerea*) and implications for management: a case study from Carnac Island, Western Australia. *Tourism in Marine Environments*, **2**(1), 129–132.
- Orsini, J.P., Shaughnessy, P.D., and Newsome, D. (2006). Impacts of human visitors on Australian sea lions (*Neophoca cinerea*) at Carnac Island, Western Australia: Implications for tourism management. *Tourism in Marine Environments*, **3**(2), 101–115.

- Osborn, L.S. (1985). *Population dynamics, behavior, and the effect of disturbance on haulout patterns of the harbor seal *Phoca vitulina richardsi**. Thesis, University of California, Santa Cruz, California, USA.
- Osinga, N., Nussbaum, S.B., Brakefield, P.M., and de Haes, H.A.U. (2012). Response of common seals (*Phoca vitulina*) to human disturbances in the Dollard estuary of the Wadden Sea. *Mammalian Biology*, **77**(4), 281–287.
- Osterrieder, S.K., Salgado Kent, C., Anderson, C.J.R., Parnum, I.M., and Robinson, R.W. (2015a). Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (*Neophoca cinerea*). *Journal of Mammalogy*, **96**(5), 988–997.
- Osterrieder, S.K., Salgado Kent, C., and Robinson, R.W. (2015b). Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia. *Endangered Species Research*, **28**, 259–274.
- Page, B., McKenzie, J., McIntosh, R., Baylis, A., Morrissey, A., Calvert, N., Haase, T., Berris, M., Dowie, D., Shaughnessy, P.D., and Goldsworthy, S.D. (2004). Entanglement of Australian sea lions and New Zealand fur seals in lost fishing gear and other marine debris before and after government and industry attempts to reduce the problem. *Marine Pollution Bulletin*, **49**, 33–42.
- Palka, D.L., Read, A.J., Westgate, A.J., and Johnston, D.W. (1996). Summary of current knowledge of harbour porpoises in US and Canadian Atlantic waters. *Report of the International Whaling Commission*, **46**, 559–565.
- Palka, D.L., Rossman, M.C., VanAtten, A.S., and Orphanides, C. (2008). Effect of pingers on harbour porpoise (*Phocoena phocoena*) bycatch in the US Northeast gillnet fishery. *Journal of Cetacean Research and Management*, **10**(3), 217–226.
- Pallottino, F., Menesatti, P., Costa, C., Paglia, P., De Salvador, F.R., and Lolletti, D. (2010). Image analysis techniques for automated hazelnut peeling determination. *Food and Bioprocess Technology*, **3**, 155–159.
- Parra, G.J. and Corkeron, P.J. (2001). Feasibility of using photo-identification techniques to study the Irrawaddy dolphin, *Orcaella brevirostris* (Owen in Gray 1866). *Aquatic Mammals*, **27**(1), 45–49.
- Parsons, E.C.M. (2012). The negative impacts of whale-watching. *Journal of Marine Biology*, **2012**, 9.

- Paterson, R., Paterson, P., and Cato, D.H. (1994). The status of humpback whales *Megaptera novaengliae* in East Australia thirty years after whaling. *Biological Conservation*, **70**, 135–142.
- Pauli, B.D. and Terhune, J.M. (1987a). Meteorological influences on harbour seal haul-out. *Aquatic Mammals*, **13**(3), 114–118.
- Pauli, B.D. and Terhune, J.M. (1987b). Tidal and temporal interaction on harbour seal haul-out patterns. *Aquatic Mammals*, **13**(3), 93–95.
- Pavez, G., Muñoz, L., Barilari, F., and Sepúlveda, M. (2014). Variation in behavioral responses of the South American sea lion to tourism disturbance: Implications for tourism management. *Marine Mammal Science*, **31**(2), 427–439.
- Payne, R., Rowntree, V., Perkins, J.S., Cooke, J.G., and Lankester, K. (1990). Population size, trends and reproductive parameters of right whales (*Eubalaena australis*) off Peninsula Valdes, Argentina. *Report of the International Whaling Commission*, 271–278.
- Pennycuik, C.J. (1978). Identification using natural markings. In B. Stonehouse (Ed.), *Animal marking*, chap. 16. MacMillan Press, London, London: MacMillan, pp. 147–159.
- Pennycuik, C.J. and Rudnai, J. (1970). A method of identifying individual lions *Panthera leo* with an analysis of the reliability of identification. *Journal of Zoology*, **160**, 497–508.
- Perry, A.L., Low, P.J., Ellis, J.R., and Reynolds, J.D. (2005). Climate change and distribution shifts in marine fishes. *Science*, **308**, 1912–1915.
- Peters, K.D., Ophelkeller, K., Bott, N.J., Deagle, B.E., Jarman, S.N., and Goldsworthy, S.D. (2015). Fine-scale diet of the Australian sea lion (*Neophoca cinerea*) using DNA-based analysis of faeces. *Marine Ecology*, **36**, 347–367.
- Peterson, J.C.B. (1972). An identification system for zebra (*Equus burchelli*, gray). *African Journal of Ecology*, **10**(1), 59–63.
- Pinheiro, J.C. and Bates, D.M. (2000). *Mixed-effects models in S and S-PLUS*. Springer.
- R Development Core Team (2014). R: A language and environment for statistical computing.

- Read, A.J. (2013). Development of conservation strategies to mitigate the bycatch of harbor porpoises in the Gulf of Maine. *Endangered Species Research*, **20**, 235–250.
- Reder, S., Lydersen, C., Arnold, W., and Kovacs, K.M. (2003). Haulout behaviour of High Arctic harbour seals (*Phoca vitulina vitulina*) in Svalbard, Norway. *Polar Biology*, **27**, 6–16.
- Reebs, S.G. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries*, **12**, 349–371.
- Ribeiro, J., Bentes, L., Coelho, R., and Gonçalves, J.M.S. (2006). Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuarine, Coastal and Shelf Science*, **67**, 461–474.
- Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution*, **43**(1), 223–225.
- Richardson, K.C. and Gales, N.J. (1987). Functional morphology of the alimentary tract of the Australian sea-lion, *Neophoca cinerea*. *Australian Journal of Zoology*, **35**(3), 219–226.
- Richardson, W.J., Greene Jr, C.R., Malme, C.I., and Thomson, D.H. (1995). *Marine mammals and noise*. Academic Press, San Diego, CA.
- Riedman, M. (1990). *The pinnipeds: Seals, sea lions, and walruses*. University of California Press, Berkeley, CA.
- Roberts, C.M., McClean, C.J., Veron, J.E., Hawkins, J.P., Allen, G.R., McAllister, D.E., Mittermeier, C.G., Schueler, F.W., Spalding, M., Wells, F., and Vynne, C. (2002). Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295**(5558), 1280–1284.
- Robertson, B.C. (2015). Is management limiting the recovery of the New Zealand sea lion *Phocarctos hookeri*? *Polar Biology*, **38**(4), 539–546.
- Robertson, B.C., Chilvers, B.L., Duignan, P.J., Wilkinson, I.S., and Gemmell, N.J. (2006). Dispersal of breeding, adult male *Phocarctos hookeri*: implications for disease transmission, population management and species recovery. *Biological Conservation*, **127**, 227–236.
- Rosas, F.C.W., Pinedo, M.C., Marmontel, M., and Haimovici, M. (1994). Seasonal movements of the South American sea lion (*Otaria flavescens*, shaw) off the Rio Grande do Sul coast, Brazil. *Mammalia*, **58**(1), 51–60.

- Rosenbaum, H.C., Maxwell, S.M., Kershaw, F., and Mate, B. (2014). Long-range movement of humpback whales and their overlap with anthropogenic activity in the South Atlantic Ocean. *Conservation Biology*, **28**(2), 604–615.
- Roux, J.P. and Hes, A.D. (1984). The seasonal haul-out cycle of the fur seal *Arctocephalus tropicalis* (Gray, 1872) on Amsterdam Island. *Mammalia*, **48**(3), 377–389.
- Sala, E. and Knowlton, N. (2006). Global marine biodiversity trends. *Annual Review of Environment and Resources*, **31**, 93–1022.
- Salgado Kent, C.P. and Crabtree, B. (2008). The effectiveness of an established sanctuary zone for reducing human disturbance to Australian sea lions (*Neophoca cinerea*) at Carnac Island, Western Australia. *Tourism in Marine Environments*, **5**(1), 29–42.
- Sato, K., Tsuchiya, Y., Kudoh, S., and Naito, Y. (2003). Meteorological factors affecting the number of Weddell seals hauling-out on the ice during the molting season at Syowa Station, East Antarctica. *Polar Bioscience*, **16**, 98–103.
- Schneider, D.C. and Payne, P.M. (1983). Factors affecting haul-out of harbor seals at a site in Southeastern Massachusetts. *Journal of Mammalogy*, **64**(3), 518–520.
- Schumann, N., Gales, N.J., Harcourt, R.G., and Arnould, J.P.Y. (2013). Impacts of climate change on Australian marine mammals. *Australian Journal of Zoology*, **61**, 146–159.
- Schusterman, R.J. (1972). Visual acuity in pinnipeds. In H.E. Winn and B.L. Olla (Eds.), *Behavior of marine animals*, vol. 2. Plenum Press, New York, USA, pp. 469–492.
- Schusterman, R.J. and Balliet, R.F. (1971). Aerial and underwater visual acuity in the California sea lion (*Zalophus californianus*) as a function of luminance. *Annals of the New York Academy of Sciences*, **188**, 37–46.
- Seber, G.A.F. (1982). *The estimation of animal abundance and related parameters*. Macmillan Publishing Company, London, UK, 2 ed.
- Seber, G.A.F. (1986). A review of estimating animal abundance. *Biometrics*, **42**, 267–292.
- Selvaggi, E., Vedder, L., Haaften van, J.L., Wensvoort, P., and Consiglio, C. (2001). Effect of disturbance on daily rhythm and haul out behavior in the harbor seal

- (*Phoca vitulina*) in the tide estuary of Dollard. Proceedings of the Fifteenth Annual Conference of the European Cetacean Society, Rome, Italy, 6-10 May 2001, 92-97.
- Sepúlveda, M., Quiñones, R.A., Carrasco, P., and Álvarez Pérez, M.J. (2012). Daily and seasonal variation in the haul-out behavior of the South American sea lion. *Mammalian Biology*, **77**, 288–292.
- Sepúlveda, M., Oliva, D.P., and Palma, F.J. (2001). Daily and annual circarhythms activity in the South American sea lion *Otaria flavescens* (carnivora: Otariidae) at the central zone of Chile. *Revista de Biología Marina y Oceanografía*, **36**(2), 181–187.
- Seuront, L. and Cribb, N. (2011). Fractal analysis reveals pernicious stress levels related to boat presence and type in the Indo-Pacific bottlenose dolphin, *Tursiops aduncus*. *Physica A*, **390**, 2333–2339.
- Shaughnessy, P.D., Dennis, T.E., and Berris, M. (2007). Predation on Australian sea lions *Neophoca cinerea* by white sharks *Carcharodon carcharias* in South Australia. *Australian Mammalogy*, **29**, 69–76.
- Shaughnessy, P.D., Goldsworthy, S.D., Hamer, D.J., Page, B., and McIntosh, R.R. (2011). Australian sea lions *Neophoca cinerea* at colonies in South Australia: distribution and abundance, 2004 to 2008. *Endangered Species Research*, **13**, 87–98.
- Shaughnessy, P.D., Nicholls, A.O., and Briggs, S.V. (2008). Do tour boats affect fur seals at Montague Island, New South Wales. *Tourism in Marine Environments*, **5**(1), 15–27.
- Sigler, M.F., Womble, J.N., and Vollenweider, J.J. (2004). Availability to Steller sea lions (*Eumetopias jubatus*) of a seasonal prey resource: a prespawning aggregation of eulachon (*Thaleichthys pacificus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 1475–1484.
- Sikes, R.S., Gannon, W.L., Care, T.A., and of the American Society of Mammalogists, U.C. (2011). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, **92**, 235–253.
- Sinclair, E.H. and Zeppelin, T.K. (2002). Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). *Journal of Mammalogy*, **83**(4), 973–990.

- Slooten, E. (2007). Conservation management in the face of uncertainty: effectiveness of four options for managing Hector's dolphin bycatch. *Endangered Species Research*, **3**, 169–179.
- Slooten, E. (2013). Effectiveness of area-based management in reducing bycatch of the New Zealand dolphin. *Endang*, **20**, 121–130.
- Slooten, E. and Davies, N. (2012). Hector's dolphin risk assessments: old and new analyses show consistent results. *Journal of the Royal Society of New Zealand*, **42**(1), 49–60.
- Slooten, E. and Dawson, S.M. (2010). Assessing the effectiveness of conservation management decisions: likely effects of new protection measures for Hector's dolphin (*Cephalorhynchus hectori*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, **20**, 334–347.
- Slooten, E., Rayment, W., and Dawson, S. (2006). Offshore distribution of Hector's dolphins at Banks Peninsula, New Zealand: Is the Banks Peninsula Marine Mammal sanctuary large enough? *New Zealand Journal of Marine and Freshwater Research*, **40**(2), 333–343.
- Southwell, C. (2003). Haul-out behaviour of two Ross seals off eastern Antarctica. *Antarctic Science*, **15**(2), 257–258.
- Southwell, C. (2005). Optimising the timing of visual surveys of crabeater seal abundance: haulout behaviour as a consideration. *Wildlife Research*, **32**, 333–338.
- Stachowicz, J.J., Bruno, J.F., and Duffy, J.E. (2007). Understanding the effects of marine biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 739–766.
- Stevens, M.A. and Boness, D.J. (2003). Influences of habitat features and human disturbance on use of breeding sites by a declining population of southern fur seals (*Arctocephalus australis*). *Journal of Zoology*, **260**, 145–152.
- Stevick, P.T., Pallsbøll, P.J., Smith, T.D., Bravington, M.V., and Hammond, P.S. (2001). Errors in identification using natural markings: rates, sources, and effects on capture-recapture estimates of abundance. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1861–1870.
- Stirling, I. (1968). Diurnal movements of the New Zealand fur seal at Kaikoura. *New Zealand Journal of Marine and Freshwater Research*, **2**, 375–377.

- Stirling, I. (1972). Observations on the Australian sea lion, *Neophoca cinerea* (Peron). *Australian Journal of Zoology*, **20**, 271–279.
- Stone, G.S. and Yoshinaga, A. (2000). Hector's dolphin *Cephalorhynchus hectori* calf mortalities may indicate new risks from boat traffic and habituation. *Pacific Conservation Biology*, **6**(2), 162–170.
- Strong, P. and Morris, S.R. (2010). Grey seal (*Halichoerus grypus*) disturbance, ecotourism and the Pembrokeshire Marine Code around Ramsey Island. *Journal of Ecotourism*, **9**(2), 117–132.
- Summers, C.F. and Witthames, S.R. (1987). The value of tagging as a marking technique for seals. In B. Stonehouse (Ed.), *Animal marking: recognition marking of animals in research*. Proceedings of the RSPCA Symposium, pp. 63–70.
- Suryan, R.M. and Harvey, J.T. (1999). Variability in reactions of Pacific harbor seals, *Phoca vitulina richardsi*, to disturbance. *Fishery Bulletin*, **97**(2), 332–339.
- Szaniszlo, W.R. (2005). *California sea lion (Zalophus californianus) and Steller sea lion (Eumetopias jubatus) interactions with vessels in Pacific Rim National Park Reserve: Implications for marine mammal viewing management*. Thesis, University of Victoria, Canada.
- Taillier, S. (2014). Great white shark probably choked to death on sea lion, authorities say. URL <http://www.abc.net.au/news/2014-07-17/great-white-shark-probably-choked-on-a-sea-lion2c-authorities-/5604410>.
- Taylor, B.L. and Gerrodette, T. (1993). The uses of statistical power in conservation biology: the vaquita and northern spotted owl. *Conservation Biology*, **7**(3), 489–500.
- Taylor, B.L., Martinez, M., Gerrodette, T., Barlow, J., and Hrovat, Y.N. (2007). Lessons from monitoring trends in abundance of marine mammals. *Marine Mammal Science*, **23**(1), 157–175.
- Thompson, P.M., Fedak, M.A., McConnell, B.J., and Nicholas, K.S. (1989). Seasonal and sex-related variation in the activity patterns of common seals (*Phoca vitulina*). *Journal of Applied Ecology*, **26**, 521–535.
- Thompson, P.M. and Harwood, J. (1990). Methods for estimating the population size of common seals, *Phoca vitulina*. *Journal of Applied Ecology*, **27**, 924–938.

- Topelko, K.N. and Dearden, P. (2005). The shark watching industry and its potential contribution to shark conservation. *Journal of Ecotourism*, **4**(2), 108–1021.
- Tregenza, N.J.C., Berrow, S.D., Hammond, P.S., and Leaper, R. (1997). Harbour porpoise (*Phocoena phocoena* l.) by-catch in set gillnets in the Celtic Sea. *ICES Journal of Marine Science*.
- Tripovich, J.S., Hall-Aspland, S., Charrier, I., and Arnould, J.P.Y. (2012). The behavioural response of Australian fur seals to motor boat noise. *PloS ONE*, **7**(5), e37228.
- Troy, S., Middleton, D., and Phelan, J. (1997). On capture, anaesthesia and branding of adult male New Zealand fur seal *Arctocephalus forsteri*. In M. Hindell and C. Kemper (Eds.), *Marine mammal research in the southern hemisphere*, vol. 1: Status, Ecology and Medicine. Surrey Beatty and Sons, Devon, UK, pp. 179–183.
- Udevitz, M.S., Jay, C.V., and Cody, M.B. (2005). Observer variability in pinniped counts: ground-based enumeration of walruses at haul-out sites. *Marine Mammal Science*, **21**(1), 108–120.
- Ullas Karanth, K. and Nichols, J.D. (1998). Estimation of tiger densities in india using photographic captures and recaptures. *Ecology*, **79**(8), 2852–2862.
- Valpine, P.d. (2014). The common sense of P values. *Ecology*, **95**(3), 617–621.
- Van Der Hoop, J.M., Moore, M.J., Barco, S.G., Cole, T.V., Daoust, P.Y., Henry, A.G., McAlpine, D.F., McLellan, W.A., Wimmer, T., and Solow, A.R. (2013). Assessment of management to mitigate anthropogenic effects on large whales. *Conservation Biology*, **27**(1), 121–133.
- Vincent, C., Meynier, L., and Ridoux, V. (2001). Photo-identification in grey seals: legibility and stability of natural markings. *Mammalia*, **65**(3), 363–372.
- Wakefield, C.B. (2010). Annual, lunar and diel reproductive periodicity of a spawning aggregation of snapper *Pagrus auratus* (Sparidae) in a marine embayment on the lower west coast of Australia. *Journal of Fish Biology*, **77**(6), 1359–1378.
- Walker, G.E. and Ling, J.K. (1981). Australian sea lion *Neophoca cinerea* (peron 1816). In S.H. Ridgway and R.J. Harrison (Eds.), *Handbook of marine mammals. The walrus, sea lions, fur seals and sea otter*, vol. 1. Academic Press, pp. 99–118.
- Walker, K.A., Mellish, J.A.E., and Weary, D.M. (2009). Behavioural responses of juvenile Steller sea lions to hot-iron branding. *Applied Animal Behaviour Science*, **122**(1), 58–62.

- Walker, K.A., Trites, A.W., Haulena, M., and Weary, D.M. (2012). A review of the effects of different marking and tagging techniques on marine mammals. *Wildlife Research*, **39**(1), 15–30.
- Watkins, W.A. (1986). Whale reactions to human activities in Cape Cod waters. *Marine Mammal Science*, **2**(4), 251–262.
- Watts, P. (1992). Thermal constraints on hauling out by harbour seals (*Phoca vitulina*). *Canadian Journal of Zoology*, **70**, 553–560.
- Watts, P. (1993). Possible lunar influence on hauling-out behavior by the Pacific harbour seal (*Phoca vitulina richardsi*). *Marine Mammal Science*, **9**(1), 68–73.
- Watts, P. (1996). The diel hauling-out cycle of harbour seals in an open marine environment: correlates and constraints. *Journal of Zoology*, **240**, 175–200.
- Whitehead, H. (1990). Computer assisted individual identification of sperm whale flukes. *Report of the International Whaling Commission*, **12**, 71–77.
- Wickens, P.A., Japp, D.W., Shelton, P.A., Kriel, F., Goosen, P.C., Rose, B., Augustyn, C.J., Bross, C.A.R., Penney, A.J., and Krohn, R.G. (1992). Seals and fisheries in South Africa - competition and conflict. *South African Journal of Marine Science*, **12**(1), 773–789.
- Winter, A., Foy, R.J., and Wynne, K. (2009). Seasonal differences in prey availability around a Steller sea lion haulout and rookery in the Gulf of Alaska. *Aquatic Mammals*, **35**(2), 145–162.
- Womble, J.N., Sigler, M.F., and Willson, M. (2009). Linking seasonal distribution patterns with prey availability in a central-place forager, the Steller sea lion. *Journal of Biogeography*, **36**, 439–451.
- Womble, J.N., Willson, M., Sigler, M.F., Kelly, B.P., and VanBlaricom, G.R. (2005). Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. *Marine Ecology Progress Series*, **294**, 271–282.
- Wood, S.M. (2006). *Generalized additive models: an introduction with R*. Chapman and Hall/CRC, Boca Raton, Florida.
- Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B., Lotze, H.K., Micheli, F., Palumbi, S.R., and Sala, E. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science*, **314**(5800), 787–790.

- Wright, A.J., Soto, N.A., Baldwin, A.L., Bateson, M., Beale, C.M., Clark, C., Deak, T., Edwards, E.F., Fernández, A., Godhino, A., Hatch, L.T., Kakuschke, A., Lusseau, D., Martineau, D., Romero, M.L., Weilgart, L.S., Wintle, B.A., Notarbartolo-di Sciara, G., and Martin, V. (2007). Do marine mammals experience stress related to anthropogenic noise? *International Journal of Comparative Psychology*, **20**, 274–316.
- Würsig, B. and Jefferson, T.A. (1990). Methods of photo-identification for small cetaceans. *Report of the International Whaling Commission*, **12**, 43–52.
- Würsig, B. and Würsig, M. (1977). The photographic determination of group size, composition, and stability of coastal porpoises (*Tursiops truncatus*). *Science*, **198**, 755–756.
- Yates, F. (1934). Contingency tables involving small numbers and the χ^2 test. *Supplement to the Journal of the Royal Statistical Society*, **1**(2), 217–235.
- Young, C., Gende, S.M., and T., H.J. (2014). Effects of vessels on harbor seals in glacier bay national park. *Tourism in Marine Environments*, **10**, 5–20.
- Zuur, A.F., Ieno, E.N., and Smith, G.M. (2007). *Analysing ecological data*. Springer Science & Business Media, New York, USA.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., and Smith, G.M. (2009). *Mixed effects models and extensions in ecology with R*. Springer.

Appendix A

CHAPTER 2 PUBLICATION



Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (*Neophoca cinerea*)

SYLVIA K. OSTERRIEDER,* CHANDRA SALGADO KENT, CARLOS J. R. ANDERSON, IAIN M. PARNUM, AND RANDALL W. ROBINSON

Centre for Marine Science and Technology, Department of Imaging and Applied Physics, Curtin University, Building 301, Wark Avenue, Bentley, Western Australia 6102, Australia (SKO, CSK, IMP)

Institute for Sustainability and Innovation, College of Engineering and Science, Victoria University, Building 6, McKechnie St., St. Albans, 3021 Victoria, Australia (SKO, RWR)

Department of Ecology and Evolutionary Biology, University of Michigan, 830 North University, 2019 Kraus Natural Science Building, Ann Arbor, MI 48109, USA (CJRA)

* Correspondent: sylvia.osterrieder@gmail.com

Reliable methods for identification of individual animals are advantageous for ecological studies of population demographics and movement patterns. Photographic identification, based on distinguishable patterns, unique shapes, or scars, is an effective technique already used for many species. We tested whether photographs of whisker spot patterns could be used to discriminate among individual Australian sea lion (*Neophoca cinerea*). Based on images of 53 sea lions, we simulated 5,000 patterns before calculating the probability of duplication in a study population. A total of 99% (± 1.5 SD) of patterns were considered reliable for a population of 50, 98% (± 1.7 SD) for 100, 92% (± 4.7 SD) for 500, and 88% (± 5.7 SD) for 1,000. We tested a semiautomatic approach by matching 16 known individuals at 3 different angles (70°, 90°, and 110°), 2 distances (1 and 2 m), and 6 separate times over a 1-year period. A point-pattern matching algorithm for pairwise comparisons produced 90% correct matches of photographs taken on the same day at 90°. Images of individuals at 1 and 2 m resulted in 89% correct matches, those photographed at different angles and different times (at 90°) resulted in 48% and 73% correct matches, respectively. Our results show that the Chamfer distance transform can effectively be used for individual identification, but only if there is very little variation in photograph angle. This point-pattern recognition application may also work for other otariid species.

Key words: Australian sea lion, individual identification, pattern recognition, pinnipeds, whisker spots

© The Author 2015. Published by Oxford University Press on behalf of American Society of Mammalogists.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Many aspects of ecological studies are significantly enhanced by the persistent identification of individuals, achieved for example by capture–recapture models in population-based studies (Nichols 1992). Behavioral studies focusing on individual differences rely on the recognition of individuals and the ability to follow them through time. Microchips, tags, or artificial marks (e.g., through branding) can be applied to aid in distinguishing among individuals (Summers and Withames 1978; Walker et al. 2012). Such methods involve capturing and handling animals, in many cases causing significant stress, can have adverse effects on the animals (Troy et al. 1997; Walker et al. 2012), and increase risk to the researchers themselves. In several species, methods use natural marks for noninvasive individual identification, often through photographic comparison. Identification is

based on recognizing unique marks, patterns, shapes of certain body parts, or scars. This is possible with unique fur patterns, such as stripes or spots in tigers (*Panthera tigris*), cheetahs (*Acinonyx jubatus*), or zebras (*Equus quagga*—Peterson 1972; Ullas Karanth and Nichols 1998; Kelly 2001; Hiby et al. 2009). In some phocids, spot patterns in fur have been used to recognize individuals (Hiby and Lovell 1990; Karlsson et al. 2005). Shapes or outlines of distinctive appendages have successfully been used for individual identification, for example dolphin dorsal fins, whale flukes, badger tails, and sea lion flippers (Würsig and Würsig 1977; Whitehead 1990; McConkey 1999; Dixon 2003). Scars may also be useful to assist identification in pinnipeds (Forcada and Aguilar 2000; Vincent et al. 2001), but often change over time, for example when animals molt

(McConkey 1999). On occasion, identification of whisker spot patterns has assisted in identification of individuals (Beentjes 1989; Miththapala et al. 1989). Australian sea lions (*Neophoca cinerea*) are an endangered species, lacking information on population estimates and demographics for many of their colonies (Goldsworthy and Gales 2008). Photo-identification would therefore be a useful tool to gain more knowledge on their population demographics and beneficial for appropriate management and their conservation. Australian sea lions, however, do not have distinctive patterns in coloration, and readily visible long-term scars are absent for the majority of individuals. Hence, it is highly advantageous to establish a noninvasive and replicable technique for individual identification of Australian sea lions. Pennyquick and Rudnai (1970) first developed and described a method using whisker spot patterns to identify individual lions (*Panthera leo*) successfully. Anderson et al. (2007) then tested a similar method for polar bears (*Ursus maritimus*), finding that of 50 individual polar bears whisker spot patterns analyzed, 98% contained enough information to reliably identify individuals. For pinnipeds, no such feasible method has been developed yet which allows effective identification of individuals in the long term.

Computer-aided photo-identification can increase the efficiency and accuracy of individual recognition and is particularly advantageous for studies on larger populations (e.g., Mizroch et al. 1990). A practical tool may also reduce the costs of a manual-matching research program significantly. The specific objectives of this project were therefore to: 1) establish whether the variability of whisker spot patterns in Australian sea lions is large enough to reliably use them for individual identification and 2) develop and test the accuracy of pattern recognition on Australian sea lion whisker spot patterns. The development of a noninvasive photo-identification method for Australian sea lions would also provide greater confidence in its potential for noninvasive identification in similar species.

MATERIALS AND METHODS

Study areas and collection of photographs.—Method testing was based on photographs of known individual Australian sea lions in captivity and in the wild. Images of captive sea lions comprised 3,036 photographs of 16 individuals, taken by zoos and aquaria including Adelaide Zoo, Pet Porpoise Pool in Coff's Harbour, SEALIFE (previously UnderWater World) in Mooloolaba, and Taronga Zoo in Sydney. Lateral photographs were taken between 1 March 2013 and 25 November 2014 of each sea lion's right muzzle at estimated angles of 70°, 90°, and 110° from its anterior, at ranges of 1 and 2 m. An angle of 90° means that the profile view of the animal is perpendicular to the camera. Photo sessions were repeated at approximately 10, 30, 60, 180, and 360 days after the 1st photo session to test the method against any ontogenic changes (Table 1) in whisker spot patterns in Australian sea lions. There was minor variability in the timing of photo sessions with some missed due to shortage of zoo staff, busy schedules, or failure of sea lions to follow trainer instructions when taking photographs.

Table 1.—Number of individuals and number of photographs taken of the right muzzle of captive Australian sea lions (*Neophoca cinerea*) on different days throughout 1 year.

	Day 1	Day 10	Day 30	Day 60	Day 180	Day 360	Total
Individuals	15	11	15	10	9	5	16
Photographs	396	430	515	580	565	550	3,036

Field-based photographs of wild Australian sea lions were obtained to increase the sample size of unique individuals. Images from 15 breeding and haul-out islands were included. Selecting a wide variety of locations allowed individuals of both sexes and various age classes to be sampled. Haul-out islands were located in the Perth Metropolitan area in Western Australia and included Seal, Carnac, Penguin, Little, and Dyer Islands, and Burns Rocks. Breeding islands included Haul-off Rock, Red Islet, Middle Doubtful, Glennie, Wickham, Houtman Abrolhos Islands, as well as Anvil and Ford Islands in the eastern group of islands of the Recherche Archipelago off the southwest coast off Albany and Esperance, and Beagle Island off Jurien Bay, Western Australia (Fig. 1; Table 2; Gales et al. 1992). From these locations, a total of 5,766 whisker photographs of Australian sea lions were taken during 127 field trips between the 8 June 2012 and 15 February 2014 using a Canon EOS 550D with a 100–400mm zoom lens (Canon, Tokyo, Japan; Table 2). We approached focal animals slowly and carefully, up to a minimum distance of 5 m to minimize disturbance. Photographs of sea lion muzzles in the field were taken from the closest range possible—approximately 5–50 m (5–10 m is minimum distance the public is recommended to maintain from a sea lion). A maximum range of 50 m was selected as beyond this, photographs were found to be less reliable and blurred in a study on polar bear identification using whisker spot patterns (Anderson et al. 2007).

During each field trip, lateral (90°) photographs of sea lion muzzles were taken, if possible from its left and right side. Photographed sea lions were either sitting in the water, swimming with their head raised above the waterline, or hauled out on land. Individuals could be distinguished from each other during a single field day (based on their haul-out locations and movements), and the total number of individuals photographed calculated. Due to unconfirmed movement patterns, the total number of individuals over all field days is unknown. To ensure that unique individuals were tested, a selection of photographs was made from the 5,766 wild Australian sea lion images. The photographs selected were either taken from multiple locations within a region on a single field day, with the assumption that animals did not have time to move between field sites during the window of field work, or at breeding islands with very large distances between them (i.e., an island near Albany versus an island near Jurien, Western Australia) where there is evidence of site fidelity (Campbell et al. 2008).

After the selection process, photographs remained from 37 unique wild Australian sea lions, for many of which, multiple photographs existed. Not all photographs from the original catalog of 8,802 images (3,036 and 5,766 images from captive and wild Australian sea lions, respectively) were of sufficient quality to be used in testing, therefore a further selection was required.

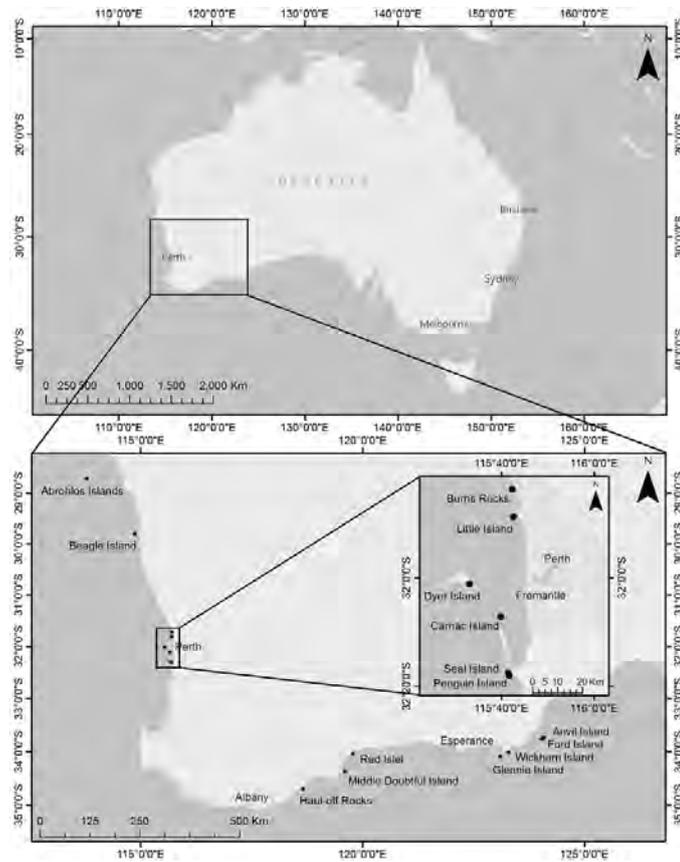


Fig. 1.—Locations of islands where photographs of Australian sea lions (*Neophoca cinerea*) in the wild were obtained.

In all cases, selection was based on user interpreted quality, i.e., in focus, not tilted and taken at the correct angle (70° , 90° , and 110° for captive individuals and 90° for wild individuals). Captive individual photographs were only included if taken at a range of 1 and 2 m and photographs of wild individuals only included between 5 and 50 m. In general, suitable photographs of wild individuals were available for 1 side of the muzzle, with more high-quality photographs from the sea lions' right-hand side. Thus, only images of the right-hand side of the sea lions were used in this study to resemble feasible sampling for usage on wild sea lions. The net result was a library of photographs for analysis, comprising 608 images of 53 individuals: 515 images of 16 captive individuals (including all 3 orientations and 2 ranges) and 93 images of 37 wild individuals (at 90°).

This work was conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth). Research on live animals followed American Society of Mammalogists guidelines (Sikes et al. 2011).

Preparation of photographs for reliability testing and matching.—Photographs were cropped in Adobe PhotoShop Elements 11 (Adobe PhotoShop Elements 2012) to eliminate superfluous parts of the photograph. In this study, a semiautomated pattern recognition software, originally developed for identifying polar bears using their whisker spots, was adapted for application on sea lions (Anderson et al. 2010; Fig. 2). The original program was mostly automated, only requiring the user to manually choose 3 reference points (Anderson et al. 2010). The region of whisker spot patterns in a photograph was automatically extracted and used to match individuals against a database. Due to low and variable contrast between the fur and whisker spots in Australian sea lions (Australian sea lions vary in fur color between sexes as well as change fur color when maturing—Walker and Ling 1981), automated whisker spot extraction was not possible, so individual whisker spots were selected manually in the program (see Fig. 3 for an example of whisker spot patterns).

Once the 3 reference points (inner corner of the eye, corner of the nostril, and outer end of the mouth; Fig. 2) and whisker spot locations were marked on the photograph, the program

Table 2.—Number of field days and photographs, which were taken of the right side of wild Australian sea lion (*Neophoca cinerea*) muzzles on various islands in Western Australia.

Location	Field days	Photographs
Seal Island	54	2,360
Penguin Island	4	28
Carnac Island	22	1,264
Dyer Island	13	192
Little Island	9	266
Burns Rocks	13	122
Haul-off Rocks	2	100
Middle Doubtful Island	1	190
Red Islet	1	108
Glennie Island	1	90
Wickham Island	1	96
Recherche Archipelago	1	70
Beagle Island	3	640
Abrolhos Islands	2	2,883
Total	127	5,766

standardized the location of the chosen whisker spot points by applying an affine transformation, such that the eye is located at spatial coordinate (0, 0), the nose is at (1, 0), and corner of the mouth is at (0.5, 0.5). These coordinate values serve to align the whisker spot patterns from different photographs (Fig. 3). These reference points were chosen based upon their ease of distinction compared to other potential reference points.

The overall methodology required 4 steps to prepare the data for reliability and matching tests. There were 6 additional steps for testing reliability of the patterns, and 3 additional steps for matching the whisker spot patterns (Fig. 4 for a flow chart). These methods for the additional steps are described below.

Variability of whisker spot patterns in Australian sea lions.—A set of 53 good-quality photographs, 1 each from 16 captive and 37 wild unique individual Australian sea lions, were selected to determine whether individual whisker spot patterns were unique enough to reliably identify individual sea lions in a population. Assessing the variability of whisker spot patterns involved investigating spot locations relative to a normalized grid laid over the standardized photograph of the muzzle and identifying whether spots were “present” or “absent” in each of the cells within that grid. The first step was to select the dimensions of each cell in the grid. The grid cell height and width were chosen using the maximum vertical and horizontal distances, respectively, between the same whisker spots on multiple photographs of the same individuals. The greatest value for each of these 2 dimensions was taken from 23 photographs of 10 individuals. These individuals were selected because there were 2–3 high-quality photographs available of each.

The grid was applied to 1 photograph from each of the 53 individuals. The cells were then tested for pairwise independence of whisker spots being present/absent, and 1 of 2 dependent cells removed from the analysis (as per Pennycuick 1978; Anderson et al. 2007, 2010). To test for mutual independence, the joint probability of 2 cells having a value of whisker spots “present” was compared to the independent probability of 2 cells having a value of whisker spots “present.” The probability of a whisker spot present in the cell was tested for each pair of cells. A set of

events (such as the presence of whisker spots) is classed as mutually independent if the joint probability for every subset of events (cells) within the set is equal to the product of their individual probabilities (Anderson et al. 2007). The “joint probability” (called the observed) was calculated as the proportion of each of 2 adjacent cells having whisker spots present. The individual probability (called the expected) was calculated as the product of the 2 cell probabilities. Observed and expected probabilities were also calculated for cells having a value of “absent.” To test whether there was a significant difference between observed and expected probabilities, whisker spots for the sample were simulated 5,000 times based on their original probability distribution for the 53 individuals’ patterns. Once dependent cells were removed, the probability of occurrence and information content were calculated for each individual as per Pennycuick (1978) and Anderson et al. (2007). First the frequency of whisker spot occurrence in each cell was calculated as $f_i = n_i/N$, where n_i is the number from the sample having a whisker spot in the cell and N is the number of individuals in the sample.

The probability of occurrence was taken as:

$$P = f_a \times f_b \times f_c \times \dots \times (1 \times f_q) \times (1 \times f_r) \times (1 \times f_s) \times \dots, \quad (1)$$

where a, b, c , etc. are cells with spots, and q, r, s , etc. are cells without spots. The information content was calculated as $I = -\log_2(P)$. As simulations can vary between passes, calculations were conducted 50 times. The mean and SDs from these calculations are presented.

The probability of duplication, that means that at most one individual has a specific whisker spot pattern, in population sizes of 50, 100, 500, and 1,000 were calculated based on the probability of occurrence of the spot pattern in the study population (as in Pennycuick 1978 and Anderson et al. 2007; Table 3). This was calculated as:

$$(1 - P)^M + MP(1 - P)^{M-1} \quad (2)$$

where M is the number of individuals in a population and P is the probability of a particular pattern occurring in a population.

Code written in Matlab R2013a (Moler 2013) was used to carry out all analyses and produce all figures presented in the results.

Pattern recognition using Chamfer distance transform.—Four catalogs of photographs were created from the complete library of 515 photographs of captive individuals to include only those pertinent for the 4 test scenarios. The “catalogs” consisted of matching photographs of the individuals taken on: 1) the same day at 90° (90 photographs), 2) the same day at different angles (70°, 90°, and 110°; 46 photographs), 3) the same day at 90° at 1- and 2-m distances (28 photographs), and 4) different days (the 1st photo session, and 10, 30, 60, 180, and 360 days from the 1st photo session) at 90° angle (64 photographs; Table 4). An adaptation of the Chamfer distance transform (Borgefors 1986) was used to compute the similarity score between 2 images based on the location of their whisker spots (point pattern). The similarity score between 2 standardized point patterns is calculated as



Fig. 2.—Adapted software interface to build a library and match whisker patterns using Chamfer distance transform. Whisker spots in the image are marked with black circles and reference points with white circles. The matching scores with other marked photographs are displayed on the left.

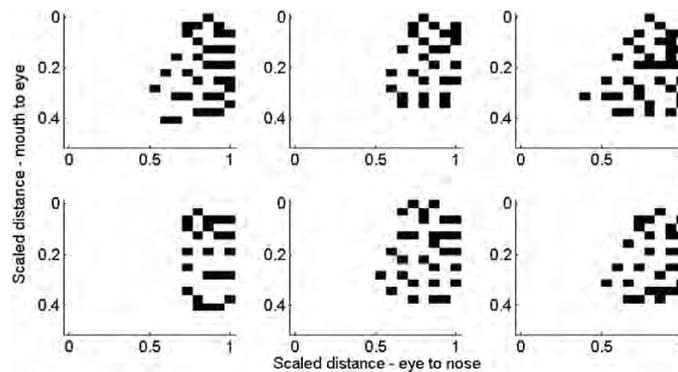


Fig. 3.—Example of marked cells where whiskers are present on grids overlaid over the muzzles of 6 captive individual Australian sea lions (*Neophoca cinerea*). Black cells are where whisker spots are present and empty cells where spots are absent. The coordinate [0,0] is the position of the inner corner of the eye, and [1,0] the reference point on the nostril.

follows: For each point in the 1st pattern, the Euclidian distance to the nearest point in the second pattern is calculated and distances then averaged. The same procedure is carried out in reverse. Both averaged distance scores are averaged together to produce a similarity score between the 2 point patterns where lower scores indicate higher similarity between 2 patterns. In addition, the algorithm calculates the similarity score many times, each time shifting one of the patterns by a small distance (chosen by the user), called the step size, and uses the smallest of these scores as the final similarity score. This “shifting” accounts for misalignments of point patterns caused by different facial angles of the animals. The software calculates the similarity score between the “candidate” sea lion being matched and every sea lion already in the database (or “library”). Users can cross-check the photographs visually to confirm or reject whether the candidate sea lion has been matched to one in the library.

Software settings and pairwise matching.—Catalog 1 photographs (images from the same day at 90°) were used to determine the best software settings to maximize correct matching

results and were then used for all catalogs. Boxplots of Catalog 1 with different settings were displayed to compare the distribution and the overlap of scores for matching and nonmatching individuals. An offset (i.e., the “shifting” to account for misalignments of spots in different photographs of the same individual) of 0.07 and step size (i.e., how much a pattern is shifted during the matching process) of 0.005 resulted in the best similarity scores. Best similarity scores in this case mean less variation in score distributions and the least overlap in matching and nonmatching scores. Pairwise matching was conducted between all photographs within each catalog and provided the similarity scores for each scenario based on the Chamfer distance transform. The distributions of scores for correct and incorrect matches for each individual to all other photographs in the catalogs were compared using boxplots for each of the 4 catalogs.

RESULTS

The grid size best suited to discriminating between individuals was found to be 0.0625 cell width and 0.025 cell height, and

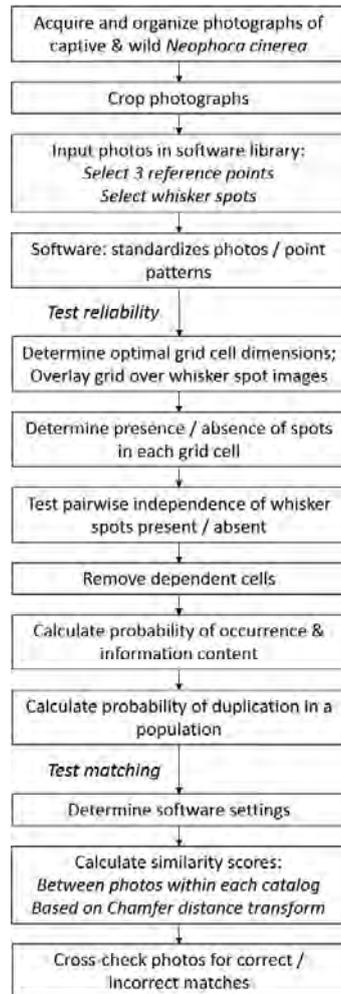


Fig. 4.—Flow chart presenting the entire process of testing the method of using whisker spot patterns for individual Australian sea lion (*Neophoca cinerea*) identification.

after testing for pairwise independence of whisker spots being present/absent (Fig. 5), 1 of 2 dependent cells were removed from the analysis. Applying these to test the whisker spot variability and pattern recognition algorithm produced the following results.

Variability of whisker spot patterns in Australian sea lions.—“Dependent” cells were mostly located close to the nose. The cells with the highest probability of whisker spots being present were those close to the nose (between coordinates $x = 0.9$, $y = 0.1$, and $x = 1$, $y = 0.4$; Fig. 6). Cells with the highest information content were those with lower frequencies of occurrence (Fig. 6; Pennycuik 1978). Once dependent cells were

Table 3.—The probability (P) of a spot pattern occurring, calculated as: $(1 - P)^M + MP(1 - P)^{M-1}$, and the corresponding information content (I) for a range of population sizes (M).

Population size	Probability of occurrence	Information content (bits)
50	$\leq 3 \times 10^{-3}$	> 8.38
100	$\leq 1.49 \times 10^{-3}$	> 9.39
500	$\leq 2.969 \times 10^{-4}$	> 11.72
1,000	$\leq 1.4862 \times 10^{-4}$	> 12.72

Table 4.—Sample sizes of photographs from 16 captive Australian sea lions (*Neophoca cinerea*) used for testing matches for photographs taken: 1) during the same day at 90°; 2) during the same day at 70°, 90°, and 110°; 3) during the same day at 1- and 2-m distances; and 4) during different sessions at 90°.

Name of individual	Same day at 90°	Same day at different angles	Same day at different distances	Different days at 90°
Abby	3	3	2	3
Ady	5	2		6
April	4	3	2	4
Cindy	3	3		3
Lexie	14	3	2	6
Liette	5	3	2	4
Malie	11	3	2	6
Maxine	6	3	2	3
Miri	11	3	2	
Miya	4	3	2	6
Nala	7	3	2	6
Nikki	2	3	2	3
Orson	6	2	2	4
Portia	5	3	2	3
Rocky	2	3	2	3
Teiko	2	3	2	4
Total	90	46	28	64

removed, 99.0% (± 1.5 SD) were considered reliable for a population size of 50 and 98.2% (± 1.7) for a population size of 100 (Fig. 7). Reliability estimates dropped to 92.2% (± 4.7) for a population size of 500, and 88.2% (± 5.7) for a population size of 1,000 (Fig. 7).

Pattern recognition algorithm and application.—Overall, most similarity scores calculated in the adapted software using the Chamfer distance transform were lower for photographs matched correctly than those matched incorrectly (Fig. 8), where a lower score denotes a better match of 2 images (Fig. 9). Similarity scores of pairwise comparisons of photographs of 16 captive animals (in zoos) taken on the same day at a 90° angle (scenario 1, Fig. 8a) resulted in 90% correct matches. Eighty-nine percentage of photographs taken at 1- and 2-m distances at 90° were correctly matched (Fig. 8c), whereas photographs taken from different angles had 48% correct matches (Fig. 8b). Comparisons of photographs that were taken at different times over a year (at 90°) yielded 73% correct matches by the adapted software (Fig. 8d). The percentage of correct matches over time did not appear to be related to the time period between photographs.

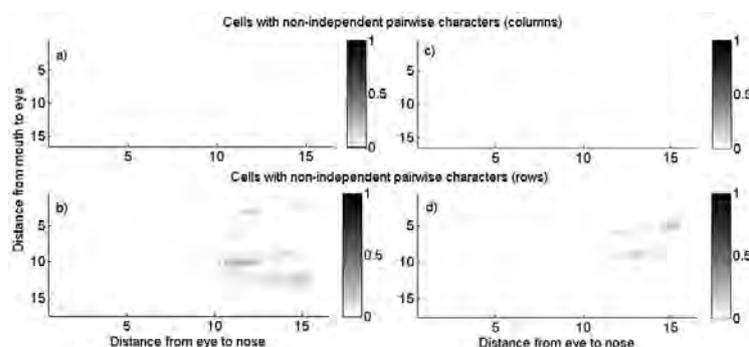


Fig. 5.—Pairwise probabilities of cells having whiskers present a) within columns (cells above and below each other) and b) within rows (cells right and left of each other). Pairwise probabilities of cells having whiskers absent c) within columns and d) within rows.

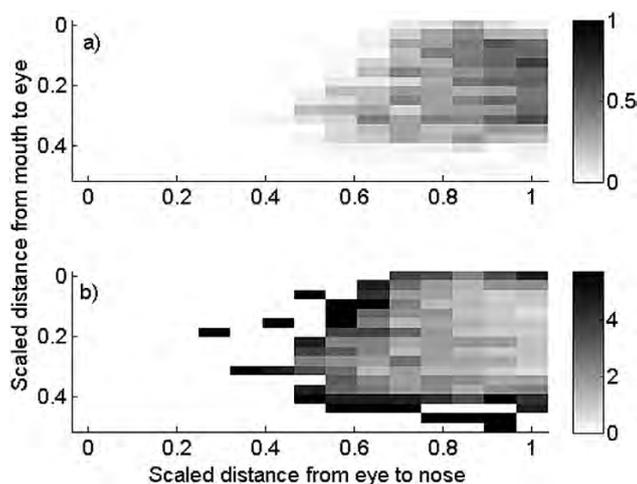


Fig. 6.—a) Frequency of occurrence and information content of whisker spots in grid cells based on 53 individual Australian sea lions (*Neophoca cinerea*), b) without removal of dependent cells, visually illustrating to the reader the locations on the muzzle where dependent cells were predominantly present.

DISCUSSION

Variability of whisker spot patterns in Australian sea lions.—Based on the information content of whisker spot patterns calculated here, there is sufficient variability in Australian sea lions for reliable matching in a relatively small population of 50 individuals. For populations of 1,000 individuals, the reliability estimates decrease and probability of duplication of a whisker spot pattern increases. In polar bears, whisker spot patterns were estimated to contain more information and populations of 1,000 individuals were estimated to be able to be matched with 99% reliability (Anderson et al. 2007). Our results are similar to the results estimated for variations in whisker spot patterns in lions, which were 92% reliable for a population size of 50 and 64% for a population of 1,000 (Pennycuik and Rudnai 1970). Similarly, whisker spot patterns in leopards were reliable for

smaller populations. Out of 21, 19 had enough information at 95% reliability level, whereas only 15 out of 21 were reliably identifiable at 99% (Miththapala et al. 1989). The main variable that can affect the estimated percentage of individuals considered to be reliable (having an information content above the minimum required for the study population size) is the cell size. For smaller cell sizes, the information content increases, and so does the percent of individuals considered to be reliable, since small differences in whisker spot positions can be detected (Pennycuik 1978). However, if the angle at which the photograph is taken shifts significantly, error in correct whisker spot cell allocation increases significantly. We therefore used a cell size that was equivalent to the maximum distance between the same whisker spots photographed multiple times on the same individuals. Having done this, the authors recognize that there

is an untestable assumption that the largest value of maximum distances between the same whisker spots on multiple photographs of the same individuals reflects the maximum shift in angle of photographs taken among different individuals.

Pattern recognition algorithm and application.—Overall, from the photographs taken in a controlled environment (captive animals photographed by zoo keepers) on the same day, the Chamfer distance transform performed relatively well with 90% correct matches. The factor most affecting correct matching was the angle at which the photographs were taken,

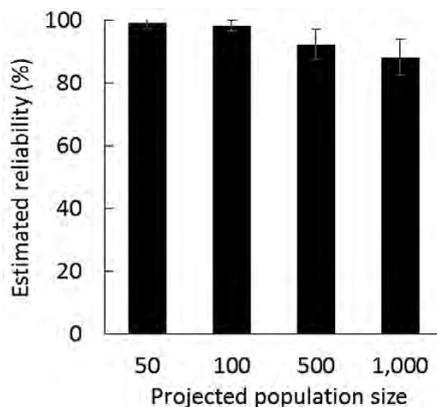


Fig. 7.—Percentage of reliable whisker spot patterns estimated from 50 repeated simulations for a population of 50, 100, 500, and 1,000 individuals, with *SD* (whiskers).

in agreement with Anderson et al.'s (2010) study which found that similarity scores increased (i.e., had poorer matches) with increasing deviance from an angle of 90°. A spot pattern technique to identify cheetahs also performed significantly poorer when photographs were taken from different angles (Kelly 2001). We suspect that the poorer performance (73% correct matches) of photographs taken during sessions 10, 30, 60, 180, and 360 days after the 1st session is likely due to slight variation in angles from which the photographs were taken. This result highlights the need for very good-quality photographs, taken at the same angle regardless of individual or location, when using this approach. As wild Australian sea lions are difficult to identify without the use of invasive methods, in the wild, it was impossible to ground truth whether multiple photographs of the same individuals were taken over time. Sea lions in captivity for this study were already mature, thus testing changes in growth stage has not been possible. Australian sea lions in controlled environments were photographed at 1- and 2-m distances to test this method with the highest quality photographs. Distance did not alter matching success compared to 90° photographs at the same distance. We believe that photographs taken at greater distances will not alter matching success when high-quality photographs focused on the muzzle are used. Wild individuals are not permitted to be approached closer than 5 m for safety reasons and to minimize human disturbance. Furthermore, photos of captive Australian sea lions were taken with cameras available to the respective institute, whereas wild individuals were photographed with a 100–400 mm zoom lens, with greater performance over increased distances.

The manual selection process of marking all whisker spots means that the matching process is slower than using the

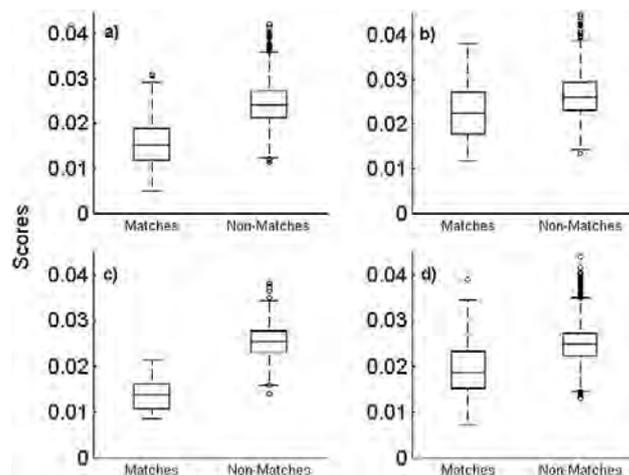


Fig. 8.—Box and whisker plots of averaged similarity scores of “matches” and “non-matches” of whisker spots of 16 individual captive Australian sea lions (*Neophoca cinerea*) for a) 90° angle, b) 70°, 90°, and 110° angles, c) 1- and 2-m distance at 90° angle, and d) 10, 30, 60, 180, and 360 days from the first session at a 90° angle. “Matches” include comparisons of different photographs of the same individuals, whereas “non-matches” are comparisons of photographs from an individual to those from all other individuals. The median is displayed as a black line, 90th percentiles as vertical boxes, 75th and 25th percentiles as range bars, and outliers as black circles.

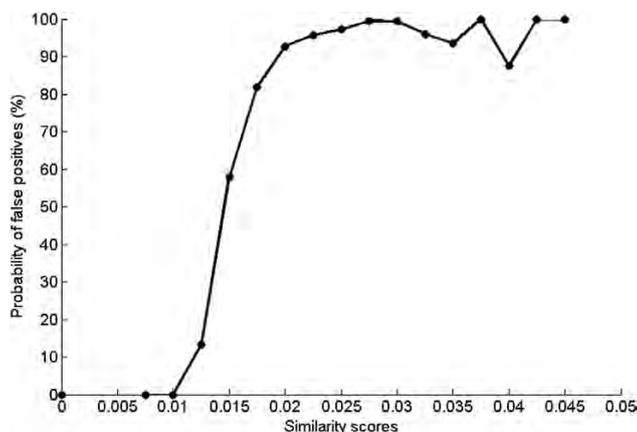


Fig. 9.—Probability of correct/incorrect matches based on the similarity scores of whisker spot patterns of 16 captive Australian sea lions (*Neophoca cinerea*) at a 90° angle ($n = 90$).

original design of the software on polar bears or lions (Anderson et al. 2010). In matching through visual inspection, biases and error can be introduced by a person's perception and level of experience (Oliveira-Santos et al. 2010). Matching through visual inspection is also labor-intensive, can be expensive, and may be exposed to human error. The positive performance of the semiautomated processing illustrates that the software can decrease labor and improve cost efficiency. Verification of the semiautomated matching process could be conducted by laying a grid over whisker spot positions in matched photographs and comparing the grid locations of the whisker spots manually to confirm positive matches.

Application and recommendations.—The approach using an adapted Chamfer distance transform has sufficient reliability to be applied to a small population size, when photographs are taken at 90°, without tilt, and are of high contrast and quality. However, we believe that keeping photographs taken at other angles and suboptimal quality photographs on record in the library may improve the chance of reidentifying an individual (Kelly 2001; Hillman et al. 2003; Arzoumanian et al. 2005). Information content for pattern matching can be increased by adding other features to improve identification, such as forehead spot patterns in leopards (Miththapala et al. 1989). Pinniped flipper shape and nicks can be individually specific and offer an additional feature for discriminating individuals. This was previously found to be the most useful feature in identifying Hooker sea lions (*Phocarctos hookeri*—McConkey 1999). However, a limiting factor in photographing all flippers of Australian sea lions is their tendency to tuck them under the body or cover them with sand, thus this information was not collected. As photograph angle was the greatest cause of reduced matching success, we recommend exploring the effectiveness of the Groth algorithm for pattern matching as an alternative technique as for whale sharks (*Rhincodon typus*—Arzoumanian et al. 2005). This approach compensates for distortion in patterns using geometric relationships between spots,

similar to how astronomers identify star constellations and the position of stars in relation to other stars (Groth 1986).

In summary, this new technique for identifying Australian sea lions can be used for small populations or resident communities. Australian sea lions often occur in small colonies that are distant from each other (Goldsworthy and Gales 2008). In conjunction with capture–recapture models to estimate colony size, this method can be used for assessment of localized habitat use and residency in localized areas. Determining the population or resident community size and their areas of use can then be fed into management and conservation of the species, in particular in allocating and defining management zones for high human use areas. The method also provides a way of monitoring these animals over long time periods without the need for capturing and invasively marking the animals. Finally, this point-pattern recognition application may also work for other otariid species.

ACKNOWLEDGMENTS

This project was made possible with the significant support provided by Adelaide Zoo, Pet Porpoise Pool in Coff's Harbour, SEALIFE (previously UnderWater World) in Mooloolaba, and Taronga Zoo in Sydney through the contribution of all captive sea lion photographs. Thanks goes to M. Perry for maintenance of the research vessel. The Department of Parks and Wildlife (Western Australia) provided substantial logistical support to and from islands as well as photographs of wild sea lions. P. Collins based at Department of Parks and Wildlife, Albany, organized several field trips off Albany and Esperance and kindly provided accommodation. The Department of Fisheries and Cockburn Volunteer Sea Search and Rescue contributed through transport to and from islands. The Fremantle Sailing Club provided support through boat ramp access. Many volunteers have allocated their time for considerable assistance in the field. A. Camacho assisted in project management. Wild Encounters Rockingham has contributed by disseminating

information regarding the project to the public and has assisted with public outreach. Finally, this project was supported by funding from the Australian Marine Mammal Centre (2012/19) and the Western Australian Government's State NRM Program.

LITERATURE CITED

- ADOBE PHOTOSHOP ELEMENTS. 2012. Adobe Photoshop Elements 11. Adobe Systems, San Jose, California.
- ANDERSON, C. J. R., J. D. ROTH, AND J. M. WATERMAN. 2007. Can whisker spot patterns be used to identify individual polar bears? *Journal of Zoology (London)* 273:333–339.
- ANDERSON, C. J. R., N. D. VITORIA LOBO, J. D. ROTH, AND J. M. WATERMAN. 2010. Computer-aided photo-identification system with an application to polar bears based on whisker spot patterns. *Journal of Mammalogy* 91:1350–1359.
- ARZOUMANIAN, Z., J. HOLMBERG, AND B. NORMAN. 2005. An astronomical pattern-matching algorithm for computer-aided identification of whale sharks (*Rhincodon typus*). *Journal of Applied Ecology* 42:999–1011.
- BEENTJES, M. P. 1989. Haul-out patterns, site fidelity and activity budgets of male Hooker's sea lions (*Phocarcetos hookeri*) on the New Zealand mainland. *Marine Mammal Science* 5:281–297.
- BORGEFORS, G. 1986. Distance transformation in digital images. *Computer Vision, Graphics, and Image Processing* 34:344–371.
- CAMPBELL, R. A., N. J. GALES, G. M. LENTO, AND C. S. BAKER. 2008. Islands in the seas: extreme female natal site fidelity in the Australian sea lion, *Neophoca cinerea*. *Biology Letters* 4:139–142.
- DIXON, D. R. 2003. A non-invasive technique for identifying individual badgers *Meles meles*. *Mammal Review* 33:92–94.
- FORCADA, J., AND A. AGUILAR. 2000. Use of photographic identification in capture-recapture studies of Mediterranean monk seals. *Marine Mammal Science* 16:767–793.
- GALES, N. J., A. J. CHEAL, G. J. POBAR, AND P. WILLIAMSON. 1992. Breeding biology and movements of Australian sea lions, *Neophoca cinerea*, off the west coast of Western Australia. *Wildlife Research* 19:405–416.
- GOLDSWORTHY, S., AND N. J. GALES. 2008. *Neophoca cinerea*. The IUCN Red List of Threatened Species. Ver. 2011.2. www.iucn-redlist.org. Accessed 23 November 2011.
- GROTH, E. J. 1986. A pattern-matching algorithm for two-dimensional coordinate lists. *Astronomical Journal* 91:1244–1248.
- HIBY, L., AND P. LOVELL. 1990. Computer aided matching of natural markings: a prototype system for grey seals. Report of the International Whaling Commission 12:57–61.
- HIBY, L., P. LOVELL, N. PATIL, N. SAMBA KUMAR, A. M. GOPALASWAMY, AND K. ULLAS KARANTH. 2009. A tiger cannot change its stripes: using a three-dimensional model to match images of living tigers and tiger skins. *Biology Letters* 5:383–386.
- HILLMAN, G. R., ET AL. 2003. Computer-assisted photo-identification of individual marine vertebrates: a multi-species system. *Aquatic Mammals* 29:117–123.
- KARLSSON, O., L. HIBY, T. LUNDBERG, M. JÜSSI, I. JÜSSI, AND B. HELANDER. 2005. Photo-identification, site fidelity, and movement of female gray seals (*Halichoerus grypus*) between haul-outs in the Baltic Sea. *Ambio* 34:628–634.
- KELLY, M. J. 2001. Computer-aided photograph matching in studies using individual identification: an example from Serengeti cheetahs. *Journal of Mammalogy* 82:440–449.
- MCCONKEY, S. D. 1999. Photographic identification of the New Zealand sea lion: A new technique. *Journal of Marine and Freshwater Research* 33:63–66.
- MITHTHAPALA, S., J. SEIDENSTICKER, L. G. PHILLIPS, S. B. U. FERNANDO, AND J. A. SMALLWOOD. 1989. Identification of individual leopards (*Panthera pardus kotiya*) using spot pattern variation. *Journal of Zoology (London)* 218:527–536.
- MIZROCH, S. A., J. A. BEARD, AND M. LYNDE. 1990. Computer assisted photo-identification of humpback whales. Report of the International Whaling Commission 12:63–70.
- MOLER, C. 2013. Matlab R2013a. MathWorks, Natick, Massachusetts.
- NICHOLS, J. D. 1992. Capture-recapture models. *BioScience* 42:94–102.
- OLIVEIRA-SANTOS, L. G. R., C. A. ZUCCO, P. C. ANTUNES, AND P. G. CRAWSHAW JR. 2010. Is it possible to individually identify mammals with no natural markings using camera-traps? A controlled case-study with lowland tapirs. *Mammalian Biology-Zeitschrift für Säugetierkunde* 75:375–378.
- PENNYCUICK, C. J. 1978. Identification using natural markings. Pp. 147–159 in *Animal marking* (B. Stonehouse, ed.). MacMillan Press, London, United Kingdom.
- PENNYCUICK, C. J., AND J. RUDNAL. 1970. A method of identifying individual lions *Panthera leo* with an analysis of the reliability of identification. *Journal of Zoology (London)* 160:497–508.
- PETERSON, J. C. B. 1972. An identification system for zebra (*Equus burchelli*, Gray). *African Journal of Ecology* 10:59–63.
- SIKES, R. S., W. L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 92:235–253.
- SUMMERS, C. F., AND S. R. WITTHAMES. 1978. The value of tagging as a marking technique for seals. Pp. 63–70 in *Animal marking: recognition marking of animals in research* (B. Stonehouse, ed.). Proceedings of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Symposium. MacMillan Press, London, United Kingdom.
- TROY, S., D. MIDDLETON, AND J. PHELAN. 1997. Marine mammal research in the southern hemisphere. Pp. 179–183 in *Marine mammal research in the Southern Hemisphere* (M. Hindell and C. Kemper, eds.). Surrey Beatty and Sons, Devon, United Kingdom.
- ULLAS KARANTH, K., AND J. D. NICHOLS. 1998. Estimation of tiger densities in India using photographic captures and recaptures. *Ecology* 79:2852–2862.
- VINCENT, C., L. MEYNIER, AND V. RIDOUX. 2001. Photo-identification in grey seals: legibility and stability of natural markings. *Mammalia* 65:363–372.
- WALKER, G. E., AND J. K. LING. 1981. Handbook of marine mammals. The walrus, sea lions, fur seals and sea otter. Pp. 99–118 in (S. H. Ridgway and R. J. Harrison, eds.). Academic Press, London, United Kingdom.
- WALKER, K. A., A. W. TRITES, M. HAULENA, AND D. M. WEARY. 2012. A review of the effects of different marking and tagging techniques on marine mammals. *Wildlife Research* 39:15–30.
- WHITEHEAD, H. 1990. Computer assisted individual identification of sperm whale flukes. Report of the International Whaling Commission 12:71–77.
- WÜRSIG, B., AND M. WÜRSIG. 1977. The photographic determination of group size, composition, and stability of coastal porpoises (*Tursiops truncatus*). *Science* 198:755–756.

Submitted 2 October 2014. Accepted 22 November 2014.

Associate Editor was Jeanette A. Thomas.

CHAPTER 3 PUBLICATION

Difficulties identifying Australian sea lions (*Neophoca cinerea*) in the wild using whisker spot patterns

Sylvia K. Osterrieder^{A,B,C}, Iain M. Parnum^A, Chandra P. Salgado Kent^A and Randall W. Robinson^B

^ACentre for Marine Science and Technology, Department of Imaging and Applied Physics, Curtin University, Bentley, WA 6102, Australia.

^BInstitute for Sustainability and Innovation, College of Engineering and Science, Victoria University, Building 6, McKechnie Street, St Albans, Vic. 3021, Australia.

^CCorresponding author. Email: sylvia.osterrieder@gmail.com

Abstract. Individual identification is a beneficial tool in behavioural and ecological research. In mark–recapture studies, for example, it can improve abundance, residency and site fidelity estimates. Two non-invasive, photo-identification approaches, using whisker spot patterns, were tested to identify wild individual Australian sea lions (*Neophoca cinerea*). The Chamfer distance transform algorithm has shown promising results when applied to captive individuals. An alternative matching method using row/column locations of whisker spots, previously applied to lions (*Panthera leo*) was also tested. Resighting wild *N. cinerea* in this study proved unfeasible with both methods. Excessive variation between photographs of the same individual was found when applying the Chamfer distance transform, and similarity between photograph-pairs appeared to decrease with increasing time between photographs. Insufficient variation among *N. cinerea* row/column pattern was detected to successfully discriminate among individuals, averaging 39 mystacial spots (range 30–46, $n = 20$) in seven rows and 9–10 columns. Additionally, different observers marking the same photographs introduced considerable variation. Colour difference (red, green and blue colour levels) between the whisker spots and surrounding fur affected marking spot locations significantly, increasing uncertainty when contrast decreased. While other pattern-matching algorithms may improve performance, accurate identification of spot locations was the current limitation.

Additional keywords: pattern matching, photo-identification.

Received 14 December 2015, accepted 4 July 2016, published online 5 August 2016

Introduction

Ecological studies of animal residency, movement patterns, population estimates and demographics significantly benefit from identification of individuals within a population. For instance, reidentifying individuals allows animal behaviours and movements to be tracked over time. By obtaining a robust sample of these reidentifications (or resights) from a population, accurate population and demographic parameter estimates can be obtained using mark–recapture statistical approaches (Seber 1982).

In species that have unique morphology or markings, such as scars or fur patterns, photo-identification has been a useful, non-invasive tool for identifying individuals (e.g. Würsig and Würsig 1977; Hiby and Lovell 1990; Whitehead 1990; Würsig and Jefferson 1990; Gowans and Whitehead 2001; Parra and Corkeron 2001; Dixon 2003; Ardovini *et al.* 2008). In species lacking such readily recognisable features, unique marks have been applied more invasively. These methods, including tagging or branding individuals, usually require

capturing and handling animals to apply the marks (Troy *et al.* 1997; Walker *et al.* 2012) and can cause stress and a risk of infection of the marked area. In some species, however, less prominent features have been found applicable to use as alternative non-invasive marks to identify individuals. For instance, in lions (*Panthera leo*) and polar bears (*Ursus maritimus*), whisker spot patterns have successfully been used to identify individuals over long-term periods (Pennycuik and Rudnai 1970; Anderson *et al.* 2007; Anderson *et al.* 2010). Whisker spot patterns have also assisted in identifying individual leopards (*Panthera pardus kotiya*) (Miththapala *et al.* 1989) and Hooker's sea lions (*Phocarcetus hookeri*) (Beentjes 1989).

Whisker spot patterns have been considered 'unique', having sufficient information to identify 92% of *P. leo* and 98% of *U. maritimus* of the populations examined (Pennycuik and Rudnai 1970; Anderson *et al.* 2007). In *P. pardus kotiya*, 91% of examined individuals were distinguishable by their whisker spot patterns. However, using additional patterns in

fur was suggested to increase the probability of distinguishing individuals (Miththapala *et al.* 1989).

Two different approaches have been applied for species in which whisker spot patterns have been used for individual identification. In *P. leo*, the locations of whisker spots in the top row relative to those in the second row (on both sides of the muzzle) have been used (Pennycuik and Rudnai 1970). The second row has been used as a reference row to determine the location of the top whisker spots in its quadrants, which has been achieved by drawing the top two rows on gridded paper (Pennycuik and Rudnai 1970; Kays and Patterson 2002; Ogotu *et al.* 2006). Similarly, the location of whisker spots in three rows in relation to a reference row have been used in *P. pardus kotiya* (Miththapala *et al.* 1989). In contrast, no such consistent reference row is available in *U. maritimus*, and instead, all whisker spots available have been used for pattern matching. In fact, using all available whisker spots increased the information content available for identifying an individual (i.e. including all whiskers made the pattern more distinctive). The method used in *U. maritimus* is based on comparing whisker spot patterns, by standardising them using three reference points. Differences between two whisker spot patterns are then determined using a Chamfer distance transform algorithm, comparing the distances between standardised whisker spots within one pattern to those in another pattern. A distance score indicates how similar two patterns are to each other. The lower the distance score, the higher the similarity between two patterns, i.e. the higher the probability of a resighting (Anderson *et al.* 2007). Following this method, a recent study showed that in controlled conditions, using captive Australian sea lions (*Neophoca cinerea*), whisker spot patterns contain sufficient information for individual identification in small populations, and matching success was relatively high (Osterrieder *et al.* 2015a). This method has, however, not been tested for reidentifying individuals in the wild.

N. cinerea is an endangered species with several populations decreasing, and is endemic to South and Western Australia. For many *N. cinerea* colonies, there is limited information on population trends and demographics (Goldsworthy 2015). This is often insufficient for their effective long-term conservation. There are six haul-out islands in the Perth metropolitan area that are used only by male *N. cinerea*, predominantly outside the breeding season (Gales *et al.* 1992). Little is known about the abundance, residency and movement patterns of *N. cinerea* residing in this area. The high variability of *N. cinerea* hauling out at a given time means that careful planning is required for count data to be used as abundance indices (Gales *et al.* 1992; Osterrieder *et al.* 2015b). Identifying individual *N. cinerea* would therefore be a valuable tool with which a better understanding of the subpopulation's movement and residency patterns can be gleaned to inform management and conservation strategies and improve their effectiveness.

The overall aim of this study was to apply and test the performance of the Chamfer distance transform method to identify and resight individual *N. cinerea* in the wild. This study also explored the potential of applying a method similarly to that used in *P. leo* as an alternative, potentially simpler approach, based on row/column locations of whisker spots. The aims were achieved by: (1) mapping out the composition

of whisker spot patterns from a sample of wild *N. cinerea*, (2) testing whisker spot pattern matching using the Chamfer distance transform and row/column mapping as a photo-identification technique for wild *N. cinerea* in the Perth metropolitan area, (3) identifying whisker spot occurrence of row/column locations and uncertainty in detection by a single observer, (4) quantifying the variation in detecting whisker spots among multiple observers, and (5) identify whether consistency in identifying the presence of spots among observers was associated with colour differences between whisker spots and surrounding fur.

Materials and methods

Study sites

Male *N. cinerea* are known to haul out on six islands in the Perth metropolitan area, Western Australia, between breeding seasons (see fig. 1 in Osterrieder *et al.* 2015a). Most islands are small and have fewer than 10 *N. cinerea* hauling out at one time (Department of Parks and Wildlife (DPAW), unpubl. data). However, over 30 *N. cinerea* have been documented to haul out during peak periods on the two most-used haul-out islands in this area: Seal (32.29°S, 115.69°E) and Carnac (32.12°S, 115.66°E) Islands (Gales *et al.* 1992; Osterrieder *et al.* 2015b). The relatively large numbers of *N. cinerea* on Seal and Carnac Islands provide a greater opportunity to obtain a relatively large photo-ID sample size (per unit effort) than at other islands. Therefore, Seal and Carnac Islands were selected as the study sites. The breeding islands in the Houtman Abrolhos Islands (~28.7°S, 113.7°E) are ~60 km off Geraldton, Western Australia and ~400 km north-west of the haul-out islands in the Perth metropolitan area. The Houtman Abrolhos Islands were added as study sites to increase the sample size of whisker spot photographs of wild individual *N. cinerea*.

Collection and selection of photographs

Whisker spot photographs were taken during 35 field days in the Perth metropolitan area (16 days at Carnac Island and 19 at Seal Island, between 20 November 2013 and 6 June 2014), and during two field days at the Houtman Abrolhos Islands (9 and 10 April 2013). *N. cinerea* were approached with care to limit disturbance and were not approached closer than 5–10 m, which is the required minimum distance the public is to maintain according to guidelines (DEC 2007; DEC and Fisheries 2011). The lateral sides of the muzzles (90° to the camera's direction of view) were photographed as described in Osterrieder *et al.* (2015a). Lateral photographs of *N. cinerea* muzzles were taken at a range between 5 and ~50 m using a Canon EOS 550D with a 100–400-mm zoom lens (Canon, Tokyo, Japan). Beyond 50 m, the quality of photographs has been shown to be too poor to identify *U. maritimus* and used as a guide in this study (Anderson *et al.* 2007). Photographs were taken as close to 90° as possible, without tilt and of the left and right sides of the muzzles when possible. To aid in correctly identifying two photographs of an individual as a match (a resighting), photographs of the whole body of an individual were taken when possible in addition to the whisker spot photographs. These supplementary photographs may contain additional information for the individual, such as scarring, age and overall size.

To map out the general composition of whisker spot patterns, photographs of *N. cinerea*'s muzzles were chosen from a single field day, 15 February 2014, in the Perth metropolitan area to ensure that the same individuals were not resampled (unknowingly on different days). It was assumed unlikely that hauled-out (i.e. typically resting) *N. cinerea* would haul-out and travel between Seal and Carnac Islands, which are ≈ 19 km apart, within the 7-h timeframe that the photographs were taken. The 15 February was selected because the largest number of individuals were photographed on Carnac and Seal Islands on that day. Similarly, photographs of *N. cinerea* at the Houtman Abrolhos Islands were added from only one day, 10 April 2013, to increase the sample size without resampling from the same location. It is not known whether *N. cinerea* move between Houtman Abrolhos Islands and the Perth metropolitan area. The distance of ≈ 400 km between these study sites is relatively large. In addition, the Perth region consists only of what could possibly be a group (the males) of the population at the Houtman Abrolhos Islands. While the time between sampling dates of ≈ 10 months is relatively large (allowing time to travel between locations), the likelihood of the same *N. cinerea* being photographed twice was assumed to be small.

Photographs were selected for analysis by removing poor quality photographs. Photographs that were poor quality were defined as those that were out of focus, taken at a different angle than the approximate 90° to the animal's muzzle, or were tilted. Whisker spots were not always clearly visible to be marked and confidence in the accuracy of marking was reduced accordingly. In extreme cases, such as a muzzle completely covered by sand, the photograph was discarded. In other studies, poor-quality photographs have been shown to result in comparatively poor matching results due to low visibility of natural marks (Gowans and Whitehead 2001; Stevick *et al.* 2001; Kelly 2001; Hastings *et al.* 2008) – whisker spots in this case. For most *N. cinerea*, it was possible to photograph only one side of their muzzle, and therefore photographs of the side having the largest sample size were used.

The total number of individuals photographed was recorded on each field day, based on their haul-out locations and movements during the period of photographing. However, individual *N. cinerea* were not distinguishable from each other on different field days by field personnel, and the total number of individuals over all field days was unknown. 'Individuals' referred to in the section below describing pairwise matching were the individuals photographed on any single day (which could be the same individual on a different day, but is considered a 'different individual' for describing methods for matching).

Application of the Chamfer distance transform method for identification

Preparation of photographs for matching using the Chamfer distance transform method

All procedures to prepare selected photographs for the matching process followed the description in Osterrieder *et al.* (2015a). Photographs were cropped in Adobe PhotoShop Elements 11 to include the required section of each image. Photographs were then added to a database ('library') in software previously used for whisker spot identification in *U. maritimus*

(Anderson *et al.* 2010), and adapted for use for *N. cinerea* (Osterrieder *et al.* 2015a). Three reference points, consisting of the inner corner of the eye, the corner of the nostril and the edge of the mouth, and all visible whisker spots were marked manually (Osterrieder *et al.* 2015a). Where an exact spot location was difficult to determine, the corresponding whisker was traced back as close to the origin as possible to identify the whisker spots.

The software standardised all photographs to each other using the selected reference points, so that the eye was at a spatial coordinate of (0, 0), the nose at (1, 0) and the mouth at (0.5, 0.5) as in Osterrieder *et al.* (2015a).

Pairwise matching of photographs method

The software calculates distance scores between pairwise matched individual photographs, based on a Chamfer distance transform (Anderson *et al.* 2010; Osterrieder *et al.* 2015a). The lower the distance score, the greater the similarity between two matched patterns. In this study, it was often possible to take several photographs of an individual in a single approach. Multiple photographs were taken to maximise the likelihood that one or more photographs met the required conditions of angle and tilt (Osterrieder *et al.* 2015a). These photographs were all placed in a software library and all photographs taken that could be confirmed as being of the same individual (e.g. multiple approaches of an individual that had not moved throughout the day, or multiple photographs from a single approach to one individual) placed into an individual's folder. Each individual's photographs in the final library were pairwise matched with other individuals, producing a distance score for each comparison. The software tests two groups of photographs (all those of Individual A with all those of Individual B) and provides the best matching score (i.e. that with the lowest distance score) for the pair.

The same software settings used by Osterrieder *et al.* (2015a) were used for this study, which included an offset of 0.07 and step size of 0.005. The offset accounts for misalignments of whisker spots in different photographs by shifting spots from a pattern to align with spots of a pattern being matched to improve the match. The step size determines the amount a pattern can be shifted during the matching process.

Thresholds to identify matching pairs of photographs using the Chamfer distance transform method

Distance scores were calculated for photographs of individuals taken on a single day to obtain the range of scores for confirmed 'matches'. Distance scores from comparisons of photographs of different individuals on the same day were calculated to obtain the range of scores for confirmed 'non-matches'. Due to the non-normal distribution of the data according to a Shapiro–Wilk normality test, a Mann–Whitney U-test was used to test for differences of distance scores of confirmed 'non-matches' and confirmed 'matches'. These distance scores were also used to identify a threshold of scores that would likely represent correct positive matches. On the basis of these scores, the probability of pairwise matches being correct matches or non-matches was calculated and plotted, and the distance score for 50% probability of a match estimated. Pairs with distance scores below the 50% probability (of not

being a match; i.e. scores indicating more similar patterns) were considered 'more likely' to be true matches, and were selected for manual (visual) inspection to confirm or reject the match. The 50% probability threshold was selected to allow for a thorough manual verification of potential pairs that exhibited a higher probability of being a match. As a preliminary stop-go test, the manual checks were conducted only on pairs more likely to be a match. It was deemed that if the method was not successful in matching individuals with scores $\geq 50\%$ probability of being a match, it would be less likely to be successful using the remaining photographs. Scores larger than the threshold were discarded as they were more likely to be false matches.

Variability between photographs of the same individual using the Chamfer distance transform method

To assess variability among sequences of photographs of the same individual, the distance scores of pairwise matched photographs of a single individual taken throughout the day were compared. This allowed the investigation of variability between photographs taken within short (within seconds) succession, to assess the impact of repositioning (altering position or orientation of either camera or subject and potentially light conditions) on the matching performance.

Manual verification of photographs to identify correct matches

Two steps were used to manually compare individual *N. cinerea* and their patterns, which resulted in distance scores below the 50% probability threshold (described above). For both steps selected photographs of individuals were compared by manual inspection by an observer, and identified as the same or different individual based on qualitative evaluation. If identified as the same individuals, then this match was considered a resight.

- (1) First, photographs were searched for little marks, spots and scars (other than whisker spot patterns), and identifying features compared across photographs. Age variation of individuals was also compared between photographs, and if the age variation was too large for the time difference between the photographs, these were considered as different individuals (i.e. an individual photographed as an adult cannot be photographed as a juvenile a few months later). Male *N. cinerea* change their fur colour when maturing, which therefore gives an indication of the individual's age. The age category (adult, subadult or juvenile) was determined based on fur colour and size of an animal (Gales *et al.* 1992; Jefferson *et al.* 2011; Osterrieder *et al.* 2015b) and the date and location of the sighting was documented.
- (2) Whisker spot patterns were compared through manual inspection, by plotting the coordinates of the whisker spots of matched photographs on top of each other in different colours on a gridded space. In addition to this, whisker spot patterns were compared by comparing cells used for estimating information content in whisker spot patterns by Osterrieder *et al.* (2015a). Grids with 0.0625 cell width and 0.025 cell height were previously determined to be the best-fitting grid size for this method and species (Osterrieder *et al.* 2015a).

Following manual verification of 'Matches' (confirmed resighting), 'Non-matches' (confirmed rejections) and 'Unconfirmed matches' (no confirmed resightings or rejections), a Mann-Whitney U-test was used to test for differences of distance scores between these categories. Additionally, comparisons of distance scores were conducted between individuals with wet and dry muzzles. Mann-Whitney U-tests (exact distribution method in cases of small sample sizes) were applied to test for variations in distance scores of 'Matches', 'Non-matches', and 'Unconfirmed matches' in pairs of wet-wet, wet-dry and dry-dry pairs of photographs. Mann-Whitney U-tests were applied due to the non-normal distribution of the data according to a Shapiro-Wilk normality test.

Identification using row-column locations

Processing of photographs, analysing occurrence of whisker spots

All visible whisker spots on the selected photographs were manually marked in Adobe PhotoShop Elements 11. Lines tracing the rows and columns of whiskers were added to facilitate allocation of whisker spots to rows and columns (Fig. 1). This was carried out by manually fitting a line through the points in which the spots most closely aligned. The average and standard deviation, as well as minimum and maximum numbers of whiskers occurring in each row and column was then calculated, and a general schematic of the composition of whisker spot patterns (i.e. in rows and columns) was drawn based on these results.

In addition to documenting the presence and absence of whisker spots at each row/column location (e.g. presence/absence of a spot in Row A, Column 2) (Fig. 1), a qualitative score was allocated based on whether there was uncertainty as to whether

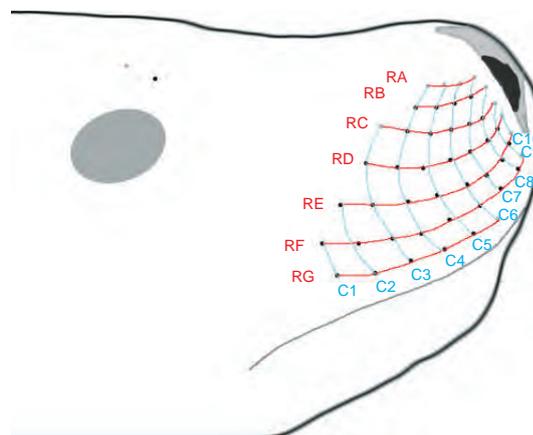


Fig. 1. General whisker spot sketch based on 20 individual wild *N. cinerea*, ordered from Row A (RA) to G (RG) and Columns 1 (C1) to 10 (C10). Black spots indicate those present in >80% of photographs, grey spots indicate those in 30–80% of photographs. Rows A–G are illustrated by red lines and Columns 1–10 by light blue lines. Whiskers may also occur above the eye.

a row/column location contained a spot. Uncertainty in a spot's presence occurred as a result of spots being hidden by fur and the corresponding emerging whiskers being thin and short, or whiskers overlapping and masking the spots and whiskers being evaluated (Osterrieder *et al.* 2015a). In cases where presence or absence of a spot or whisker could not be definitively determined, it was marked as an uncertain.

The frequency of individuals having spots at each row/column location was quantified to identify areas of consistency and variation among individuals. The frequency was mapped as the percentage of whisker spots occurring at each row/column location of the total photographs evaluated. The frequency in the uncertainty of determining the presence of whisker spots at each row/column location was also mapped to identify areas of high certainty. A cross-correlation was conducted to pair-wise compare all different patterns to determine differences and matches between all whisker spot patterns. Cross-correlation was also carried out after removing all locations showing an arbitrary chosen value of >10% uncertainty of a row/column location showing a spot.

Observer variability

Observer bias has been shown to affect the results of photo-identifying individuals (Oliveira-Santos *et al.* 2010). It is reasonable to assume, however, that multiple observers in various studies over time would apply this photo-identification method if this approach of whisker spot pattern matching proved useful. Therefore, different operators were asked to select all whisker spots in selected photographs (selection criteria described below) to examine the consistency in marking the spots. The lead observer in the overall study, plus five experienced marine mammal observers marked all visible whisker spots on each of 10 selected photographs (independently from each other). Photographs included varying whisker spots and fur colouration, animals at different stages of maturation, wet and dry muzzles, and two images of a single individual taken at different times (the photographs were marked randomly, and those of the same individual over time were not marked immediately after each other). As training, the observers were supplied one additional photograph before marking the following images. All observers had a minimum of three years' experience working in marine biology projects and three had been involved in photo-identification of other marine mammals previously.

Analysing uncertainties of marking whisker spots

N. cinerea possess different fur colours between sexes, and while both sexes change fur colour as they mature from pups to juveniles, males also change fur colour as they progress from juveniles to adults. As a result, the difference between the colour of the whiskers, whisker spots, and fur vary depending upon the age and sex of the animals. In comparison, *U. maritimus* and *P. leo* exhibit light fur colours and dark whisker spots throughout their lives and between sexes.

To determine the impact of fur colour in selecting whisker spots, the colours (Red, Green and Blue) of whisker spots were compared with surrounding fur. There are various metrics that could be calculated to quantify the contrast. The authors chose

to do this by calculating the Root-Mean-Squared (RMS) difference of the Red (R), Green (G) and Blue (B) levels between the 'spot' and surrounding 'fur'. Calculating the RMS of the RGB levels to quantify contrast has been used successfully in various applications (such as in Pallottino *et al.* 2010; to determine hazelnut peeling). This study used the following implementation, Eqn (1):

$$RMS = \sqrt{\frac{1}{3} \sum (R_{spot} - R_{fur})^2 + (G_{spot} - G_{fur})^2 + (B_{spot} - B_{fur})^2}$$

where R_{spot} is the mean value of the red component (0–255) of the spot and R_{fur} is the mean value of the red component of the fur, with the same principle applied to green (G_{spot} and G_{fur}) and blue (B_{spot} and B_{fur}) components. The mean levels were calculated from user-defined areas of the spot and surrounding fur. The user attempted to define a similar ratio of spot area to surrounding fur area throughout the markings to limit bias. The spot itself was used as a mask to exclude those pixels in the calculation of the RGB mean levels of the fur. To calculate the RGB mean levels, six whisker spots were chosen in a stratified approach from each of the 10 photographs marked by six observers. The rationale of the stratified approach was to choose three of these whisker spots marked by 5–6 observers and three spots marked by 1–4 observers. To ensure that pooling of whisker spots marked by 1–2 and 3–4 observers was validated, a Mann–Whitney U-test was performed, and groups not significantly different from each other were subsequently pooled. The RMSs of the RGB mean levels were then compared by applying a Mann–Whitney U-test between whisker spots marked by most or all observers with those marked by low number of observers. The Mann–Whitney U-test was used due to the non-normal distribution of most groups according to a Shapiro–Wilk normality test applied beforehand. Where possible, spots for colour extraction were chosen if no other whisker was overlaying the area and the whisker spot was clearly visible. Where these spots were not available the pixels comprising overlaying whiskers were included in the analysis as this contributed to the variation in RGB levels that the observer used to discriminate between to identify the whisker spot.

All analyses were carried out and figures produced in Matlab R2014a. This work was conducted under a Department of Parks and Wildlife permit (no. SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth).

Results

Composition of whisker spot patterns

Photographs of a total of 76 *N. cinerea* were selected to determine the general composition of whisker spot patterns, including 44 *N. cinerea* from the Houtman Abrolhos Islands, 17 from Seal Island and 14 from Carnac Island. A larger number of good-quality photographs were available of the right side of *N. cinerea*, and therefore were selected for analyses. Of the photographs of the right side of the animals, good-quality

photographs of whisker spot patterns were available from a total of 20 individuals: eight from Houtman Abrolhos, four from Seal Island, and eight from Carnac Island.

The general whisker spot pattern composition of *N. cinerea* consisted of seven distinct rows of mystacial whisker spots (Rows A–G in Fig. 1), ordered in 9–10 columns (Columns 1–10 in Fig. 1). Some individuals also had whiskers above the eye. Based on 20 individuals, the mode of whisker spot numbers in *N. cinerea* was 40, ranging from 30 to 46, and on average 38.8 (± 3.4 , s.d.). There were 0–2 whiskers above the eye, called superciliary whiskers.

Application of the Chamfer distance transform method for identification

A total of 2884 photographs of *N. cinerea* were taken for individual identification between November 2013 and June 2014 – 1171 on Carnac Island and 1713 photographs on Seal Island, to test the application of spot pattern matching in wild *N. cinerea* based on the Chamfer distance transform algorithm (Osterrieder et al. 2015a). As before, a greater number of individuals were photographed from the right-hand side. Therefore, only photographs taken from this side were considered for matching. A rigorous selection due to the stringent criteria for photographs, to be at 90° without any tilt, for successfully matching captive individuals (Osterrieder et al. 2015a), resulted in a library with 135 photographs of 66 *N. cinerea*. Of these, 25 individuals were photographed on Carnac Island and 41 on Seal Island. Multiple photographs existed for 45 individuals, with up to five photographs for each of these.

Identifying similarity thresholds for matches

Distance scores varied significantly ($Z = 21.8697$, $P < 0.001$) between multiple photographs of the same (confirmed match, number of scores = 332) and different (confirmed non-match, number of scores = 1080) individuals taken on the same day (Fig. 2). Comparison of these distance scores resulted in an approximate 50% probability of a false positive match (a score at which there is an approximately equal probability of the two

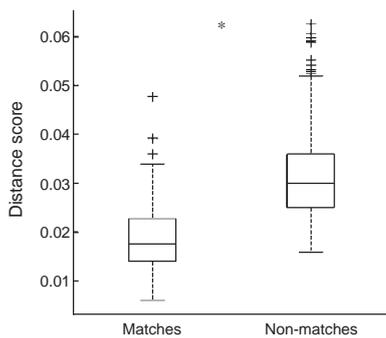


Fig. 2. Box-whisker plots of the distance scores of confirmed ‘matches’ (same individual, no. of scores = 332) and confirmed ‘non-matches’ (different individuals, no. of scores = 1080) of whisker spot patterns photographed on the same day. The median is shown as a black line, 25th and 75th percentiles as vertical boxes, 90th percentiles as range bars, and outliers as black crosses.

whisker spot patterns coming from the same individual as from two different individuals) at a score of 0.02 (Fig. 3). Therefore, all compared photographs with distance scores ≤ 0.02 were manually checked to identify whether they were a correct match and considered to be of a resighted individual.

Variability between photographs of the same individual

Distance scores ($n = 105$) of multiple photographs of the same individual (49 individuals) resulted in an average score of 0.01747 (s.d. = 0.006434). These distance scores varied considerably, also when two matched photographs of the same individual were taken within a few seconds of each other (Fig. 4, higher scores mean they were less similar). The high variability in scores, with several scores exceeding the threshold of 0.02,

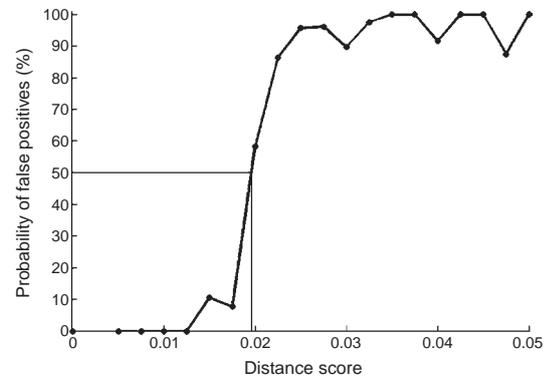


Fig. 3. Probability of confirmed matches and confirmed non-matches based on the distance scores of whisker spot patterns calculated using the Chamfer distance transform algorithm. Distance scores of pattern comparisons are based on multiple photographs of an individual taken on a single day and of known different individuals on the same day ($n = 135$ photographs of 66 individual sea lions).

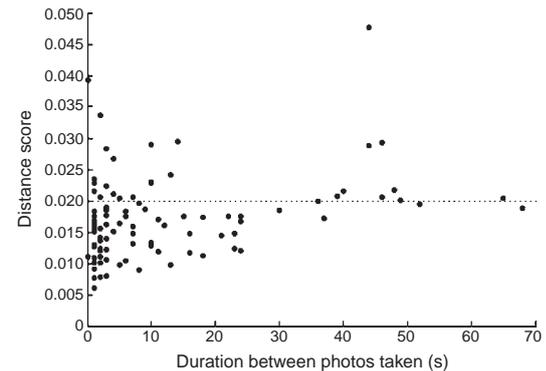


Fig. 4. Distance scores of whisker spot patterns between pairs of photographs of the same individuals taken within 70 s of each other. Ten scores beyond 70 s were excluded from the figure for display purposes (four data points around 150–250 s, four data points around 800 s, and two data points around 1400 s).

occurred up to a time difference of 50 s between tested photographs, after which the sample size was very small and therefore not presented ($n=10$ after 70 s, maximum duration between photographs = 23.48 min). Although taken within a short succession of each other, distance scores of >0.02 between photographs occurred throughout the entire period of 70 s, showing that an individual cannot be identified reliably in the wild (Fig. 4).

Manual verification

Out of the total 66 tested individuals, 128 comparisons involving 38 individuals (unconfirmed matches or unconfirmed non-matches) had distance scores ≤ 0.02 , with up to 19 potential matches (i.e. resightings) per individual. After manually (visually) inspecting these 128 comparisons to verify or reject the match (example in Fig. 5), 82 of the matches with a distance score of ≤ 0.02 were rejected and classed as confirmed 'non-matches'. Rejections were mainly based on the age gap of the individuals matched being impossible (e.g. a bull being resighted as a subadult). There were also a few individuals photographed on the same day and documented as a different individual. Scars large enough to be easily noticed were rarely seen and scars were therefore not helpful for manual verification of potential matches. None of the potential matches, based on their whisker spot patterns, from different days that were visually inspected could be

confirmed as a 'match' (i.e. a resighting). Therefore the remaining 46 matches could neither be confirmed nor rejected as resightings, and were classed as 'unconfirmed' matches (example in Fig. 6). The 10 lowest distance scores ranged from 0.0147 to 0.0162, and resulted in five confirmed non-matches and five unconfirmed matches. The plotted patterns, neither as filled cells in a grid nor as dots on top of each other, supported a clear decision as to whether a *N. cinerea* had been sighted previously (Fig. 6, example of a 'match' and 'non-match' in Figs 7 and 8). The distance scores of rejected matches, i.e. confirmed non-matches, were similar to the scores assigned to unconfirmed matches (Fig. 9) ($Z=0.3358$, $P>0.05$). The confirmed non-matches and unconfirmed matches plotted as cells or spots on a scaled grid proved too variable to decipher the pattern visually to be able to confirm a resighted individual.

Variability between photographs of wet and dry individuals

The library to test the effects of whether the individual had wet or dry fur included photographs of 18 wet individuals, of which nine individuals resulted in 66 distance scores of ≤ 0.02 when compared in pairs. These matches included 14 pairs of both wet individuals and 52 pairs of which one individual was wet and the other dry. Ten of the wet-wet matches could be confirmed as non-matches and four resulted in unconfirmed matches, whereas of the

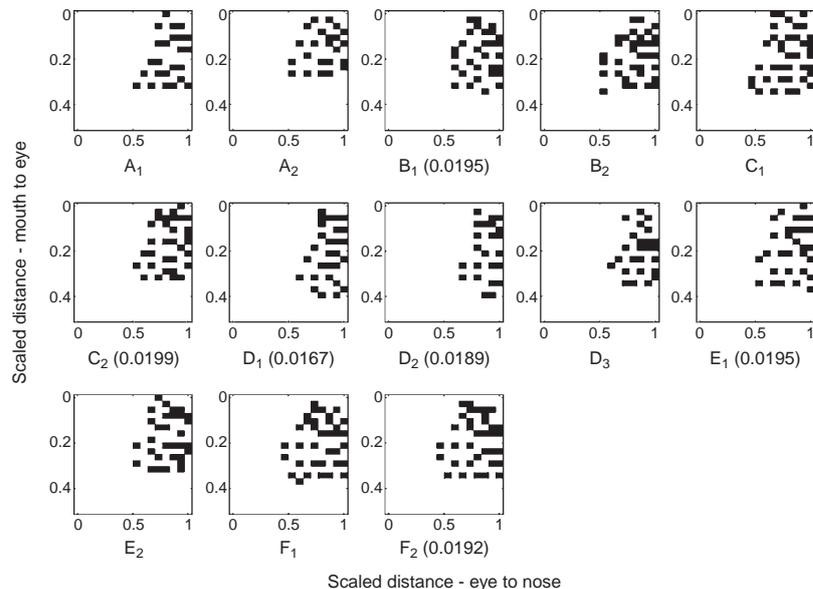


Fig. 5. Standardised whisker spot patterns of Individuals A–F, index numbering the good photographs available for each individual. Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Example of Individual A (bold) pairwise matched with all individuals in the final library. B–F are individuals, each returning at least one spot pattern matched with a distance score ≤ 0.02 (numbers in parentheses show distance scores to A1, the lowest score resulted for the combination of individuals). Panels without distance scores show spot patterns of other photographs available to aid the matching process. Individuals C and E are unconfirmed matches and individuals B, D and F confirmed non-matches with individual A (based on age classes). Only spots between the reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5] are shown.

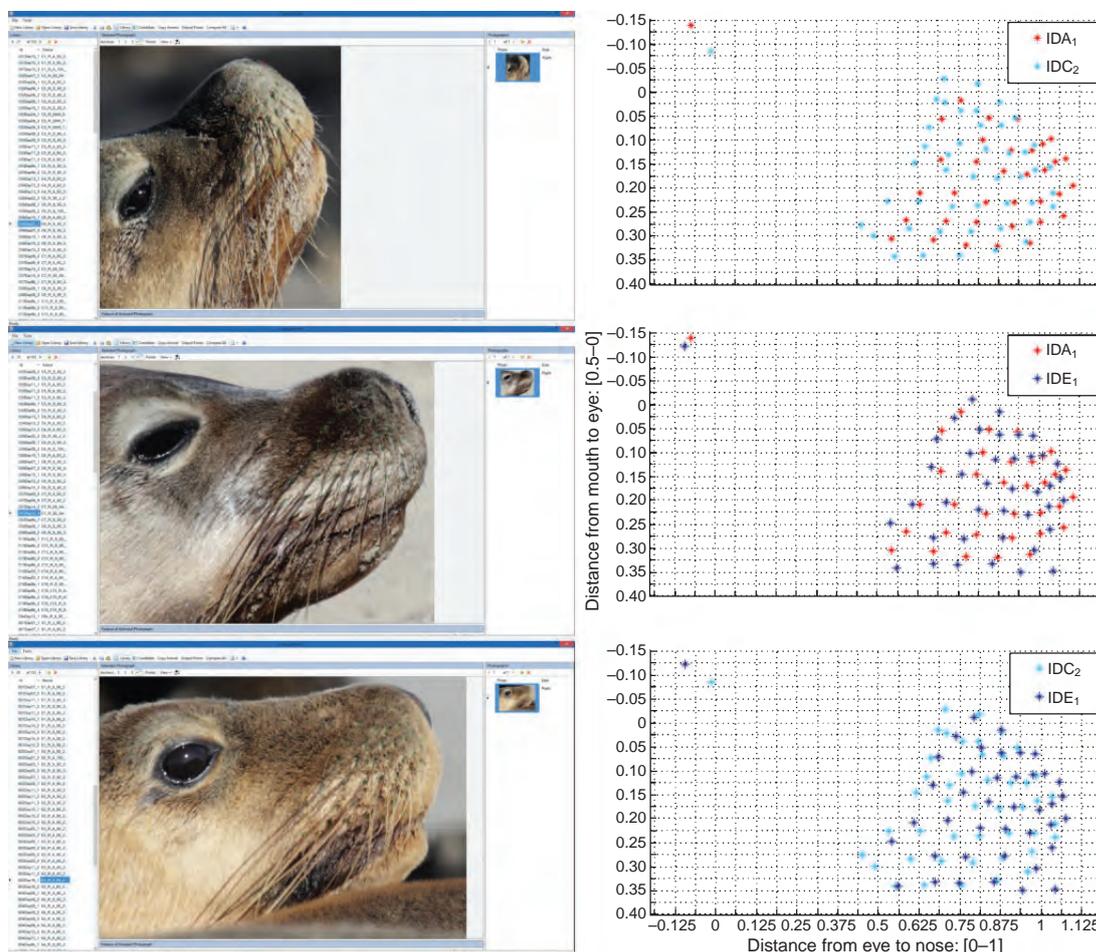


Fig. 6. ‘Unconfirmed’ matches of A1 (top photograph) with C2 (middle photograph) and E1 (bottom photograph) after visual inspection of all potential matches (distance score ≤ 0.02) in the library (Fig. 4). Standardised spot patterns were plotted on top of each other to aid visual comparison of A1 and C2 (top grid) and A1 and E1 (bottom grid), including spots beyond reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5]. Photographs by S. Osterrieder.

wet–dry comparisons, 38 pairs were confirmed non-matches and 14 pairs unconfirmed matches (Fig. 10). Pairwise comparison of dry individuals resulted in 62 matched pairs with distance scores ≤ 0.02 , of which 36 were non-matches and 26 pairs remained unconfirmed matches. The variation of the distance scores was similar amongst each group of comparison of wet–wet ($W = 34.0$, $P > 0.05$, exact distribution method due to small sample size), wet–dry ($Z = 0.1654$, $P > 0.05$) and dry–dry ($Z = 0.5434$, $P > 0.05$) individuals (Fig. 10).

Identification using row–column locations

Of 57 confirmed mystacial whisker spot locations (i.e. at least one of the 20 individuals had a whisker spot present at these

locations), 32 locations occurred in 18–20 individuals (90–100%) (Fig. 11a). The remaining whisker spots were located in the lower and upper column ranges of each row (Fig. 11a). Marking the locations of visual uncertainties of whisker spots’ presence, indicated highest uncertainties of whisker spots occurring towards the lower and upper column ranges of each row, particularly Columns 9 and 10 and Rows A, B and F (Fig. 11b). Of the cells in the mystacial rows and columns, 35% had more than 20% uncertainty, and 40% had more than 10% uncertainty (Fig. 11). Whisker spots occurring in ≥ 5 individuals showed a decreasing trend in uncertainty (i.e. whisker spots occurring in most individuals had least uncertainty). Whisker spots occurring in < 5 individuals had variable uncertainty of a whisker spot’s presence (Fig. 11c). A cross-correlation between all pair-wise

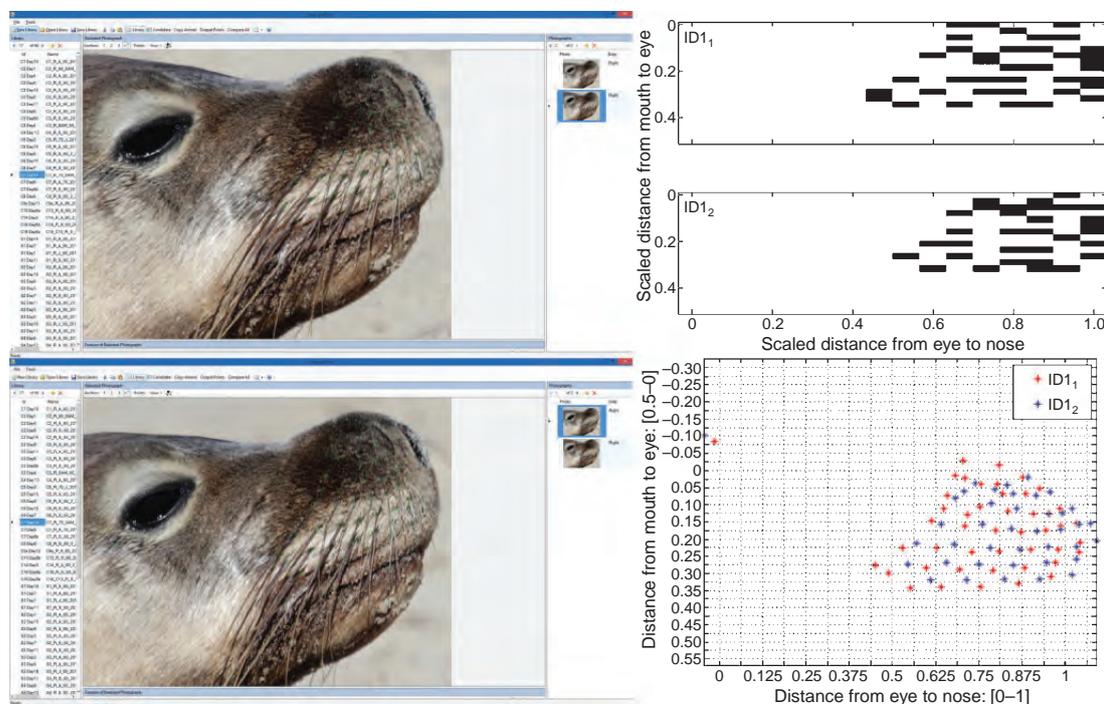


Fig. 7. Example of a confirmed match of two photographs of the same individual taken 1 s apart with a distance score of 0.0182 (top ID11 and bottom ID12, photographs). The standardised marked cells of ID11 and ID12 indicate where whiskers are present on grids overlaid over the muzzle (top right). Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Only spots between the reference points are displayed. The bottom right grid shows the standardised spot patterns of ID11 and ID12 plotted on top of each other, including spots beyond the reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5]. Photographs by S. Osterrieder.

matched individuals resulted in 20 out of 20 individuals having different whisker spot compositions of marked spots from each other. After removing all whisker spot locations with a nominally chosen uncertainty of >10% across all individuals, 12 out of the 20 individuals showed distinct whisker spot compositions based on gridded locations.

Observer variability

There was considerable variation in the number of whisker spots marked by the first author and five additional observers for 10 photographs of nine individual *N. cinerea* when comparing the different observers' marking of the same individual photographs (Figs 12, 13, Table 1). Rematching of the same individual appears unlikely if the two photographs are analysed by different observers (Fig. 13, Table 1). Although ID 8 showed the highest variation in terms of the number of whiskers selected, ID 8 also showed the most consistent marking amongst all observers according to the distance scores, followed by ID 2 (relatively low distance scores) (Fig. 13). IDs 8 and 2 are both wet individuals with black fur colour and light-coloured whiskers and light fur colour and dark whisker spots, respectively (Table 1). ID 1 (another wet individual), followed

by IDs 5 and 6 (both dry individuals), individuals with similar brown-coloured fur, whiskers and whisker spots, showed the highest inconsistency in marking between the observers according to the distance scores (Fig. 13, Table 1). IDs 4, 5 and 6 displayed the greatest range of whisker spot numbers between observers and high standard deviations in the average number of whiskers marked (Fig. 13, Table 1). Row A, followed by Rows B and G, were the most inconsistently marked rows, as were Columns 9 and 10 (Fig. 12), indicating that visibility of the whisker spots was lower than in Rows C–F in the first eight columns.

Up to four eye whiskers were marked on most individuals by at least one observer (except for on one photograph where none were marked by all observers). On one photograph, ID 8, each observer marked 2–3 whiskers above the eye (Table 1). Up to five additionally selected mystacial whisker spots were marked outside of the row/column locations. However, only one of these additional whisker spots was marked by all observers. All other additional whisker spots varied considerably between observers, and usually only one or two observers selected the same additional whisker spot. At times, the shadows of overlapping whiskers were marked, which is why these additional whisker spots were analysed separately.

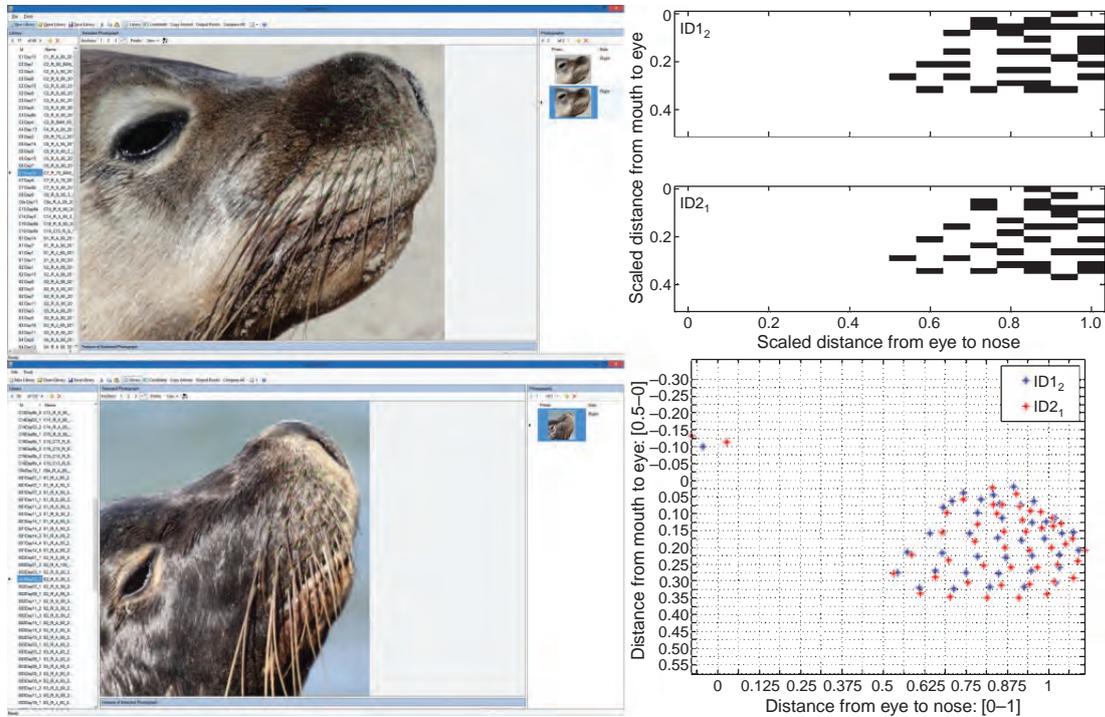


Fig. 8. Example of a confirmed non-match of two photographs of different individuals with a distance score of 0.0188 (top ID12 and bottom ID21 photographs). The standardised marked cells of ID12 and ID21 indicate where whiskers are present on grids overlaid over the muzzle (top right). Black cells represent cells containing whisker spots, empty cells mark the absence of spots. Only spots between the reference points are shown. The bottom right grid shows the standardised spot patterns of ID12 and ID21 plotted on top of each other, including spots beyond reference points of the corner of the inner eye [0,0], nostril [1,0] and edge of mouth [0.5,0.5]. Photographs by S. Osterrieder.

RGB levels of fur and whisker spots

The mean difference in Root-Mean-Squared (RMS) of RGB levels between whisker spots and surrounding fur was significantly greater for spots that were consistently marked by most or all observers (5–6 of the six observers, number of spots = 30) averaging 50.33 (± 30.0 , s.d.) compared with spots identified less consistently, by 1–4 observers, which averaged 26.61 (± 19.8 , s.d.) (number of spots = 30; $Z = 3.1417$, $P < 0.002$).

Discussion

Identification of individuals from their whisker spot patterns was non-trivial. The variation between photographs of potential resightings was similar to the variation between photographs of different individuals. Two techniques were tested to assess performance in correctly resighting individuals; however, under the conditions of this survey, neither exhibited satisfactory confidence in resightings.

Variation between photographs of the same individual, which were taken in short succession, increased with time between photographs. This suggests that movements of *N. cinerea* and the photographer alter the relative orientation between them and

the resulting identified spot pattern, such that rematching was not possible with the Chamfer distance transform method. The reduction in matching performance with time implies that as time passes it becomes more difficult to return to the original relative orientation, possibly also changing light conditions between photographs. Here, time (i.e. duration between photographs) was effectively acting as a proxy for differences in orientation between animal and photographer and the potential differences in lighting conditions between photographs taken in succession. While a direct relationship between these effects and time has not been established, the premise is that as time between photographs increases, so does the likelihood of difference in, for example, orientation, position, muzzle lighting and, in the extreme, subject state (for example, a dry subject photographed entering the water and getting wet before the second photograph, or rolling around covering itself in sand before the second photograph). A reduction in performance was also observed in tests with captive animals, although with a reduction from 90% to 73%, it was not as pronounced as in this study of wild animals (Osterrieder *et al.* 2015a).

Although individual *N. cinerea* showed variation in their whisker spot pattern composition, the numbers of whiskers in

each row and column were less variable than for *P. leo* (Pennycuick and Rudnai 1970). In *P. leo*, the number of whiskers occurring in the top row varied between zero and five, with 13 possible positions compared with the reference row below and thus this variation could be used to identify individuals. Additionally, the use of both sides of the muzzle provided sufficient information for a reliable photo-identification method (Pennycuick and Rudnai 1970). In *U. maritimus*, there was no consistent row for this type of testing (Anderson *et al.* 2007). In this study, there was a subtle difference from findings in *U. maritimus* in that there was no row in *N. cinerea* that exhibited a relatively high variability in occurrence in whisker spot location that could also be consistently marked. Therefore, the reason was

similar to *U. maritimus* in that applying the pattern matching method used in *P. leo* was inapplicable to *N. cinerea*, under the conditions in this study.

When applying the Chamfer distance transform approach to wild individuals, the probability of 50% of the matches being false positives was determined to be 0.02, 33% higher than the 0.015 determined for captive individuals by Osterrieder *et al.* (2015a). Photographs taken in controlled environments therefore showed less variation than when taken in the wild. However, as mentioned above, success rates decreased when matching photographs taken over time of captive *N. cinerea*, showing that repositioning caused differences in spot patterns even in controlled environments (Osterrieder *et al.* 2015a). Orientation of photographer and *N. cinerea* to each other appeared to be too difficult to duplicate with reliable accuracy for matching purposes, both in the wild and in controlled surroundings.

In controlled environments and with trained individuals, 70% of photographs taken on different days were matched correctly using the Chamfer distance transform approach. In comparison, unconfirmed matches and confirmed non-matches of wild individuals were not significantly different in their distance scores. Although only good-quality photographs were used, small changes between photographs in *N. cinerea* position (how they are holding their heads or lying on the sand), different light conditions or slight variations in photograph angle appear to introduce too much variation to enable resightings in the wild.

Observer bias was significant in this study, resulting in rematching even the same photographs unlikely if analysed by different observers. The selection of the exact locations of the reference points, particularly at the end of the mouth, varied considerably among observers, contributing to variation in detected whisker spot patterns when marked by multiple observers. Three of the observers commented that marking spots and determining exact locations of reference points was

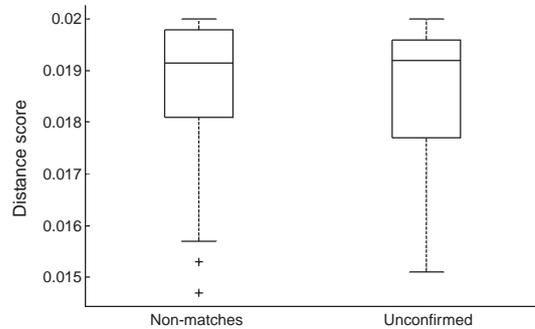


Fig. 9. Box-whisker plots of the distance scores of 'Non-matches' (confirmed rejections) and 'Unconfirmed' (no confirmed resightings or rejections) of whisker spot patterns with distance scores of ≤ 0.02 . The median is shown as a black line, 25th and 75th percentiles as vertical boxes, 90th percentiles as range bars, and outliers as black crosses.

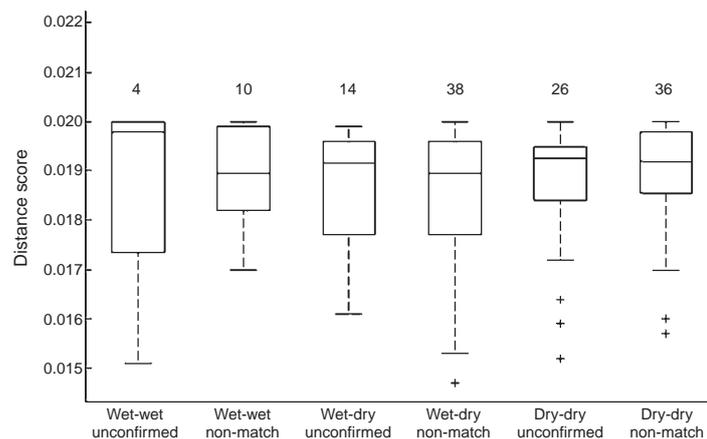


Fig. 10. Box-whisker plots of the distance scores of ≤ 0.02 between whisker spot patterns of wet and dry individuals and their combinations, which resulted in 'Unconfirmed' (no confirmed resightings or rejections) and 'Non-matches' (confirmed rejections). The median is shown as a black line, 25th and 75th percentiles as vertical boxes, 90th percentiles as range bars, and outliers as black crosses. Numbers on top of each box show the number of distance scores for each category.

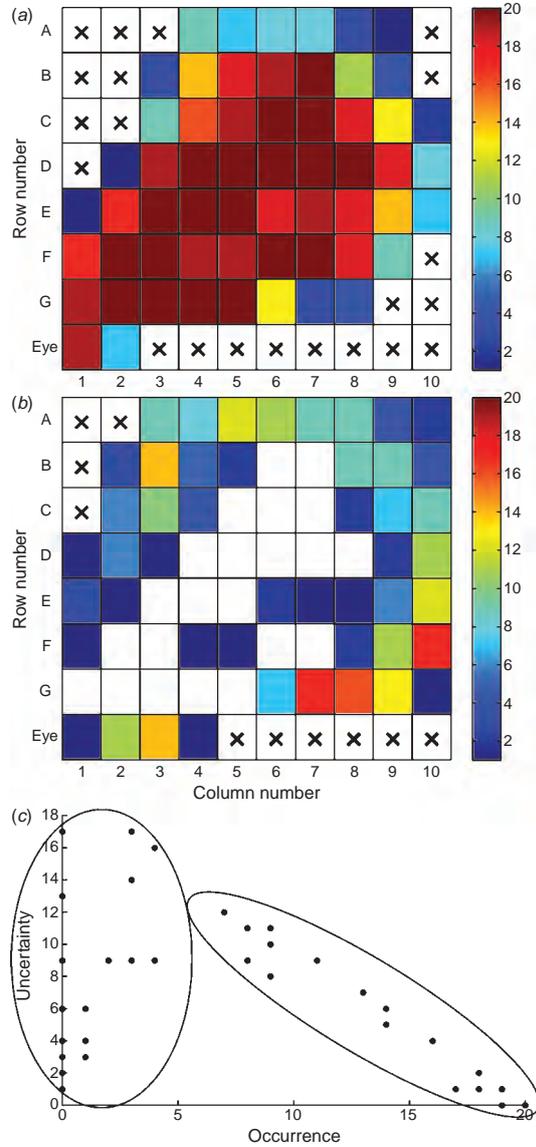


Fig. 11. Top and middle panels: (a) no. of whisker spots' presence based on 20 wild individuals ('x' indicates locations in which whiskers are absent), and (b) uncertainty of whisker spots' presence (white indicates 0 uncertainty, and 'x' indicates locations in which whiskers were absent with 0 uncertainty). The bottom row (Eye) represents the number of whiskers occurring above the eye. Bottom panel: (c) uncertainty of a whisker spot's presence Frequency of mystacial whisker spots occurring (e.g. uncertainty of 10 means that in 10 individuals it was uncertain if a whisker spot was present) as a function of mystacial whisker spots occurring (e.g. an occurrence of 15 means that 15 individuals had this whisker spot) plotted for 20 individuals (excluding superciliary whiskers).

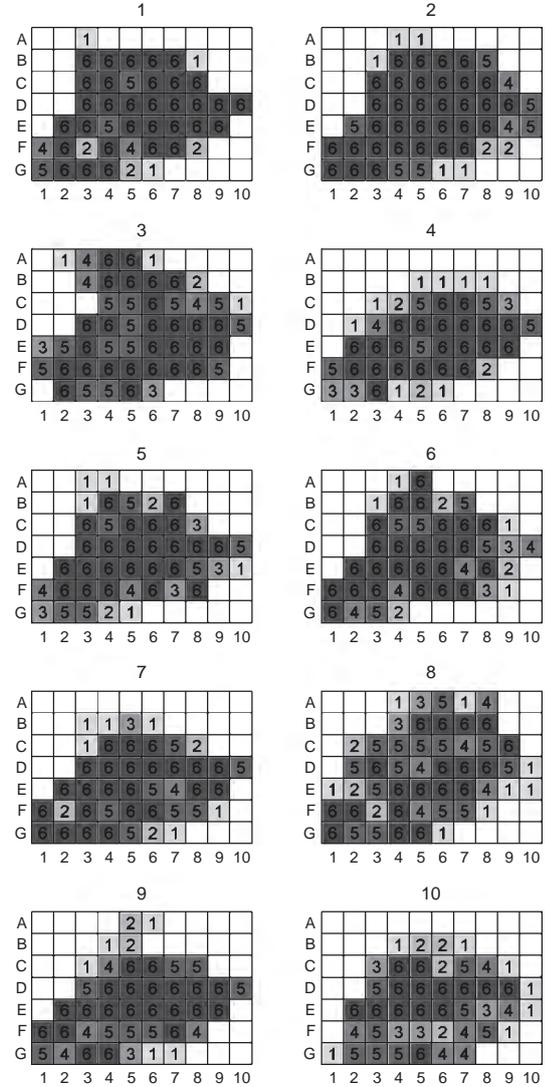


Fig. 12. Number of observers ($n=6$) that marked whisker spots in Rows A–G in Columns 1–10; where 10 whisker spot patterns (IDs 1–10) were marked by the first author and five observers experienced in marine science, and the numbers of selected whisker spot locations summed (i.e. 1: one of the observers marked a whisker spot at this location; 6: all of the observers marked a whisker spot at this location). Note that Panels 4 and 9 were patterns from two different photographs of the same individual.

difficult. Photographs in which *N. cinerea* had closed eyes made the first reference point (the corner of the eye) more difficult to distinguish, and best guesses in these cases may have resulted in inaccuracy in standardisations of the images. Applying the row/column approach, however, showed that the areas of highest

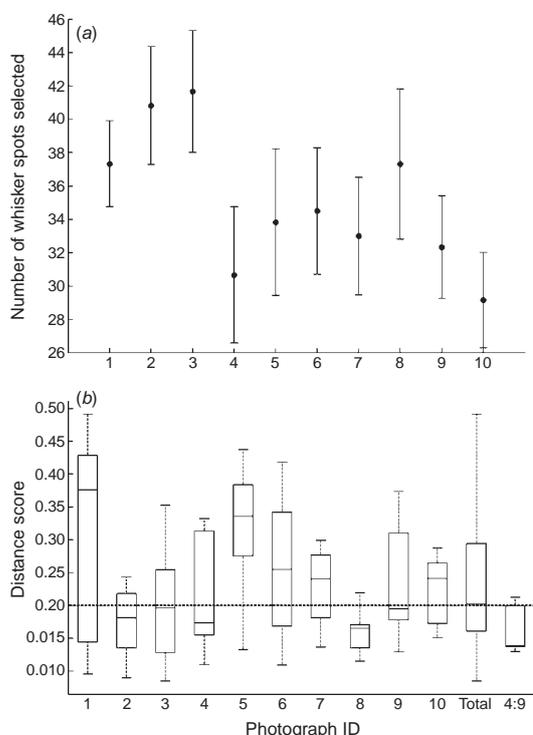


Fig. 13. (a) Average (dot), and standard deviation (lower and upper error bars) of whisker spots marked by six observers on 10 different photographs (IDs 4 and 9 are different photographs from the same individual). (b) Box-whisker plot of distance scores for selected whisker spot patterns by six different observers of the same photographs (IDs 1–10). 'Total' shows the distribution of distance scores from all pairs (IDs 1–10). IDs 4 and 9 are photographs of the same individual, taken on the same day, and '4:9' shows the distribution of distance scores from each observer matching the two photographs of the same individual.

uncertainties detected by the main observer were the same areas where inconsistencies were found between multiple observers. Thus, the difficulty in marking the whisker spots was independent of those found in marking the reference points. Whisker spots located on the periphery of the populated area of the muzzle presented the highest uncertainty of all spots when marked by the main observer. At the same locations, inconsistencies in detections of whisker spots occurred among observers. Several studies have shown variations in photo-identifying individual animals when different observers analysed the photographs (e.g. Friday *et al.* 2000; Anderson *et al.* 2007; Oliveira-Santos *et al.* 2010). Differences may occur when observers are less experienced (Friday *et al.* 2000) or when photographs are poor in quality. In this study all observers were trained scientists and were given detailed instructions for marking, and only good-quality photographs were used. This supports the premise that variation between different individual animals may be too small and even the best photographs too variable to identify *N. cinerea* in the wild in conditions similar to those of this study.

Variability in marking photographs can also occur when the unique mark, or part of it, is inconspicuous or too similar to other marks present (Friday *et al.* 2000; Anderson *et al.* 2007; Oliveira-Santos *et al.* 2010). Comparing the distinctiveness of a whisker spot within the surrounding fur showed that whisker spots selected by most or all observers were more visible on an RGB scale than whisker spots selected by few observers. This association suggests that whisker spots may not have been clearly discernible at certain locations, particularly the mystacial whiskers in the top row, above the top row, and in the columns close to the nostril. Due to the inconsistency in spot detection among observers, Anderson *et al.* (2007) suggested identifying unambiguous spots to ensure consistent selection of whisker spots, in addition to using only high-quality photographs. For methods that use the relative location of these whisker spots taken at slightly different angles does not affect accuracy). However, if the method relies on the presence and absence of spots within rows and columns, removal of inconsistent and uncertain columns and rows for individual identification would reduce the

Table 1. Number of whiskers marked by six different observers on 10 photographs (IDs 1–10)

Range (minimum–maximum), mode, mean (\pm s.d.) as well as the number of whiskers marked above the eye, and additional whiskers marked outside of the row/column arrangement. Characteristics for each *N. cinerea* are given as the fur and whisker spot colour as well as wet or dry condition of the fur and any other notable feature (and whisker colours generally light to dark brown unless stated otherwise). Note that IDs 4 and 9 were two photographs of the same individual

ID	No. of whiskers (range)	No. of whiskers (mode)	No. of whiskers (mean \pm s.d.)	No. of whiskers eye (range)	No. of additional whiskers (range)	Characteristics: fur, whisker spot colour, wet/dry, other
ID 1	35–41	35	37.3 \pm 2.6	0–4	0–5	Dark brown, dark brown/black, wet
ID 2	37–45	38	40.8 \pm 3.5	0–1	0–1	Light brown, dark brown/black, brown, wet
ID 3	37–46	41	41.6 \pm 3.7	0–2	0–3	Dark brown, dark brown/black, dry
ID 4	27–38	27	30.7 \pm 4.1	0–2	0–3	Light to dark brown, dark brown/black, dry
ID 5	28–40	32	33.8 \pm 4.4	0–3	0–2	Brown, light to dark brown, dry, little bit sand
ID 6	30–41	34	34.5 \pm 3.8	0–3	1–5	Brown, light to dark brown, dry
ID 7	31–40	31	33.0 \pm 3.5	0	0–2	Light brown, light to dark brown, dry
ID 8	33–43	43	37.3 \pm 4.5	2–3	0–2	Black, black, wet, white whiskers
ID 9	29–38	31	32.3 \pm 3.1	0–2	0–3	Light to dark brown, dark brown/black, dry
ID 10	26–34	30	29.2 \pm 2.9	0–1	0–2	Light to dark brown, dark brown/black, dry

probability of a whisker spot pattern being unique. The highest individual variability in whisker spot patterns occurred in the areas of highest inconsistency in whisker spot selection (Osterrieder *et al.* 2015a). However, some of this variation among individuals in Osterrieder *et al.* (2015a) may have been caused by inconsistent selection of whisker spots. Nonetheless, most individuals in this study still showed unique patterns based on row/column locations after removing areas of high uncertainties, suggesting that differences in whisker spot patterns do occur in wild *N. cinerea*. There was high variability in uncertainty of spots with low numbers of occurrences, with some whisker spots showing low uncertainty when occurring, and adding to the variation of spot patterns detected. The rate of unique pattern composition, 60% in 20 individuals, however, was lower than found in photo-identification methods in other species, e.g. 98% in 50 individuals in *U. maritimus* and 92% in 25 *P. leo* (Pennycuik and Rudnai 1970; Anderson *et al.* 2007) and while the latter methods differed slightly from this study, the difference in performance is tangible.

The contrast between whisker spots and fur is one of the most obvious differences between *N. cinerea* and *U. maritimus*, *P. leo* and *P. pardus kotiya* (Pennycuik and Rudnai 1970; Miththapala *et al.* 1989; Anderson *et al.* 2007). In *P. leo* and *P. pardus kotiya*, the black whisker spots are highly visible against the contrasting pale fur colour, and the authors of the studies indicated that there was little ambiguity in identifying their presence and absence. With the greater accuracy of selecting presence and location of whisker spots, smaller differences between individual patterns are also easier to detect and greater information can be gleaned from smaller areas. Among individual *N. cinerea* in this study, there was a high variation in fur and whisker spot colours. This study showed that the colouration in *N. cinerea* impacted the consistency of marking whisker spots significantly, with whisker spots with higher colour variability to the surrounding fur being marked by more observers.

The difference in RGB levels between the whisker spots and surrounding fur was calculated conservatively in that only spots that were perceived by the main observer were used. However, whisker spots in *N. cinerea* can also be covered or partly covered by fur, decreasing the area with colour differences available to be detected, especially in those areas with smaller and thinner whiskers (Ling 1977). Different light conditions can obscure whisker spot marking as well by introducing glare or by darkening sections of the muzzle on the photograph. Sand on the muzzle of *N. cinerea* complicated marking as well, but muzzles predominantly covered in sand were excluded from analysis. Due to these difficulties, some spots may have been missed on some photographs and manually marking of spot patterns may have also introduced some variation. Some colour combinations were particularly difficult, specifically in cases where whiskers and whisker spots blended in with the fur colour. The whisker spot to fur colour difference appeared to be more pronounced in wet than in dry individuals, with the whisker spot selection in two out of three wet individuals showing consistency between the observers. The photograph of the third wet individual showed some sun glare in the top rows, contributing to the difficulties of selecting the whisker spots. Although wet individuals were marked with greater consistencies between observers, there was still some marking variation in the

columns towards the nostril as well as in the lower rows. However, distance scores of wet and dry individuals did not differentiate between these more easily marked matches, which could not be ground truthed either.

In harbour seals (*Phoca vitulina*), the use of multiple photographs for each individual in an automated matching process using spot patterns in their fur increased matching success significantly (Hastings *et al.* 2008). It is possible that the use of multiple photographs improves matching for photographs with small variations (for example, minor changes in lighting or shade, environmental conditions or animal–photographer composition). However, in this study, while many individuals had more than one good-quality photograph available, the matching did not appear to be improved with greater numbers of photographs. Furthermore, a larger number of photographs had to be discarded due to the stringent selection required. The low success rate of suitable photographs obtained also presents limitations in this method in the field.

Pinnipeds have well developed whiskers in regards to their length and thickness, and are generally ordered in rows and columns as is typical of mammals (Ling 1966, 1977; Miller 1975; Ahl 1986). The numbers of mystacial whiskers per individual recorded in this study was between values reported from two previous studies, which reported 26 and 44–48 whiskers for *N. cinerea*, respectively (data obtained from unknown sample sizes: Ling 1977, 1992). Only one of the individuals marked by six observers resulted in a mode as low as 27 mystacial whiskers, and 45–46 whiskers were recorded as maximum values in this study. Compared with those of *U. maritimus* and *P. leo*, the whiskers of *N. cinerea* are more pronounced and frequently overlap spots in rows below (Pennycuik and Rudnai 1970; Ling 1977). This further explains the difficulty in selecting some whisker spots in the lower rows – particularly Row G. Two and three superciliary whiskers have previously been recorded, which is consistent with this study although they were detected by only a few observers (Ling 1977, 1992). Similar to the infrequently selected mystacial whiskers, superciliary whiskers often appeared inconspicuous within the surrounding fur and were often missed.

Whisker spots outside the row/column locations add information to patterns, making them more distinctive. However, additional mystacial whisker spots, apart from one on a single individual, were not usually selected by most observers and increased the inconsistency in whisker spot detection. For additional whisker spots to be useful, they need to be distinctive and readily detectable by observers. On some occasions, the shadows of whiskers gave the impression of additional whiskers occurring; and on other occasions, very small and short whiskers growing adjacent to the main whisker identified for that row/column were marked. These detections added to the inconsistency of marking whisker spots. More experience in whisker spot selection with varying colour combinations of fur, whisker spots and whiskers and light conditions could reduce inconsistencies due to colour contrast. However, difficulties in selecting whisker spots on photographs taken under environmental conditions such as obscuring sand, light and glare would still affect consistency, which could perhaps be removed by considering these as poor-quality photographs. This would, however, considerably limit the number of photographs

available, or extend the time required to acquire photographs of sufficient quality.

The use of the Chamfer distance transform approach on spots that can be consistently identified and are of high colour contrast may overcome some limitations experienced in the previous test of its application to wild *N. cinerea*. However, the variability in patterns resulting from a smaller number of spots may potentially limit the method to small colonies. If only consistent spots were used and there was sufficient uniqueness in the relative distances between them, approaches that correct for distortion could have some success. The Groth algorithm has successfully been used for pattern matching in whale sharks (*Rhincodon typus*). It is based on using triangles, matching the dimensions of all possible triangles between selected spots, and may improve matching results between photographs taken with slight angle variations (Groth 1986; Arzoumanian *et al.* 2005). The visual confirmation of potential resightings by either comparing plotted spot patterns alone or by including the whole body was unsuccessful in this study. An alternative method would need to overcome uncertainties of confirming matches or be sufficiently robust so that visual checks would be unnecessary. Other possible discrimination methods could include using other features such as flippers (McConkey 1999; McNally 2001). However, the hind flippers are rarely observed spread out and were deemed unfeasible to be used for photo-identification (by the authors). Hence, this method, previously used in Hooker's sea lions (*Phocarcos hookeri*), was not tested here. It may be possible to test whether a single front flipper contains enough information for photo-identification. In general, the authors have observed that animals spend long periods with their flippers tucked under the body, or buried in sand. Hence, a considerable amount of effort (and time) may be required for *N. cinerea* to move and stretch or lift one of its front flippers into an appropriate position for photographing.

An automated software developed for using the outline of sea lion flippers to match sea lions by their flipper shapes provides a list of the most likely matches (Gope *et al.* 2005). In testing the software, Gope *et al.* (2005) found that 27 incorrect matches were suggested before the correct image on the list was suggested as a match. Thus, the software was reported to support individual identification with required visual double-checking. If confirmations of potential matches by eye were robust and feasible, and there was sufficient information in flipper shapes to apply to large populations, this method may then be more viable than using whisker spot patterns. The whisker spot patterns showed large variation between photographs of the same individual under the conditions and with animals tested in this study.

Acknowledgements

This project was made possible by significant field support by many volunteers. In addition, this project would not have been possible without the substantial logistical support to and from the islands provided by the Department of Parks and Wildlife (Western Australia). The Department of Fisheries and Cockburn Volunteer Sea Search and Rescue also contributed by providing transport to and from islands. Malcom Perry maintained the research vessel, and boat ramp access was provided by the Fremantle Sailing Club. Thanks to Miles Parsons for editing and improving first drafts of the manuscript. Finally, this project was supported by funding from the Australian

Marine Mammal Centre (2012/19) and the Western Australian Government's State NRM Program.

References

- Ahl, A. S. (1986). The role of vibrissae in behavior: a status review. *Veterinary Research Communications* **10**, 245–268. doi:10.1007/BF02213989
- Anderson, C. J. R., Roth, J. D., and Waterman, J. M. (2007). Can whisker spot patterns be used to identify individual polar bears? *Journal of Zoology* **273**, 333–339. doi:10.1111/j.1469-7998.2007.00340.x
- Anderson, C. J. R., Vitoria Lobo, N. d., Roth, J. D., and Waterman, J. M. (2010). Computer-aided photo-identification system with an application to polar bears based on whisker spot patterns. *Journal of Mammalogy* **91**, 1350–1359. doi:10.1644/09-MAMM-A-425.1
- Ardevini, A., Cinque, L., and Sangineto, E. (2008). Identifying elephant photos by multi-curve matching. *Pattern Recognition* **41**, 1867–1877. doi:10.1016/j.patcog.2007.11.010
- Arzoumanian, Z., Holmberg, J., and Norman, B. (2005). An astronomical pattern-matching algorithm for computer-aided identification of whale sharks (*Rhincodon typus*). *Journal of Applied Ecology* **42**, 999–1011. doi:10.1111/j.1365-2664.2005.01117.x
- Beentjes, M. P. (1989). Haul-out patterns, site fidelity and activity budgets of male Hooker's sea lions (*Phocarcos hookeri*) on the New Zealand mainland. *Marine Mammal Science* **5**, 281–297. doi:10.1111/j.1748-7692.1989.tb00341.x
- DEC (2007). Shoalwater Islands Marine Park Management Plan 2007–2017. Department of Parks and Wildlife, Perth.
- DEC, and Fisheries (2011). Shoalwater Islands Marine Park. Marine parks. WA's submerged wonders. Recreation guide. Department of Environment and Conservation & Department of Fisheries, Perth.
- Dixon, D. R. (2003). A non-invasive technique for identifying individual badgers *Meles meles*. *Mammal Review* **33**, 92–94. doi:10.1046/j.1365-2907.2003.00001.x
- Friday, N., Smith, T. D., Stevick, P. T., and Allen, J. (2000). Measurement of photographic quality and individual distinctiveness for the photographic identification of humpback whales, *Megaptera novaeangliae*. *Marine Mammal Science* **16**, 355–374. doi:10.1111/j.1748-7692.2000.tb00930.x
- Gales, N. J., Cheal, A. J., Pobar, G. J., and Williamson, P. (1992). Breeding biology and movements of Australian sea lions, *Neophoca cinerea*, off the west coast of Western Australia. *Wildlife Research* **19**, 405–416. doi:10.1071/WR9920405
- Goldsworthy, S. D. (2015). *Neophoca cinerea*. The IUCN Red List of Threatened Species, Version 2015.2. Available at: www.iucnredlist.org [accessed 5 August 2015].
- Gope, C., Kehtarnavaz, N., Hillman, G., and Würsig, B. (2005). An affine invariant curve matching method for photo-identification of marine mammals. *Pattern Recognition* **38**, 125–132. doi:10.1016/j.patcog.2004.06.005
- Gowans, S., and Whitehead, H. (2001). Photographic identification of northern bottlenose whales (*Hyperoodon ampullatus*): sources of heterogeneity from natural marks. *Marine Mammal Science* **17**, 76–93. doi:10.1111/j.1748-7692.2001.tb00981.x
- Groth, E. J. (1986). A pattern-matching algorithm for two-dimensional coordinate lists. *The Astronomical Journal* **91**, 1244–1248. doi:10.1086/114099
- Hastings, K. K., Hiby, L. A., and Small, R. J. (2008). Evaluation of a computer-assisted photograph-matching system to monitor naturally marked harbor seals at Tugidak Island, Alaska. *Journal of Mammalogy* **89**, 1201–1211. doi:10.1644/07-MAMM-A-151.1
- Hiby, L., and Lovell, P. (1990). Computer aided matching of natural markings: a prototype system for grey seals. *Report of the International Whaling Commission* **12**, 57–61.
- Jefferson, T. A., Webber, M. A., and Pitman, R. L. (2011). 'Marine Mammals of the World: A Comprehensive Guide to Their Identification.' (Academic Press.)

- Kays, R. W., and Patterson, B. D. (2002). Mane variation in African lions and its social correlates. *Canadian Journal of Zoology* **80**, 471–478. doi:10.1139/z02-024
- Kelly, M. J. (2001). Computer-aided photograph matching in studies using individual identification: an example from Serengeti cheetahs. *Journal of Mammalogy* **82**, 440–449. doi:10.1644/1545-1542(2001)082<0440:CAPMIS>2.0.CO;2
- Ling, J. K. (1966). The skin and hair of the southern elephant seal, *Mirounga leonina* (Linn.). I. The facial vibrissae. *Australian Journal of Zoology* **14**, 855–866. doi:10.1071/ZO9660855
- Ling, J. K. (1977). Vibrissae of marine mammals. In 'Functional Anatomy of Marine Mammals. Vol. 3'. (Ed. R. J. Harrison.) pp. 387–415. (Academic Press: London)
- Ling, J. K. (1992). *Neophoca cinerea*. *Mammalian Species* **392**, 1–7.
- McConkey, S. D. (1999). Photographic identification of the New Zealand sea lion: a new technique. *Marine and Freshwater Research* **33**, 63–66. doi:10.1080/00288330.1999.9516857
- McNally, N. C. W. (2001). New Zealand sea lion abundance, demographics and movements in southern New Zealand. University of Otago, Dunedin, New Zealand
- Miller, E. H. (1975). A comparative study of facial expressions of two species of pinnipeds. *Behaviour* **53**, 268–284. doi:10.1163/156853975X00227
- Miththapala, S., Seidensticker, J., Phillips, L. G., Fernando, S. B. U., and Smallwood, J. A. (1989). Identification of individual leopards (*Panthera pardus kotiya*) using spot pattern variation. *Journal of Zoology* **218**, 527–536. doi:10.1111/j.1469-7998.1989.tb04996.x
- Ogutu, J. O., Piepho, H.-P., Dublin, H. T., Reid, R. S., and Bhola, N. (2006). Application of mark–recapture methods to lions: satisfying assumptions by using covariates to explain heterogeneity. *Journal of Zoology* **269**, 161–174.
- Oliveira-Santos, L. G. R., Zucco, C. A., Antunes, P. C., and Crawshaw, P. G. Jr (2010). Is it possible to individually identify mammals with no natural markings using camera-traps? A controlled case-study with lowland tapirs. *Mammalian Biology-Zeitschrift für Säugetierkunde* **75**, 375–378. doi:10.1016/j.mambio.2009.08.005
- Osterrieder, S. K., Salgado Kent, C., Anderson, C. J. R., Parnum, I. M., and Robinson, R. W. (2015a). Whisker spot patterns: a noninvasive method of individual identification of Australian sea lions (*Neophoca cinerea*). *Journal of Mammalogy*. doi:10.1093/jmammal/gyv102
- Osterrieder, S. K., Salgado Kent, C., and Robinson, R. W. (2015b). Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia. *Endangered Species Research* **28**, 259–274. doi:10.3354/esr00690
- Pallottino, F., Menesatti, P., Costa, C., Paglia, P., De Salvador, F. R., and Lolletti, D. (2010). Image analysis techniques for automated hazelnut peeling determination. *Food and Bioprocess Technology* **3**, 155–159. doi:10.1007/s11947-009-0211-1
- Parra, G. J., and Corkeron, P. J. (2001). Feasibility of using photo-identification techniques to study the Irrawaddy dolphin, *Orcaella brevirostris* (Owen in Gray 1866). *Aquatic Mammals* **27**, 45–49.
- Pennycuik, C. J., and Rudnai, J. (1970). A method of identifying individual lions *Panthera leo* with an analysis of the reliability of identification. *Journal of Zoology* **160**, 497–508. doi:10.1111/j.1469-7998.1970.tb03093.x
- Seber, G. A. F. (1982). 'The Estimation of Animal Abundance and Related Parameters.' 2nd edn. (Macmillan Publishing Co.: London.)
- Stevick, P. T., Pallsbøll, P. J., Smith, T. D., Bravington, M. V., and Hammond, P. S. (2001). Errors in identification using natural markings: rates, sources, and effects on capture–recapture estimates of abundance. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1861–1870.
- Troy, S., Middleton, D., and Phelan, J. (1997). Marine mammal research in the Southern Hemisphere. In 'Marine Mammal Research in the Southern Hemisphere. Vol. 1: Status, Ecology and Medicine'. (Eds M. Hindell and C. Kemper.) pp. 179–183. (Surrey Beatty: Devon, UK.)
- Walker, K. A., Trites, A. W., Haulena, M., and Weary, D. M. (2012). A review of the effects of different marking and tagging techniques on marine mammals. *Wildlife Research* **39**, 15–30. doi:10.1071/WR10177
- Whitehead, H. (1990). Computer assisted individual identification of sperm whale flukes. *Report of the International Whaling Commission* **12**, 71–77.
- Würsig, B., and Jefferson, T. A. (1990). Methods of photo-identification for small cetaceans. *Report of the International Whaling Commission* **12**, 43–52.
- Würsig, B., and Würsig, M. (1977). The photographic determination of group size, composition, and stability of coastal porpoises (*Tursiops truncatus*). *Science* **198**, 755–756. doi:10.1126/science.198.4318.755

CHAPTER 4 PUBLICATION



Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia

Sylvia K. Osterrieder^{1,2,*}, Chandra Salgado Kent¹, Randall W. Robinson²

¹Centre for Marine Science and Technology, Curtin University, Bentley, Western Australia 6102, Australia

²Institute for Sustainability and Innovation, College of Engineering and Science, Victoria University, Footscray Park, Victoria 3011, Australia

ABSTRACT: Pinnipeds spend significant time hauled out, and their haul-out behaviour can be dependent on environment and life stage. In Western Australia, male Australian sea lions *Neophoca cinerea* haul out on Perth metropolitan islands, with numbers peaking during aseasonal (~17.4 mo in duration), non-breeding periods. Little is known about daily haul-out patterns and their association with environmental conditions. Such detail is necessary to accurately monitor behavioural patterns and local abundance, ultimately improving long-term conservation management, particularly where, due to lack of availability, typical pup counts are infeasible. Hourly counts of *N. cinerea* were conducted from 08:00 to 16:00 h on Seal and Carnac Islands for 166 d over 2 yr, including 2 peak periods. Generalised additive models were used to determine effects of temporal and environmental factors on *N. cinerea* haul-out numbers. On Seal Island, numbers increased significantly throughout the day during both peak periods, but only did so in the second peak on Carnac. During non-peak periods there were no significant daytime changes. Despite high day-to-day variation, a greater and more stable number of *N. cinerea* hauled out on the significantly smaller beach of Seal Island during 1 peak. Overall, numbers hauled out were associated with temperature and tidal height, but not wind speed. Relative percentages of age classes hauled out also varied with time of breeding cycle. Due to high variability in haul-out behaviour in space and time, and its association with environmental conditions, we conclude that counts for monitoring relative abundance in management decisions should be conducted systematically, using robust survey designs with relatively large sample sizes.

KEY WORDS: Haul-out pattern · Australian sea lion · *Neophoca cinerea*

INTRODUCTION

Hauling out is a behaviour displayed by pinnipeds where animals temporarily leave the water to spend time on land between periods of activity. Hauling out onto land is important for pinnipeds during specific periods of their life cycle, such as breeding and moulting, but most pinniped species also haul out for other reasons. For example, they may travel considerable distances to foraging grounds and may forage at their aerobic dive limits (e.g. Arnould & Hindell 2001, Costa & Gales 2003, Chilvers et al. 2005), thus

hauling out may help conserve energy and contribute to recuperation (Riedman 1990). Pinnipeds may also haul out to avoid predation (LeBoeuf et al. 1982), as shown by an inverse relationship between successful great white shark (*Carcharodon carcharias*) attacks and distance from a haul-out island (Hammerschlag et al. 2006) or increased instances of haul-out behaviour in the presence of killer whales *Orcinus orca* (London et al. 2012). Haul-out sites are also commonly used for social interactions and thermoregulation (e.g. Ling et al. 1974, Marlow 1975, Krieber & Barrette 1984, Riedman 1990).

*Corresponding author: sylvia.osterrieder@gmail.com

© The authors 2015. Open Access under Creative Commons by Attribution Licence. Use, distribution and reproduction are unrestricted. Authors and original publication must be credited.

Publisher: Inter-Research · www.int-res.com

The duration and frequency of hauling out can be specific to species, population or individual. Duration of haul out may be longer during the breeding and pupping season of some pinniped species (e.g. Lake et al. 1997, Southwell 2005), which may be related to variations between female and male haul-out duration and pattern (Thompson et al. 1989, Reder et al. 2003, Southwell 2003). Accordingly, within a population of the same species, age and sex, as well as timing within the breeding, pupping or moulting cycles, can influence haul-out patterns (Thompson et al. 1989, Reder et al. 2003, Bengtson & Cameron 2004).

Previous studies of seals and sea lions have shown considerable differences between species in diurnal and seasonal fluctuations in haul-out numbers (Thompson et al. 1989, Sepúlveda et al. 2001, 2012, Reder et al. 2003). Some phocids and otariids have shown lower numbers in the morning, with peaks in the afternoon (Stirling 1968, Lake et al. 1997, Carlens et al. 2006), though the timing of the peaks can vary between seasons (Lake et al. 1997). Some seals, for example, hauled out more over midday in some seasons, but less at the same time of day during others, such as harbour seals *Phoca vitulina* in Scotland, where there was a greater probability of hauling out around midday in summer (Cunningham et al. 2009). Diurnal haul-out patterns can also vary between locations (Cunningham et al. 2009), a trait observed in South American sea lions *Otaria flavescens* (Rosas et al. 1994, Sepúlveda et al. 2001, 2012).

Several studies have shown that variations in numbers of pinnipeds hauled out can be affected by weather conditions, such as temperature, cloud cover or wind speeds (Schneider & Payne 1983, Watts 1992, Carlens et al. 2006, Andrews-Goff et al. 2010). Additionally, oceanographic factors, such as tidal heights, times or currents may play a significant role in haul-out patterns (Stirling 1968, Pauli & Terhune 1987b, Thompson et al. 1989, Watts 1992). There may also be a combined effect of several environmental conditions combined with time of day and season (e.g. Schneider & Payne 1983, Pauli & Terhune 1987a,b, Thompson et al. 1989, Lake et al. 1997, Reder et al. 2003, Carlens et al. 2006, Mogren et al. 2010).

Australian sea lions *Neophoca cinerea* have been listed as Endangered on the IUCN Red List and are endemic to South and Western Australia (Goldsworthy 2015). *N. cinerea* have an unusual breeding cycle, unique among pinnipeds. Breeding takes place aseasonally on average every 17.4 mo (range: 16.0–19.9 mo) and asynchronously between breeding locations, during a period of 5 to 9 mo (Ling & Walker 1978, Gales et al. 1992, 1994, Higgins 1993, Goldswor-

thy et al. 2008). Haul-out sites in the Perth metropolitan area (Western Australia), approximately 250 km south of the closest breeding islands at Jurien Bay, are known to be used exclusively by males (Gales et al. 1992). Male *N. cinerea* travel significant distances away from breeding colonies, presumably to maximise foraging success and efficiency (Gales et al. 1992). Thus, access to these non-breeding haul-out sites is critical to the health of male sea lions and successful reproduction of the species. Management to reduce human impacts at key haul-out sites where animals rest is often implemented (e.g. Gales 1995, Lovasz et al. 2008), especially in areas of high human visitation such as the Perth metropolitan area. To determine the effectiveness of management guidelines, monitoring of animals using the islands is often undertaken by conducting counts over time (Cassini et al. 2004, Salgado Kent & Crabtree 2008). These counts can be used as estimates of relative abundance on which to base trends. Male numbers at the Perth haul-out islands peak aseasonally, aligned with the non-breeding season (Ling & Walker 1978, Gales et al. 1992, 1994, Higgins 1993, Goldsworthy et al. 2008). While these peaks can be predicted, little is known about the extent and variability in daily use of these islands, or how they vary with environmental conditions. Variation in the proportion of age classes on these islands is also unknown.

Counts are often used for management purposes to monitor numbers of animals hauled-out on islands over time. Based on the resulting counts, measures, such as establishing sanctuary zones in areas used by large number of animals, may be implemented to minimise human disturbance (e.g. Gales 1995, Cassini et al. 2004). However, single counts used as an indicator of numbers of animals using a haul-out site can be subject to significant inaccuracies when there is large day-to-day variability (Southwell 2005). Consequently, information on the extent of use of these islands and its associated variability with environmental conditions is critical for accurately monitoring changes in behaviour and relative abundance and is essential for the long-term conservation management of *N. cinerea* in the region.

To enhance current knowledge and thereby improve future monitoring practices and conservation management of *N. cinerea*, we aimed to identify whether *N. cinerea* had a consistent pattern in daily haul-out behaviour. We recorded haul-out numbers over a 2 yr period on 2 main haul-out islands in the Perth metropolitan area, and assessed whether hauling out was associated with local environmental conditions.

MATERIALS AND METHODS

Study sites

Male *Neophoca cinerea* are known to regularly haul out on 6 islands off the Perth metropolitan coast in Western Australia (WA, Fig. 1). On these 6 male-only haul-out islands, Seal (32.29° S, 115.69° E) and Carnac (32.12° S, 115.66° E) Islands are used by the largest number of *N. cinerea*, with up to 28 and 45 ind. hauled out during the Perth peak season,

respectively (Gales et al. 1992), and were therefore chosen as the field sites for this study. Approximately 15 *N. cinerea* can be found hauled out at Burns Rocks and Little Island combined, mostly less than 10 at Dyer Island during the Perth peak season, and 1 or 2 can be found infrequently on Penguin Island (Department of Parks and Wildlife [DPaW] unpubl. data). It is highly likely that sea lions move between the haul-out islands given their proximity; however, this has rarely been documented (Gales et al. 1992).

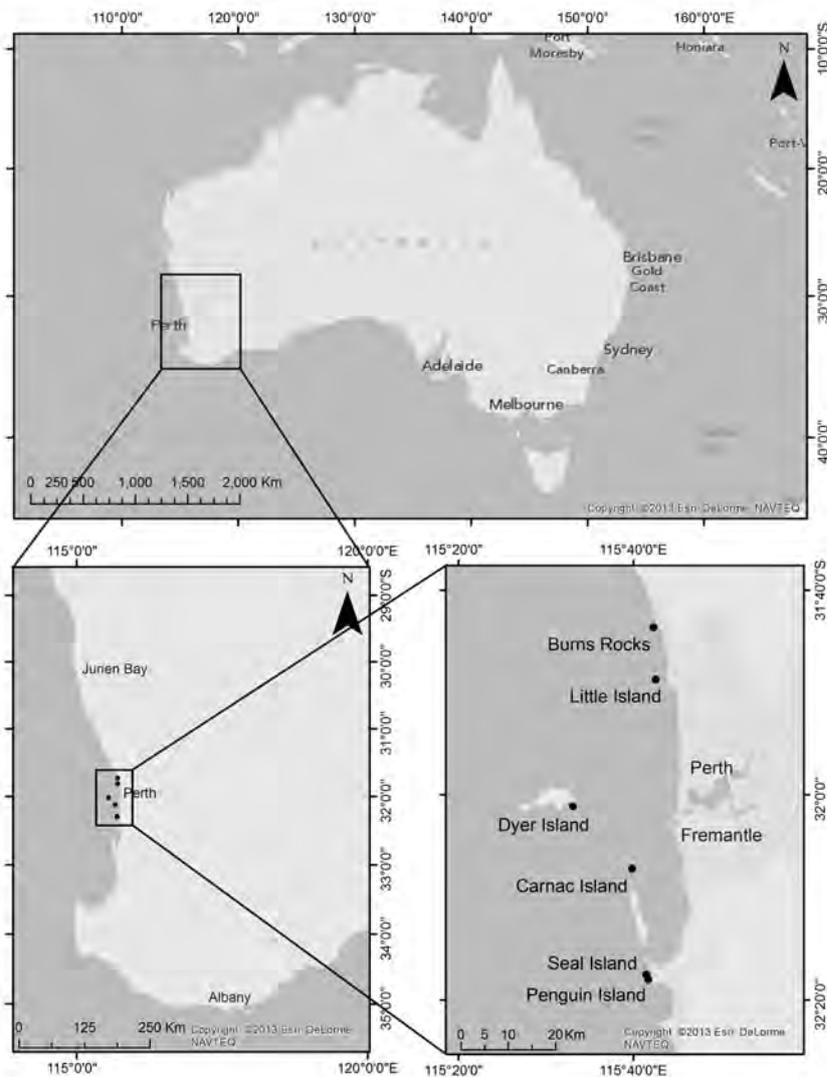


Fig. 1. Haul-out sites of *Neophoca cinerea* in the Perth metropolitan area

Seal Island is located in the Shoalwater Islands Marine Park, approximately 0.9 km offshore from Shoalwater (ca. 45 km south of Perth, capital city of WA, Fig. 1). Seal Island is a sanctuary zone, managed as a 'look, but don't take' area and, as such, landing on the island is prohibited (Department of Environment and Conservation [DEC] 2007, DEC & Fisheries 2011). The island provides a sandy beach of approximately 0.27 ha on the eastern side of the island (estimated from a Google Earth, 2014 image from 1 January 2014) with shrubs at the edges, small rocky overhangs and caves north and south of the beach, as well as along the southern bay. Apart from the beach area, the shores of the island are rocky. *N. cinerea* mainly use the beach and occasionally use adjacent shrubs or caves to haul out.

Carnac Island, approximately 10 km south-west of Fremantle (ca. 15 km south of Perth, Fig. 1) is classified as an A class nature reserve. Access to most of the island is prohibited, but the southern part of the eastern beach is available for public access during the day (Department of Conservation and Land Management [CALM] 2003). *N. cinerea* mainly haul out on the sandy beach of ca. 0.78 ha (estimated from a Google Earth, 2014 image from 1 January 2014) located on the east side of the island. The beach is fringed with shrubs and small bushes. There are some rocks, small rocky overhangs and caves to the north and south of the main beach where *N. cinerea* have been observed occasionally.

Data collection

Neophoca cinerea counts

Counts of *N. cinerea* on the main haul-out beaches of Seal and Carnac Islands were undertaken over 166 d between June 2012 and April 2014. The study was designed so that days on which counts were undertaken occurred approximately once or twice a week over most of the study period; however, on occasion, sampling was less frequent. During each day of counts, all *N. cinerea* within view of the observer were counted hourly, primarily between 08:00 and 16:00 h. *N. cinerea* not in view at the time of counting, but known to be present during the count (i.e. seen when entering a cave as well as when exiting) were also included in the counts. Counts were made every hour, except during inclement weather or when there were technical difficulties that caused some counts to be missed during the day or some days cut short. Counts on Seal Island were conducted over 78 d and on Carnac Island over 88 d.

On Seal Island, the observer was located strategically, where the entire beach was within view, and counts were conducted with the aid of binoculars (Nikon Eagleview 8–24 × 25). At Carnac Island, a live camera (AVT284 IP Camera with remote Pan, Tilt, and Zoom capability and 22× optical zoom), owned and serviced by DPaW, with a radio link to the local office (using a Proxim 8150 PTP microwave radio link), was used. This radio link allowed remote control over the camera's viewing direction and zoom (Salgado Kent & Crabtree 2008). The camera is located on a vantage point overlooking the eastern beach and was panned from north to south along the beach to count the *N. cinerea*. The zoom was used to aid counts when necessary, particularly to distinguish *N. cinerea* from some rocks on the far, southern part of the beach. This method was considered to accurately reflect counts that would have been made at a strategic vantage point at the site, based on the results of a study comparing simultaneous *in situ* beach counts and counts made with a previous model of this camera on the island (Salgado Kent & Crabtree 2008).

There were several cases when these methods were not used, due to logistical constraints (for example, in instances when a vessel was not available to travel to Seal Island or the live camera was not working at Carnac Island). On Seal Island, between July and August 2012 as well as on the 9 October 2012, a vantage point on the mainland (on the coast of Shoalwater, WA) with the entire beach area on Seal Island in view was used. Counts from this location were conducted using either a telescope (115 mm Tasco reflecting with either a 25, 20 or 10 mm eye piece with 36×, 45× or 90× magnification, respectively) or a spotting scope (Televid 77 with 20× to 60× zoom). The vantage point on the mainland (at 32.2855° S, 115.7035° E) was approximately 1.4 km away from Seal Island, and the telescope and spotting scopes were considered to give sufficient magnification to count *N. cinerea* easily and accurately. On Carnac Island, between the end of November 2013 and the end of April 2014 (with the exception of 8 and 13 January 2014), counts were conducted directly from the vantage point where the live camera was located.

During all counts (with the exception of the first 6 mo of the study; i.e. counts before 22 October 2012), animals were visually classified into age groups by S. K. O., either as juvenile, sub-adult, adult or otherwise unknown, following the description of Jefferson et al. (2011). Juvenile males were identified based on their silvery grey backs and light brown or fawn lower fronts. They are also lighter in colour than sub-

adults and adults. Sub-adult males are larger and darker than juveniles and most show a white ring around their eyes which diminishes as they grow into bulls. Bulls are usually dark brown with a light creamy coloured crown. A number of *N. cinerea* present in the Perth metropolitan area are in intermediate stages of becoming either a sub-adult or adult and it can be difficult to distinguish between the age classes. For classification purposes here, younger *N. cinerea*, with undersides darker in colouration than juveniles and showing darker spots, were classed as sub-adults. Distinguishing between sub-adults and adults was based on size and the light coloured crown. If the individual showed a pronounced white eye ring and the light coloured crown was at the initial stages of showing through, it was classified as a sub-adult; however, if a full white crown was visible it was classified as an adult. For most *N. cinerea*, the age classification was straightforward. However, if no decision could be made on an individual *N. cinerea* was obscured sufficiently so that no age class could be determined, the class was recorded as unknown. Counts and age classifications were made by the same observer to avoid observer variability (Udevitz et al. 2005).

Environmental data

Beaufort condition and cloud cover (in percentage) were recorded qualitatively at the time each count was made. Quantitative measures of air temperature, wind speed, wind direction, precipitation and atmospheric pressure at sea level were accessed through the Bureau of Meteorology (BOM) half-hourly weather station measurements made on Garden Island. This station was the closest to the study sites; located between Seal and Carnac Islands at a range of approximately 5.5 and 13.8 km from the islands, respectively. Weather station readings within 30 min from the start time of *N. cinerea* counts were taken to represent those at the time counts were made. Tide levels (from the lowest astronomical tide) at the start of counts were interpolated based on half-hourly tidal measurements from the tidal gauge at Fremantle, WA (provided by BOM). The times in which counts were conducted were recorded using a GPS set to local time (UTC +8) unless the remote-controlled camera was used, in which the current local time was accessed from www.timeanddate.com (which uses Australian Western Standard Time). The times were also expressed as decimal hours since sunrise in the final database by subtracting the time

of sunrise (accessed from www.timeanddate.com) from the local start time of counts (with minutes being divided by 60 to express decimal hours).

Statistical analysis

Modelling

Generalised additive models (GAM) with Poisson distribution and log link functions for count data were used to determine the relationship between temporal and environmental factors and numbers of *N. cinerea* hauled out at the 2 islands. GAMs were chosen to allow the inclusion of smoothers in the model as well as an autocorrelation structure to account for the dependency of subsequent counts in a short time period. Explanatory variables included survey interval (as a factor) with 8 levels (1 to 8), location as a factor with 2 levels (Seal and Carnac Islands), hours since sunrise (in decimal hours) in which the counts were made (as a continuous variable), and temperature, tide level and wind speed (as continuous variables). The entire survey time was split into 8 survey intervals, hereafter called 'periods'. Each period consisted of an average of 83.6 d (varying by a maximum of 3 d), with Period 1 starting on Day 1 of the study and Period 8 starting on Day 586 (corresponding to 6 July 2012 and 12 February 2014, respectively). Periods 1 and 7 were found to be the peak periods for *N. cinerea* counts, and Period 4 fell on the minima of the cycle. As not all intervals within the 17.4 mo breeding cycle of *N. cinerea* could be sampled multiple times, 'period' is treated as a sequential survey interval from 1 to 8 rather than a period relative to the aseasonal cycle. Thus, Periods 1 and 2 and Periods 7 and 8 represented intervals during sequential peak and post-peak periods in the aseasonal cycles, respectively. Each period included between 20 and 26 survey days (across both islands), except Period 6 (October 2013) which had 9 survey days. The autocorrelation structure used decimal days rescaled so that values were relative to the time since sunrise, reported as hours since sunrise from Day 1.

Interaction terms included time since sunrise by location and location by period. Correlation structures tested to account for temporal correlation among counts included autoregressive correlation of order 1 (AR-1), continuous AR(1), and exponential and spherical correlations (available in the R package 'mgcv' used for the GAMs, Wood 2006).

Variance inflation factors (VIF) were used to test for collinearity. All covariates showed VIFs < 3, indicating

no collinearity between covariates (Zuur et al. 2007, 2009). Sea level pressure, however, showed high non-linear correlation with air temperature and was excluded from the analysis to prevent model misfit. Air temperature was chosen as biologically more significant than sea level pressure because air temperature has shown significant influence on haul-out behaviour in previous studies (e.g. Carlens et al. 2006). Precipitation was excluded from the models because of the lack of rainy days and, therefore, the poorly balanced data set. Cloud cover was recorded as a percentage of the whole sky. This was removed from the analysis (to simplify an already complex model) as it was considered of low biological significance, since recorded clouds could have been far away and not impacting *N. cinerea* locally. Wind speed influences the sea conditions the Beaufort scale measures and we included wind speed as the more precise covariate in the model, excluding Beaufort from the analysis. Wind direction was excluded from the analysis to keep the model as simple as possible (and to avoid problems in convergence), since it was considered biologically of less significance than the other variables. The full model thus included air temperature, tide level and wind speed as environmental covariates. Temperature, wind speed and tide level showed some non-linear patterns with period, hence the validity and interpretation of models including these environmental factors were assessed carefully. Three counts lacked temperature and wind speed measurements and were thus removed from the analysis.

Data exploration and model validation

Data exploration was undertaken to identify and remove any outliers or any single exceptionally large or small values that would overly influence the model results, and to check general assumptions of GAMs. Counts from 2 days, Days 38 and 550 (within Periods 1 and 7, respectively) from Carnac Island were excluded from the model to avoid influential data in the analysis. Counts on Day 38 were exceptionally low and were made 2 d after an unusually large storm event. Day 550 had exceptionally high numbers of *N. cinerea*.

The general approach to model construction and validation was to begin with the most complex model, with all effects that were considered to be of relevance to the numbers of *N. cinerea* hauling out based on biological knowledge (Flom & Cassell 2007, Zuur et al. 2009). The model was restricted *a priori* to an acceptable level of complexity, based on a general rule of thumb of at least 20 samples per covariate

level (Harrell 2001), with the exception of Period 6 with 9 sampling days. Period 6 was included in the analysis to avoid a large data gap between Periods 5 and 7, and was interpreted carefully. To validate the model, residuals were plotted against each individual explanatory variable to ensure there were no obvious patterns. To test that the inclusion of a correlation structure accounted for dependency sufficiently with no persisting autocorrelated residuals, normalised residuals were inspected for remaining pattern using variograms (Zuur et al. 2009). Fewer counts were made before 08:00 h and after 16:30 h which appeared to influence the autocorrelation left in the normalised residuals. Therefore, only counts between 08:00 and 16:30 h (which included 0.7 until 10.9 h after sunrise) were included in the models.

Following this first complex model, submodels were created by removing insignificant explanatory terms ($p > 0.05$) with very small estimated variances one by one from the model, starting with the least significant term for model simplification (Wood 2006, Zuur et al. 2009). Each time, the resulting submodel was refitted and re-validated. Submodels were compared, and of these the final model was selected by finding the simplest validated model using Akaike's information criterion (AIC) as a guide. While identifying the simplest model that reduced the AIC by >2 units (Burnham & Anderson 2002) was the initial aim, because environmental variables were related (non-linearly) to period, selection of the lowest AIC reduced by >2 units would have meant removing significant terms from the model. We minimised AIC for model selection, while including all significant terms based on p -values for which to explain phenomena (de Valpine 2014). Smoothers fit to the variable 'time since sunrise' were straight lines, indicating linear relationships between response and explanatory variables, and did not improve the model from one using a non-smoothed 'time since sunrise' term according to the AIC. When adding a smoother to tide level in the final model selected the model could not converge; however, comparisons in previous submodels leading up to the final model fit better with tide as a non-smoothed function. Time since sunrise and tide level were therefore added without smoothing functions.

Finally, there were some submodels that could not be tested, for the simple reason that they did not converge. All submodels, regardless of which insignificant terms had been removed, showed the same explanatory variables as significant, providing confidence in the final model selected.

As a large number of *N. cinerea* used the islands during peak periods (i.e. Periods 1 and 7 in this

study), maximum counts were also compared between islands. Finally, models were not generated for identifying patterns associated with age class since data sets split by age class did not contain sufficient samples. These patterns were, however, investigated using exploratory analyses. The maximum numbers of juveniles, sub-adults, adults and unknowns counted for each day were averaged over each period. Percentages of each class were then calculated from the averages. All analyses and figures were produced using R version 3.1.1 (R Development Core Team 2014) run through RStudio Version 0.98.983 – © 2009-2013 RStudio.

RESULTS

Neophoca cinerea was observed on 163 of 166 survey days. There were 620 hourly counts made over 78 d on Seal Island and 712 counts over 88 d on Carnac Island, totalling 1332 hourly counts. Models were produced using 1227 counts, which covered all survey intervals (603 from Seal Island and 624 from Carnac Island).

The final GAM selected which produced the most parsimonious model followed the form:

$$\log(\mu_i) = \alpha + \beta_1 \times \text{TimeSinceSunrise}_i + \beta_2(\text{Period}_i \times \text{Location}_i) + \beta_3 \times \text{Tide}_i + s(\text{Temperature}_i) + e_i \quad (1)$$

where:

$$e_i = \text{Time} + \varepsilon_i \quad (2)$$

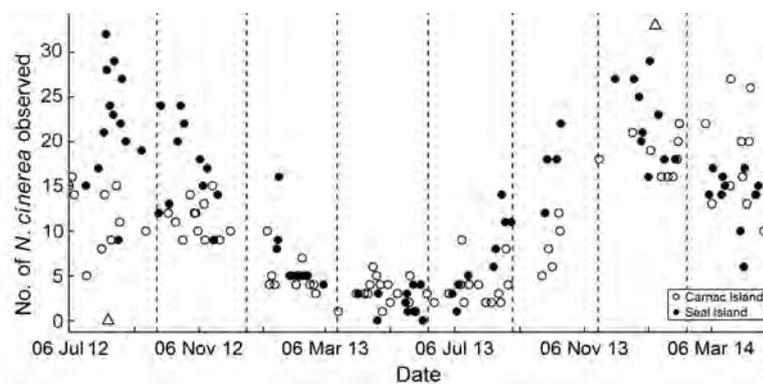
with μ being the number of *N. cinerea* observed at count i , α being the intercept, β the corrections of the slope for each covariate at count i , s the smoothing function, and e_i consisting of the correlation structure indicated by the Time + noise ε (with noise normally distributed with mean 0 and variance σ^2). Time here was expressed as decimal days, rescaled so that

values were relative to the time the sun rises (rather than relative to GMT). A spherical correlation structure resulted in the best fit, and was given by the equation in Pinheiro & Bates (2000), their Table 5.2:

$$\gamma(s, \rho) = 1 - \left[1 - 1.5 \left(\frac{s}{\rho} \right) + 0.5 \left(\frac{s}{\rho} \right)^3 \right] I(s < \rho) \quad (3)$$

with $\gamma(s, \rho)$ as the correlogram with correlation parameters ρ as the range and s as the time, and where function $I(s < \rho)$ denotes a binary variable taking value 1 when $s < \rho$ and 0 otherwise' Pinheiro & Bates (2000, p. 231). The greatest variation in numbers of *N. cinerea* on Seal and Carnac Islands was from the 17.4 mo aseasonal cycle in arrival at and departure of animals from breeding grounds. Peak numbers at both islands occurred between July and August 2012 and December 2013 and January 2014 (Periods 1 and 7, respectively, Fig. 2). The trough in numbers was between April and May 2013 (Period 4). This was reflected in the model by 'Period' being the most influential variable ($p < 0.001$, see Table S1 in the Supplement at www.int-res.com/articles/suppl/n028/p259_supp.pdf). There was a significant difference in numbers of *N. cinerea* hauling out on Seal and Carnac Islands ($p < 0.001$, Table S1), with overall numbers greater at Seal than at Carnac Island (Fig. 2). The interaction between period and location was significant ($p < 0.001$, Table S1), showing that the greater numbers at Seal Island than at Carnac occurred during Periods 1, 2, 5 and 6 (Fig. 2). Numbers of *N. cinerea* present did not show any significant difference between the 2 islands in Periods 3, 4 and 7, but numbers on Carnac Island were greater than on Seal Island in Period 8 (Fig. 2). A small increase with time since sunrise was observed for Carnac Island in the second peak season, but not in the first peak season (Fig. 3). The time since sunrise and its interaction with period significantly influenced the number of *N.*

Fig. 2. Maximum number of *Neophoca cinerea* observed on Carnac (O) and Seal Islands (●) over 166 survey days between June 2012 and April 2014, including the exceptionally high and low observations (Δ) on Carnac Island. Dashed lines mark the survey periods (survey intervals 1 to 8)



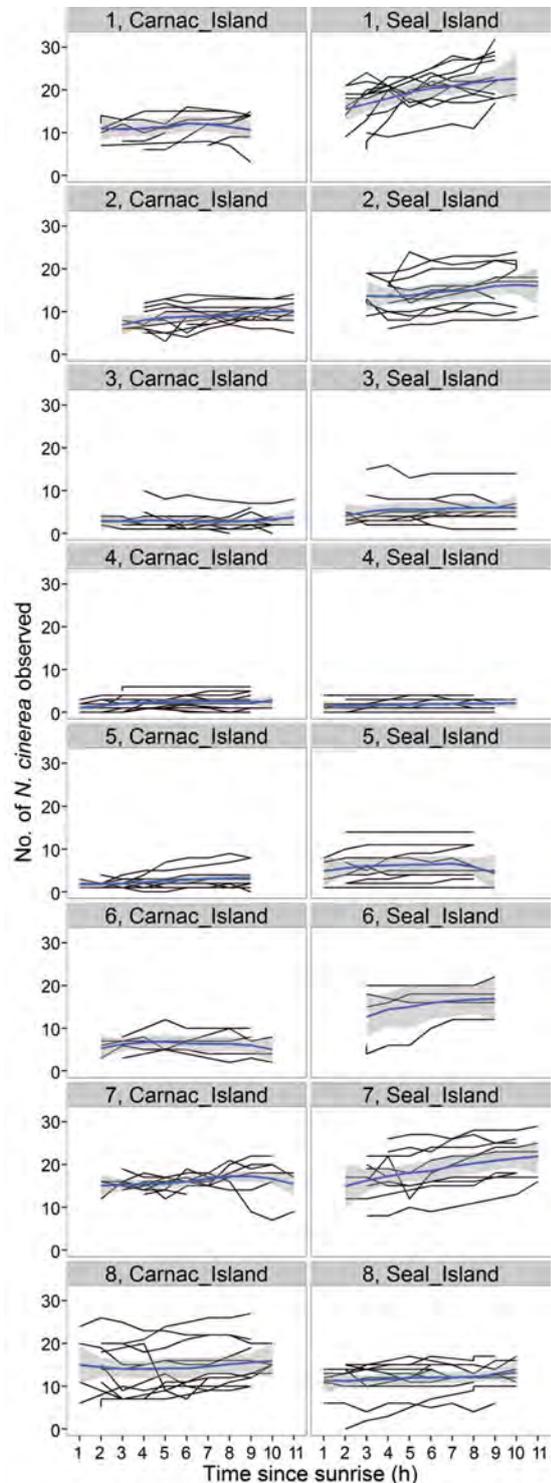
cinerea ($p < 0.001$, Table S1), with increasing numbers of *N. cinerea* hauled out over the course of a day during certain periods (Fig. 3). On Seal Island, this trend occurred during Periods 1, 6 and 7 with a slight increase during Period 2. On Carnac Island, the trend occurred during Period 7 and to a lesser extent during Periods 2 and 8. No increase over the course of a day was observed at either location during Periods 3, 4 and 5, which were the periods with the lowest numbers of *N. cinerea* present. Additionally, no trend was observed during Periods 1 and 6 on Carnac Island (Fig. 3). The interaction term between location and time since sunrise did not show any significant effect on the number of *N. cinerea* hauled out and was thus removed from the final model.

Air temperature and tide level had significant effects on the number of *N. cinerea* hauled out on Seal and Carnac Islands (Figs. 4 & 5). The numbers of *N. cinerea* on the islands decreased with increasing tide level ($p = 0.003$, Table S1, Fig. 5). The pattern was more pronounced on Seal Island than on Carnac Island (Fig. 5). On Carnac Island, a steeper decrease in *N. cinerea* numbers hauled out was only observed when the tide had reached 1.0 m above the lowest astronomical tide (Fig. 5). The numbers of *N. cinerea* increased with air temperature up to approximately 21°C ($p < 0.001$, Table S1), at which point they became comparatively stable. Smoothing function confidence intervals (95%) at temperatures below 15°C and above 27°C were large; thus, interpretation at these temperatures is unreliable (Fig. 4). Wind speed did not have a significant effect on numbers of *N. cinerea* hauled out ($p > 0.1$).

Peak periods

There were 308 counts conducted in the peak periods, 174 on Seal Island and 134 on Carnac Island over 21 and 23 d, respectively. More *N. cinerea* were observed on Seal than on Carnac Island during this period. The maximum numbers counted were 32 and 29 on Seal Island and 16 and 33 on Carnac Island in the first and second peaks, respectively. The maximum numbers of animals hauling out in each peak

Fig. 3. Number of *Neophoca cinerea* observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represents counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The 2 exceptionally high and low observation days on Carnac Island were excluded)



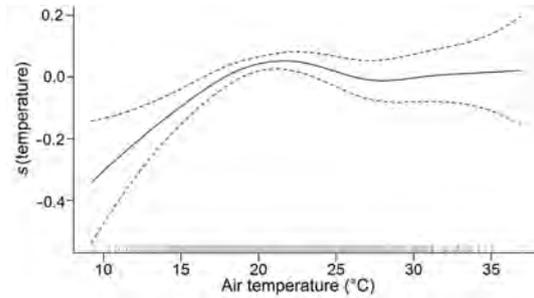


Fig. 4. Smoothing function (solid line) with 95% confidence intervals (dashed lines) fitted to air temperature (°C) estimated in the final generalised additive model (GAM). The small ticks above the x-axis represent air temperature values of the observations

season were counted on Days 35 and 550 on Seal Island and Days 3 and 556 on Carnac Island, and were 515 (16.9 mo) and 553 d (18.3 mo) apart, respectively. However, excluding the highest and most influential count, the highest count was conducted on Day 578 (19.0 mo after Day 3) with 22 ind. counted on Carnac Island. More *N. cinerea* hauled out on Carnac Island in the second than in the first peak season (Fig. 2). There was a high variation in numbers counted on different days in the peak seasons, more so on Seal than on Carnac Island (Figs. 2 & 3).

Age classes

The majority of animals on Seal and Carnac Islands consisted of sub-adults and adults, with a maximum

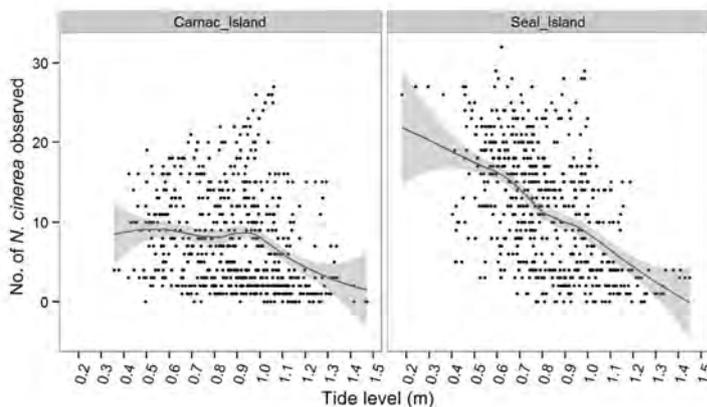


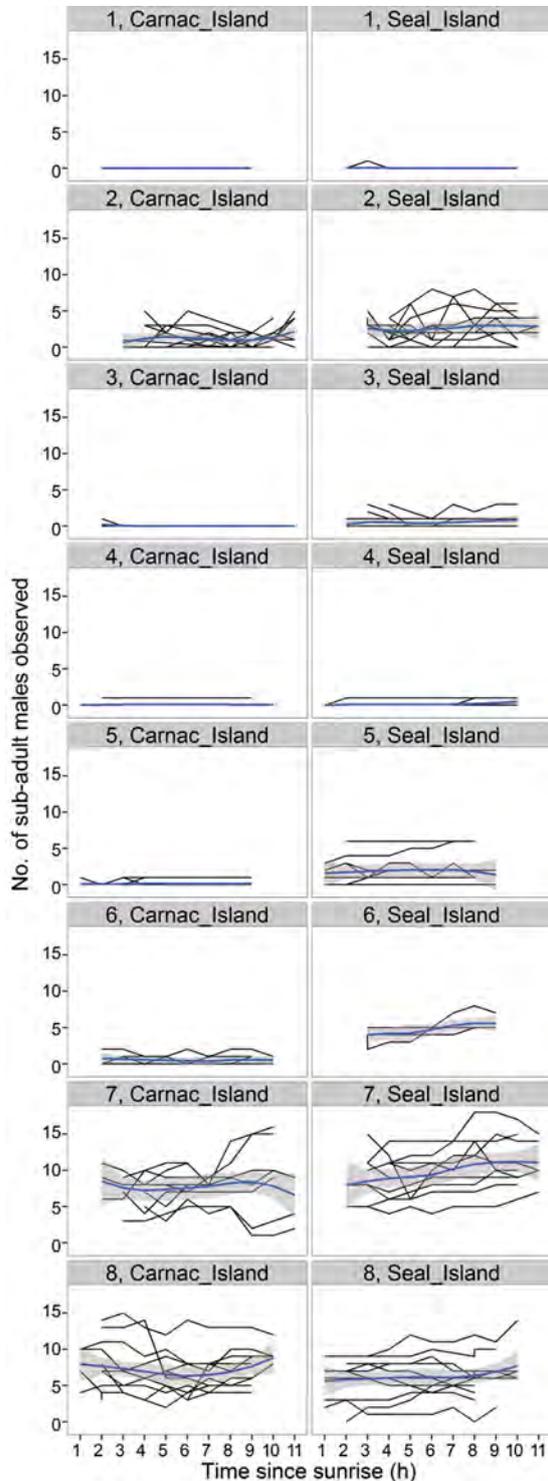
Fig. 5. Relationship between number of *Neophoca cinerea* and tide level (m) observed on Seal and Carnac Islands. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added to aid visual interpretation

of 18 sub-adults and 24 adults hauled out at any one time (Figs. 6 & 7). Juveniles were present on both Seal and Carnac Islands, but were low relative to overall numbers, not exceeding 8 ind. at any time. Juveniles did not show any visible variation in hauled out numbers throughout the day. Sub-adults increased throughout the day during Periods 6 and 7, and slightly increased during Period 8 on Seal Island. A similar increasing pattern was observed during Periods 7 and 8 on Carnac Island. An increase in numbers of *N. cinerea* was evident in Period 7 between 2 and 9 h after sunrise, but numbers decreased between 10 and 12 h after sunrise. However, the variability in these last 3 h of observations was visibly greater. Sub-adults on Carnac Island during Period 8 showed a slight decrease in numbers until 6 h after sunrise, but numbers thereafter increased. With few *N. cinerea* remaining in the area during the non-peak period, a small variation in *N. cinerea* numbers had a large influence in their percentages (indicated by the large standard deviations). Thus, patterns in haul-out behaviour of different age classes could only be detected in plots when *N. cinerea* numbers were high. Adult *N. cinerea* came ashore throughout the day during Periods 2 and 7 on both Seal and Carnac Islands and also during Periods 5 and 8 on Carnac Island. The sample size in Period 6 on Seal Island was too small and the variability of adult counts too high to identify a pattern.

The ratio of adult to sub-adult *N. cinerea* increased at both islands during the breeding season (i.e. the period with few individuals occurring around Perth). During the breeding season (Periods 2 to 6), the period-averaged, maximum daily percentage (\pm SD) of sub-adults on the islands ranged from 2% (\pm 8) to 32% (\pm 34), compared with the percentage of adults which ranged from 47% (\pm 23) to 92% (\pm 49) (Table S2 in the Supplement). When numbers of *N. cinerea* in the area increased, however, the ratio of adults to sub-adults was closer to parity, with adults ranging from 43% (\pm 26) to 47% (\pm 24) and sub-adults ranging from 49% (\pm 17) to 52% (\pm 20) (Table S2, Fig. 8).

DISCUSSION

Numbers of male *Neophoca cinerea* hauling out in Perth metropolitan waters display aseasonal cycles in



abundance, varying according to the approximately 17.4 mo breeding cycle of the species. Sampling period, in relation to the breeding season, was the most influential variable on numbers of *N. cinerea* hauled out, similar to other sea lion species (Sepúlveda et al. 2001, Marcotte 2006). Results here were similar to those of Gales et al. (1992) in that overall *N. cinerea* numbers on Perth metropolitan haul-out islands followed a 17 to 18 mo cycle, with troughs in Perth aligning with the peaks on breeding islands to the north (Jurien Bay).

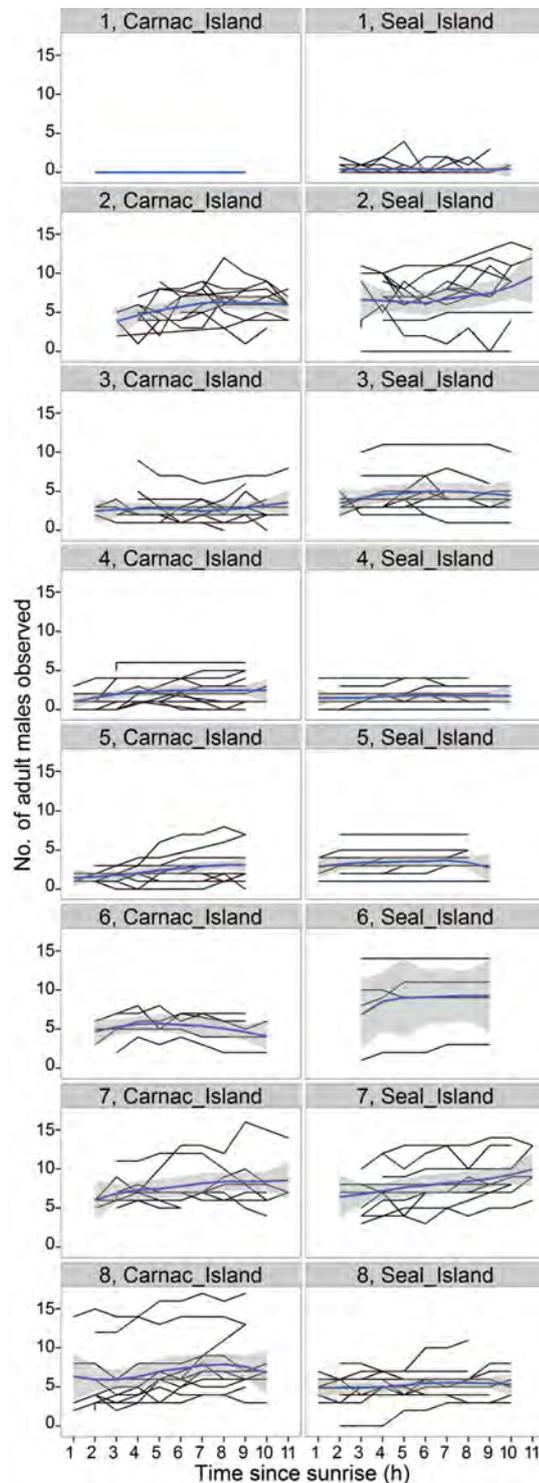
The numbers of hauled out *N. cinerea* at their peaks varied between peaks and islands; with greater numbers throughout all of the first peak and during part of the second peak at Seal Island than at Carnac Island. There are many possible reasons for these differences. While a larger beach size, potentially allowing more sea lions to haul out, has been suggested (Krieger & Barrette 1984), beach area available appeared to be unrelated in this study. The beach area on Carnac Island was estimated to be approximately 3 times larger than that at Seal Island. The intertidal region where animals hauled out at Carnac Island appeared to be equally as large as that on Seal Island. We suggest that other drivers, such as proximity to preferred foraging locations, influenced haul-out site choice. If there are shifting prey locations, we suggest that this may be reflected by changes in haul-out site selection. There is evidence of this behaviour in other pinnipeds, such as Steller sea lions *Eumetopias jubatus* in Alaska, suggested to depart to follow herring spawn and eulachon runs (Marcotte 2006), and Californian sea lions *Zalophus californianus* in California, responding to prey abundance (Ainley et al. 1982). Since breeding, and the resulting peak numbers in the Perth metropolitan area, follow a 17 to 18 mo cycle, successive peaks in the Perth area occur in different seasons. In this study, the first peak occurred in winter, and the second in summer. A seasonal change in targeted prey location is possible, and therefore a change in foraging location for the same prey or a change in target prey species in different seasons could be expected (Lowry et al. 1991, Sinclair & Zeppelin 2002, Sigler et

Fig. 6. Number of sub-adult male *Neophoca cinerea* observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represents counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The 2 exceptionally high and low observation days on Carnac Island were excluded)

al. 2004, Womble et al. 2005, 2009, Winter et al. 2009). Such instances could have resulted in Carnac Island being relatively less favourable during the second peak than the first peak. A second plausible explanation is that the numbers using the islands are directly related to human use reflected by the differing management regulations at the 2 islands. Carnac Island has direct access for recreational use on most of the beach, and despite the presence of a sanctuary zone on a section of the beach, the entire beach is used for recreation. At Seal Island, recreational users are completely and effectively restricted from landing on any part of the island, including the beach.

In the present study, the percentage of adults to sub-adults increased at both islands from close to parity to above 90%, as the abundance of *N. cinerea* dropped from peak to trough in the cycle. A range of age class distributions have been observed among pinniped species at haul-out and breeding sites elsewhere. At a Steller sea lion (*E. jubatus*) breeding island in Southeast Alaska, for example, more bulls than sub-adults hauled out consistently across the survey period (Marcotte 2006). In contrast, sub-adult Subantarctic fur seals *Arctocephalus tropicalis* dominate during most of the year at a haul-out site where no breeding occurs as well as at a breeding site on the same island during the non-breeding season (at Marion Island, close to the Antarctic Convergence; Kerley 1983). Results that are more similar to those in this study were observed for *A. tropicalis*, with a sub-adult to adult ratio of approximately 5 to 3 at a haul-out site with occasional breeding on Amsterdam Island (6% females, Roux & Hes 1984). In the current study, the changes in the composition of different age classes were consistent between the 2 islands and appeared to follow the timing of the breeding season. It is not known if age classes depart at different times from the breeding sites in this species; however, staggered departures have been observed between females and pups in *E. jubatus* (Marcotte 2006). We suggest that observations here are likely a result of the following premise: as young males grow older, they increasingly travel longer distances and stay away for longer periods (Goldsworthy et al. 2009).

Fig. 7. Number of adult male *Neophoca cinerea* observed during hourly counts on Carnac and Seal Islands during survey Periods 1 to 8. Each line represents counts conducted on a single sampling day represented in hours since sunrise. A LOESS smoother (blue line) with 95% confidence intervals (grey) has been added as a visual aid. (The 2 exceptionally high and low observation days on Carnac Island were excluded)



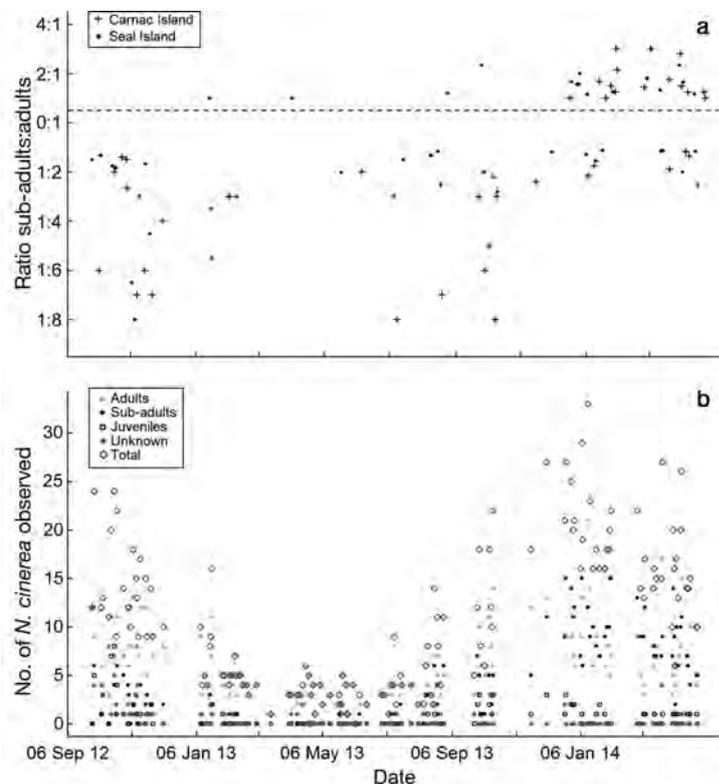


Fig. 8. Age classes of *Neophoca cinerea* observed at the time of maximum count on 166 days on Carnac and Seal Island between September 2012 and April 2014 (after the first non-breeding season, i.e. high numbers in Perth). (a) Ratio of sub-adults to adults on Carnac (black crosses) and Seal (diamonds) Islands. The dashed horizontal line marks the ratio of sub-adults to adults at 1:1. Three data points are missing due to zero sub-adults or adults present, and no ratio could be calculated. (b) Number of *N. cinerea* observed in each age classes: (grey triangles) adults; (black, solid circles) sub-adults; (white-filled circles) juveniles; (black asterisks) unknown

Day-to-day haul-out numbers of *N. cinerea* on Seal and Carnac Islands often fluctuated considerably, similar to South American *Otaria flavescens*, *E. jubatus* and Hooker's *Phocarctos hookeri* sea lions (Beentjes 1989, Rosas et al. 1994, Kucey 2005). The positive relationship between numbers of hauled out *N. cinerea* and time since sunrise each day, observed during periods of high numbers of *N. cinerea* in the study area, is not uncommon. Hooker's sea lions *P. hookeri* on the Otago Peninsula in New Zealand were found to increasingly arrive ashore mid-morning, with numbers reaching a plateau at midday before departing again around 18:00 h (Beentjes 1989). Similarly, studies of other otariids, of varying sex and age class, have

also shown a pattern of increasing numbers hauling out throughout the day, until mid-afternoon or early evening, when these numbers began to decrease (Stirling 1968, Harestad 1978, Ainley et al. 1982, Sepúlveda et al. 2001, 2012). On Carnac Island, this pattern was less prominent, and only occurred significantly during the second non-breeding season, where there were overall greater numbers of animals hauling out.

During a study of *N. cinerea* at Dangerous Reef in South Australia, animals mostly arrived and hauled out between 05:00 and 07:00 h, and departed between 18:00 and 20:00 h to forage mainly at night (Goldsworthy et al. 2009). The study, however, was on lactating adult females, rather than males. While there are variations in behaviour among species, there are also variations within different populations of the same species. This has been shown to be true for Steller sea lions *E. jubatus* which displayed no evidence of a diurnal pattern in hauling out at one site (Kucey 2005, Marcotte 2006), while Harestad (1978) indicated a clear diurnal pattern, dissimilar from the Perth findings, at another. Furthermore, *N. cinerea* in this study represent a unique cohort of juvenile, sub-adult, and adult males, reflecting the unique haul-out patterns observed. Adult male *N. cinerea* are known to forage further offshore, spend longer periods at sea, and have higher variations among individuals in distances travelled than other age and sex classes (Goldsworthy et al. 2009). The large number of adult males at Carnac and Seal Islands likely influenced the arrival times, as *N. cinerea* travelling longer distances may return later in the day. Conversely, juvenile foraging behaviour is reportedly similar to the more restricted ranges of adult females, compared to the more distant and longer duration foraging by adult males (Goldsworthy et al. 2009). The low numbers of juveniles counted on Seal and Carnac Islands suggests that daily patterns in behaviour would be mostly due to sub-adults and bulls using the islands.

While the present study showed similarities and contrasts to findings elsewhere, studies referred to

here have reported haul-out timing in relation to local time rather than relative to the time of sunrise (e.g. Stirling 1968, Sepúlveda et al. 2001, Carlens et al. 2006). We suggest that by reporting haul-out patterns relative to sunrise and sunset rather than relative to GMT, studies will be more comparable and meaningful in terms of their biological significance. Circadian rhythms of wild animals are more closely related to daily solar patterns and seasonal changes than our clocks (Reebs 2002, McCauley 2012). Also, we note, that in this study, logistical constraints limited observations to before 07:00 h and after 17:00 h. It is possible that numbers at Seal and Carnac Island decrease at dusk or later, as has been observed for *N. cinerea* in South Australia (Goldsworthy et al. 2009). *P. hookeri* and *O. flavescens* have also been reported to depart haul-out islands in the evening, likely to forage (Beentjes 1989, Sepúlveda et al. 2001, 2012). Alternatively, numbers present at Seal and Carnac Islands later in the day after observations had been made could have remained constant if diurnal foraging patterns are absent, such as reported by Costa & Gales (2003) for female *N. cinerea* on Kangaroo Island, South Australia. The few counts conducted in this study before 07:00 h did not indicate a spike in numbers of *N. cinerea* returning ashore just after sunrise as reported by Goldsworthy et al. (2009).

During the breeding season, when overall numbers of *N. cinerea* were low, there was no distinguishable diurnal pattern. This was likely due to either too few numbers of *N. cinerea* present to detect a relatively small effect, or highly variable foraging patterns (and thus arrival times) of adult males.

In our study, numbers of *N. cinerea* hauled out increased with increasing temperature up to 21°C. This association has also been observed in harbour seals *Phoca vitulina* and Weddell seals *Leptonychotes weddellii* (Watts 1992, 1996, Andrews-Goff et al. 2010). In winter months, the local waters around Perth can drop to below 16°C (BOM). Immersion in water below 17°C is energetically costly and may stop cell growth in Steller sea lions (Feltz & Fay 1966). Thus, for winter months, temperature could be expected to be a contributing factor to haul-out patterns as it benefits cell growth and recovery. Hauling out also conserves energy, reducing heat loss by spending less time in a high temperature-conducting medium, such as water (Riedman 1990). When temperature on land increases, however, pinnipeds may return to water to support thermoregulation, which may explain why there were no further increases in association beyond 21°C. However, the effect of temperature on seals' diurnal haul-

out patterns has also been observed to vary at different times of the year in *P. vitulina* in Scotland and in captive harp seals *Pagophilus groenlandicus* (Grellier et al. 1996, Watts 1996, Moulton et al. 2000).

N. cinerea decreased in numbers in the present study when tidal height increased. While this pattern was similar to that reported for Steller sea lions *E. jubatus* on islands off Alaska, tide level did not have a significant influence at other locations in Alaska (Kastelein & Weltz 1991, Kucey 2005). Tidal heights have been reported to have variable effects in different seasons in *P. vitulina* (Reder et al. 2003), and other pinnipeds, regardless of season (Thompson et al. 1989). In *E. jubatus*, more adult males entered the water during low tides, despite the tide level having little to no impact on their preferred haul-out location (Kastelein & Weltz 1991). The variation observed in other studies is comparable to that seen in *N. cinerea* in the present study, where tide level was more influential on Seal Island than on Carnac Island. While Seal Island was, overall, a preferred site over the larger beach of Carnac Island, the decrease in the number of *N. cinerea* hauling out became more prominent when tidal heights were above 1.0 m. This increase in tide would have greater impact on available intertidal and overall beach area on Seal Island than Carnac Island because of the wider beach on Carnac Island. Variation in tidal heights in this study was small compared to the 4 m tidal heights that impact some *P. vitulina* haul-out sites. Where higher proportions of *N. cinerea* haul out and tides above 4 m constrain the available size of the haul-out sites, numbers hauled out reduce (Watts 1993). Extremely high tides, combined with strong winds, can push the water over the whole beach on Seal Island; however, a large proportion of the beach on Seal Island does not appear to be impacted during typical high tides, suggesting that the association between tidal height and numbers hauled out may not be simplistic. It is not only beach availability that is affected by lunar variation, however. Localised movement of fish species in relation to tides and lunar phase have been documented in the Perth metropolitan area (Wakefield 2010), which may also be true for prey species of *N. cinerea*. Some prey species may be influenced by tide and may become easier to target during low tide (Morrison et al. 2002, Ribeiro et al. 2006), implying that *N. cinerea* might follow their food source during a time when it is easier to catch, leaving lower numbers on the haul-out islands during lower tidal heights.

Wind speed did not have a significant effect on the numbers of *N. cinerea* hauling out in this study, similar to the case for *P. vitulina* in Scotland and

Norway (Grellier et al. 1996, Reder et al. 2003), although it has been observed to affect diurnal haul-out patterns of other pinniped species (e.g. Lake et al. 1997, Sato et al. 2003). One sampling day on Carnac Island was removed from the analysis because of the unusually low number (zero) of *N. cinerea* hauling out during the peak season. Within 3 d prior to this count, a storm including time-averaged winds of up to 54 km h⁻¹ and heavy rains with up to 10.2 mm d⁻¹ precipitation passed Carnac Island and may have affected the *N. cinerea* haul-out pattern. Extreme environmental conditions have been shown to alter sea lion behaviour, for example in *E. jubatus* during stormy weather (Kenyon & Rice 1961) or *O. flavescens* after an earthquake and tsunami (Sepúlveda et al. 2012).

Finally, different methods of sampling can often bias count data. It is unlikely that the different sampling methods used here would have caused significant variations in count numbers in this type of study (Balouin et al. 2014). Salgado Kent & Crabtree (2008) have previously shown that the remotely controlled camera on Carnac Island does not produce significantly different counts to those made by an observer on the island. Though considered infrequent, individuals may have remained undetected during the few surveys when counts were conducted from the vantage point at Shoalwater. On 1 occasion, rangers aboard a DPaW vessel, near Seal Island, conducted a count on Seal Island at the same time as counts were conducted from the Shoalwater vantage point. The authors observed 19 of the 21 observed from the vessel. Two were hidden from view from the vantage point.

While sub-models resulting in the same significant explanatory variables provided confidence in the final model selected, none of the sub-models accounted for absolutely all of the autocorrelation in the residuals. A smoother through numbers of *N. cinerea* observed versus normalised residuals still explained approximately 7.6% of the variation remaining in the residuals. Nevertheless, despite modelling constraints and convergence problems experienced in modelling these complex, longitudinal data, the models provided an improvement in our current knowledge of *N. cinerea*, which is needed for management and conservation.

The variability in numbers of *N. cinerea* hauling out at Carnac and Seal Islands within a day can affect the accuracy of trend in relative abundance over time if counts are undertaken at different times each day. For monitoring trends in relative abundance, counts would be best conducted between 9 and 11 h after sunrise if this is logistically possible. If this is not pos-

sible, a similar time of day across all survey days (in relation to the time of sunrise) should be targeted, so that they are comparable between sites and years. Conducting surveys only during periods of comparable temperature and tidal conditions, in addition to comparable times, would be logistically highly restrictive, resulting in a very small sample size. We have therefore suggested maintaining consistency in the most influential variable, the time of day. However, temperature and tidal heights can be recorded so that relative abundance can be adjusted using a correction factor to improve comparability over time (Seber 1986, Huber et al. 2001). Through the systematic collection of count data during periods when haul-out behaviour is expected to be comparable, more accurate trend estimations can be obtained to improve management outcomes.

Acknowledgements. This project was made possible with the significant support provided by the Department of Parks and Wildlife (Western Australia) who made the spotting scope available to use, provided substantial logistical support to and from islands, as well as local and scientific knowledge. The Department of Fisheries and Cockburn Volunteer Sea Search and Rescue also contributed transports to and from islands. The project was also supported by Malcom Perry through maintenance of the research vessel, and the Fremantle Sailing Club who provided access to their boat ramp. Mike Todd lent us his telescope. Many volunteers have allocated substantial time in assistance in the field and data entry. We are grateful to Sven Gestauer for helping with the R coding and to Alain Zuur who gave useful advice concerning initial models. Miles Parsons provided comments on multiple drafts of the manuscript, which significantly improved its quality. Finally, this project was supported by funding from the Holsworth Grant, and was conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth).

LITERATURE CITED

- Ainley DG, Huber HR, Bailey KM (1982) Population fluctuations of California sea lions and the Pacific whiting fishery off central California. *Fish Bull* 80:253–258
- Andrews-Goff V, Hindell MA, Field IC, Wheatley KE, Charassin JB (2010) Factors influencing the winter haulout behaviour of Weddell seals: consequences for satellite telemetry. *Endang Species Res* 10:83–92
- Arnould JPY, Hindell MA (2001) Dive behaviour, foraging locations, and maternal attendance patterns of Australian fur seals (*Arctocephalus pusillus doriferus*). *Can J Zool* 79:35–48
- Balouin Y, Rey-Valette H, Picand PA (2014) Automatic assessment and analysis of beach attendance using video images at the Lido of Sète beach, France. *Ocean Coast Manage* 102:114–122
- Beentjes MP (1989) Haul-out patterns, site fidelity and

- activity budgets of male Hooker's sea lions (*Phocarctos hookeri*) on the New Zealand mainland. *Mar Mamm Sci* 5:281–297
- Bengtson JL, Cameron MF (2004) Seasonal haulout patterns of crabeater seals (*Lobodon carcinophaga*). *Polar Biol* 27: 344–349
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, NY
- Carlens H, Lydersen C, Krafft BA, Kovacs KM (2006) Spring haul-out behavior of ringed seals (*Pusa hispida*) in Kongsfjorden, Svalbard. *Mar Mamm Sci* 22:379–393
- Cassini MH, Szteren D, Fernández-Juricic E (2004) Fence effects on the behavioural responses of South American fur seals to tourist approaches. *J Ethol* 22:127–133
- Chilvers BL, Wilkinson IS, Duignan PJ, Gemmill NJ (2005) Summer foraging areas for lactating New Zealand sea lions *Phocarctos hookeri*. *Mar Ecol Prog Ser* 304:235–247
- Costa DP, Gales NJ (2003) Energetics of a benthic diver: Seasonal foraging ecology of the Australian sea lion, *Neophoca cinerea*. *Ecol Monogr* 73:27–43
- Cunningham L, Baxter JM, Boyd IL, Duck CD, Lonergan M, Boss SE, McConnell B (2009) Harbour seal movements and haul-out patterns: implications for monitoring and management. *Aquat Conserv* 19:398–407
- de Valpine P (2014) The common sense of *P* values. *Ecology* 95:617–621
- Department of Conservation and Land Management (CALM) (2003) Carnac Island Nature Reserve, Management Plan 2003. Department of Conservation and Land Management, Perth
- Department of Environment and Conservation (DEC) (2007) Shoalwater Islands Marine Park Management Plan 2007–2017. Department of Parks and Wildlife, Perth
- DEC, Fisheries (2011) Shoalwater Islands Marine Park. Marine parks. WA's submerged wonders. Recreation guide. Department of Environment and Conservation, Department of Fisheries, Perth
- Feltz ET, Fay FH (1966) Thermal requirements *in vitro* of epidermal cells from seals. *Cryobiology* 3:261–264
- Flom PL, Cassell DL (2007) Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. NorthEast SAS Users Group 20th Annual Conference, 11–14 November 2007, Baltimore, MD
- Gales NJ (1995) Hooker's sea lion recovery plan (*Phocarctos hookeri*). Threatened species recovery plan series no. 17. Threatened Species Unit, Department of Conservation, Wellington
- Gales NJ, Cheal AJ, Pobar GJ, Williamson P (1992) Breeding biology and movements of Australian sea lions, *Neophoca cinerea*, off the west coast of Western Australia. *Wildl Res* 19:405–416
- Gales NJ, Shaughnessy PD, Dennis TE (1994) Distribution, abundance and breeding cycle of the Australian sea lion *Neophoca cinerea* (Mammalia: Pinnipedia). *J Zool (Lond)* 234:353–379
- Goldsworthy SD (2015) *Neophoca cinerea*. The IUCN Red List of Threatened Species Version 2015.2. www.iucnredlist.org (accessed 5 August 2015)
- Goldsworthy SD, Shaughnessy PD, McIntosh RR, Kennedy C, Simpson J, Page B (2008) Australian sea lion populations at Seal Bay and the Seal Slide (Kangaroo Island): continuation of the monitoring program. South Australian Research and Development Institute, Government of South Australia, Adelaide
- Goldsworthy SD, Page B, Shaughnessy PD, Hamer D and others (2009) Innovative solutions for aquaculture planning and management: addressing seal interactions in the finfish aquaculture industry. South Australian Research and Development Institute, Adelaide
- Grellier K, Thompson PM, Corpe HM (1996) The effect of weather conditions on harbour seal (*Phoca vitulina*) haulout behaviour in the Moray Firth, northeast Scotland. *Can J Zool* 74:1806–1811
- Hammerschlag N, Martin RA, Fallows C (2006) Effects of environmental conditions on predator–prey interactions between white sharks (*Carcharodon carcharias*) and Cape fur seals (*Arctocephalus pusillus pusillus*) at Seal Island, South Africa. *Environ Biol Fishes* 76:341–350
- Harestad AS (1978) Diurnal activity of Northern sea lions, *Eumetopias jubatus* (Schreber). *Syesis* 11:279–280
- Harrell FE Jr (2001) Regression modeling strategies. Springer, New York, NY
- Higgins LV (1993) The nonannual, nonseasonal breeding cycle of the Australian sea lion, *Neophoca cinerea*. *J Mammal* 74:270–274
- Huber HR, Jeffries SJ, Brown RF, Delong RL, Vanblaricom G (2001) Correcting aerial survey counts of harbor seals (*Phoca vitulina richardsi*) in Washington and Oregon. *Mar Mamm Sci* 17:276–293
- Jefferson TA, Webber MA, Pitman RL (2011) Marine mammals of the world: a comprehensive guide to their identification. Academic Press, London
- Kastelein RA, Weltz FC (1991) Distribution and behaviour of Steller sea lions (*Eumetopias jubatus*) in Prince William Sound, Alaska, June 1989. *Aquat Mamm* 17:91–97
- Kenyon KW, Rice DW (1961) Abundance and distribution of the Steller sea lion. *J Mammal* 42:223–234
- Kerley GIH (1983) Comparison of seasonal haul-out patterns of fur seals *Arctocephalus tropicalis* and *A. gazella* on Subantarctic Marion Island. *S Afr J Wildl Res* 13:71–77
- Krieger M, Barrette C (1984) Aggregation behaviour of harbour seals at Forillon National Park, Canada. *J Anim Ecol* 53:913–928
- Kucey L (2005) Human disturbance and the hauling out behaviour of Steller sea lions (*Eumetopias jubatus*). MSc thesis, University of British Columbia, Vancouver
- Lake SE, Burton HR, Hindell MA (1997) Influence of time of day and month on Weddell seal haul-out patterns at the Vestfold Hills, Antarctica. *Polar Biol* 18:319–324
- LeBoeuf BJ, Riedman M, Keyes RS (1982) White shark predation on pinnipeds in California coastal waters. *Fish Bull* 80:891–895
- Ling JG, Walker GE (1978) An 18-month breeding cycle in the Australian sea lion? *Search* 9:464–465
- Ling JK, Button CE, Ebsary BA (1974) A preliminary account of grey seals and harbor seals at Saint-Pierre and Miquelon. *Can Field-Nat* 88:461–468
- London JM, Ver Hoef JM, Jeffries SJ, Lance MM, Boveng PL (2012) Haul-out behavior of harbor seals (*Phoca vitulina*) in Hood Canal, Washington. *PLoS ONE* 7:e38180
- Lovasz T, Croft DB, Banks P (2008) Establishing tourism guidelines for viewing Australian sea lions *Neophoca cinerea* at Seal Bay Conservation Park, South Australia. In: Lunney D, Munn A, Meikle M (eds) Too close for comfort: contentious issues in human-wildlife encounters. Royal Zoological Society of New South Wales, Mosman, p 225–232
- Lowry MS, Stewart BS, Heath CB, Yochem PK, Francis JM (1991) Seasonal and annual variability in the diet of Cal-

- ifornia sea lions *Zalophus californianus* at San Nicolas Island, California, 1981–86. *Fish Bull* 89:331–336
- Marcotte M (2006) Steller watch: timing of weaning and seasonal patterns in numbers and activities of Steller sea lions (*Eumetopias jubatus*) at a year-round haulout site in southeast Alaska. MSc thesis, University of British Columbia, Vancouver
- Marlow BJ (1975) The comparative behaviour of the Australasian sea lions, *Neophoca cinerea* and *Phocarcos hookeri* (Pinnipedia: Otariidae). *Mammalia* 39:159–230
- McCauley RD (2012) Fish choruses from the Kimberley, seasonal and lunar links as determined by long term sea noise monitoring. Acoustics, Development and the Environment, 21–23 November 2012, Fremantle, Australian Acoustical Society. *Proc Acoustics* 2012:441–446
- Mogren HG, Lindstrøm U, Nilssen KT, Haug T (2010) Haulout behaviour of harbour seals (*Phoca vitulina*) during breeding and moult in Vesterålen, Norway. *NAMMCO Sci Publ* 8:303–312
- Morrison MA, Francis MP, Hartill BW, Parkinson DM (2002) Diurnal and tidal variation in the abundance of the fish fauna of a temperate tidal mudflat. *Estuar Coast Shelf Sci* 54:793–807
- Moulton VD, Miller EH, Ochoa-Acuña H (2000) Haulout behaviour of captive harp seals (*Pagophilus groenlandicus*): incidence, seasonality, and relationships to weather. *Appl Anim Behav Sci* 65:367–378
- Pauli BD, Terhune JM (1987a) Meteorological influences on harbour seal haul-out. *Aquat Mamm* 13:114–118
- Pauli BD, Terhune JM (1987b) Tidal and temporal interaction on harbour seal haul-out patterns. *Aquat Mamm* 13:93–95
- Pinheiro JC, Bates DM (2000) *Mixed-effects models in S and S-PLUS*. Springer, New York, NY
- R Development Core Team (2014) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna
- Reder S, Lydersen C, Arnold W, Kovacs KM (2003) Haulout behaviour of High Arctic harbour seals (*Phoca vitulina vitulina*) in Svalbard, Norway. *Polar Biol* 27:6–16
- Reebbs SG (2002) Plasticity of diel and circadian activity rhythms in fishes. *Rev Fish Biol Fish* 12:349–371
- Ribeiro J, Bentes L, Coelho R, Gonçalves JMS (2006) Seasonal, tidal and diurnal changes in fish assemblages in the Ria Formosa lagoon (Portugal). *Estuar Coast Shelf Sci* 67:461–474
- Riedman M (1990) *The pinnipeds: seals, sea lions, and walruses*. University of California Press, Berkeley, CA
- Rosas FCW, Pinedo MC, Marmontel M, Haimovici M (1994) Seasonal movements of the South American sea lion (*Otaria flavescens*, Shaw) off the Rio Grande do Sul coast, Brazil. *Mammalia* 58:51–60
- Roux JP, Hes AD (1984) The seasonal haul-out cycle of the fur seal *Arctocephalus tropicalis* (Gray, 1872) on Amsterdam Island. *Mammalia* 48:377–389
- Salgado Kent CP, Crabtree B (2008) The effectiveness of an established sanctuary zone for reducing human disturbance to Australian sea lions (*Neophoca cinerea*) at Carnac Island, Western Australia. *Tourism Mar Environ* 5:29–42
- Sato K, Tsuchiya Y, Kudoh S, Naito Y (2003) Meteorological factors affecting the number of Weddell seals hauling-out on the ice during the molting season at Syowa Station, East Antarctica. *Polar Biosci* 16:98–103
- Schneider DC, Payne PM (1983) Factors affecting haul-out of harbor seals at a site in southeastern Massachusetts. *J Mammal* 64:518–520
- Seber GAF (1986) A review of estimating animal abundance. *Biometrics* 42:267–292
- Sepúlveda M, Oliva DP, Palma FJ (2001) Daily and annual circarhythms activity in the South American sea lion *Otaria flavescens* (Carnivora: Otariidae) at the central zone of Chile. *Rev Biol Mar Oceanogr* 36:181–187
- Sepúlveda M, Quiñones RA, Carrasco P, Álvarez-Pérez MJ (2012) Daily and seasonal variation in the haul-out behavior of the South American sea lion. *Mamm Biol* 77: 288–292
- Sigler MF, Womble JN, Vollenweider JJ (2004) Availability to Steller sea lions (*Eumetopias jubatus*) of a seasonal prey resource: a prespawning aggregation of eulachon (*Thaleichthys pacificus*). *Can J Fish Aquat Sci* 61: 1475–1484
- Sinclair EH, Zeppelin TK (2002) Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). *J Mammal* 83:973–990
- Southwell C (2003) Haul-out behaviour of two Ross seals off eastern Antarctica. *Antarct Sci* 15:257–258
- Southwell C (2005) Optimising the timing of visual surveys of crabeater seal abundance: haulout behaviour as a consideration. *Wildl Res* 32:333–338
- Stirling I (1968) Diurnal movements of the New Zealand fur seal at Kaikoura. *NZ J Mar Fresh* 2:375–377
- Thompson PM, Fedak MA, McConnell BJ, Nicholas KS (1989) Seasonal and sex-related variation in the activity patterns of common seals (*Phoca vitulina*). *J Appl Ecol* 26:521–535
- Udevitz MS, Jay CV, Cody MB (2005) Observer variability in pinniped counts: ground-based enumeration of walruses at haul-out sites. *Mar Mamm Sci* 21:108–120
- Wakefield CB (2010) Annual, lunar and diel reproductive periodicity of a spawning aggregation of snapper *Pagrus auratus* (Sparidae) in a marine embayment on the lower west coast of Australia. *J Fish Biol* 77:1359–1378
- Watts P (1992) Thermal constraints on hauling out by harbour seals (*Phoca vitulina*). *Can J Zool* 70:553–560
- Watts P (1993) Possible lunar influence on hauling-out behavior by the Pacific harbour seal (*Phoca vitulina richardsi*). *Mar Mamm Sci* 9:68–73
- Watts P (1996) The diel hauling-out cycle of harbour seals in an open marine environment: correlates and constraints. *J Zool (Lond)* 240:175–200
- Winter A, Foy RJ, Wynne K (2009) Seasonal differences in prey availability around a Steller sea lion haulout and rookery in the Gulf of Alaska. *Aquat Mamm* 35:145–162
- Womble JN, Willson MF, Sigler MF, Kelly BP, VanBlaricom GR (2005) Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. *Mar Ecol Prog Ser* 294:271–282
- Womble JN, Sigler MF, Willson MF (2009) Linking seasonal distribution patterns with prey availability in a central-place forager, the Steller sea lion. *J Biogeogr* 36:439–451
- Wood SM (2006) *Generalized additive models: an introduction with R*. Chapman and Hall/CRC, Boca Raton, FL
- Zuur AF, Ieno EN, Smith GM (2007) *Analysing ecological data*. Springer, New York, NY
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York, NY

Editorial responsibility: Clive McMahon, Darwin, Australia

Submitted: March 12, 2015; Accepted: June 25, 2015
 Proofs received from author(s): September 21, 2015

CHAPTER 5 PUBLICATION

Responses of Australian sea lions, *Neophoca cinerea*, to anthropogenic activities in the Perth metropolitan area, Western Australia

SYLVIA K. OSTERRIEDER^{a,b,*}, CHANDRA SALGADO KENT^a and RANDALL W. ROBINSON^b

^a*Centre for Marine Science and Technology, Department of Imaging and Applied Physics, Curtin University, Bentley, Australia*

^b*Institute for Sustainability and Innovation, College of Engineering and Science, Victoria University, Melbourne, Australia*

ABSTRACT

1. Tourist-based activities, partly due to their rapid increase, have raised concerns regarding the impacts of anthropogenic activity on marine fauna. Documented effects on pinnipeds in proximity to humans include changes in behaviour, site use and potentially higher aggression levels towards people. Effects vary considerably between populations and sites, thus requiring separate assessment of human impacts on activity and energy budgets.

2. Responses of the endangered Australian sea lion, *Neophoca cinerea*, to human visitation were recorded from November 2013 through April 2014. Exposure levels and response types to anthropogenic activities were assessed at two easily accessible locations with different management schemes, Seal (landing prohibited) and Carnac (landing permitted) islands, Western Australia. Exposure levels were measured as both stimulus type (i.e. 'People', 'Paddlers', 'Small', 'Medium', and 'Large vessels', 'Tour vessels', and 'Jet skies'), and people ('Direct', 'Attract', 'Interact', 'View', 'Incidental', 'Water', 'Low-level'), and vessel activities ('Interact', 'Approach/Follow', 'Anchor noise', 'Engine noise', 'Close to beach', 'Moderate/Fast travel', 'Slow travel', 'Transit', 'Drift/At anchor', 'Aircraft noise').

3. Exposure levels varied significantly between the islands in numbers, stimuli type, duration and minimum approach distances. The instantaneous behaviours of 'Lift head', 'Interact' and 'Sit' were the most frequent responses. 'Aggressive' and 'Retreat' responses, the highest disturbance levels measured, occurred on Carnac approximately once per day, but rarely on Seal Island. 'Aggressive' behaviour towards 'People' was observed only on Carnac Island and elicited only by 'People'. 'People', 'Tour vessels', and scenic 'Aircrafts' on both islands as well as 'Jet skis' on Carnac Island had the highest probability of triggering responses. Owing to their relatively high visitation at Seal Island, 'Paddle powered vessels', followed by 'Tour vessels' elicited the highest number of responses, compared with 'People', 'Small', and 'Medium vessels' at Carnac Island. The majority of responses occurred when any stimulus type was at short-range (≤ 10 m), and 'People' 'Viewing' *N. cinerea* elicited most. Vessels triggered more responses at larger ranges than 'People'.

4. To limit close-range access to *N. cinerea*, one possibility is to close the beach at Carnac Island to human visitation and increase the minimum approach distance by vessels and 'People' by installing marker buoys at least 15 m from the shore.

Copyright © 2016 John Wiley & Sons, Ltd.

Received 28 October 2015; Revised 14 March 2016; Accepted 26 March 2016

KEY WORDS: coastal; island; disturbance; endangered species; habitat management; mammals; pinnipeds; anthropogenic activities

*Correspondence to: Sylvia Osterrieder, Centre for Marine Science and Technology, Department of Imaging and Applied Physics, Curtin University, Building 301, Wark Avenue, Bentley, Western Australia 6102, Australia. Email: sylvia.osterrieder@gmail.com

INTRODUCTION

Conservation of animal populations requires accurate knowledge of the potential impacts that anthropogenic activities may have on their health and survival. Impacts from anthropogenic activities are wide-ranging, and the level of severity depends upon factors including the type of activity, duration and their proximity to the animals. Marine ecotourism such as whale and dolphin watching is increasing in popularity and often includes direct (swimming with) and indirect (observational) interaction. More recently, excursions to observe other fauna such as seals and sea lions at haul-out locations, have also increased. In the Southern Hemisphere alone, 1.3 million people visit pinnipeds every year (estimated average from 1995 to 2000), with an annual value of US \$12.6 million (Kirkwood *et al.*, 2003). Activities range from swimming with seals and sea lions to watching them from boats, planes or land (Boren *et al.*, 2002; Kirkwood *et al.*, 2003; Lovasz *et al.*, 2008; Cowling *et al.*, 2014).

The Australian sea lion, *Neophoca cinerea*, endemic to Western and South Australia (Gales *et al.*, 1994; Goldsworthy *et al.*, 2008), is classified as Vulnerable by the Environment Protection and Biodiversity Conservation (EPBC) Act 1999 and by the two states in which it occurs (National Parks and Wildlife Act 1972, South Australia; Wildlife Conservation Act 1950, Western Australia), and is listed as Endangered on the IUCN Red List of Threatened Species (Goldsworthy, 2015). In Australia, *N. cinerea* is increasingly targeted by marine tourism. Seal Bay at Kangaroo Island in South Australia is the most popular location to watch *N. cinerea* in the wild, receiving up to 110,000 visitors in any one year since the 1950s (Kirkwood *et al.*, 2003). Haul-out islands off Port Lincoln, South Australia and Jurien Bay, north of Perth in Western Australia (WA) also receive large numbers of visitors (Kirkwood *et al.*, 2003).

Pinniped tourism is very popular and economically beneficial (Kirkwood *et al.*, 2003). However, there is public and scientific concern that these activities may have detrimental effects on the health of marine wildlife populations (Gerrodette and Gilmartin, 1990). Various studies have

endeavoured to document behavioural changes, for example aggressive displays, avoidance or habituation, physiological responses, and direct threats to the survival of animals, such as entanglement and increased risk of boat strikes (Gerrodette and Gilmartin, 1990; Constantine, 1999; Stevens and Boness, 2003; Newsome and Rodger, 2008; French *et al.*, 2011). There is evidence that animals may reduce time spent resting or hauling out, possibly affecting their energy budget; or may leave pups unattended, which, while currently untested, could potentially increase pup mortality (Kovacs and Innes, 1990; Jansen *et al.*, 2010). Assessing the magnitude of effects is complex as age, sex, degree of exposure, and stage in the breeding cycle may influence responses to disturbance and level of impact (Boren *et al.*, 2002; Cowling *et al.*, 2015). Furthermore, most studies have been limited to assessing short-term (over the course of a day) and immediate responses of individuals, rather than long-term impacts (over months and years) on the population. This is probably a result of the challenges involved in long-term monitoring owing to long-term required funding, and the non-trivial nature of disentangling the effects of human disturbance from changes in a complex environment.

To reduce the impact of human/pinniped interactions, various regulations and guidelines have been initiated (e.g. spatial and temporal restrictions or limitations in vessel speed and visitor numbers) to both maintain the health of the marine environment and to protect animals and tourists during interactions (Orams, 1999). In Australia, several patrolled marine parks and sanctuary zones, with limited access for visitors to view and interact with animals, have been established (Gales, 1995; Kirkwood *et al.*, 2003; Cassini *et al.*, 2004; Salgado Kent and Crabtree, 2008; Young *et al.*, 2014). Where close approaches are allowed, there are guidelines recommending safe distances for viewing and for reducing disturbance to pinnipeds. Although regulations and guidelines are in place for management, the scientific basis for these management decisions in relation to *N. cinerea* is limited (DEC, 2007; Lovasz *et al.*, 2008; Salgado Kent and Crabtree, 2008; Young *et al.*, 2014).

In metropolitan waters around Perth, for example, moving/approaching *N. cinerea* slowly and keeping a minimum distance of 5–10 m are recommended (DEC and DoF, 2011). At this location, the largest numbers of *N. cinerea* haul out on Seal and Carnac islands, which are two of the six main local haul-out sites (Gales *et al.*, 1992). Owing to the close proximity of the islands to Perth (~2 million people) and their ease of access, both islands are heavily used for tourism and recreational activities, including viewing *N. cinerea* in the wild (Orsini and Newsome, 2005). On Seal Island, located within a marine park, landing by either vessels or people is prohibited. In comparison, the beach on Carnac Island is divided into two different zones with only the sanctuary zone off limits to the public. While the designation of the zone was based on the area used most often by *N. cinerea* in a study in 2005 (Orsini and Newsome, 2005), a follow-up study, 2 years later, showed that *N. cinerea* used the beach outside the sanctuary zone just as frequently (Salgado Kent and Crabtree, 2008). It was determined that the most effective approach for reducing disturbance on Carnac Island was to expand the sanctuary zone over the entire beach (Salgado Kent and Crabtree, 2008).

Beyond the study at Carnac Island, the effectiveness of small sanctuaries, or no-go zones, in reducing disturbance in the Perth metropolitan area, and other areas, is not accurately known (Gormley *et al.*, 2012; Hartel *et al.*, 2015). Owing to the competing interests in use and access to the islands by conservationists, recreational users, and commercial operators, as well as the underlying need for conservation of the species, the effectiveness of sanctuary zone size and applied management strategies must be assessed (Salgado Kent and Crabtree, 2008). Establishing baseline data and determining impacts of various types of use is critical for improving the design of reserves and management outcomes (Kelleher, 1999).

This study investigates the responses of *N. cinerea* to anthropogenic activity at two sites (Seal Island and Carnac Island) with different management strategies. Various activity types were documented as pinnipeds have been shown to respond differently to varying stimuli (Cassini,

2001; Boren *et al.*, 2002; Jansen *et al.*, 2010). Specifically, stimulus types (i.e. vessel types and people), their activities, and *N. cinerea*'s response behaviours were categorized, and recorded. Distances between the stimuli and responding *N. cinerea* were also recorded. Thus, the influence of stimulus types, their activities, and distances on *N. cinerea* behaviour were investigated.

The specific objectives of this study were to: (1) compare the numbers of vessels/people present, and to quantify their activities at Seal and Carnac islands, two islands with contrasting management types, to provide context to the response of *N. cinerea* for wider application; (2) compare the frequency and level of disturbance to *N. cinerea* at the two islands, in relatively close proximity to urban areas (0.9 and 10 km); and (3) assess the influence of anthropogenic activity types and their proximity to the animals through measurements of the frequency and level of *N. cinerea* responses. Understanding the key impacts of tourism on *N. cinerea* behaviour is necessary for improved, scientific-based, long-term management, and where necessary, recovery plans for endangered species, such as *N. cinerea*, on both a local, regional and national scale. To do this requires an understanding of the context within which the animals have been observed (Objectives 1 and 2).

METHODS

Study sites

Six islands are known to be used as haul-out sites by male *N. cinerea* off the Perth metropolitan coast, Western Australia (Figure 1). Of the six islands, the islands included in this study – Seal Island (–32.29° S, 115.69° E) and Carnac Island (–32.12° S, 115.66° E; Figure 1) – have the largest proportions of *N. cinerea* hauling out; more than 30 during the peak season (Osterrieder *et al.*, 2015). Usually less than 10 *N. cinerea* haul out at the other metropolitan haul-out islands (Department of Parks and Wildlife, unpublished data).

Seal Island is a sanctuary zone where landing is not permitted, located in the Shoalwater Islands Marine Park, ≈0.9 km from the coast and ≈45 km south of Perth (Figure 1). Here, *N. cinerea* can be

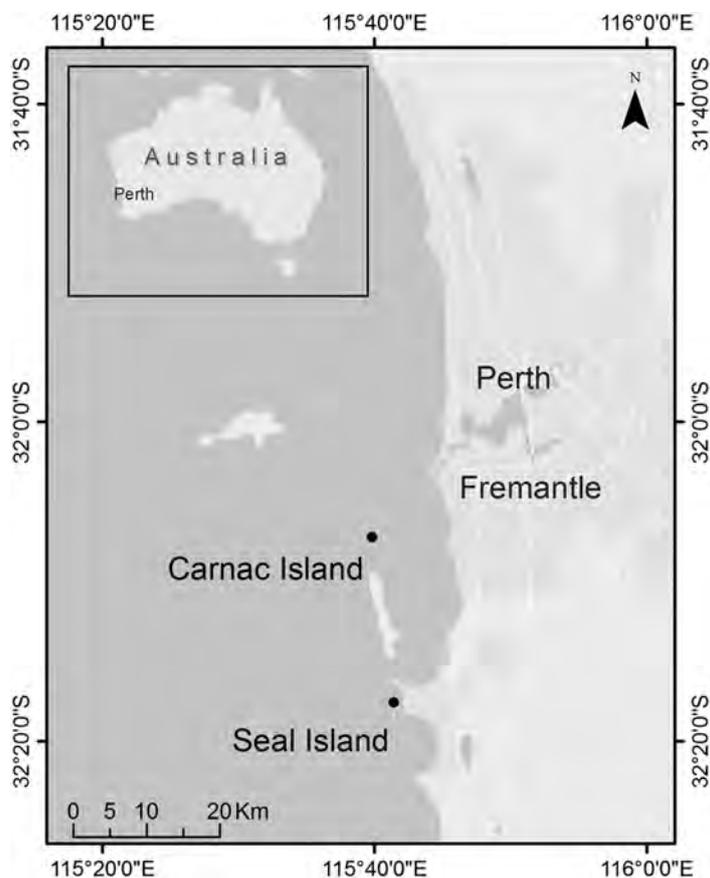


Figure 1. Seal Island and Carnac Island, largest *N. cinerea* haul-out sites in the Perth metropolitan area, Western Australia.

viewed on a kayaking or boat tour and the sanctuary zone's 'look, but don't take' area offers the highest level of protection allowing boating, snorkelling and nature appreciation activities, but prohibits fishing (DEC and DoF, 2011). *Neophoca cinerea* predominantly haul out on the beach of ≈ 0.27 ha (estimated from a Google Earth, 2014 image from 1 January 2014) on the eastern side of the island. They also haul out adjacent to the shrubs or caves at the southern bay on occasion, but have not been seen to haul out on the other sides of the island that comprise mostly rocky outcrops.

Carnac Island (≈ 10 km south-west of the Fremantle coast and 15 km south of Perth; Figure 1) is an A class nature reserve, with part of the island designated as a sanctuary zone. Access

to most of the island is prohibited, but the southern part of the eastern beach is available for public access during the day (CALM, 2003). The eastern beach is ≈ 0.78 ha (estimated from a Google Earth, 2014 image from 1 January 2014) and *N. cinerea* mainly haul out on this sandy beach. Charter and tour vessels travel to Carnac Island, though less frequently than to Seal Island. Both, Seal and Carnac islands, can also be easily accessed by private recreational vessels.

Experimental design

Count data for Seal and Carnac islands, either conducted by an observer located on the islands, or remotely using a locally installed, live video

camera, were collected over a period of two years, between June 2012 and April 2014 (Figure 2(a)). Within this period, individual responses to anthropogenic activities were recorded over 5 months, from the end of November 2013 until the end of April 2014.

Observations at Seal Island were predominantly made from a vantage point located on the island with a view of the entire beach, using either the naked eye or binoculars (Nikon Eagleview 8–24 × 25). Transfer issues restricted travel to Seal Island between July and August 2012 and on 9 October 2012 (Figure 2(a) and (b)). As a result, observations during those times were made from a

vantage point on the mainland (32.2855° S, 115.7035° E), with the entire beach area in view, using either a telescope (115 mm Tasco reflecting with either a 25 mm, 20 mm or 10 mm eye piece with 36×, 45× or 90× magnification, respectively) or a spotting scope (Televid 77 with 20× to 60× zoom).

Data for Carnac Island were predominantly collected remotely, via an at the time of operation live, remote controlled camera (AVT284 IP Camera with remote Pan, Tilt, and Zoom capability and 22× optical zoom) with a radio link to a local Department of Parks and Wildlife office (using a Proxim 8150 PTP microwave radio link). The camera was located overlooking the eastern

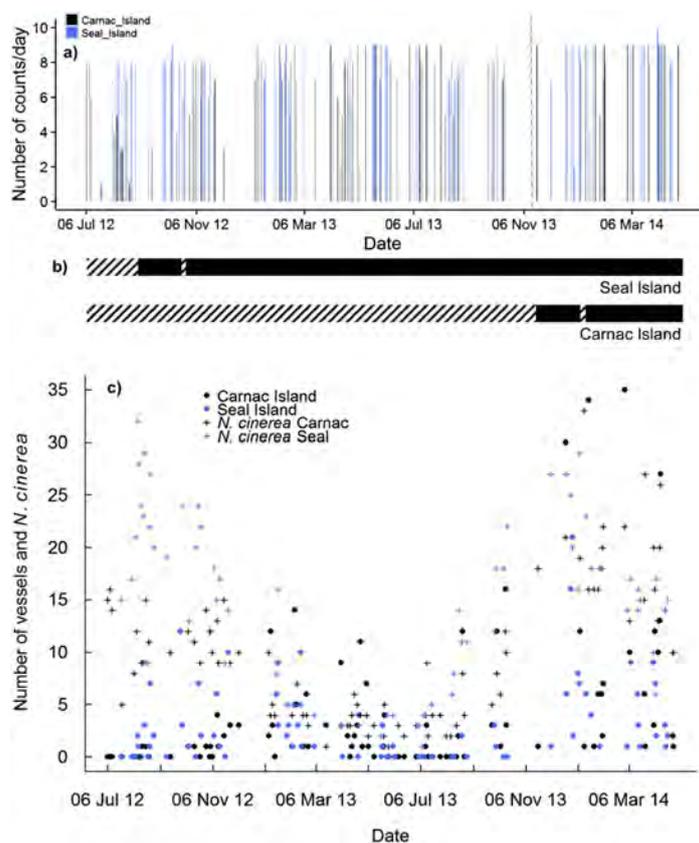


Figure 2. (a) Sampling frequency with number of counts conducted per sampling day (Carnac Island is demarcated in black, Seal Island in blue, and dashed line at end of November 2013 indicates the start of the collection of disturbance data). (b) Sampling method used throughout the sampling period displayed in Figure 2(a) (black stripes = remote, including Seal Island observations from the vantage point in Shoalwater using the telescope or spotting scope, and Carnac Island observations with the remote controlled, live camera). (c) Maximum number of vessels (○) and *N. cinerea* (+) observed on Carnac Island (black) and Seal Island (blue) each day during 166 survey days between June 2012 and April 2014.

beach. Direct observations made on Carnac Island from the same vantage point as the camera were conducted between the end of November 2013 and the end of April 2014, with the exceptions of the 8 and 13 January 2014 (Figure 2(a) and (b)). To minimize disturbance caused by the researchers upon arrival, the vantage points on Seal and Carnac islands were approached from a small bay at the back of the beach (Seal Island), or by landing in gaps between *N. cinerea* (Carnac Island), always remaining as far away from *N. cinerea* as possible. While on-island, observations were always conducted from a range >20 m, movement minimized (e.g. no sudden standing up) and conversation kept to a level thought to be inaudible at the ranges where *N. cinerea* had hauled out.

Remote observations were limited to counts of vessels and 'People' (i.e. people in the water or on the beach, not attached to any floatation device, and herein classified as 'People') to ensure comparable and accurate data were collected. More detailed behavioural data were collected only when observers were on the islands. The telescope and spotting scope were considered to give sufficient magnification for easy and accurate counts, and the remotely operated camera has previously been shown to reflect counts accurately (Salgado Kent and Crabtree, 2008).

Counts of vessels, 'People' and *N. cinerea*

All vessels approaching or passing within approximately 400 m of the beach were counted by one to three observers during island-based monitoring. In addition, counts were made of all

'People' and *N. cinerea* within view, either on land or in the water. Counts were generally conducted during 5 to 10 min scans, and were made every hour primarily between 08:00 and 16:00 h, with the exceptions of inclement weather or when technical difficulties cut some days short. *Neophoca cinerea* known to be present during the count but temporarily out of view (i.e. sighted when going behind rocks and again when coming back into view) were also recorded.

Hourly counts conducted remotely were carried out by panning from north to south, from one side of the beach to the other, to count vessels, 'People' and *N. cinerea* (Salgado Kent and Crabtree, 2008). The zoom on the live camera was used to aid counts when necessary, particularly to distinguish *N. cinerea* from some rocks on the far, southern part of the beach.

Behavioural responses to anthropogenic activities

An observer recorded arrival and departure times of anthropogenic stimulus types (e.g. vessels, and 'People', Table 1), including the time 'People' entered or left the water or the beach, on a dictaphone. These arrival and departure times were used to calculate the total number of 'People' and vessels, except on five days when high activity and numbers of vessels at Carnac Island (up to a maximum of 36 vessels and 20 'People' at any one time) made this unfeasible. During these periods, counts were conducted every 5 to 15 min instead to determine totals and numbers of *N. cinerea* present during each behavioural response taken from the nearest count. At all other times on

Table 1. Definition of stimulus categories

Stimulus type	Description
People	People in the water or on the beach, not attached to a floatation device or vessel. On Carnac 'People' occurred in the water and on the beach. On Seal Island people were restricted (legally) to the water (except for on 10 occasions when people accessed the island illegally)
Paddle powered/(Paddlers)	Small vessels ≤3 m in length with no engine (e.g. kayak, paddleboard, canoe, row boat, body board)
Small vessels	Vessels up to 6 m in length (e.g. recreational/fishing vessels, dinghies)
Medium vessels	Vessels >6 m and ≤15 m in length with a single deck (including government department vessels and power/speed boats)
Large vessels	Vessels >15 m in length or fitted with multiple decks (e.g. charter boats, catamarans, party boats, commercial dive vessels, and sailing boats)
Tour vessels	Vessels visiting the islands with the aim of observing <i>N. cinerea</i> (these were usually medium sized vessels on Seal Island and large vessels on Carnac Island)
Jet ski	Jet propelled personnel water craft
Aircraft	Planes (usually scenic and military) and helicopters

Carnac Island and at all times on Seal Island vessel numbers were accounted for at each response. During these ‘busy’ periods, particular attention was paid to those closest to *N. cinerea* and vessels involved in activities anticipated to have greater impacts (e.g. varying the engine throttle or playing music) to capture detailed behavioural response information. Overall documented responses of interactions anticipated to have ‘lower’ impacts were not affected. Rather, the more detailed information was used separately – for focal behavioural response analysis.

‘People’s and vessels’ activities (Tables 2 and 3), including the times the activities were undertaken, were also recorded. Groups of ‘People’ were defined as one or more closely-spaced humans displaying similar or associated behaviour. Groups of vessels (such as several kayaks travelling in close proximity) were considered in the same way.

Vessel categories included ‘Paddle powered’, ‘Small’, ‘Medium’, ‘Large’ and ‘Tour vessels’ as well as ‘Jet skis’, and ‘Aircrafts’ (Table 1). Vessel activities included 10 categories ranging from

Table 2. Definition of human activities associated with vessels ordered from highest to lowest anticipated impact

Activity classification	Description
Interact	Vessels interacting with <i>N. cinerea</i> , including: animals following a vessel, swimming or porpoising around a vessel
Approach/Follow <i>N. cinerea</i>	Vessels which are seeking to interact with <i>N. cinerea</i> by approaching for a better view, driving in circles around <i>N. cinerea</i> or following/chasing <i>N. cinerea</i>
Anchor noise	Setting or retrieving the anchor with associated rattling noise of the anchor chain and splashing when dropping the anchor
Engine noise	Activities producing higher level of engine noise than when travelling, including revving engine, reversing, travelling with particularly noisy engines
Close to beach	Activities within the vicinity of the beach, including approaching, staying close to or leaving the beach, and landing on the beach
Moderate/fast travel	Travelling at moderate to fast speeds (including rapid circles)
Slow travel	Travelling at a slow speeds (such as paddling)
Transit	Approaching, passing, leaving or returning to the vicinity of the island, including paddle powered vessels placed in the water from a vessel anchored off Carnac Island
Drift/At anchor	Activities with no or low movement and/or noise levels associated with them, including drifting, vessels anchored, or no activity
Aircraft noise	Planes or helicopters flying overhead

Table 3. List of categories used for recording anthropogenic activities in the order of the highest to lowest anticipated impact levels (if different activities were performed at the same time, the highest activity was recorded). Abbreviated activity names used in text and figures are marked in bold

Activity	Description
Direct /Invasive Interaction	Invasive activities in direct contact or attempting direct contact with <i>N. cinerea</i> , including touching <i>N. cinerea</i> directly or with a tool (e.g. stick), feeding <i>N. cinerea</i> (including throwing fish towards <i>N. cinerea</i>), throwing objects towards <i>N. cinerea</i> , and splashing water at <i>N. cinerea</i>
Deliberately Attracting Attention	Activities seeking <i>N. cinerea</i> 's attention and provoking responses, without <i>N. cinerea</i> 's engagement, including splashing water (to attract <i>N. cinerea</i> , but not splashing directly at them), imitating <i>N. cinerea</i> noises (barking), clapping, honking, and banging vessel, hitting paddles on the water's surface, following <i>N. cinerea</i> (usually swimming), circling <i>N. cinerea</i> (e.g. standing/crowding around <i>N. cinerea</i> in a circle), yelling, screaming, whistling, loud talking, laughing, loud speaker systems on vessels, playing music, barking dog, jumping into the water
Mutual Interaction	Interacting, people and <i>N. cinerea</i> engaged with each other, i.e. people playing with <i>N. cinerea</i> (in the water), such as mimicking <i>N. cinerea</i> behaviour and achieving a similar response from the <i>N. cinerea</i>
Viewing	Activities involved in viewing <i>N. cinerea</i> such as standing and observing <i>N. cinerea</i> , taking photos, being in close proximity to <i>N. cinerea</i> , approaching, passing or leaving <i>N. cinerea</i> , retreating from aggressive <i>N. cinerea</i>
Incidental Activities	Activities not aimed to provoke <i>N. cinerea</i> responses, typically occurring on the beach or in shallow (knee-deep) water, including playing on the beach, dragging a boat onto the beach or into the water, picnicking, talking at a conversation level (including humans on a vessel), walking on the beach, in the wash zone or in shallow water (but not passing <i>N. cinerea</i>)
Water-related Activities	Activities undertaken in the water (more than knee-deep) including swimming, snorkelling, diving, playing in water (but not interacting with <i>N. cinerea</i>), entering and leaving water from the boat, fishing
Low-level Recreational Activities	Activities, not fitting in previous categories and only when of low level noise, and without rapid movements such as ‘quiet’, i.e. barely audible talking humans not moving or moving little on the boat or beach,

activities anticipated to have a low impact, such as 'Drifting' and 'At anchor', to those anticipated to have a high impact such as 'Interactions with *N. cinerea*' (Table 2). Activities undertaken by 'People' fell into one of seven categories ranging from 'Low-level' recreational activities to 'Direct', invasive interactions (Table 3). *Neophoca cinerea*'s behavioural responses to the activities were classed as 'Aggressive', 'Retreat', 'Enter water', 'Interact', 'Travel', 'Sit', 'Lift head', 'Move head', 'Look', and 'No response' (Table 4). If multiple activities occurred at the same time (e.g. standing close to and watching *N. cinerea* – 'Viewing' activity) and clapping hands or screaming ('Attract' activity), the activity with the highest anticipated impact was recorded ('Attract' in this example; Table 3). Similarly, if a *N. cinerea* responded with multiple behavioural responses (e.g. 'Moving its head' to look towards the stimulus and 'Sitting' up at the same time) the highest response level was recorded ('Sit' in this case; Table 4). Ethograms were compiled based on proven techniques from previous studies (Beentjes, 1989; Cassini *et al.*, 2004; Salgado Kent and Crabtree, 2008; Bowles and Anderson, 2012), and adjusted to capture those relevant to this study. For each interaction, numbers of *N. cinerea* responding, frequency of responses and *N. cinerea*'s behavioural response types were recorded. *Neophoca cinerea* do not have readily identifiable patterns and do not often have scars which aid discrimination among individuals. Therefore, on some rare occasions, during periods when greater numbers were hauling out and multiple individuals responded to

the different stimuli, it was not always possible to assign responses to particular individuals.

Whenever possible, distances and angles from the observer to the stimuli (vessel or 'People'), from the observer to the *N. cinerea* closest to the stimulus, and from the observer to any *N. cinerea* responding to anthropogenic activities (regardless of the distance) were measured using laser rangefinder and compass (TruPulse 360 R with accuracies of ± 0.5 m in distance to high quality targets such as *N. cinerea* and stimuli types, and $\pm 1^\circ$ azimuth). The distance between the stimulus and the nearest *N. cinerea* (unless another *N. cinerea* was responding to the stimulus which was then measured) was calculated using basic trigonometry. Distances were not measured on 18 January 2014 at Carnac Island, on 28 December 2013 and 3 April 2014 at Seal Island, nor after 10:05 h on 8 March 2014 on Seal Island because of the unavailability of the rangefinder or the lack of functioning replacement batteries. *Neophoca cinerea* in the water did not typically present a sufficiently reflective target for the rangefinder and could not be measured. When appropriate, distance from the closest vessel or 'People' to the closest *N. cinerea* was estimated in *N. cinerea* body lengths (≈ 2 m) and was used for estimating distances up to 10 m. 'People' within arm's reach of a *N. cinerea* were recorded as at 1 m and those touching a *N. cinerea*, as 0 m. Distances were measured when *N. cinerea* responded to groups of vessels or 'People' (in the water or on beaches) and when groups were seen to approach *N. cinerea*.

Table 4. Definitions of response types of *N. cinerea* responses to vessel and human activities, in order from highest to lowest level anticipated responses (if different responses occurred in combination with each other, the most severe was recorded)

<i>N. cinerea</i> response	Description
Aggression	Aggressive behaviour displayed towards a stimulus (e.g. gaping or lunging at 'People')
Retreat	<i>N. cinerea</i> walking or swimming away from vessels or 'People' to deliberately increase the distance between vessel/'People'
Enter water	<i>N. cinerea</i> entering water, including running into water
Interact	Socializing with vessels or 'People', includes behaviours such as porpoising, spy hopping, following and swimming in circles around vessels or 'People'
Sit	<i>N. cinerea</i> sitting upright, including when near or facing a stimulus
Travel	<i>N. cinerea</i> swimming or walking in a specific direction (e.g. swimming or walking past 'People')
Lift head	<i>N. cinerea</i> lifting its head off the sand, such as when looking at a stimulus
Move head	<i>N. cinerea</i> moving its head by turning its head and looking around when sitting up or after lifting its head, such as when looking at vessels or 'People'
Look	Opening or moving eyes to look at a stimulus (i.e. vessels or 'People')

Analytical approach

Overall numbers of each vessel type and 'People', and numbers of *N. cinerea* were based on data collected over the entire study period. However, for comparing responses of *N. cinerea* with anthropogenic disturbance at Carnac and Seal islands, a subset of data was used from the same period at both islands (from 20 November 2013 to 27 April 2014), to ensure observations had comparable seasonal conditions. Observation effort at the two islands differed by 8 h (equivalent to approximately one survey day of 20), thus effort was accounted for by normalizing the frequency of activities and responses at each of the islands to an hourly rate. 'Aircrafts' were considered in analyses of the total numbers of groups of stimulus types visiting and in the total number of responses elicited by anthropogenic activities. However, owing to their relatively low overall numbers and different types of behaviours, they were excluded from all other analysis. All analyses and figures were produced using R version 3.2.0 (R2014) run through RStudio Version 0.98.1103 – © 2009–2014 RStudio, Inc.

Number of vessels and 'People'

The number and composition of different stimulus types at Carnac and Seal islands were compared using Pearson's Chi² tests with Yates' continuity correction (Yates, 1934). While sampling effort was approximately 6% greater on Seal Island than on Carnac Island, Chi² tests are robust with unequal sample sizes (McHugh, 2013). Furthermore, the difference in sampling effort between the islands was small.

The duration of visits and minimum approach distances of stimulus types at Carnac and Seal islands were compared using Kruskal–Wallis tests (Kruskal and Wallis, 1952). Comparisons of duration and minimum approach distances between Carnac Island and Seal Island for the different vessel types and 'People' were also analysed using Kruskal–Wallis tests.

For all analyses on numbers of each stimulus type, duration of their visits, and their minimum approach distances, multiple tests were conducted using the entire dataset and several subsets of the

data. Therefore, the family-wise error rate (the probability of rejecting at least one null hypothesis erroneously) could be expected to increase since the tests are no longer independent. A sequential Bonferroni correction on the *P*-values considered as significant was therefore applied (Rice, 1989). Eight Chi² tests were performed on the exposure of *N. cinerea* to the number of stimuli and were considered significant when *P* < 0.006. Duration and minimum approach distance of different vessel types and 'People' were considered significant when *P* < 0.006 and *P* < 0.005 to account for the eight and nine Chi² tests conducted, respectively.

Behavioural responses to anthropogenic activities

On occasion, individual *N. cinerea* responded several times to a single stimulus, sometimes in quick succession, such as 'Lifting their head' to 'Look' at the stimulus and then 'Sitting up' within a few seconds or minutes. If the same individual (*N. cinerea* A, for example) responded to the same stimulus within a 5 min period only one response, the behaviour considered to represent the greatest response, was used in analyses. If, however, *N. cinerea* A responded to a different stimulus at a different location, or a different individual (*N. cinerea* B) responded to the same stimulus as *N. cinerea* A, these were counted as separate responses. Once the 5 min period was completed, a response to the original stimulus by *N. cinerea* A was counted as a new response. During a subsample of 310 responses, the number of repeat responses (i.e. responses to the same stimulus by the same individual) occurring more than 5 min after the initial responses and prior to the stimulus departing the area occurred <3% of the time. Ongoing 'Interactions' between humans and *N. cinerea* can feasibly extend over 5 min (for example a *N. cinerea* may follow a vessel or play with a person for a prolonged period). Continued 'Interactions' of this sort (also exceeding 5 min) with one stimulus group were analysed as a single 'Interaction' response.

The number of *N. cinerea* responses to each stimulus type were compared among each other and between islands. In addition, a comparison of number of responses for each behaviour type was

made among the stimulus types and the two islands. Either the Chi² test, or in cases with small sample sizes Fisher's exact test (Fisher, 1922; Yates, 1934), were used. A sequential Bonferroni correction was carried out and *P*-values of $P < 0.005$, $P < 0.005$, $P < 0.008$ and $P < 0.007$ were considered as significant for analyses of the number of responses for each behavioural type level to: groups of vessels vs. groups of 'People' regardless of the location, a stimulus regardless of the type (vessels and 'People' combined) at Carnac Island vs. Seal Island, groups of 'People' at Carnac Island vs. Seal Island, and groups of vessels at Carnac Island vs. Seal Island, respectively (Rice, 1989). To assess whether the percentage of *N. cinerea* responding (of those hauled out at any one time) was related to the number of vessels and 'People' visiting the island at that time, a linear regression was applied to the data and the corresponding R² value was calculated.

To investigate the influence of stimulus activities (regardless of whether they were on a vessel, swimming, or on land), *N. cinerea* behavioural responses to each activity level were calculated per hour of sampling effort and plotted. Response behaviours per hour of sampling effort were also calculated for each stimulus type and plots were used for comparisons.

Response distances

The relationship between stimulus range and frequency of occurrence of a response was investigated through histograms. To ensure all possible errors were accounted for across all ranges between stimulus and *N. cinerea* (maximum error ranges over all measured distances averaged 1.77 m (± 0.96 SD) due to triangulation error), and for ease of viewing, the distances were analysed in 5 m bins. This was also plotted for the stimulus groups and activity types.

RESULTS

Numbers of vessels, 'People' and *N. cinerea*

Vessels, 'People' in the water or on the beach, and *N. cinerea* were observed on 127, 57 and 163 days, respectively, during a total of 166 survey days

(Figure 2). On Seal Island, 619 hourly counts were made during 78 days, and on Carnac Island 709 hourly counts were conducted on 88 days.

Between 20 November 2013 and 27 April 2014, when behavioural responses to anthropogenic activities were recorded from observation points on the islands, 134 h of observations were conducted over 19 days on Carnac Island and 142 h during 20 days on Seal Island. Eight of the days spent on each island were weekend days or public holidays. Over the 6-month period, a maximum of 35 and 21 vessels and a maximum of 19 and six 'People' were recorded at any one time on Carnac and Seal islands, respectively (Figure 2). During this period, 402 and 521 groups of vessels and 164 and 38 groups of 'People' were observed on Carnac and Seal islands, respectively. Owing to the high number of vessels visiting during 5 days at Carnac Island, several vessels were unaccounted for and the total number of groups of vessels on Carnac Island is therefore probably an underestimate by an order of tens of vessels (cf. Orsini, 2004).

Significantly more vessels visited the islands than 'People' ($P < 0.0001$, Table 5), and both varied significantly. The total number of groups of vessels was greater on Seal Island than on Carnac Island; however, the number of groups of vessels on Carnac Island was underestimated on 5 days. The composition of vessel types during these days was similar to the overall composition of vessel types on the remaining days. It is likely that the sample accurately represents the data, hence, the total number of vessels is reported with the inclusion of the 5 days. The exposure of *N. cinerea* to different vessel types differed between Seal and Carnac islands (Table 5, Figure 3). While 'Small vessels', 'Paddle powered', and 'Tour vessels' visited Seal Island most frequently, Carnac Island was mostly visited by 'Medium' and 'Large vessels' ($P < 0.0001$ for each vessel type except for 'Large vessels', Table 5, Figure 3). 'Large vessels' were only observed on Seal Island on one occasion. Carnac Island was visited by more than four times as many groups of 'People' as Seal Island ($P < 0.0001$, Table 5).

The duration of time stimulus source spent in proximity to *N. cinerea* varied significantly among

RESPONSES OF *N. CINEREA* TO ANTHROPOGENIC ACTIVITIES

Table 5. Results of Chi² and Kruskal–Wallis tests comparing the number of groups of vessels of different types and ‘People’ visiting, the duration of visits, and the minimum approach distances to *N. cinerea* at Carnac and Seal islands. Numbers in bold represent significant values, type of test added as + Chi² test or * Kruskal–Wallis rank sum test, and X² or KW-X², respectively, in brackets following the *P*-value. (Sample size for ‘Large vessels’ were too small for calculations and not included.)

	<i>P</i> -value: Number stimuli Carnac vs. Seal	<i>P</i> -value: Duration stimuli Carnac vs. Seal	<i>P</i> -value: Min approach distance stimuli Carnac vs. Seal
Overall	<2.2 ⁻¹⁶ (91.895) ⁺	3.608 ⁻⁰⁷ (25.893)*	<2.2 ⁻¹⁶ (179.29)*
People	<2.2 ⁻¹⁶ (78.594) ⁺	0.4378 (0.602)*	0.687 (0.162)*
Paddle powered	<2.2 ⁻¹⁶ (147.63) ⁺	0.6561 (0.198)*	0.310 (1.034)*
Jet ski	0.00343 (8.565) ⁺	0.9746 (0.00101)*	0.371 (0.801)*
Small vessel	<2.2 ⁻¹⁶ (105.98) ⁺	0.000933 (10.956)*	3.065 ⁻⁰⁶ (21.775)*
Medium vessel	5.971 ⁻¹⁶ (65.447) ⁺	0.0319 (4.606)*	0.434 (0.609)*
Tour vessel	<2.2 ⁻¹⁶ (94.308) ⁺	NA	< 0.005 (7.881)*
Carnac only, stimuli overall	NA	0.000203 (26.219)*	<2.2 ⁻¹⁶ (17.349)*
Seal only, stimuli overall	NA	0.0487 (11.141)*	9.111 ⁻¹⁰ (50.89)*
Bonferroni corrected <i>P</i> -value for significance	0.006	0.006	0.005

⁺Chi² test

*Kruskal–Wallis rank sum test

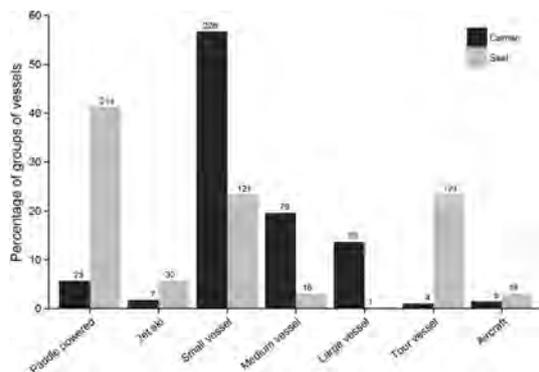


Figure 3. Percentage of groups of vessels observed visiting Carnac Island and Seal Island. Percentages are of the total at each island rather than the total combined at both islands. Values on top of each bar display the number of times each vessel type was observed (with Carnac Island having 134 h, and Seal Island 142 h of sampling effort between November 2013 and end April 2014).

stimulus types at Carnac Island ($P = 0.0002$, Table 5, Figure 4), but not at Seal Island ($P = 0.05$) spending on average 0.56 h (± 0.79 SD) at Carnac Island and 0.23 h (± 0.30 SD; $P < 0.0001$) at Seal Island. At Carnac Island, the variation among vessel types was greater with ‘Jet skis’ staying the shortest periods (on average 6 min), and ‘Tour vessels’ and ‘Large vessels’ staying up to several hours at Carnac Island; longer than any vessel type at Seal Island. The sample size, however, was too small to test for differences (Figure 4).

Minimum approach distances varied significantly among vessel types and ‘People’ on each island

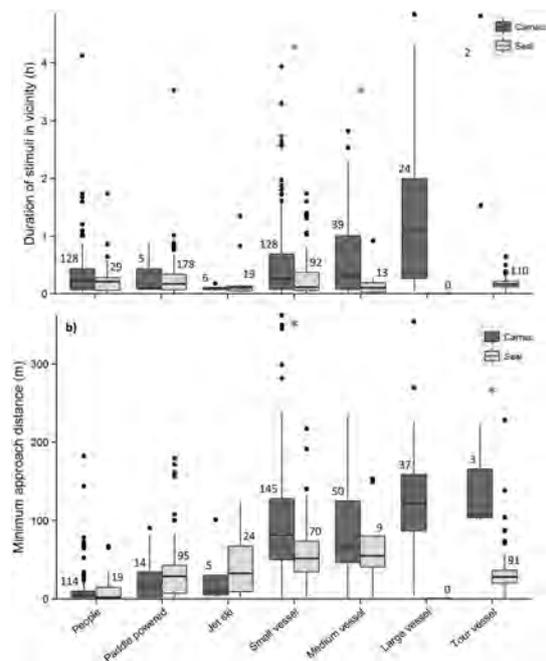


Figure 4. (a) Duration (h) and (b) minimum approach distance (m) of ‘People’ and vessels staying in the vicinity of Carnac Island and Seal Island. Values next to each bar display sample size of recorded approach and departure times for groups of vessels and ‘People’ observed (with Carnac Island having 134 h and Seal Island having 142 h sampling effort between November 2013 and end April 2014). * = significant differences between the islands.

($P < 0.0001$, Table 5, Figure 4) as well as between Seal and Carnac islands ($P < 0.0001$). The average distance to which groups of vessels approached

N. cinerea was 57.5 m (± 64.1 SD) and 34.4 m (± 33.5 SD) on Carnac Island and Seal Island, respectively. 'People' approached *N. cinerea* more closely than any other stimuli type at both islands, on average 10.8 m at Carnac Island and 15.7 m at Seal Island.

Number of responses

In total, 1348 responses were recorded. Of those, 1300 responses were associated with vessels and 'People', and 48 with 'Aircrafts'. Of the responses to 'Aircrafts', nine occurred on Carnac Island and 39 on Seal Island (Figure 3). 'Aircrafts' were not included in statistical analysis owing to their combination of relatively small sample size, lack of determined range, and non-distinct noise source direction. Of the responses to vessels, 250 occurred at Carnac Island and 568 on Seal Island. The total numbers of responses to 'People' were 373 on Carnac Island and 109 on Seal Island, significantly more than to vessels in relation to the total number of stimuli ($P < 0.0001$, Table 6).

Overall, 40% and 39% of all groups of vessels on Carnac Island and Seal Island, respectively, elicited one or more responses from one or more *N. cinerea* (Figure 5). 'Aircrafts' flying over or past the islands, triggered responses in 67% and 81% of their passes from one or more *N. cinerea* at Carnac Island and Seal Island, respectively. *Neophoca cinerea* responded to 66% and 74% of all groups of 'People' at Carnac Island and Seal Island,

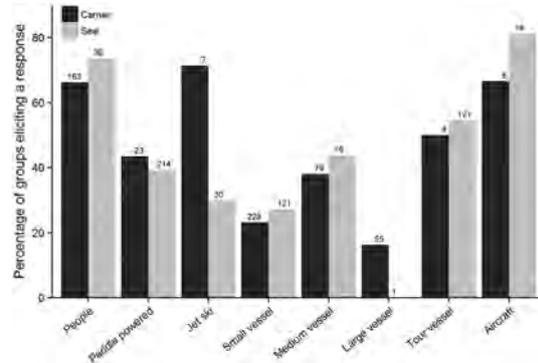


Figure 5. Percentage of groups for the different stimulus types (different vessels and 'People') that elicited one or more responses from one or more *N. cinerea* (with Carnac Island having 134 h and Seal Island having 142 h observation effort between November 2013 and end April 2014). Values on top of each bar display the sample size of groups of vessels or 'People'.

respectively. The percentage of different vessel types that triggered responses in one or more *N. cinerea* varied little between the islands apart from 'Jet skis' and 'Large vessels'. A regression applied to assess whether the percentage of *N. cinerea* responding (of those hauled out at any one time) was related to the number of vessels and 'People' visiting the island at the time, did not reveal a linear relationship (Seal Island: adjusted $R^2_{\text{People}} = 0.01$, adjusted $R^2_{\text{vessels}} = 0.0007$; Carnac Island: adjusted $R^2_{\text{People}} = 0.006$, adjusted $R^2_{\text{vessels}} = 0.001$). There was also no obvious

Table 6. Results of χ^2 and Fisher's exact tests comparing the number of *N. cinerea* responses elicited by groups of vessels and 'People' at Carnac and Seal islands. Numbers in bold represent significant values, type of test added as χ^2 test or * Fisher's exact tests, and X² or odds ratio, respectively, in brackets following the *P*-value. (Aggressive responses were not observed at Seal Island, and therefore not included analyses.)

	<i>P</i> -value: Number responses vessels vs. 'People'	<i>P</i> -value: Number responses Carnac vs. Seal	<i>P</i> -value: Number responses to 'People' Carnac vs. Seal	<i>P</i> -value: Number responses to vessels Carnac vs. Seal
Overall	<2.2 ⁻¹⁶ (74.755)*	0.317 (0.999) [†]	0.491 (0.474) [†]	2.116 ⁻⁰⁵ (13.725) [†]
Retreat	3.516 ⁻⁰⁸ (0.0696)*	4.234 ⁻⁰³ (8.181) [†]	NA	NA
Enter water	3.01 ⁻¹¹ (44.17) [†]	0.353 (0.862) [†]	1.225 ⁻⁰⁴ (0.1589)*	0.561 (0.337) [†]
Interact	2.511 ⁻¹⁴ (58.085) [†]	0.994 (5.106 ⁻⁰⁵) [†]	0.581 (0.305) [†]	0.0305 (4.68) [†]
Travel	2.372 ⁻⁰³ (0.274)*	1 (2.672 ⁻²⁸) [†]	0.439 (0.604)*	0.718 (0.131) [†]
Sit	6.926 ⁻⁰⁸ (29.085) [†]	0.625 (0.239) [†]	0.818 (0.0530) [†]	3.451 ⁻⁰³ (8.552) [†]
Lift head	3.78 ⁻⁰³ (8.386) [†]	0.983 (4.524 ⁻⁰⁴) [†]	0.61 (0.260) [†]	0.274 (1.199) [†]
Move head	1.314 ⁻⁰³ (14.622) [†]	0.63 (0.232) [†]	NA	0.273 (1.201) [†]
Look	3.257 ⁻⁰³ (0.240)*	0.714 (0.135) [†]	NA	NA
Bonferroni corrected <i>P</i> -value for significance	0.005	0.005	0.008	0.007

[†] χ^2 test
*Fisher's exact tests
NA: sample size too small for calculation

non-linear pattern associated with the total number of vessels or 'People' at either island.

Response distances

Out of the total 1300 responses (623 and 677 on Carnac Island and Seal Island, respectively, excluding aircrafts), the distance between a stimulus and a responding *N. cinerea* was measured for 482 responses; 280 on Carnac Island and 202 on Seal Island. For the remaining 973 responses, a number of factors inhibited measuring the response distance; including lack of available range finder on survey, multiple *N. cinerea* responding either simultaneously or in short succession, multiple active stimuli, or a fast-moving stimulus. Responses were triggered between 0 and 345 m, with a mean of 29.6 m (± 39.18 SD). The majority of responses of *N. cinerea* elicited by vessels or 'People' were observed from the shortest ranges (≤ 10 m) to *N. cinerea*, and decreased with increasing distance, most prominently at Carnac Island (Figure 6). The number of responses measured between 0 and 5 m at Carnac Island was double that at Seal Island, and *N. cinerea* appeared to respond to vessels and 'People' at greater distances at Carnac Island (Figure 6). Responses triggered from medium ranges (15–50 m), were predominantly due to 'Tour vessels' and to a lesser extent to 'Paddle

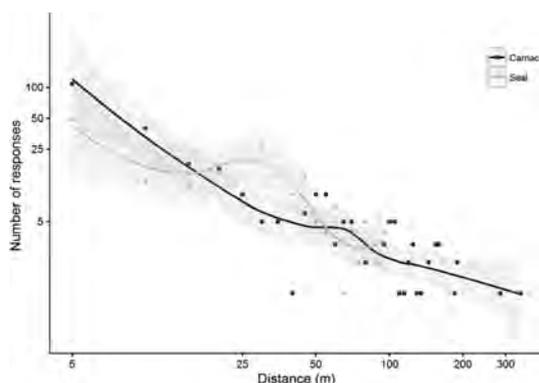


Figure 6. Number of *N. cinerea* responses occurring at 5 m binned distances at Carnac Island ($n = 280$) and Seal Island ($n = 202$), displayed on log 10 transformed axis. Loess smoothers for Carnac Island (black) and Seal Island (grey) with 95% confidence intervals were added to aid visual interpretation.

powered vessels' at Seal Island, and 'Small vessels' at Carnac Island (Figure 7).

All activities carried out by 'People' (regardless of whether on board, in the water or on the beach) potentially induced a response within 10 m, and the probability of a response increased within 5 m range (Figure 8). Overall, the shortest ranges causing the highest number of responses were from 'Viewing' ($11.9 \text{ m} \pm 11.27 \text{ SD}$) and 'Low-level' activities ($41.4 \text{ m} \pm 43.61 \text{ SD}$), followed by 'Interacting' ($5.73 \text{ m} \pm 1.77 \text{ SD}$). The number of responses decreased with increasing distances for most activities (Figure 8). Distance had less effect than activity when humans were involved in 'Attracting' greater numbers of response occurrences at longer distances. Although the frequency of responses to 'Low-level' activities decreased with increasing distance, many responses were still triggered beyond 30 m.

Response behaviours

'Lift head', 'Interaction' and 'Sit' were the most frequent behavioural responses triggered by both vessels and 'People' (Figure 9). All behavioural responses were more likely caused by 'People' than vessels on a per visit basis ($P < 0.005$ for each response level; Table 6, Figure 9). The number of responses provoked by 'People' did not vary significantly between Carnac and Seal islands ($P = 0.5$, Table 6). 'Aggressive' behaviours, however, occurred only at Carnac Island, in response to 'People'. 'Retreat' behaviours occurred mainly at Carnac Island, also mostly in response to 'People' ($P = 0.004$ Carnac/Seal for 'Retreat' behaviour; Figure 9). At Carnac Island, 'Viewing' elicited the most responses, however, on Seal Island 'People' involved in 'Interact', 'Attract', 'Viewing' or 'Water' activities all elicited responses (Figure 10). 'Lift head' accounted for half of the total number of responses at Seal Island provoked by vessels and was triggered at a rate of about 1 h^{-1} at Carnac Island and $>2 \text{ h}^{-1}$ at Seal Island (Figure 9). The relationships between the different types of response to each stimulus group are shown in Table 6 and displayed in Figures 10 and 11. 'Small' and 'Medium vessels' elicited most responses at Carnac Island. Of the vessel activities and anthropogenic activities on

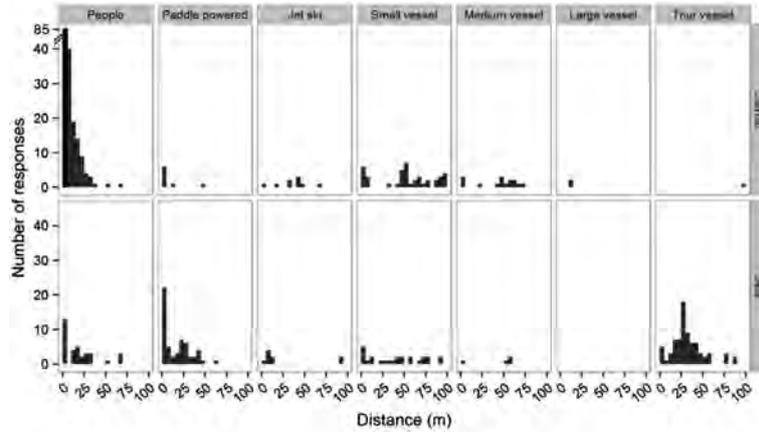


Figure 7. Number of *N. cinerea* responses elicited by groups of ‘People’ and vessels at Carnac Island and Seal Island, in 5 m bins and truncated at 100 m.

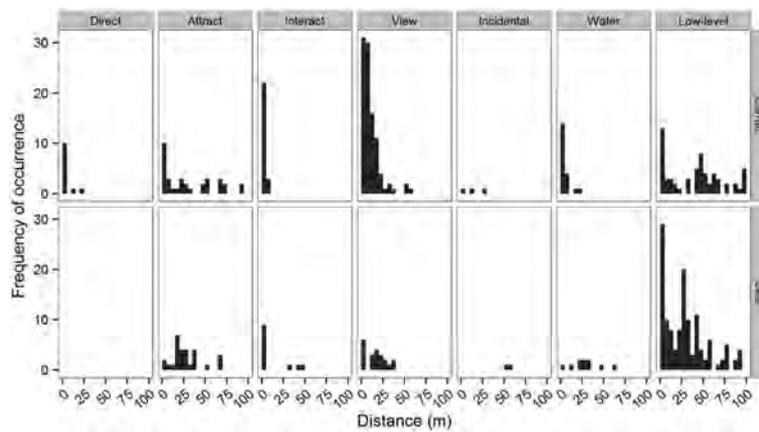


Figure 8. Frequency of *N. cinerea* responses elicited by ‘People’s’ activities on board, in the water and on the beach at Carnac Island and Seal Island, in 5 m bins and truncated at 100 m.

vessels at Carnac Island, ‘Anchor’ and ‘Engine’ noises elicited most responses in *N. cinerea* (Figure S2). At Seal Island, most vessel related responses were triggered by ‘Low-level’ activities (Figure S2).

DISCUSSION

Neophoca cinerea regularly respond to anthropogenic activities and the response type and frequency can be dependent on the stimulus itself, its range and the activity. In this study, anthropogenic stimulus and activity types varied at two differently managed islands. While response

levels were, in general, similar at both locations, the most severe behavioural response levels, ‘Aggressive’ and ‘Retreat’, occurred mostly at Carnac Island, predominantly elicited from approaches by ‘People’ and probably because of their proximity (≤ 10 m) to *N. cinerea*. The majority of responses were generated from stimuli that achieved the closest range and decreased with increasing range. Responses elicited at greater ranges were more likely to occur when stimuli were undertaking activities associated with elevated noise levels or actions directed at attracting *N. cinerea*’s attention.

RESPONSES OF *N. CINEREA* TO ANTHROPOGENIC ACTIVITIES

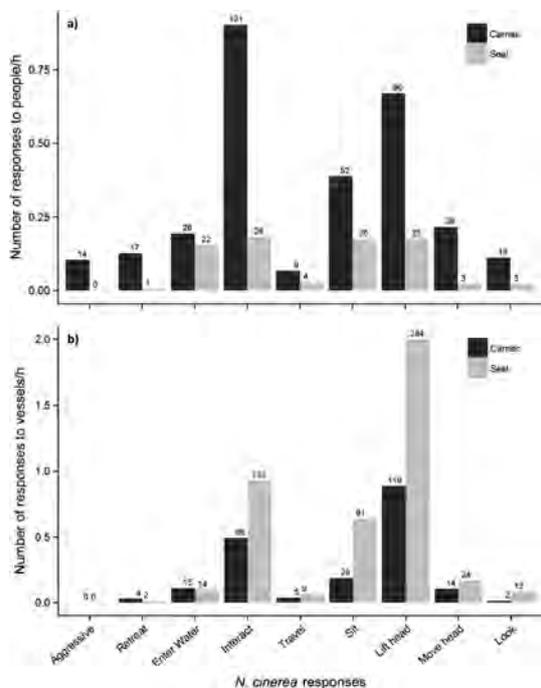


Figure 9. Number of *N. cinerea* responses per hour of sampling elicited by (a) 'People' and (b) vessels at Carnac Island and Seal Island (excluding 'Aircrafts'). Numbers above each bar indicate the total number for each behaviour observed (Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).

Distance has been identified in many studies as the main factor in altering pinniped behaviour, eliciting stronger responses when disturbance occurred within closer ranges (Cassini, 2001; Boren *et al.*, 2002; Labrada-Martagón *et al.*, 2005; Szanislo, 2005; Shaughnessy *et al.*, 2008; Strong and Morris, 2010; Pavez *et al.*, 2014; Young *et al.*, 2014). Here, 'Viewing' activities were associated with low levels of noise, as any discernible sound (e.g. screaming, banging objects or splashing water) reclassified the activity to a higher level. 'Viewing' elicited one of the highest rates of response (apart from 'Low-level' activities) and were mostly conducted at relatively short ranges where animals could perceive them without auditory cue (Schusterman and Balliet, 1971; Schusterman, 1972). In phocids, such close range approaches of various stimulus types caused Saimaa ringed seals (*Phoca hispida saimensis*) (Niemi *et al.*, 2013) and harbour seals (*Phoca vitulina*) at various locations to exhibit avoidance behaviour and enter the water (Allen *et al.*, 1984; Suryan and Harvey, 1999; Henry and Hammill, 2001; Jansen *et al.*, 2010; Anderson *et al.*, 2012; Osinga *et al.*, 2012). In this study, more than 40% of all responses elicited by 'People' were attributed to 'Viewing' activities with most of these approaches being classified as

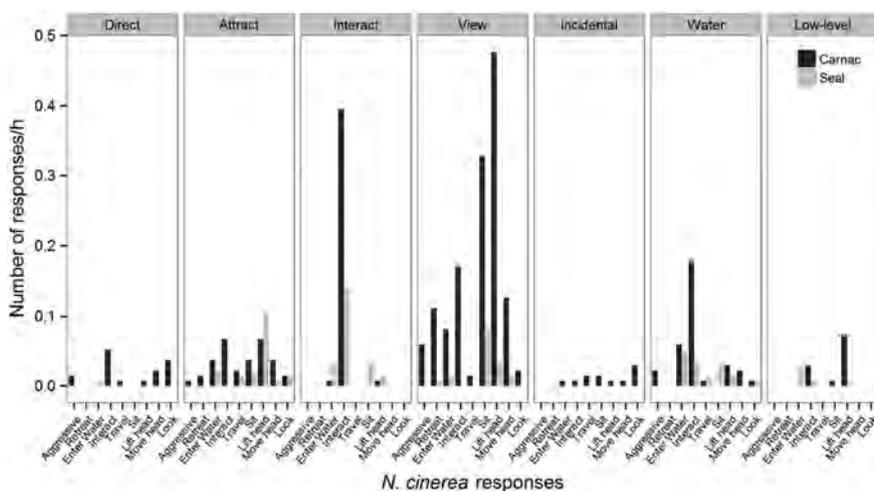


Figure 10. Number of *N. cinerea* responses elicited per hour as a result of groups of 'People' undertaking different activities at Carnac Island and Seal Island (Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).

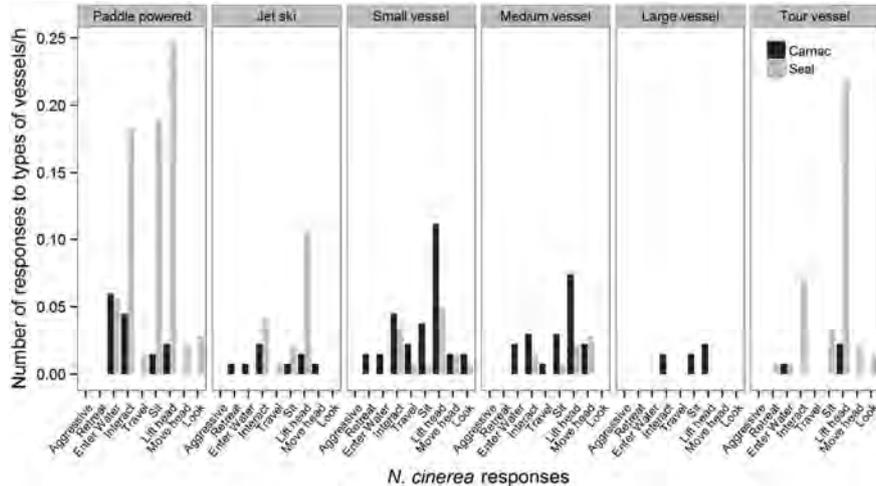


Figure 11. Number of *N. cinerea* responses per hour of sampling elicited by different vessel types at Carnac Island and Seal Island (excluding 'Aircrafts'; Carnac: 134 h, Seal: 142 h sampling effort between November 2013 and end April 2014).

breaches of the required 5 m minimum distance. These findings are consistent with a study of *N. cinerea* at Seal Bay, South Australia, which exhibited elevated response rates, including aggressive and avoidance behaviours, when approached within 10 m and even more so when approached within 5 m (Lovasz *et al.*, 2008).

While distance has a significant effect on responses elicited, human behaviour also has been noted to significantly contribute to disturbance of otariids (*Arctocephalus australis*; Cassini, 2001; Labrada-Martagón *et al.*, 2005; Pavez *et al.*, 2014), and phocids. Vessel activities that involve higher in-air noise levels have been shown to have similar effects to direct, i.e. interactive human disturbance. *Neophoca cinerea* in this study 'Lifted their heads' to 'Engine' and 'Anchor' noise, similar to Australian fur seals (*Arctocephalus pusillus*) that exhibited increased response rates at higher noise levels of vessels (Tripovich *et al.*, 2012). It should be noted that the presence of noise in this study was based on its perceived presence by researchers located within proximity of the animals, and was not based on measurements of in-air noise levels. However, otariid's hearing sensitivity includes the frequency band in which much of the energy from an engine, human speech, and anchor noise occurs (Gramming *et al.*, 1988;

Richardson *et al.*, 1995; Badinoa *et al.*, 2012; Muslow *et al.*, 2014). During visits to Seal Island, the 'Tour vessel's' amplified guides were regularly audible to the researchers on the island, and probably the cause of frequent *N. cinerea* responses. This probably also contributes to the peak of responses occurring at 25–30 m at Seal Island, reflecting the most common shortest range to which the 'Tour vessel' approached. Similarly, anthropogenic activity in association with noise was identified as likely to cause disturbance in harbour seals, during a non-breeding season in Iceland (Granquist and Sigurjonsdottir, 2014). Elevated noise levels of passing 'Aircrafts', such as scenic flights resulted in responses in *N. cinerea*, similar to responses of Steller sea lions (*Eumetopias jubatus*) and *P. vitulina* to low-flying aircraft (Osborn, 1985; Henry and Hammill, 2001; Kucey, 2005; Szaniszlo, 2005).

'Aggressive' gaping and launching behaviours in *N. cinerea* towards 'People' were primarily evoked by close proximity 'Viewing' and occasionally 'Direct' invasive activities. Proximity of 'People' to *N. cinerea* occurred mainly at Carnac Island owing to easy (and non-restricted) beach access at a limited number of specific points, and was probably the main trigger of 'Aggressive' behaviours and higher numbers of 'Retreat' responses here compared with

Seal Island. Approaches from land are potentially perceived as a more immediate and greater threat than approaches by vessels, and the resulting behaviours have been observed in other pinnipeds (Stirling, 1972; Boren *et al.*, 2002; Osinga *et al.*, 2012).

While stimulus type had a significant influence on the level of *N. cinerea* responses, stimulus types varied in exposure level, minimum approach distance and duration between the two islands. Although not directly studied here, draft associated with vessel type may limit a vessel's approach range to a beach, and thus the distance at which different vessel types may trigger responses from hauled out *N. cinerea*; simply put, larger vessels did not approach as closely as smaller vessels at either island. Furthermore, the relative proximity of the island to the mainland coast also affected the type of vessel that visited the islands. The close proximity of Seal Island to shore and its location within the sheltered waters of Shoalwater Bay, allowed 'Paddlers' to access the island easily and 'Tour vessels' to offer multiple trips per day to view *N. cinerea*. Thus, the high number of 'Paddle powered' vessels able to approach the island to within a few metres may explain the high number of responses at Seal Island. In addition, 'Paddlers' elicited responses mostly during 'Low'-level activities, i.e. mostly by their presence alone, indicating range, rather than activity per se, was the driving factor. Such 'surprise' appearances (i.e. no engine noise) at close range and higher mobility have been thought to have similar impacts on pinnipeds elsewhere (Allen *et al.*, 1984; Osborn, 1985; Suryan and Harvey, 1999; Henry and Hammill, 2001). In comparison, there were fewer responses to other vessel types, particularly large vessels associated with large drafts, which on average were at greater distances from *N. cinerea*.

Carnac Island's longer distance from shore is probably the reason for the greater number of large vessels visiting compared with Seal Island. Moreover, the draft associated with larger vessels meant that they remained further from the beach than smaller vessels. Furthermore, Carnac Island has a relatively large area of sandy beach with non-restricted access by people during the day, resulting in greater numbers of people on the beach than 'no access' Seal Island. In general, 'People' approached *N. cinerea* more closely than 'Paddlers', possibly

explaining their greater probability of eliciting behavioural responses. The number of 'Paddlers' visiting Carnac Island was approximately 10 times lower than at Seal Island, maybe due to the increased distance from the mainland coast.

Apart from Carnac Island's greater distance from shore, the intention to visit Carnac Island as a 'day trip', rather than the 'stop-off' that Seal Island represents, may also explain the longer times vessels stayed at Carnac Island than at Seal Island. 'People' visit Carnac Island mainly for other recreational purposes and 'Viewing' *N. cinerea* is a secondary activity (Orsini and Newsome, 2005). Conversely, as landing on Seal Island is prohibited, viewing *N. cinerea* is the primary reason for visitation which most groups carried out for relatively short times resulting in *N. cinerea* being exposed to human activity for shorter individual periods.

The total number of vessels and 'People' can have variable influences on pinniped reactions (Jansen *et al.*, 2015). Here, the proportion of responding *N. cinerea* did not appear to vary with increasing or decreasing numbers of vessels or 'People', which is similar to some studies where response behaviours remained comparatively consistent (Kovacs and Innes, 1990; Strong and Morris, 2010). However, in other studies varying behavioural responses occurred with differing numbers of people in the vicinity, such as adult male *N. cinerea*, during the breeding season reportedly responding to individual people at greater distances than to groups of people (Lovasz *et al.*, 2008). In contrast, females and other age groups observed in the same study did not show variation when approached by people on their own or in groups. Lovasz *et al.* (2008) speculated that the breeding season may play a role in responses, but was not able to ascertain what that might be. Quite the opposite, however, has been observed in *A. australis* (Cassini *et al.*, 2004).

Long-lasting interactions between *N. cinerea* and vessels or 'People' in the water were a common occurrence in this study (26.5% of all responses), similar to *Arctocephalus forsteri* approaching kayaks or interacting with swimmers from a swim tour (Boren *et al.*, 2002; Cowling *et al.*, 2014). In contrast, at Seal Bay, South Australia, *N. cinerea* have been recorded to only rarely respond to interacting behaviours (Lovasz *et al.*, 2008). In this

study, one example of long-lasting duration occurred at Carnac Island when no other vessel or 'People' were in the vicinity. One adult and three sub-adult *N. cinerea* 'Entered the water' immediately when one of the marine park rangers removed star pickets from the beach (always remaining at >10 m range). The first sub-adult to haul out after leaving the beach did so more than 1 hour after the incident, and more than 40 min after the rangers' vessel had left. This is comparable with *Zalophus californianus* mostly re-hauling out within 10 min after disturbance ceased, though they could take up to 3.5 h (Labrada-Martagón *et al.*, 2005). Anthropogenic impacts may, therefore, have altered *N. cinerea*'s natural behaviour in this study considerably, especially when *N. cinerea* 'Entered the water' or began 'Interactions', although 'Interactions' may have occurred voluntarily.

Arctocephalus pusillus have shown increased levels of aggression among themselves when exposed to higher sound levels (Tripovich *et al.*, 2012). In contrast, similar behavioural changes as a response to noise were not observed in this study, and aggressive behaviours towards stimuli were comparatively rare. This difference may be explained by age and sex composition of the study populations, as well as timing within the breeding cycle (Boren *et al.*, 2002; Labrada-Martagón *et al.*, 2005; Tripovich *et al.*, 2012; Cowling *et al.*, 2014; Pavez *et al.*, 2014). How human impacts affect different age and sex classes is known to vary between different species of pinnipeds. Females were more sensitive to anthropogenic activities in *P. vitulina* (Selvaggi *et al.*, 2004), whereas sub-adult males were more responsive to anthropogenic activities in South American sea lions *Otaria bryonia*, and adult male *N. cinerea* elsewhere reacted at slightly greater distances than females and other age classes (Lovasz *et al.*, 2008). In *O. bryonia*, more frequent disturbance was elicited at a breeding site compared with a haul-out site, whereas female *P. vitulina* displayed less pronounced responses, appearing reluctant to leave their pups (Anderson *et al.*, 2012; Pavez *et al.*, 2014).

The high frequency of anthropogenic activities, the resulting disturbance, and the time to return to previous behaviours may have an important effect on *N. cinerea* activity and energy budgets of

individual animals. The accumulation over time of these may lead to long-term effects. *Neophoca cinerea* have a ~2.3 times higher field metabolic rate and a ~6.2 times higher basal metabolic rate than terrestrial animals of comparable size (Costa and Gales, 2003). Based on this knowledge, the energy demands on individual *N. cinerea* are relatively high. *Neophoca cinerea* are benthic foragers and their foraging trips are highly demanding and energy intensive (Costa and Gales, 2003). Hauling out may help conserve energy and contribute to recuperation between foraging trips (Riedman, 1990). Interrupting *N. cinerea*'s recovery time from strenuous foraging trips may, therefore, alter their activity budgets and increase energetic requirements. This could mean that *N. cinerea* frequently responding to anthropogenic activity while resting, must increase time spent foraging to gain sufficient energy to offset the time spent at higher activity levels, which, consequently, could result in less time spent resting. If *N. cinerea* spend less time resting between foraging trips, they may be more susceptible to disease and other threats if their fitness is reduced (Taillier, 2014). This study did not attempt to track movements of identified individuals over time or investigate impacts on overall numbers of animals hauled out. However, pinnipeds may face displacement from preferred sites and move to less suitable habitat as a result of ongoing disturbance (Allen *et al.*, 1984; Stevens and Boness, 2003; Kucey, 2005). The impact of anthropogenic activities on overall numbers of *N. cinerea* hauling out at Carnac and Seal islands is unknown, but recommended to be investigated in future studies.

Habituation to people has been suspected in *N. cinerea* at Seal Bay, South Australia. *Neophoca cinerea* show more disturbance at a rarely disturbed site compared with a long-term, frequently visited site where people are able to approach within close range (Stirling, 1972; Lovasz *et al.*, 2008). Carnac and Seal islands are both visited frequently and *N. cinerea* may show some level of habituation, especially considering the high number of interactions with vessels and 'People'. However, quantifying habituation in animals so commonly visited over such a prolonged period as occurs at the islands studied would not be trivial.

Some biases may have been introduced by the inability to equally measure all distances between responding animals and stimuli. However, the difference between the distribution of total minimum approach distances and that of response distances illustrate that the sample size across ranges was sufficient to detect the inverse relationship of response with range. Furthermore, while behavioural changes of *N. cinerea* were excluded when there was uncertainty as to whether the response was to anthropogenic activities, some responses might have been misclassified as a response to humans, when they were not. The authors, however, believe that these cases were rare and that responses were more likely underestimated. In particular, while measurements were taken of vessels, 'People', and closest *N. cinerea* during heavy visitation periods (although priority was placed on 'People', vessels in close proximity to *N. cinerea* and vessels involved in conspicuous activities), some *N. cinerea* responses or measured distances may have been missed. 'Look', for example, was often an inconspicuous behaviour, particularly if *N. cinerea* faced away from the researchers, and was therefore possibly underestimated. A previous study, conducted at Carnac Island during summer months, approximately 6 months prior to the *N. cinerea* peak season, investigated responses to people. This documented relatively high numbers of responses in the three response categories measured (lift head, sit and look) and include repeated responses to the same stimuli (Orsini, 2004; Orsini *et al.*, 2006). Hourly sampling periods, observing these responses were conducted on one *N. cinerea* at a time, totalling 240 *N. cinerea* sampling periods. The sampling method and measurements, however, differ from that of this study, in particular that the observer was positioned within close proximity to the animals and thus while detecting a greater number of low level responses, may also have contributed to them (Orsini, 2004; Orsini *et al.*, 2006).

Suggestions for management

This study showed that not only did distance play a major role in eliciting responses in *N. cinerea*, but human and vessel activity types were also contributors. These factors should be included as

primary considerations for programmes aiming at reducing disturbance. The impact of disturbance on individual energetics has not been investigated here and, similar to response levels, are likely to vary between species and location. However, it is feasible that many of the following suggestions, and indeed the current management protocols put in place by the Department of Parks and Wildlife, Western Australia, would reduce responses of *N. cinerea* if applied to haul-out locations of pinniped species elsewhere. Thus by increasing the minimum approach distance for vessels and people to 30 m, disturbance would be expected to decrease significantly as high rates in this study were observed at the current minimum approach distance restrictions of 5–10 m (DEC and DoF, 2011). The frequent breaches of the current limit was a notable feature in this study, thus enforced minimum distances may improve the effectiveness of the regulations. In a separate study in South America, fencing limited the distance people were able to access, approach, and view *A. australis* from land, and significantly decreased human disturbance, including attacks on people which were reduced from four in a month to zero (Cassini *et al.*, 2004). 'Aggressive' behaviour towards 'People' and 'Retreat' behaviours, were observed more than once a day on Carnac Island in 74% of all *N. cinerea* observation days in this study. Limiting the approach distance and/or beach access may reduce the highest response levels and lower the chances of danger to both humans and pinnipeds. Designating all of Carnac Island (rather than a section of the beach) as a sanctuary zone, as presently exists on Seal Island, may assist in reducing disturbance. It would perhaps also provide visitors with a stronger awareness of their responsibilities when interacting with wild animals. As a control measure, marker buoys installed 15 m off the waterline at low tide at Carnac and Seal islands may reduce the disturbance of *N. cinerea* thermoregulating in the wash zone during periods of higher air temperatures (Marlow, 1975; Riedman, 1990). Creating a demarcation of a boundary with buoys where vessels and 'People' should not pass may help reduce 'People' accidentally beaching their kayaks (as occurred during 50% of the field days at Seal Island) and also increase awareness of the

sanctuary zone. In addition, standardizing control measures across *N. cinerea* haul-out locations may assist in generating more consistent behaviour from the public to limit disturbance. This study has not investigated the impacts of disturbance on the energetics of *N. cinerea*. The following suggestions are therefore made based on a precautionary approach, given that the level of effects of disturbance on *N. cinerea* energetics has yet to be quantified.

In general, it is probable that most visitors are not aware they are causing a disturbance to *N. cinerea* or what effects these disturbances may have on colonies and the overall population (Orsini and Newsome, 2005). Clear signage and other forms of information and educational material, including increased direct communication from patrol officers, may improve awareness of the importance of haul-out and resting behaviours to *N. cinerea* health and body condition. Furthermore awareness of the potential impacts of noise may alter peoples' behaviours so that noise levels and overall disturbance are reduced when in close proximity to animals (Newsome and Rodger, 2008). In a previous study, the combination of approaching slower, maintaining greater ranges, and having quieter passengers reduced disturbance of *P. vitulina* by 60–80% (Hoover-Miller *et al.*, 2013).

In conclusion, this study has shown that a considerable number of responses and behavioural changes were elicited by anthropogenic activities. Significant differences occurred between Seal and Carnac islands in levels of exposure, including the exposure duration and types of stimuli, as well as in the elicited response levels. However, most responses occurred in close ranges to *N. cinerea*. If minimum approach distances in guidelines are increased, and the public is made aware that calm and quiet behaviour around Seal and Carnac islands would significantly reduce the potential impacts of anthropogenic activity, the number of responses due to disturbance may be reduced. Longer-term studies measuring the cumulative duration of interactions, assessing the effects of anthropogenic activities on *N. cinerea*'s energy budgets, and determining the impacts of fitness and habitat displacement at an individual and population level are recommended. However, it should also be noted that pinniped responses to humans varies widely between species

and that context is an important factor in the application of protocols to mitigate disturbance.

ACKNOWLEDGEMENTS

The Department of Parks and Wildlife (Western Australia) made this project possible with substantial logistical support provided including transport to and from the islands and loan of a spotting scope, as well as provision of local and scientific knowledge. Transport to and from islands was also provided by the Department of Fisheries and Cockburn Volunteer Sea Search and Rescue. The Fremantle Sailing Club provided access to their boat ramp. The project was also supported by Malcom Perry through maintenance of the research vessel and Mick Todd through loan of his telescope. Many volunteers contributed substantial time assisting in the field and entering data into spreadsheets in the office. The authors are grateful to Miles Parsons for providing editorial and analytical advice which significantly improved the quality of this manuscript, Sven Gastauer for providing advice on R code, and Julie Sims who gave useful advice on data analysis. The authors appreciate the constructive feedback and helpful advice from both reviewers and the editor to improve this manuscript. Finally, this project was supported by funding from the Holsworth Wildlife Research Endowment. This project was also conducted under a Department of Parks and Wildlife permit (number SF009371) and university animal ethics approvals (AEETH24/11 granted by Victoria University, Melbourne and AEC_2013_32 granted by Curtin University, Perth). The authors have no conflicting interests to declare.

REFERENCES

- Allen SG, Ainley DG, Page GW, Ribic CA. 1984. The effect of disturbance on harbor seal haul out patterns at Bolinas Lagoon, California. *Fishery Bulletin* **82**: 493–500.
- Anderson SM, Teilmann J, Dietz R, Schmidt NM, Miller LA. 2012. Behavioural responses of harbour seals to human-induced disturbances. *Aquatic Conservation: Marine and Freshwater Ecosystems* **22**: 113–121.
- Badino A, Borellib D, Gaggeroc T, Rizzutod E, Schenonee C. 2012. Normative framework for ship noise: present situation and future trends. *Noise Control Engineering Journal* **60**: 740–762.

- Beentjes MP. 1989. Haul-out patterns, site fidelity and activity budgets of male Hooker's sea lions (*Phocarctos hookeri*) on the New Zealand mainland. *Marine Mammal Science* **5**: 281–297.
- Boren L, Gemmell NJ, Barton KJ. 2002. Tourist disturbance on New Zealand fur seals *Arctocephalus forsteri*. *Australian Mammalogy* **24**: 85–95.
- Bowles AE, Anderson RC. 2012. Behavioral responses and habituation of pinnipeds and small cetaceans to novel objects and simulated fishing gear with and without a pinger. *Aquatic Mammals* **38**: 161–188.
- CALM. 2003. Carnac Island Nature Reserve - Management Plan 2003. Report, Department of Conservation and Land Management.
- Cassini MH. 2001. Behavioural responses of South American fur seals to approach by tourists – a brief report. *Applied Animal Behaviour Science* **71**: 341–346.
- Cassini MH, Szteren D, Fernández-Juricic E. 2004. Fence effects on the behavioural responses of South American fur seals to tourist approaches. *Journal of Ethology* **22**: 127–133.
- Constantine R. 1999. Effects of tourism on marine mammals in New Zealand. Report, Department of Conservation.
- Costa DP, Gales NJ. 2003. Energetics of a benthic diver: seasonal foraging ecology of the Australian sea lion, *Neophoca cinerea*. *Ecological Monographs* **73**: 27–43.
- Cowling M, Kirkwood R, Boren LJ, Scarpaci C. 2014. The effects of seal-swim activities on the New Zealand fur seal (*Arctophoca australis forsteri*) in the Bay of Plenty, New Zealand, and recommendations for a sustainable tourism industry. *Marine Policy* **45**: 39–44.
- Cowling M, Kirkwood R, Boren L, Scarpaci C. 2015. The effects of vessel approaches on the New Zealand fur seal (*Arctocephalus forsteri*) in the Bay of Plenty, New Zealand. *Marine Mammal Science* **31**: 501–519.
- DEC. 2007. *Shoalwater Islands Marine Park Management Plan 2007–2017*. Management Plan: Perth, Western Australia.
- DEC, DoF. 2011. Shoalwater Islands Marine Park. Marine parks...WA's submerged wonders. Recreation guide. Perth, Western Australia.
- Fisher RA. 1922. On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society* **85**: 87–94.
- French SS, González-Suárez M, Young JK, Durham S, Gerber LR. 2011. Human disturbance influences reproductive success and growth rate in California sea lions (*Zalophus californianus*). *PloS One* **6**: e17686.
- Gales NJ. 1995. Hooker's sea lion recovery plan (*Phocarctos hookeri*). In *Threatened species recovery plan*, 17. Threatened Species Unit, Department of Conservation: Wellington, New Zealand.
- Gales NJ, Cheal AJ, Pobar GJ, Williamson P. 1992. Breeding biology and movements of Australian sea lions, *Neophoca cinerea*, off the west coast of Western Australia. *Wildlife Research* **19**: 405–416.
- Gales NJ, Shaughnessy PD, Dennis TE. 1994. Distribution, abundance and breeding cycle of the Australian sea lion *Neophoca cinerea* (Mammalia: Pinnipedia). *Journal of Zoology* **234**: 353–379.
- Gerrodette T, Gilmartin WG. 1990. Demographic consequences of changed pupping and hauling sites of the Hawaiian monk seal. *Conservation Biology* **4**: 423–430.
- Goldsworthy SD. 2015. *Neophoca cinerea*. The IUCN Red List of Threatened Species 2015: e.T14549A45228341. <http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T14549A45228341.en>. Downloaded on 19 January 2016.
- Goldsworthy SD, Shaughnessy PD, McIntosh RR, Kennedy C, Simpson J, Page B. 2008. Australian sea lion populations at Seal Bay and the Seal Slide (Kangaroo Island): continuation of the monitoring program. Report, South Australian Research and Development Institute.
- Gormley AM, Slooten E, Dawson S, Barker RJ, Rayment W, du Fresne S, Bräger S. 2012. First evidence that marine protected areas can work for marine mammals. *Journal of Applied Ecology* **49**: 474–480.
- Gramming P, Sundberg J, Ternström S, Leanderson R, Perkins WH. 1988. Relationship between changes in voice pitch and loudness. *Journal of Voice* **2**: 118–126.
- Granquist SM, Sigurjonsdottir H. 2014. The effect of land based seal watching tourism on the haul-out behaviour of harbour seals (*Phoca vitulina*) in Iceland. *Applied Animal Behaviour Science* **156**: 85–93.
- Hartel EF, Constantine R, Torres LG. 2015. Changes in habitat use patterns by bottlenose dolphins over a 10-year period render static management boundaries ineffective. *Aquatic Conservation: Marine and Freshwater Ecosystems* **25**: 701–711.
- Henry E, Hammill MO. 2001. Impact of small boats on the haulout activity of harbour seals (*Phoca vitulina*) in Métis Bay, Saint Lawrence Estuary, Québec, Canada. *Aquatic Mammals* **27**: 140–148.
- Hoover-Miller A, Bishop A, Prewitt J, Conlon S, Jezierski C, Armato P. 2013. Efficacy of voluntary mitigation in reducing harbor seal disturbance. *The Journal of Wildlife Management* **77**: 689–700.
- Jansen JK, Boveng PL, Dahle SP, Bengtson JL. 2010. Reaction of harbor seals to cruise ships. *The Journal of Wildlife Management* **74**: 1186–1194.
- Jansen JK, Boveng PL, Ver Hoef JM, Dahle SP, Bengtson JL. 2015. Natural and human effects on harbor seal abundance and spatial distribution in an Alaskan glacial fjord. *Marine Mammal Science* **31**: 66–89.
- Kelleher G. 1999. *Guidelines for Marine Protected Areas*. World Commission on Protected Areas of IUCN – The World Conservation Union: Gland, Switzerland, and Cambridge, UK.
- Kirkwood R, Boren L, Shaughnessy P, Szteren D, Mawson P, Huckstadt L, Hofmeyr G, Oosthuizen H, Schiavini A, Campagna C, Berris M. 2003. Pinniped-focused tourism in the southern hemisphere: a review of the industry. In *Marine Mammals: Fisheries, Tourism and Management Issues*, Gales N, Hindell M, Kirkwood R (eds). CSIRO Publishing: Collingwood; 257–272.
- Kovacs KM, Innes S. 1990. The impact of tourism on harp seals (*Phoca groenlandica*) in the Gulf of St. Lawrence, Canada. *Applied Animal Behaviour Science* **26**: 15–26.
- Kruskal WH, Wallis WA. 1952. Haulout patterns of grey seals *Halichoerus grypus* in the Baltic Sea. *Journal of the American Statistical Association* **47**: 583–621.
- Kucey L. 2005. Human disturbance and the hauling out behaviour of Steller sea lions (*Eumetopias jubatus*). Thesis, University of British Columbia, Vancouver, Canada.
- Labrada-Martagón V, Aurióles-Gamboja D, Martínez-Díaz SF. 2005. Natural and human disturbance in a rookery of the California sea lion (*Zalophus californianus californianus*) in the Gulf of California, Mexico. *Latin American Journal of Aquatic Mammals* **4**: 175–185.

- Lovasz T, Croft D, Banks P. 2008. Establishing tourism guidelines for viewing Australian sea lions *Neophoca cinerea* at Seal Bay Conservation Park, South Australia. In *Too Close For Comfort*, Lunney D, Munn A, Meikle M (eds). Royal Zoological Society of New South Wales: Mosman, NSW, Australia; 225–232.
- Marlow BJ. 1975. The comparative behaviour of the Australasian sea lions, *Neophoca cinerea* and *Phocarcos hookeri* (Pinnipedia: Otariidae). *Mammalia* **39**: 159–230.
- McHugh ML. 2013. The Chi-square test of independence. *Biochemia Medica* **23**: 143–149.
- Muslow J, Houser DS, Finneran JJ. 2014. Aerial hearing thresholds and detection of hearing loss in male California sea lions (*Zalophus californianus*) using auditory evoked potentials. *Marine Mammal Science* **30**: 1383–1400.
- Newsome D, Rodger K. 2008. Impacts of tourism on pinnipeds and implications for tourism management. In *Marine Wildlife and Tourism Management: Insights from the Natural Social Sciences*, Higham JES, Lück M (eds). CABI: Oxfordshire; 182–205.
- Niemi M, Auttila M, Valtonen A, Viljanen M, Kunnasranta M. 2013. Haulout patterns of Saimaa ringed seals and their response to boat traffic during the moulting season. *Endangered Species Research* **22**: 115–124.
- Orams M. 1999. *Marine Tourism: Development, Impacts and Management*. Routledge: London, UK and New York, USA.
- Orsini JP. 2004. Human impacts on Australian sea lions, *Neophoca cinerea*, hauled out on Carnac Island (Perth, Western Australia): implications for wildlife and tourism management. Thesis, Murdoch University, Perth, Western Australia.
- Orsini JP, Newsome D. 2005. Human perceptions of hauled out sea lions (*Neophoca cinerea*) and implications for management: a case study from Carnac Island, Western Australia. *Tourism in Marine Environments* **2**: 129–132.
- Orsini JP, Shaughnessy PD, Newsome D. 2006. Impacts of human visitors on Australian sea lions (*Neophoca cinerea*) at Carnac Island, Western Australia: implications for tourism management. *Tourism in Marine Environments* **3**: 101–115.
- Osborn LS. 1985. Population dynamics, behavior, and the effect of disturbance on haulout patterns of the harbor seal *Phoca vitulina richardsi*. Thesis, University of California, Santa Cruz, CA, USA.
- Osinga N, Nussbaum SB, Brakefield PM, de Haes HAU. 2012. Response of common seals (*Phoca vitulina*) to human disturbances in the Dollard estuary of the Wadden Sea. *Mammalian Biology* **77**: 281–287.
- Osterrieder SK, Salgado Kent C, Robinson RW. 2015. Variability in haul-out behaviour by male Australian sea lions *Neophoca cinerea* in the Perth metropolitan area, Western Australia. *Endangered Species Research* **28**: 259–274.
- Pavez G, Muñoz L, Barilari F, Sepúlveda M. 2014. Variation in behavioral responses of the South American sea lion to tourism disturbance: Implications for tourism management. *Marine Mammal Science* **31**: 427–439.
- Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Richardson WJ, Greene CR Jr, Malme CI, Thomson DH. 1995. *Marine Mammals and Noise*, Academic Press: San Diego, CA.
- Riedman M. 1990. *The Pinnipeds: Seals, Sea Lions, and Walruses*, University of California Press: Berkeley, CA.
- Salgado Kent CP, Crabtree B. 2008. The effectiveness of an established sanctuary zone for reducing human disturbance to Australian sea lions (*Neophoca cinerea*) at Carnac Island, Western Australia. *Tourism in Marine Environments* **5**: 29–42.
- Schusterman RJ. 1972. Visual acuity in pinnipeds. In *Behavior of Marine Animals, Vol. 2*, Winn HE, Olla BL (eds). Plenum Press: New York; 469–492.
- Schusterman RJ, Balliet RF. 1971. Aerial and underwater visual acuity in the California sea lion (*Zalophus californianus*) as a function of luminance. *Annals of the New York Academy of Sciences* **188**: 37–46.
- Selvaggi E, Vedder L, Haafte van JL, Wensvoort P, Consiglio C. 2004. Effect of disturbance on daily rhythm and haul out behavior in the harbor seal (*Phoca vitulina*) in the tide estuary of Dollard. Proceedings of the Fifteenth Annual Conference of the European Cetacean Society, Rome, Italy, 6–10 May 2001, 92–97.
- Shaughnessy PD, Nicholls AO, Briggs SV. 2008. Do tour boats affect fur seals at Montague Island, New South Wales. *Tourism in Marine Environments* **5**: 15–27.
- Stevens MA, Boness DJ. 2003. Influences of habitat features and human disturbance on use of breeding sites by a declining population of southern fur seals (*Arctocephalus australis*). *Journal of Zoology* **260**: 145–152.
- Stirling I. 1972. Observations on the Australian sea lion, *Neophoca cinerea* (Peron). *Australian Journal of Zoology* **20**: 271–279.
- Strong P, Morris SR. 2010. Grey seal (*Halichoerus grypus*) disturbance, ecotourism and the Pembrokeshire Marine Code around Ramsey Island. *Journal of Ecotourism* **9**: 117–132.
- Suryan RM, Harvey JT. 1999. Variability in reactions of Pacific harbor seals, *Phoca vitulina richardsi*, to disturbance. *Fishery Bulletin* **97**: 332–339.
- Szanişzlo WR. 2005. California sea lion (*Zalophus californianus*) and Steller sea lion (*Eumetopias jubatus*) interactions with vessels in Pacific Rim National Park Reserve: implications for marine mammal viewing management. Thesis, University of Victoria, Canada.
- Taillier S. 2014. Great white shark probably choked to death on sea lion, authorities say. URL: <http://www.abc.net.au/news/2014-07-17/greatwhite-shark-probably-choked-on-a-sea-lion-2c-authorities-/5604410>.
- Tripovich JS, Hall-Aspland S, Charrier I, Arnould JPY. 2012. The behavioural response of Australian fur seals to motor boat noise. *PLoS One* **7**: e37228.
- Yates F. 1934. Contingency tables involving small numbers and the χ^2 test. *Supplement to the Journal of the Royal Statistical Society* **1**: 217–35.
- Young C, Gende SM, Harvey JT. 2014. Effects of vessels on harbor seals in Glacier Bay National Park. *Tourism in Marine Environments* **10**: 5–20.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site.

Appendix B

CHAPTER 4 TABLES

Table A1: Results of the final GAM determining effects of temporal and environmental factors on *N. cinerea* haul-out numbers.

Parametric terms	Estimate	Std error	t-value	df ⁺	F	p-value
Intercept	2.5	0.13	18.4	–	–	<2x10 ⁻¹⁶
Period (overall)	–	–	–	7	32.6	<2x10 ⁻¹⁶
Period 2	-0.2	0.14	-1.6	–	–	0.11
Period 3	-1.2	0.21	-5.9	–	–	5.10x10 ⁻⁹
Period 4	-1.6	0.21	-7.7	–	–	3.96x10 ⁻¹⁴
Period 5	-1.3	0.20	-6.6	–	–	7.73x10 ⁻¹¹
Period 6	-0.67	0.21	-3.1	–	–	0.0022
Period 7	0.31	0.14	2.2	–	–	0.027
Period 8	0.36	0.14	2.7	–	–	0.0074
Location (overall)	–	–	–	1	19.5	1.12x10 ⁻⁵
Location Seal Island	0.56	0.13	4.4	–	–	1.12x10 ⁻⁵
Time since Sunrise	0.02	0.004	5.3	–	–	1.19x10 ⁻⁷
Tide	-0.25	0.09	-3.0	–	–	0.0032
Period*Location (overall)	–	–	–	7	7.3	1.4x10 ⁻⁸
Period 2*Location Seal Island	-0.10	0.18	-0.54	–	–	0.59
Period 3*Location Seal Island	-0.004	0.26	-0.02	–	–	0.99
Period 4*Location Seal Island	-0.79	0.33	-2.4	–	–	0.017
Period 5*Location Seal Island	0.25	0.25	1.0	–	–	0.32
Period 6*Location Seal Island	0.50	0.26	1.8	–	–	0.077
Period 7*Location Seal Island	-0.36	0.17	-2.1	–	–	0.036
Period 8*Location Seal Island	-0.84	0.17	-4.9	–	–	1.12x10 ⁻⁶
Smoother Temperature	–	–	–	4.6	5.34	0.00014
				(edf ⁺⁺)		

⁺ df = degrees of freedom

⁺⁺ edf = estimated degrees of freedom

Table A2: Proportion of juvenile, sub-adult, adult and unknown *Neophoca cinerea* hauling out on Seal and Carnac Islands in Period 1 to 8 (n = sample size of field days on the particular island and season).

Period	Island (n)	Juveniles (% \pm SD)	Sub-adults (% \pm SD)	Adults (% \pm SD)	Unknown (% \pm SD)
1	Carnac (11)	NA	NA	NA	100 (\pm 0)
1	Seal (13)	NA	NA	NA	100 (\pm 0)
2	Carnac (12)	3.7 (\pm 4.5)	15.4 (\pm 14.1)	61.8 (\pm 19.9)	19.1 (\pm 19.5)
2	Seal (11)	19.1 (\pm 14.8)	17.0 (\pm 11.5)	47.3 (\pm 22.7)	16.5 (\pm 20.2)
3	Carnac (10)	0.0 (\pm 0.0)	2.0 (\pm 6.3)	92.0 (\pm 39.1)	6.0 (\pm 9.7)
3	Seal (10)	6.0 (\pm 10.4)	11.9 (\pm 16.9)	76.1 (\pm 35.5)	6.0 (\pm 10.4)
4	Carnac (16)	0.0 (\pm 0.0)	2.0 (\pm 8.0)	92.0 (\pm 49.4)	6.0 (\pm 17.4)
4	Seal (10)	0.0 (\pm 0.0)	9.5 (\pm 20.1)	85.7 (\pm 70.3)	4.8 (\pm 15.1)
5	Carnac (13)	0.0 (\pm 0.0)	4.1 (\pm 10.0)	89.8 (\pm 55.8)	6.1 (\pm 11.6)
5	Seal (9)	14.3 (\pm 21.4)	31.7 (\pm 34.1)	54.0 (\pm 24.5)	0.0 (\pm 0.0)
6	Carnac (5)	12.2 (\pm 14.9)	14.6 (\pm 5.5)	68.3 (\pm 22.2)	4.9 (\pm 6.7)
6	Seal (4)	14.3 (\pm 3.3)	31.4 (\pm 5.7)	54.3 (\pm 26.6)	0.0 (\pm 0.0)
7	Carnac (10)	2.5 (\pm 3.6)	49.2 (\pm 17.2)	47.2 (\pm 24.3)	1.0 (\pm 3.2)
7	Seal (10)	6.3 (\pm 5.2)	50.0 (\pm 12.6)	43.3 (\pm 12.1)	0.0 (\pm 0.0)
8	Carnac (11)	3.1 (\pm 3.0)	51.0 (\pm 17.3)	43.2 (\pm 26.2)	2.6 (\pm 7.0)
8	Seal (11)	3.9 (\pm 3.8)	52.0 (\pm 19.6)	44.1 (\pm 15.0)	0.0 (\pm 0.0)

Appendix C

CHAPTER 5 FIGURES

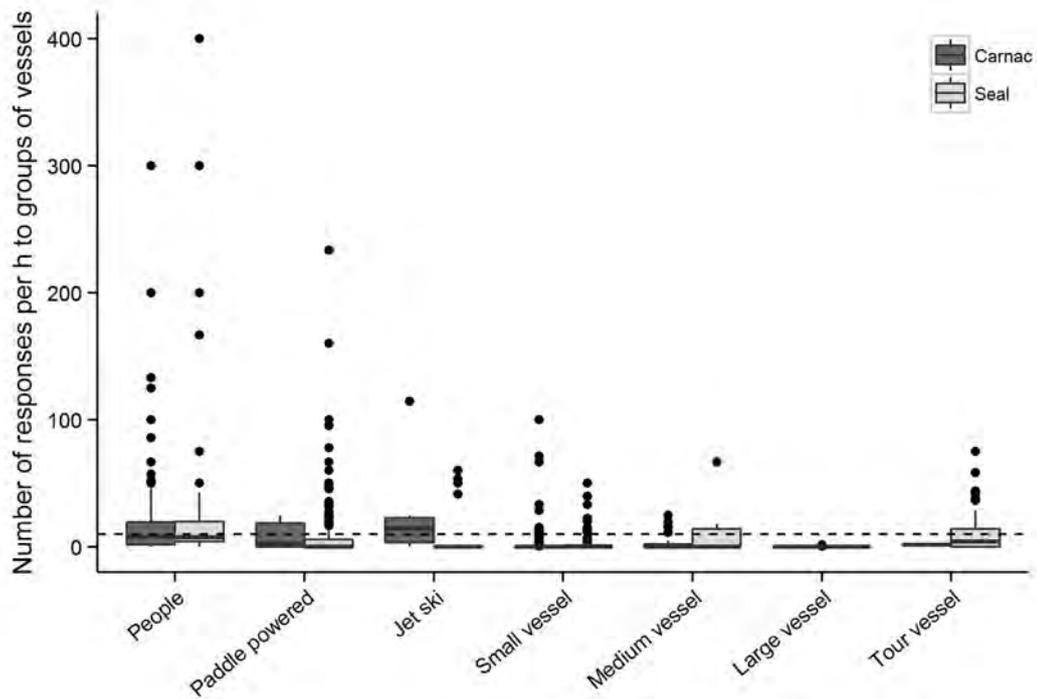


Figure A1: The interquartile ranges in the numbers of *N. cinerea* responses per hour per group for each vessel type and ‘People’ (with Carnac Island having 134 h and Seal Island having 142 h observation effort between November 2013 and end April 2014). The horizontal dotted line corresponds with 10 responses per hour.

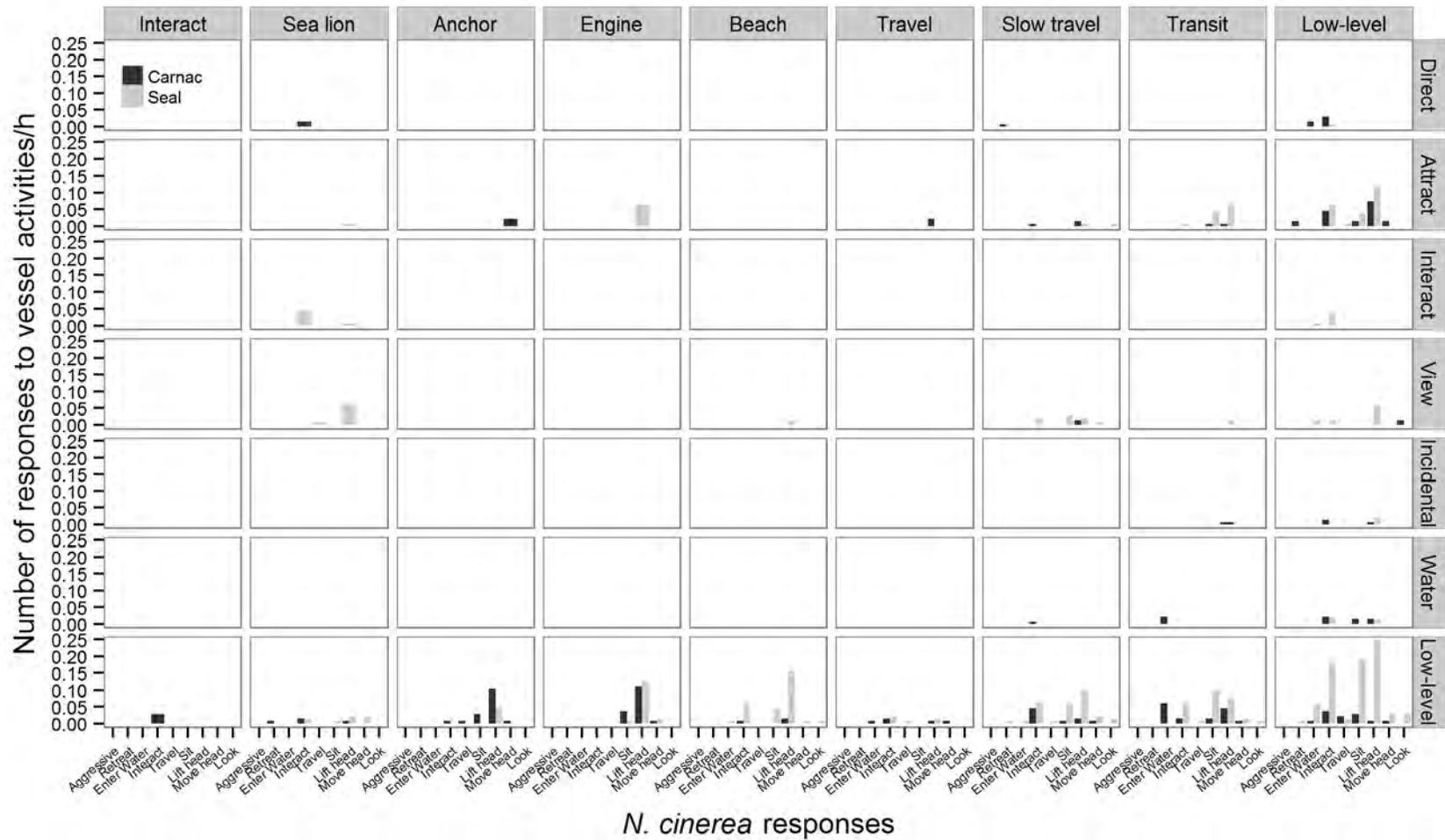


Figure A2: Number of *N. cinerea* responses per hour of sampling elicited by vessels at Carnac and Seal Island (including ‘Aircrafts’), divided into vessel activities and ‘People’ activities onboard.

Appendix D

CHAPTER 6 TABLE AND FIGURE

Table A3: Resighted individuals based on their scarring, including the date and location as well as the number of sightings and age of the individual *N. cinerea*. Location in brackets after the date: C – Carnac Island, S – Seal Island.

Individual	Dates (location) of sightings	Number of sightings	Age
1	16/08/2012 (C), 7/09/2012 (C)	2	Adult
2	23/08/2013 (S), 28/08/2013 (C)	2	Adult
3	29/08/2013 (S), 17/10/2013 (S), 6/01/2014 (S), 9/04/2014 (C), 11/04/2014 (C)	5	Sub-Adult
4	6/01/2014 (S), 7/01/2014 (S)	2	Adult





Figure A3: Photographs of resighted, scarred individuals (1-4 in Table A3) including the location and date of sighting.