Milestone 2: Report

An Investigation into the Control of Bryozoan (*Plumatella and Fredericella*) Infestation of Water Pipeline Systems

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VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

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March 2010

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1. The Milestones

Milestone 1 (1st October 2009 to 15th December 2009)

i. Utilizing the leverage from the first year of the project, prepare and submit an ARC Linkage application for Round 2 of 2010 entitled: "Prevention of Bryozoan biofouling of water pipeline systems". This application is to include Dr. Jane Sargison of the University of Tasmania as a collaborator on the project.

ii. Attend, and present the outcomes of the first year of the existing project, at the 32nd Hydrology & Water Resources Symposium, Newcastle, NSW, 1-3 December 2009.

iii. Conduct a two day field trip in December in order to collect post-chlorination samples from the NMP and live colonies for the purpose of colony-to-colony propagation. To consolidate and deliver the Milestone 5 report from the first year of the project.

iv. Provide a copy of the ARC Linkage application - submitted on the 18th October 2009.

v. Provide a copy of the presentation delivered by Dr. Andrew Barton, Dr. Robin Mitra and Professor John Orbell at the 32nd Hydrology & Water Resources Symposium. Provide a copy of the refereed publication that has been included in the proceedings.

vi. Provide the outstanding progress report in relation to Milestone 5 for the first year of the project. Details of the December '09 field trip will be included in the Milestone 5 report. Invoice for the second instalment of $20,871.00 on Tuesday 15th December 2009.

Milestone 2 (16th December 2009 to 28th February 2010)

i. Organize all literature collected to date into a comprehensive review article on freshwater Bryozoans and their biofouling characteristics. Liaise with all team members in order to prepare a draft of this review for submission to a high quality international journal.

ii. Using the live colonies collected on the December '09 field trip, initiate colony-to-colony propagation experiments in the laboratory. The identity of these colonies is to be confirmed from the morphology of their statoblasts (SEM). Continue work on statoblast-to-colony propagation.

iii. Access the commissioned GHD report and reconcile this with our investigations – liaise with Mike Chapman and Barbara Bowles, particularly in relation to a risk management approach to the project.

iv. Arrange a team meeting in February in order to discuss matters relating to experimental design. Issues for consideration include the development of a more systematic sampling protocol, methods for assessing (qualitative and quantitative) the degree of biofouling, water quality data, access to maintenance records and importantly the design and implementation of laboratory experiments to investigate alternative (to chlorine) methods for controlling Bryozoan infestation. Imperative to the testing of alternative control methods is the supply of sufficient quantities of viable Bryozoan colonies that can be challenged in the laboratory with various chemical agents and conditions.

v. Whilst progressing, the current statoblast-to-colony and colony-to-colony methods are proving to be rather sluggish at this stage of the project. Therefore, a concurrent strategy will be initiated relating to the cultivation of colonies on transportable "plates" within the Ouyen "pit". Such plates and growth media can be transported to facilities at VU for control experiments. This "field laboratory" will also allow the issue of seasonality to be conveniently investigated. The experimental design for this will be established at the February meeting scheduled for Thursday 11th February.

vi. Progress report in relation to Milestone 2 to be submitted with invoice for third instalment of $20,871.00 on Monday 1st March 2010.

Milestone 3 (1st March 2010 to 16th May 2010)

i. Finalize and submit the review described in Milestone 2 no later than the end of March.

ii. Using SEM, subject the statoblasts obtained from the December '09 and February '10 sampling to particle size analysis as part of the continuing program to investigate the seasonality characteristics of these organisms.

iii. Continue the statoblast-to–colony and colony-to-colony cultivation of the identified Bryozoan organisms in the laboratory.

iv. Concurrently design, construct and install a "field cultivation laboratory" consisting of an array of growth plates to be suspended in the "pit" at Ouyen (see Milestone 5 report).

v. Transport the plate colonies and pit water to the purpose-built facilities at the St Albans Campus of VU and establish a methodology for assessing growth status and for carrying out static

vi. Liaise with all team members to produce a draft manuscript detailing the SEM characteristics of the two NMP species also describing their geographical locations.

vii. Initiate formal discussions for the extension of the investigations to the WMP.

viii. Progress report in relation to Milestone 3 to be submitted with invoice for fourth instalment of $20,871.00 on Monday 17th May, 2010.

Milestone 4 (17th May 2010 to 30th July 2010)

i. Submit the article described in Milestone 3 to a high quality international journal by the end of May.

ii. Conduct a two day field trip in June in order to collect seasonal samples and to monitor and collect samples from the field laboratory. Evaluate the field laboratory and process all samples as in Milestone 3.

iii. Continue the static testing and acquire data in relation to the relative effects of various control agents.

iv. Draft a technical paper based on our field sampling experience for publication in appropriate journal.

v. Design, acquire and commission laboratory scale equipment whereby cultivated colonies may be systematically challenged with various control agents under flow conditions.

vi. Progress report in relation to Milestone 4 to be submitted with invoice for fifth instalment of $20,871.00 on Friday 30th July 2010

Milestone 5 (31st July 2010 to 30th September 2010)

i. Attend and present (JO and/or RM) at the International Bryozoan Association conference in Kiel, Germany, from 1-7 August, 2010.

ii. Conduct a two day field trip in late August in order to collect seasonal samples and to monitor and collect samples from the field laboratory.

iii. Continue static testing and the acquiring of data in relation to the relative effects of various control agents both under static and flow conditions.

iv. Evaluate the field laboratory and process all samples as in Milestone 3.

v. Submit the technical paper described in Milestone 4 to an appropriate journal.

vi. Make recommendations on the relative effectiveness of various control agents towards Bryozoans relative to chlorine.

vii. Progress report in relation to Milestone 5 to be submitted on Thursday 30th September 2009.

Table 1: Timetable depicting progress of the project in relation to the YEAR 2 milestones.

Milestones	Oct 2009	Nov 2009	Dec 2009	Jan 2010	Feb 2010	March 2010	April 2010	May 2010	June 2010	July 2010	Aug 2010	Sept 2010
MS 1												
MS2												
MS3												
MS4												
MS5												

1.1. Reappraisal of Milestone 1: salient points

1. Utilizing the leverage from the first year of the project, prepare and submit an ARC Linkage application for Round 2 of 2010 entitled: "Prevention of Bryozoan biofouling of water pipeline systems". This application is to include Dr. Jane Sargison of the University of Tasmania as a collaborator on the project.

AUSTRALIAN RESEARCH COUNCIL
Linkage - Projects
Linkage Projects 2010 - Round 2

LP

PROJECT ID: LP100200562

First Investigator: Prof John Orbell

Admin Org: Victoria University

Total number of sheets contained in this Proposal: 74

Information on this form and its attachments is collected in order to make recommendations to the Minister on the allocation of financial assistance under the Australian Research Council Act 2001 and for post award reporting. The information collected may be passed to third parties for assessment purposes. It may also be passed to the National Health and Medical Research Council, the Department of Foreign Affairs and Trade, the Department of Innovation, Industry, Science and Research, the Department of the Environment, Water, Heritage and the Arts, the Department of Education, Employment and Workplace Relations, the Department of Agriculture, Fisheries and Forestry and the Department of Veterans' Affairs for the purpose of checking eligibility. In other instances, information contained in this Proposal can be disclosed without your consent where authorised or required by law.

Fig 1. The proposal entitled 'Prevention of Bryozoan biofouling of water pipeline systems' that was submitted as an ARC Linkage application for Round 2 of 2010.

Achieved : The above mentioned ARC Linkage application for Round 2 of 2010 has been duly submitted. Recently the rejoinders in response to the report from Assessors 1 and 2 (Refer to Appendix 1 for the Assessor reports and the corresponding response) were also submitted by Prof. John Orbell.

2. Attend, and present the outcomes of the first year of the existing project, at the 32nd Hydrology & Water Resources Symposium, Newcastle, NSW, 1-3 December 2009.

Fig 2. Delivery of the presentation at the 32nd Hydrology and Water Resources Symposium 2009 on Wednesday the 2nd of December 2009 at the Waratah Room by (a) Dr Andrew Barton, (b) Dr Robin Mitra and (c) Professor John Orbell (d) the conference paper was provided in a CD.

Achieved : The outcomes from the first year of the existing project was successfully presented at the 32nd Hydrology and Water Resources Symposium 2009. The refereed publication was disseminated by the symposium committee to all the participants within a CD.

3. Conduct a two day field trip in December in order to collect post-chlorination samples from the NMP and live colonies for the purpose of colony-to-colony propagation. To consolidate and deliver the Milestone 5 report from the first year of the project.

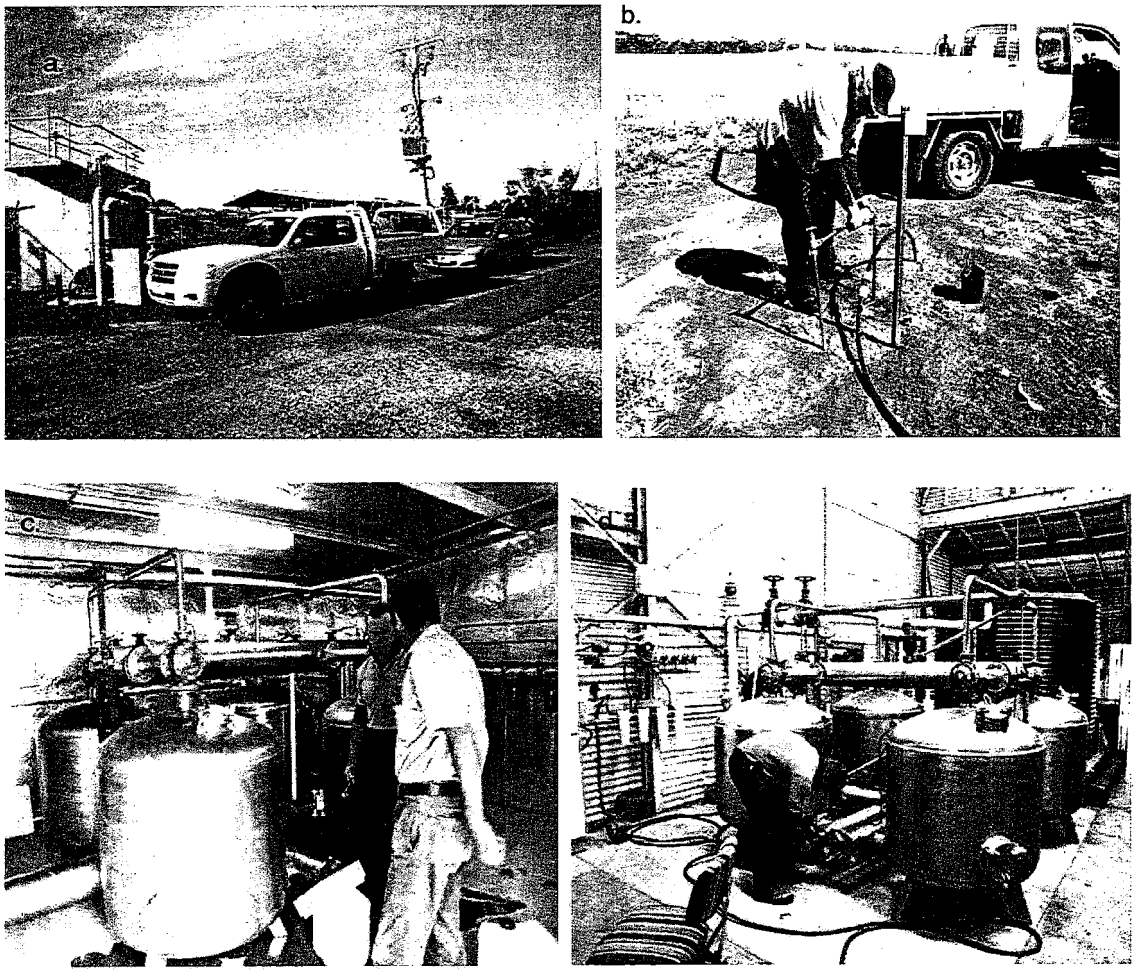

Fig 3. The fourth sampling tour program commenced with a meeting at the GWM Head Office in Horsham between Robin Mitra, John Orbell, Andrew Barton, Steven Briggs and Darcy Prior on the afternoon of the 8th of December. Freshwater Bryozoan samples were collected from the four prime sampling sites (a) Ouyen. (b) Kiamal, (c) Piangil and (d) Nyah on the 9th of December.

4. Provide a copy of the ARC Linkage application - submitted on the 18th October 2009.

5. Provide a copy of the presentation delivered by Dr. Andrew Barton, Dr. Robin Mitra and Professor John Orbell at the 32nd Hydrology & Water Resources Symposium. Provide a copy of the refereed publication that has been included in the proceedings.

6. Provide the outstanding progress report in relation to Milestone 5 for the first year of the project. Details of the December '09 field trip will be included in the Milestone 5 report. Invoice for the second instalment of $20,871.00 on Tuesday 15th December 2009.

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December 2009

Achieved : All of the above were duly included in the Milestone 5 report submitted in December 2009.

2. Addressing Milestone 2

2.1. Milestone 2 (16th December 2009 to 28th February 2010)

i. Organize all literature collected to date into a comprehensive review article on freshwater Bryozoans and their biofouling characteristics. Liaise with all team members in order to prepare a draft of this review for submission to a high quality international journal.

A massive literature hunt was launched during the initial stage of the project which resulted in a comprehensive collection of journal articles on freshwater Bryozoans. Refer to the list in Appendix 2. These articles were either downloaded from the database of the Victoria University library or those that were unavailable in the database were ordered through the library via the Interlibrary Loan Services http://www.clic.edu.au/victoria. After consulting some of the review journal articles published by established experts on both marine and freshwater Bryozoans such as Lacourt (1968), Mukai (1982), Okamura and Hatton-Ellis (1995), Brood (1998), Wood (2001) and Ryland (2005), the journal articles in our collection were categorized under the following sub-headings :-

1. Laboratory cultivation, culture, nutrition and sampling.
2. Biology, ecology, development, dispersal and distribution.
3. Identification of new species of freshwater Bryozoans.
4. Studies relating to dormant buds: stato/floato/sessoblasts
5. Studies relating to Bryozoan biofouling and control
6. General Introduction to invertebrates and Bryozoa
7. Phylogeny, taxonomy, population genetics and evolution in Bryozoa.
8. Miscellaneous

The updated list has been circulated to all team members, however the preparation of the draft for the review article has not commenced. A meeting among the team members is desired in order to structure the initial draft.

ii. Using the live colonies collected on the December '09 field trip, initiate colony-to-colony propagation experiments in the laboratory. The identity of these colonies is to be confirmed from the morphology of their statoblasts (SEM). Continue work on statoblast-to-colony propagation.

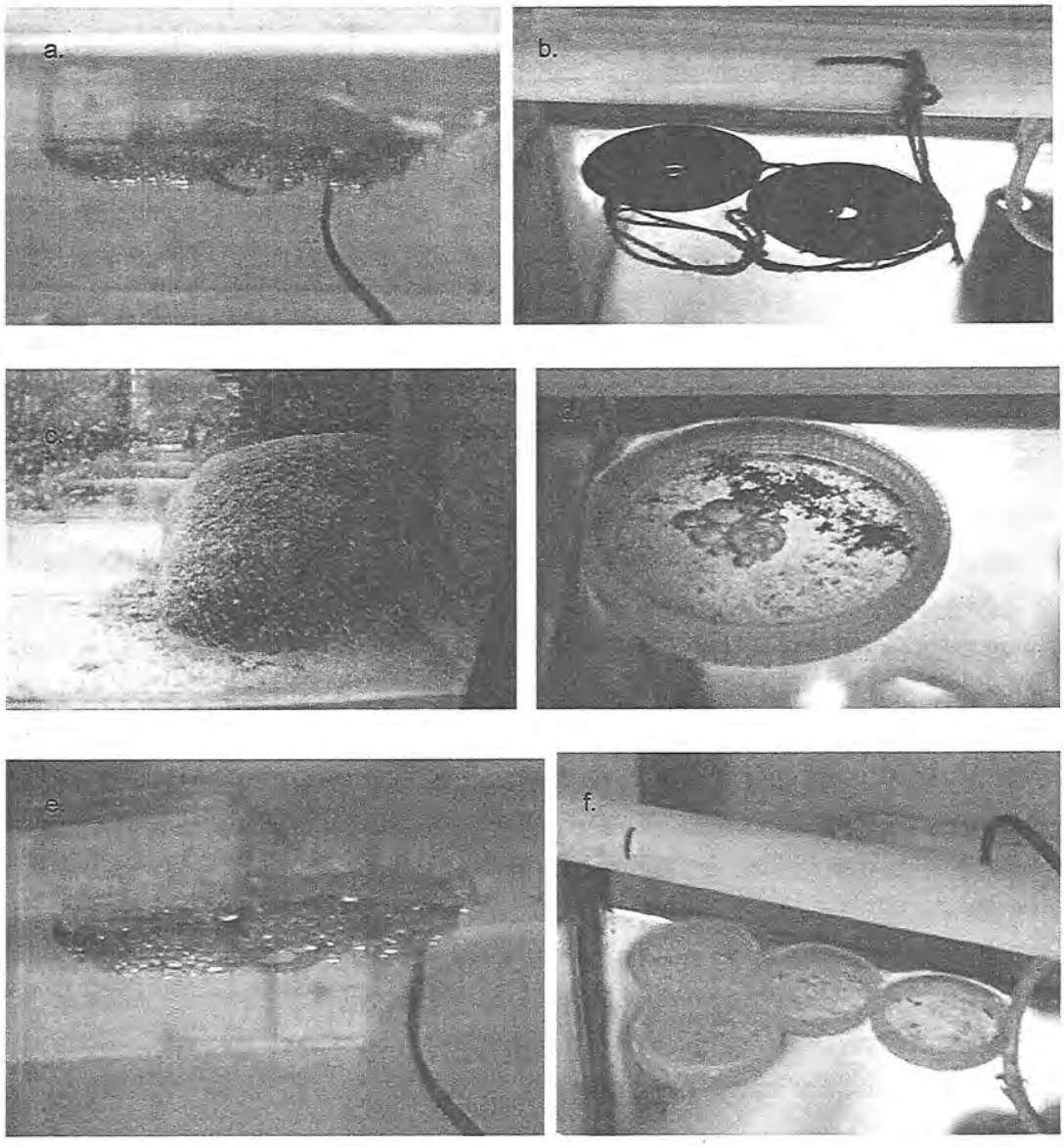


Fig 4. (a) and (e) Plastic filters that were collected from the original site at Kiamal and Potter/Wood Road Kiamal depicting Bryozoan growth. (b) Top view of the two filters that were set afloat in the Kiamal growth tanks. (c) Bryozoan colonies in the Nyah growth tanks. (d) A few columns of Bryozoan colonies within which statoblasts were observed and set afloat in the Piangil tank (f) Isolated stato/sesso/floatoblasts set afloat in the Ouyen tank.

2.1.1. Colony-to-colony propagation experiments

From the many sampling tours that were undertaken during the course of the investigation it is now becoming apparent that the Bryozoan colonies do not tend to survive more than two days outside their ecological niche - especially in sealed sampling containers. Hence it is now preferable that all the four prime sampling sites viz. Ouyen, Kiamal, Piangil and Nyah be covered in a single day with the colonies being taken to the laboratory at St Albans immediately and stored at room temperature

overnight. The following morning, the adhering debris can be rinsed off the colonies which are then set out in petri dishes. The body wall of the colonies are composed of a non living external ectocyst that make up the zooecium (Wood 1973) and hence there is no way of telling whether the colonies are alive or dead until and unless lophophores, the food gathering organ of the zooid, with its expanding and retracting tentacles are observed under a microscope or a characteristic stench of decaying animal proteins from dying colonies is sensed upon opening of the sample bottle. However, at this point of time, it is deemed appropriate that the freshly collected colonies be laid out on plastic lids of specimen containers and left afloat in the moist milieu of the growth tanks supplemented with a mixture of cured and source water. Prior to this, the colonies were immersed in source water and put out in petri dishes at room temperature. This approach not only necessitated replacement with fresh water every 2-3 days but the colonies were also in the peril of drying up if neglected. With the exception of the freshwater Bryozoan *Lophopus crystallinus* all other species especially those belonging to the genus *Plumatella* and *Fredericella* require protection from settling particles (Wood and Okamura 2005) and the accumulation of ammonia within the colonies. Therefore it is considered more appropriate that they are cultured in the 'top-down' orientation for which the trick lies in sticking the growing tips of the dividing colonies to the bottom of the petri dishes and then flipping the petri dish up side down and setting it afloat. Bryozoan colonies entwined within the filters seem to have served the purpose well by skirting the problem of having to adhere the colonies to a substratum, especially when they are floated upside down in the tanks allowing the colonies to grow in the 'top-down' orientation **Figs 4a** and **4e**. To date, the growing tips of the colony branches from the Kiamal filters have not been isolated and allowed to grow on a substrate. However it seems most appropriate that rather than clipping the growing tips of the colonies and placing them in petri dishes, a method is being sought in which the growing tips are not only left undamaged but are concomitantly in constant contact with the underside of the petri dishes in which they may be left to divide and proliferate. The method is presented as a schema in **Fig 5.**

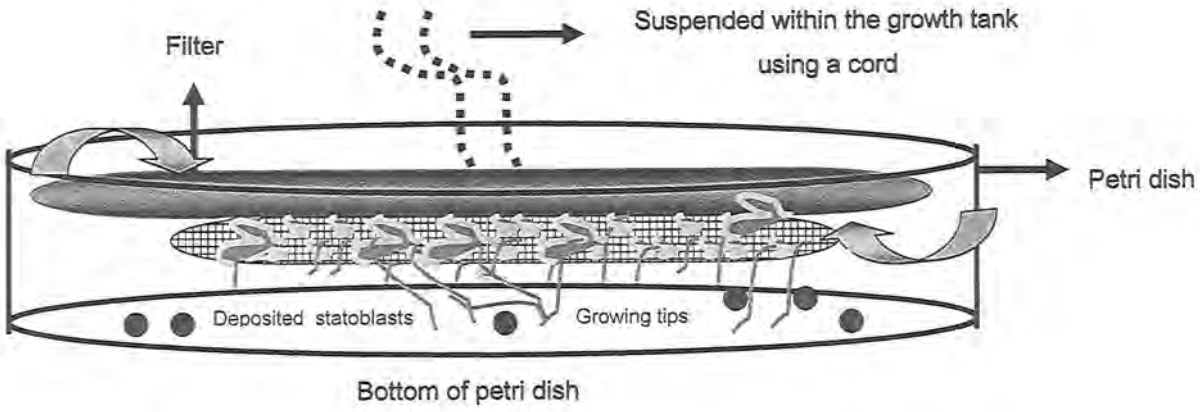


Fig 5. Filter floated within a petri dish so that the growing tips of the Bryozoan colonies are in constant contact with the bottom of the petri dish. The whole device is then set afloat within the growth tank.

2.1.2. Statoblast-to-colony propagation experiments

Many hundreds of dormant or quiescent asexual structures such as statoblasts of the Fredericellid type as well as sessoblasts and floatoblasts of the Plumatellid type were isolated especially from the samples that were collected from Piangil and Nyah. A portion of these asexual buds were put out for germination, some of it were send to Bryo Technologies in the U.S. for identification purposes (Refer to Appendix 3) whilst the rest was used for Scanning Electron Microscopy (SEM) analyses. From the samples that were collected during the December 2009 sampling tour, it was noteworthy that the samples obtained from Nyah was dominated by *Fredericella* type statoblasts whilst that of the Piangil by *Plumatella* floatoblasts.

Documented studies have revealed that Bryozoan statoblasts are known to undergo an obligate period of dormancy that may last for 3 to 5 weeks or more (Wood 2005) and according to Bushnell and Rao (1974) for one day to many weeks. It is also becoming more apparent that the germination of these statoblasts may be dependent on seasonality i.e. the time of the year these dormant buds were collected whether in autumn or in spring and also whether these statoblasts have endured the vicissitudes of a long winter season and ready to germinate again (Bushnell and Rao 1974). It is now suspected that perhaps 'overwintering' is programmed in them and statoblasts that were synthesized within the zooids and collected in autumn would remain dormant even when the most conducive conditions are provided in the laboratory to promote germination. However in agreement with Wood (2005), it is deemed proper that the freshly isolated statoblasts from recently collected samples be subjected to unfavourable conditions for a certain period of time, such as exposure to drying or freezing conditions prior to them being put out for germination at a more favourable condition. The method of drying the statoblasts and then immersing them in source water mixed with 'aged water' from the goldfish aquarium had worked previously (Refer to Milestone 4 report), albeit the germination rate was low.

However, currently, a slight modification to the approach was introduced. The isolated statoblasts were dried in the plastic lids of specimen containers and then set afloat in the moist environment of the growth tanks Fig 4f. Unlike the petri dishes that sink to the bottom of the tanks, the plastic lids on the other hand, remains afloat without sinking. Some of the dying colonies that were studded with statoblasts were also carefully isolated laid out in the plastic lids and set afloat within the growth tanks Fig 4d. However in spite of all the different approaches thus undertaken, till date, the statoblasts that were isolated from the colonies collected during the summer (9th December 2009) are still dormant. It is also noteworthy to mention here that no attempts have been made to attach the statoblasts through artificial means to the substrate (plastic lids in our case) using pastes like oxyphosphate zinc cement that is used in dental work (Mukai 1974) to prevent them from falling off when set afloat in the reverse top down position. To harvest more statoblasts for the prime purpose of identification/germination/ SEM analyses, some of the colonies are deliberately left behind in the sampling containers and

placed in the cool room at 5^{0}C for some time with the intention that the dying colonies will release more statoblasts.

2.1.3. Detection of two new species

From a recent report obtained from Bryo Technologies dated the 16th of February (Refer to Appendix Three) it is now evident, that other than the existence of *Plumatella emarginata* and *Fredericella australiensis* that were previously identified from the Northern Mallee Pipeline system (Refer to Milestone 2 report) there is also the presence of two other *Plumatella* species viz. *Plumatella vaihiriae* Fig 6, and *Plumatella casmiana* Fig 7. Three samples from three different locations (a) Ouyen Backwash (b) Piangil membrane filter and (c) Nyah membrane filter containing Bryozoan colonies were sent to Bryo Technologies with the intention of detecting *Plumatella reticulata* and *Fredericella sultana* because a floatoblast resembling that of *Plumatella reticulata* with a network of raised reticulations was observed during our first Scanning Electron Microscopy (SEM) work at Melbourne University (Refer to Milestone 5 report Fig 24). *Plumatella vaihiriae* was detected from the Ouyen backwash sample and *Plumatella casmiana* was detected from the sample scraped off the Piangil membrane filter during the December 2009 sampling tour.

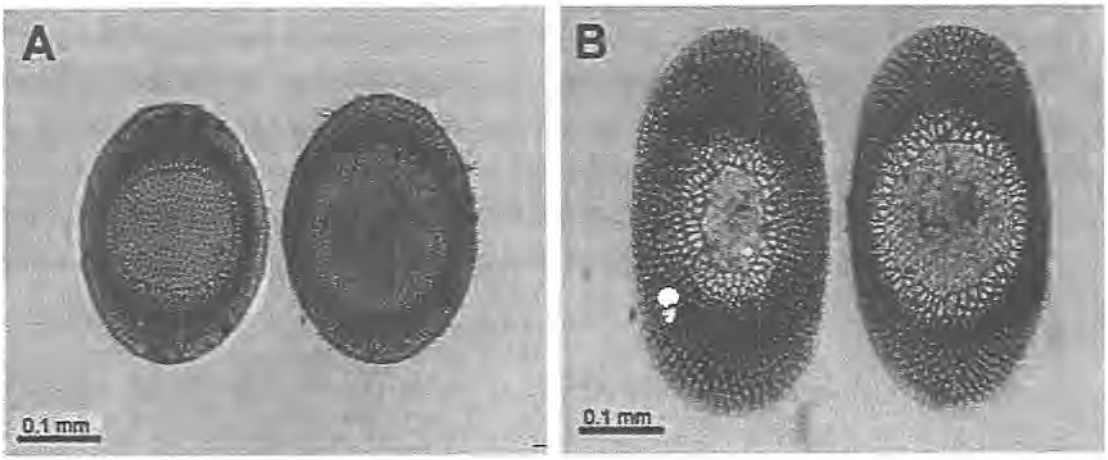


Figure 2. Plumatellid statoblast valves from Ouyen Backwash: (a) *Plumatella vaihiriae*: (B) *Plumatella emarginata*.

Fig 6. Detection of *Plumatella vaihiriae* and *Plumatella emarginata* from the Ouyen backwash. sample.

2.1.4. *Plumatella vaihiriae* (Hastings 1924)

Plumatella vaihiriae was initially described as *Hyalinella vaihiriae* by Hastings (1929) when it was first discovered within the material collected from Lake Vaihiria in Tahiti. Since then, *Plumatella vaihiriae* has now been found in Utah (Rogick 1942), in Hawaii (Baily-Brock and Hayward 1984) and in Argentina (Cazzaniga 1988). Larcourt (1986) reported the occurrence of *Plumatella vaihiriae* in

Australia but his finding remained unconfirmed (Wood and Marsh 1999) until it was detected by Dr Timothy Wood at the Brisbane Waterworks (unpublished) and later at Ouyen in the Northern Mallee pipeline (Refer to Appendix Three for the report) during the course of our December 2009 sampling tour. According to Dr Timothy Wood, *Plumatella vaihiriae* is believed to be of Asian origin which is rapidly expanding in North America and Europe. *Plumatella vaihiriae* is a notorious biofouler with the ability to clog pipes and filters and the free statoblasts are suspected of interfering with the ultraviolet disinfection of wastewater (Wood and Marsh 1999). Like all other species of *Plumatella,* it is known to produce both types of statoblasts, the much smaller, free and buoyant floatoblast **Fig 6**, and the large and sessile sessoblast that remains cemented to the submerged surfaces (Wood and Marsh 1999)

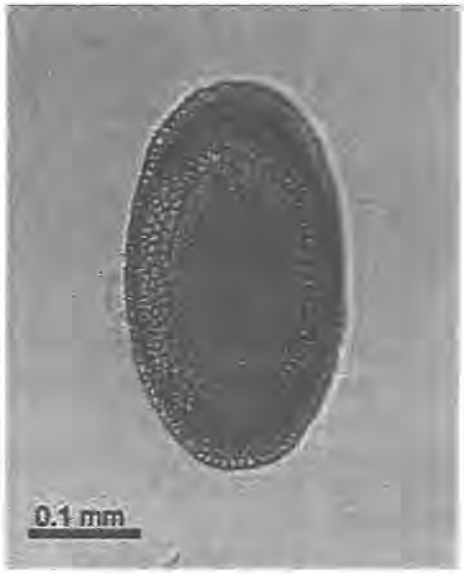


Figure 3. Statoblast of *Plumatella casmiana* from Piangil Membrane Filter.

Fig 7. Detection of *Plumatella casmiana* in the sample scraped off the Piangil membrane filter.

2.1.5. *Plumatella casmiana* (Oka 1907)

Plumatella casmiana was originally described by Oka in 1907 from Kasumigaura, Japan and later by Mary Rogick (1941; 1943) from North America (Hirose and Mawatari 2007). Unlike the other members of the Plumatellid family, species belonging to *Plumatella casmiana* , are known to produce two types of free statoblasts (a) a capsulated floatoblast with a typical Plumatellid structure and function that exhibits a well developed periblast surrounding the capsule (b) a leptoblast lacking an internal capsule and enclosing a fully developed zooid within a membranous periblast (Wood and Okamura 2005). The distinguishing features of a leptoblast are that it has a very narrow annulus area and hence is weakly buoyant and the valves are not completely fused together. Leptoblasts are known to germinate immediately upon their release from the colony. The *Plumatella casmiana* statoblast depicted in **Fig 7**, has a long and oval fenestral area with a medium sized annulus area and probably

cannot be categorized as a leptoblast. The floatoblast morphology seems to be a bit complicated here especially when the leptoblast is taken into consideration as there is a distinction in the features of the periblast and the capsule (Hirose and Mawatari 2007).

2.1.6 The *Fredericella* Dilemma in Northern Mallee pipeline system

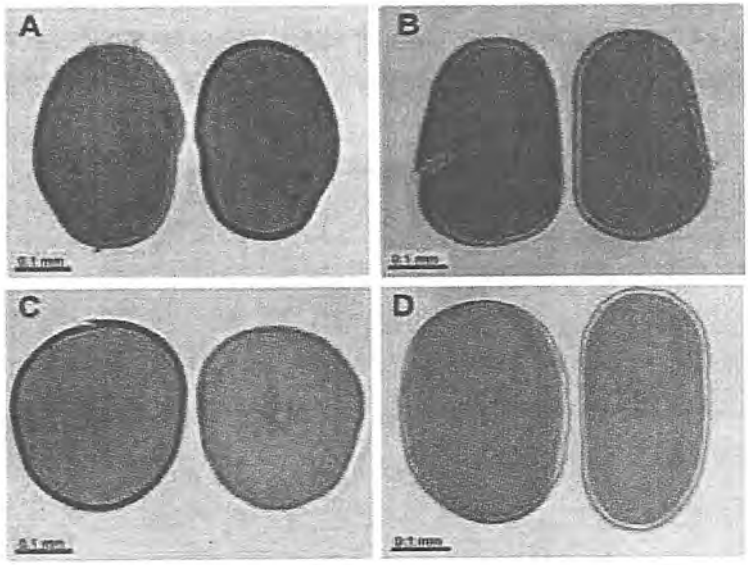


Figure 1. Fredericellid statoblast valves: A-C are from the Ouyen Backwash; D is *Fredericella sultana* from Bough Beech Reservoir in Kent, UK (for comparison).

Figure 4. Statoblast valves of *Fredericella sutraliensis* from Nyah Membrane Filter.

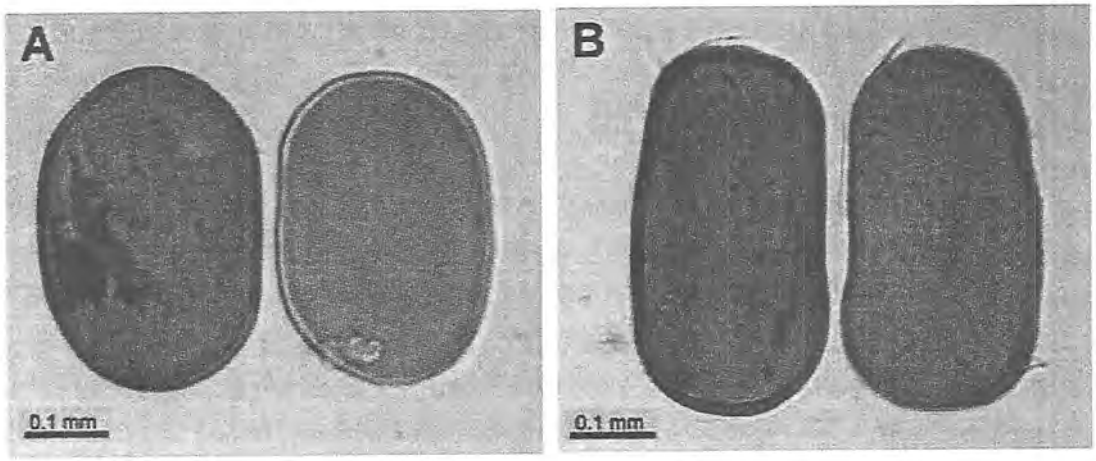


Fig 8. There is a possibility that the two species *Fredericella australiensis* and *Fredericella sultana* are hybridizing into a novel species *Fredericella sutraliensis* (?), a phenomenon hitherto never encountered nor reported before amongst freshwater Bryozoans.

From the report obtained from Bryotechnologies dated the 16th of February 2010 (see Appendix Three), it is now becoming apparent that the Fredeicellid statoblasts obtained from the Northern Mallee Pipeline (NMP) are undergoing some sort of transformation which has been tentatively attributed as hybridization (interspecies ?) by Dr Timothy Wood. According to Dr Wood (personal communication) the statoblasts that were obtained and identified from the Kiamal filter and the Ouyen strainer last year exhibited the true characteristics of *Fredericella australiensis* (Refer to reports from Bryo Technologies dated the 4th and 24th of February 2009 incorporated in the appendix of Milestone 2 report) Fig 9.

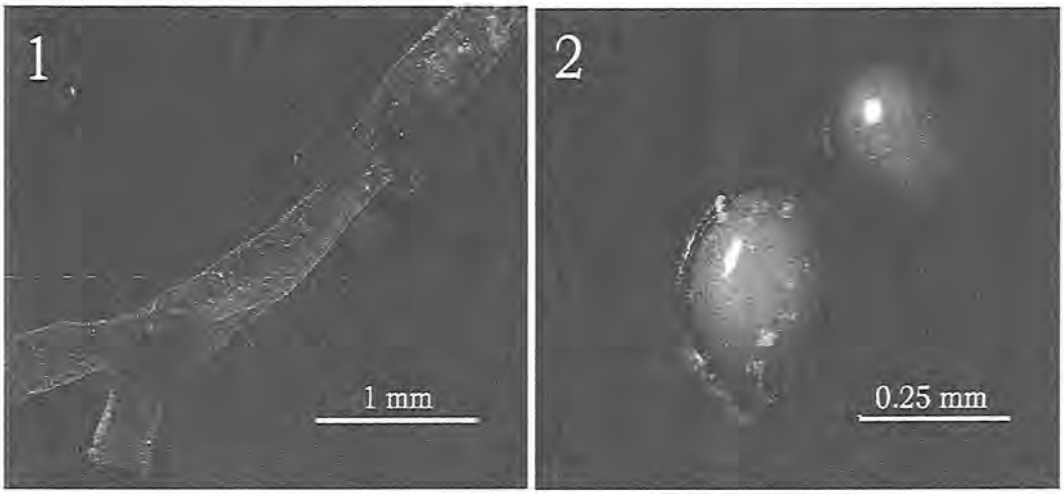


Fig 9. Statoblasts of *F.australiensis* from the Kiamil sample depicting a smooth and shiny surface and variable size / shape (identified from samples collected in December 2008).

The Fredericellid statoblasts, on the other hand, that were examined from the December 2009 sampling trip from the Northern Malle Pipeline system, Fig 8 seem to exhibit a combination of characteristics that are common to both *Fredericella australiensis* and *Fredericella sultana* and hence were designated as *Fredericella sutraliensis.* Interspecies hybridization between closely related species is not an uncommon phenomenon in nature and the possibility of interspecies hybridization between *Fredericella australiensis* and *Fredericella sultana* cannot be altogether ruled out. According to Dr Tim Wood (personal communication) the line of distinction between *Fredericella australiensis* and *Fredericella sultana* is a thin one which is largely based on the statoblast dimensions and till date there are no established dimensional criteria to resolve the difference between the two species. The statoblast shape is heavily influenced by the zooecium which is composed mainly of the body wall and a secreted non living external ectocyst (Wood 1973). The zooecium size in turn is influenced by many environmental aspects such as the availability of nutrition, temperature and a host of other factors. This is a novel dilemma which stretches beyond SEM analysis and possibly warrants inference through the techniques involving DNA fingerprinting which of course is beyond the scope of

the present project. Keeping the above in view, the discussion section on our forthcoming paper on the identification of freshwater Bryozoans using SEM technology requires to be addressed in a more judicious manner.

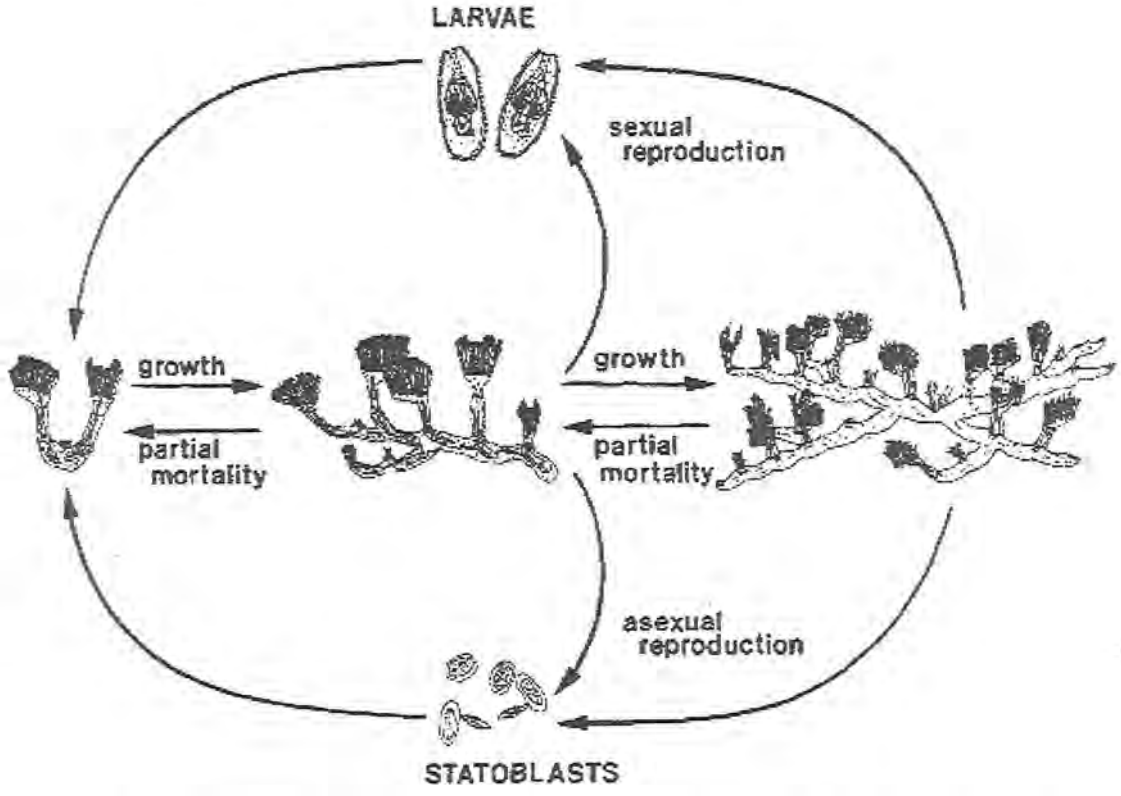


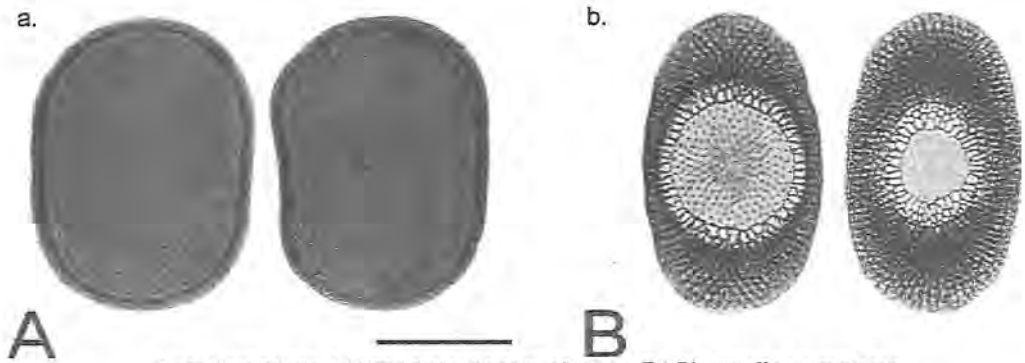
Fig 10. The life history of freshwater Bryozoans encompasses both the sexual mode via the production of larvae and the asexual mode through production of small encapsulated structures known as statoblasts (Okamura and Hatton-Ellis 1995).

Normally a high degree of clonal reproduction is observed amongst freshwater Bryozoans and the dissemination of the genotype (genetic constitution) among the local habitats is basically achieved through colony growth, fission, fragmentation of columns (as observed in the members of the *Fredericella)*, rarely through larvae, and most conspicuously by the production of encapsulated structures known as statoblasts within which is enclosed the undifferentiated primordial tissue and the associated nutritive yolk (Okamura and Hatton-Ellis 1995). A single primary zooid may give rise to a colony through budding in which the zooids are more or less autonomous and these autonomous zooids may in turn produce meiotically reduced gametes which may engage in sexual reproduction through the fusion of the two gametes known as syngamy to form a zygote (2n). The zygote will develop into a free swimming larva for a brief period of time and then undergo metamorphosis to give rise to a primary zooid. If the gamete from one zooid fertilizes that of another zooid from the same colony then a phenomenon known as autogamy occurs and all the zooids in the colony tend to have

the same genotype. Conversely, if fertilization occurs between zooids from different colonies the mode of reproduction is termed at amphimictic. However when the conditions becomes unfavourable the Bryozoans do not produce larvae but copious amounts of dormant asexual buds known as statoblasts to survive the harsh conditions, and this mode of reproduction has been deemed functionally equivalent to apomixis (Bell 1982). However the phenomenon of interspecies hybridization between two different species although belonging to the same genus has never been hitherto reported from the freshwater Bryozoans.

2.1.7 Scanning electron microscopy (SEM)

One of the modern approaches relating to the establishment of Phylogenetic relationships among freshwater Bryozoans is through the use of mitochondrial ribosomal DNA sequences of 12S and 16S rDNA genes (Okuyama *et al.,* 2006). The first comprehensive molecular phylogeny of Bryozoa based on the combined analysis of two nuclear genes 18S rDNA and 28S rDNA and the mitochondrial gene COI has been carried out by Fuchs *et al.* (2009). However, most Phylactolaemate systematists still regard statoblast morphology as one of the primary diagnostic features in distinguishing freshwater Bryozoans at the species level (Bushnell and Rao 1979). Not only are the statoblasts helpful in identifying the different species within a genus but also aid to distinguish the dorsal and the ventral valves of the floatoblasts in the members of the genus *Plumatella* through the size of the float area known as the annulus and the central region termed as the fenestra. The dorsal valve generally has a greater float area and a smaller central fenestral area compared to that of the ventral valve (Refer to Fig 11b, depicting the ventral and dorsal valve of *Plumatella emarginata*). The distinction between the dorsal and the ventral valves are however not possible in the members of the genus Fredericella because the statoblasts are devoid of external micro-architecture and is usually endowed with a thin peripheral annulus or lamella (Bushnell and Rao 1979) – the characteristic thickened rim observed in *Fredericella australiensis* ?



Statoblast valves. (A) *Fredericella australiensis*; (B) *Plumatella emarginata*.

Fig 11. (a) Statoblasts valves of *Fredericella australiensis* and (b). *Plumatella emarginata* (Bryo Technologies Report dated the 14th of March 2010).

The scanning electron microscopy technique was first used by Bushnell and Rao (1974) to reveal the micro-architectural details of the statoblasts. As mentioned in Milestone 5 report, the statoblasts, especially those that of the floatoblasts and sessoblasts of the Plumatellidae are sclerotized structures that often bear minute micro- ornamentations in the form of rounded prominences called tubercles or raised reticulations thereby offering the possibility of distinguishing individual species (Wood 1979) of a particular genus. The first trial run for identification of freshwater Bryozoans at the species level using SEM analysis with approximately 100 Plumatellid floatoblasts and sessoblasts as well as 100 Fredericellid statoblasts were carried out on the 25th of March 2010 at Melbourne University under the guidance of Dr Simon Crawford. Presented below are some of the SEM images that were obtained from the first trial run.

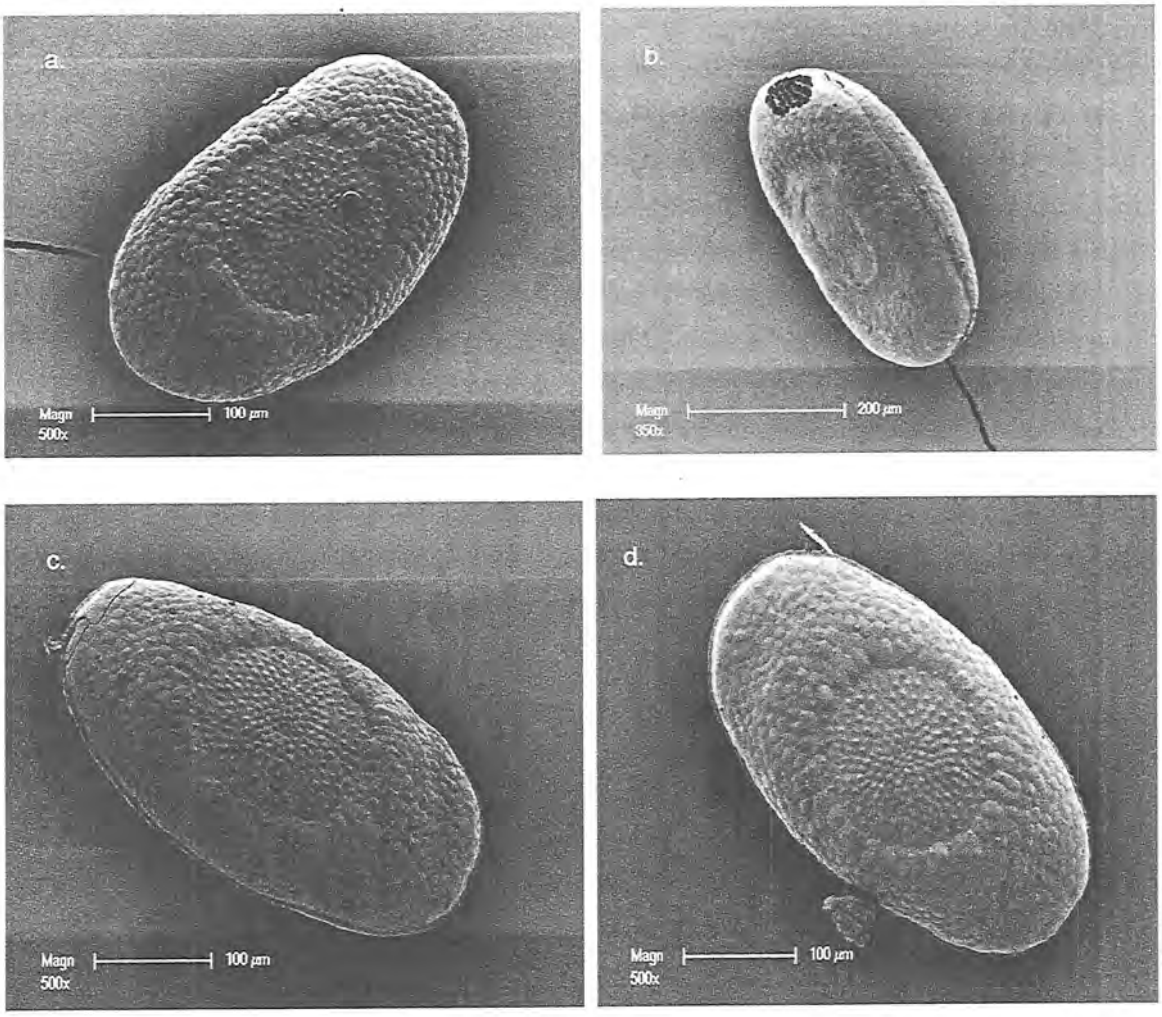


Fig 12. Floatoblasts of *Plumatella emarginata* (a) (c) and (d) ventral valve bearing distinct tubercles on the large fenestral area (b) dorsal valve with flat and characteristic flat and smooth fenestral area and smooth surface.

Plumatella emarginata is a cosmopolitan species of freshwater bryozoan (Mukai *et al.,* 1990). One of the distinguishing characteristics in the floatoblasts belonging to the species *Plumatella emarginata* is the presence of distinct tubercles on the fenestral region of the ventral valve **Figs 12a, 12c and 12d,** which is much less apparent on the dorsal valve **Fig 12b.**

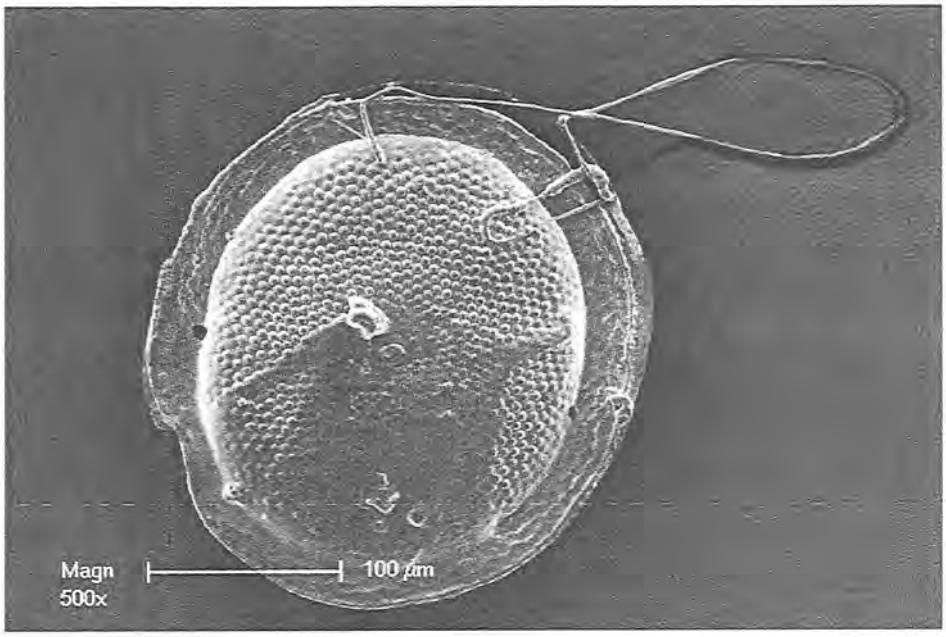


Fig 13. Inferred as a leptoblast (free floatoblast ?) of *Plumatella casmiana* because of its large fenestral area and a somewhat narrow annulus as well as the presence of tubercules in the fenestral area.

Refer to details on *Plumatella casmiana* under section 2.1.5. According to Wood and Okamura (2005) the size of the dorsal fenestra in *Plumatella casmiana* is more than half the length of the free floatoblast. Hirose and Mawatari (2007) describes the capsuled floatoblast of *Plumatella casmiana* as having a well developed periblast but the presence of tubercles is weak on the surface of fenestra. The leptoblast, on the other hand lacks the internal capsule and is endowed with a narrow annulus as depicted in **Fig 13.** Martinovic-Vitanovic *et al.* (2010) is also of the opinion that the presence of a thin wall is the unique characteristic of the leptoblast of *P. casmiana*. However SEM images by Massard and Geimer (1995) show the presence of tuberculation and reticulation in the leptoblasts of *Plumatella casmiana* (Refer to Appendix four for the publication) as seen in **Fig. 13.** The following images were obtained from a trial run and more details would be required for the confirmation at the species level for the final SEM images. An endeavour would be made to seek a second opinion from Dr Timothy Wood if possible for the final SEM images that would be developed with publication in view.

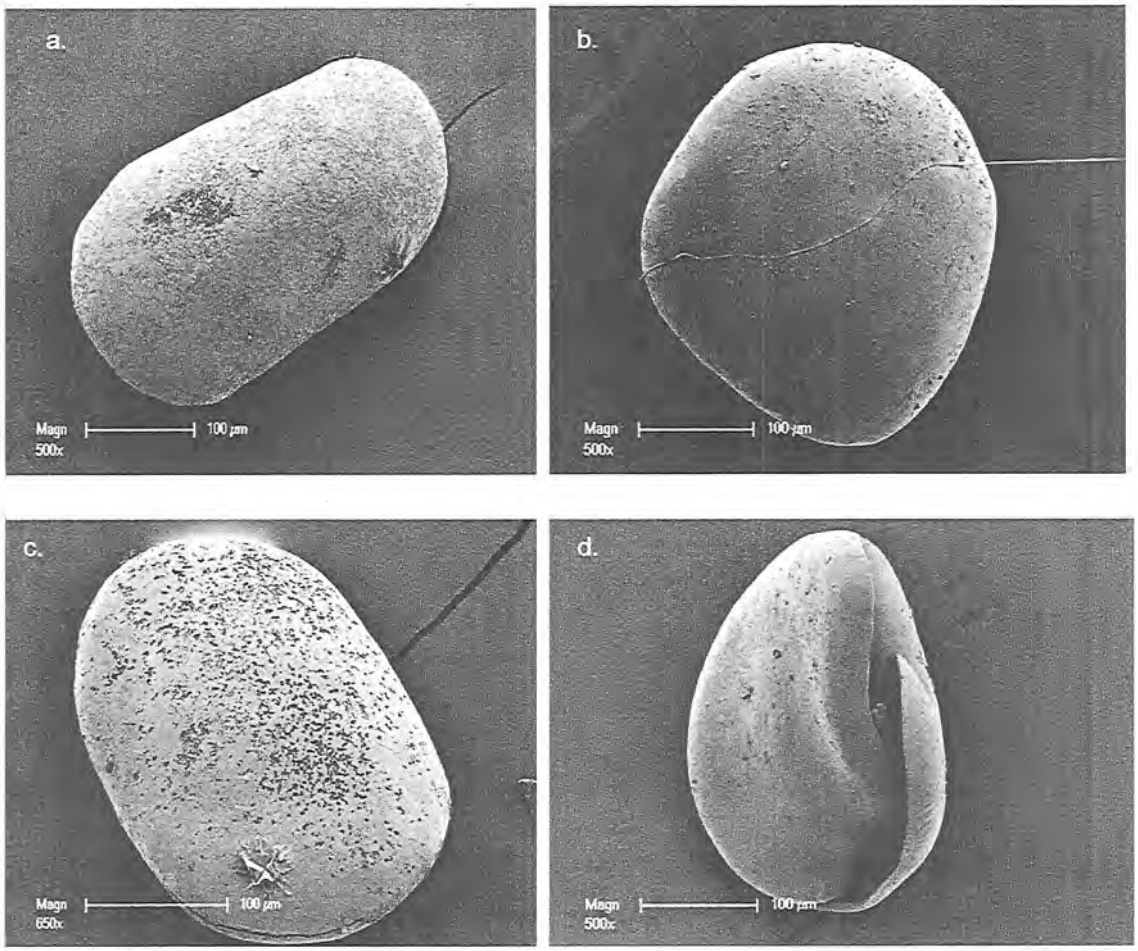


Fig 14. Statoblasts of the Fredericellid type devoid of exterior micro-architecture.

From the trial SEM analysis that was carried on the 25th of March 2010 micro images depicting different sizes and shapes of the Fredericellid statoblasts were obtained. According to Wood and Okamura (2005) the dimensions of the Fredericellid statoblasts are strongly influenced by the size of the tubules which in turn is strongly influenced by the environmental conditions such as availability of nutrition, temperature etc. If the tubules are narrow, the statoblasts thus produced are elongated in shape and on the other hand, statoblasts produced from wider tubules are shorter and wider by nature. Unlike the floatoblasts of the genus *Plumatella*, most of the Fredericellid statoblasts tend to remain confined within the tubules that adhere to the substratum (Wood and Okamura 2005). However free floating statoblasts that are not confined within tubules has also been observed in samples especially in those that were collected from the Piangil pump station. According to Wood *et al.* (1998) **Fig 15a**, the statoblasts of *F.australiensis* is usually rounded with a characteristic thickened rim. This thickened rim has been observed under the light microscope at St Albans campus, in most rounded statoblasts **Fig 15c** but unlike Wood (1998) **Fig 15b**, we have not been able to observe the thickened rim in SEM images of Fredericella statoblasts as yet .

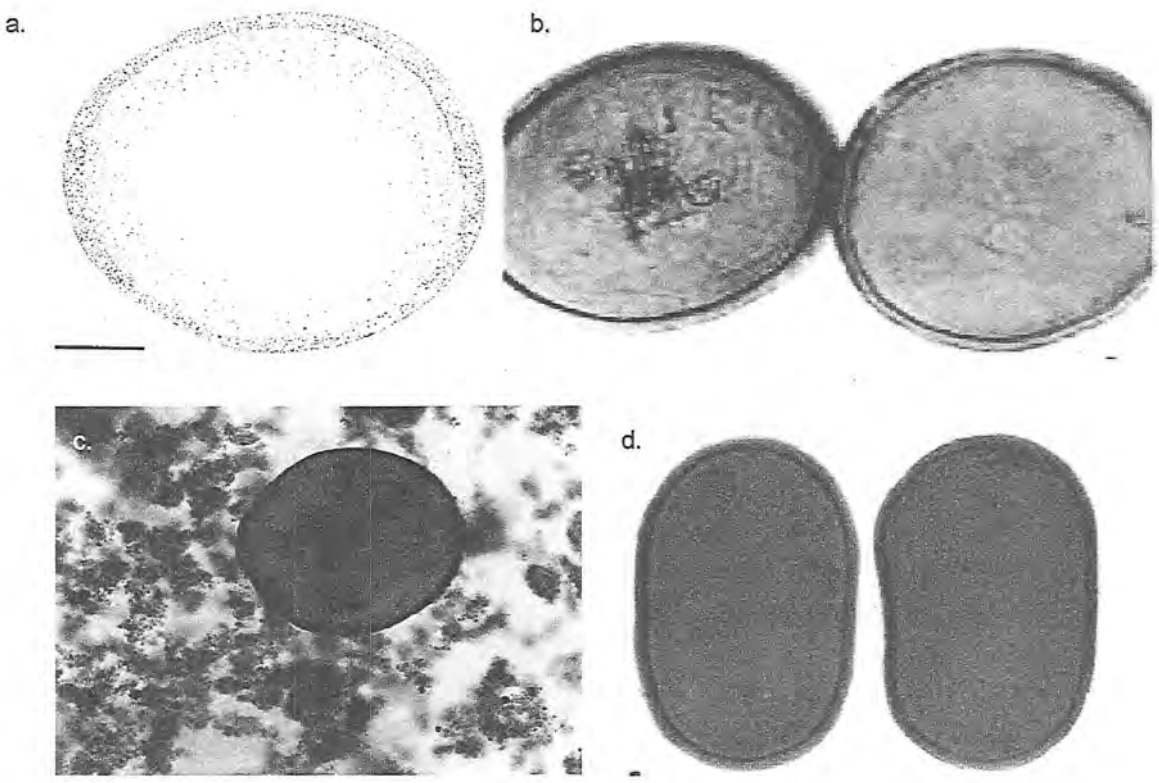


Fig 15. (a) Sketch of a single, rounded statoblast valve of *Fredericella australiensis* with the characteristic thickened rim and the lack of ornamentation (Wood *et al.*,1998). (b) Statoblast valves of *Fredericella australiensis* ; light micrograph (Wood 1998). (c) Statoblasts of the Fredericellid type devoid of exterior micro-architecture with the characteristic thickened rim under light microscope photographed at St Albans (d) Fredericellid statoblasts (Bryo Technologies Report dated the 14th of March 2010).

Usually two distinct types of Fredericellid statoblasts have been observed in our samples, one with smooth and dark glossy texture **Fig 16a**, and the other round and with the characteristic thickened rim around it. **Fig 16b**.

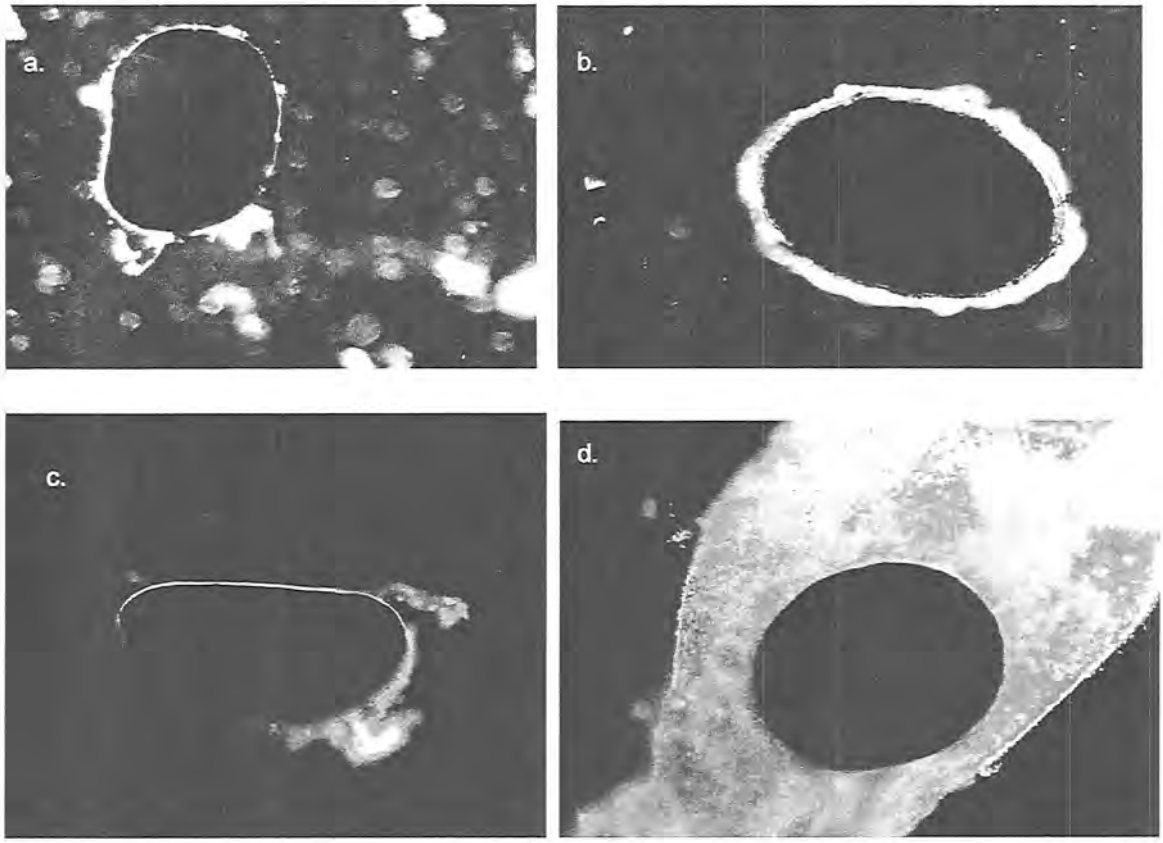


Fig 16. (a) and (d) Dark, smooth and glossy *Fredericella* statoblast devoid of both the exterior micro-architecture and the thickened rim around the edges (b) and (d) light brown hued *Fredericella* statoblasts devoid of the exterior micro-architecture but displaying the presence of the characteristic thickened rim around the edges.

The dark glossy statoblasts **Fig 16a**, have been suspected as those belonging to *Fredericella sultana* and seem to be more analogous to the European species of *Fredericella sultana* as described by Økland *&* Økland (2001). *Fredericella sultana* had been reported from Australia by Blumenbach in 1779 although there are no verifiable references relating to the occurrence of *Fredericella sultana* in Australia (Wood 1998). However, according to Wood and Okamura (2005), *Fredericella sultana* is the most common Fredericellid in Britain, Ireland and other parts of Europe and also known to occur in Asia, Australia and New Zealand, but only very rarely in North America.

2.1.8. Drawbacks of the first SEM trial run.

From the many damaged statoblasts **Fig 17.**, that were obtained from the first SEM trial run it became apparent, that the need to reduce the sonication cycles is imperative and the statoblasts be subjected to a less stringent alkaline detergent treatment prior to them being sputtered with gold. To circumvent the problem, the statoblasts for the next SEM analysis which begins on Wednesday the 24th of March 2010 at Melbourne University, have been rinsed in 5 % bleach followed by many rinses in source

water. The notion behind this initial pre-treatment with mild bleach is to remove most of the adhering debris and reduce the sonication cycles as well as the stringency of the detergent treatment in order to mitigate the damage on the sclerotized, chitinous exterior of the statoblasts.

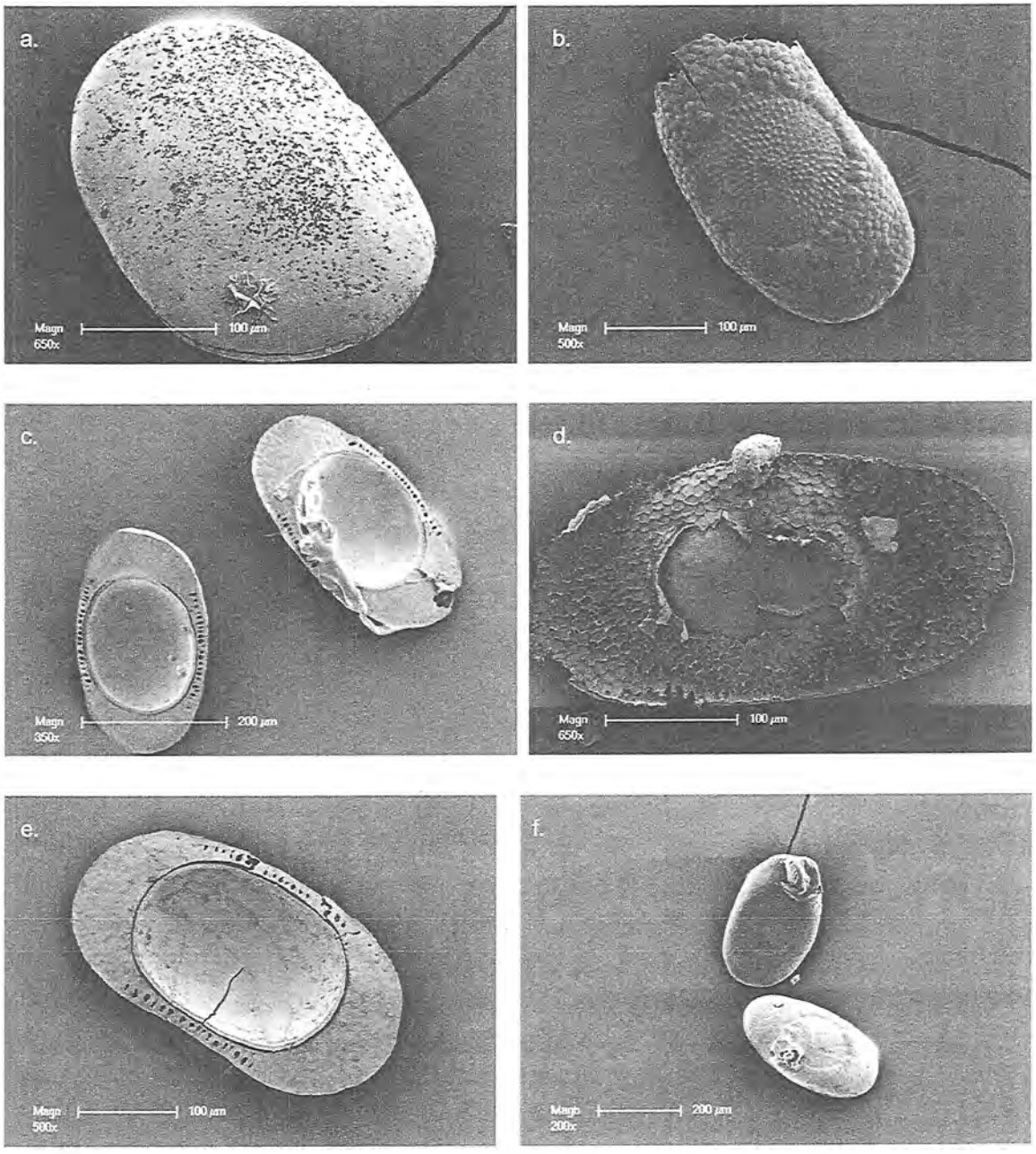


Fig 17. (a) Occurrence of minute but numerous flecks on the exterior of the Fredericellid statoblast (b) Sheared annulus zone in a Plumatellid floatoblast (c) and (e) Loss of annulus zone in Plumatellid floatoblasts exposing the chitinous capsule area which usually contains the yolky material and the embryonic tissue (d) Scarred annular zone exhibiting the underlying hollow epidermal cells also known as floats and a part of exposed capsule area (f) Damaged Plumatellid floatoblasts.

iii. Access the commissioned GHD report and reconcile this with our investigations – liaise with Mike Chapman and Barbara Bowles, particularly in relation to a risk management approach to the project.

Grampians Wimmera Mallee Water

Wimmera Mallee Pipeline

Preventative Maintenance Program

December 2009

Fig 18. The commissioned GHD report.

The GHD report was provided to us by Dr Andrew Barton. From the report **Fig 18.**, it was apparent that the WMP system is organized into several discreet supply systems referred to as SS1 through to SS7. According to the report, two biofouling freshwater Bryozoans from the genus *Plumatella* and *Fredericella* had been found to occur in SS1 and therefore there is a high probability that these organisms may colonize the SS2, SS3 and SS4 in the near foreseeable future (Anon 2009). An initial attempt has been made in the report to address the 'Bryozoan biofouling problem' which to some extent has been found to be adequate however the identification of the biofouling Bryozoans down to the species level is deemed impossible without SEM analyses. To date, liaison with Mike Chapman and Barbara Bowles from GHD has not been carried out as yet, however it is anticipated that such a meeting in the near future would be extremely profitable in steering the project towards more tangible outcomes.

iv. Arrange a team meeting in February in order to discuss matters relating to experimental design. Issues for consideration include the development of a more systematic sampling protocol, methods for assessing (qualitative and quantitative) the degree of biofouling, water quality data, access to maintenance records and importantly the design and implementation of laboratory experiments to investigate alternative (to chlorine) methods for controlling Bryozoan infestation. Imperative to the testing of alternative control methods is the supply of sufficient quantities of viable Bryozoan colonies that can be challenged in the laboratory with various chemical agents and conditions.

The following meeting is yet to be held.

v. Whilst progressing, the current statoblast-to-colony and colony-to-colony methods are proving to be rather sluggish at this stage of the project. Therefore, a concurrent strategy will be initiated relating to the cultivation of colonies on transportable "plates" within the Ouyen "pit". Such plates and growth media can be transported to facilities at VU for control experiments. This "field laboratory" will also allow the issue of seasonality to be conveniently investigated. The experimental design for this will be established at the February meeting scheduled for Thursday 11th February.

As a result of the meeting between Andrew Barton, Steven Briggs, John Orbell and Robin Mitra, on Thursday the 11th of February 2010, a 'Pit apparatus' also known as the 'Field Rig' that would hold together a set of three square settling plates was designed **Figs 19b** and **19c**. The settling plates were to be cut out of cement sheeting with dimensions of 200 x 200 mm. The plates would be placed 500 mm apart held together by strong, hollow steel tubes through which a strong nylon cord would be inserted. At the bottom the 'Field Rig' four 4 snapper sinkers each weighing 294 grams would be suspended and the whole apparatus was to be immersed into the 'Pit' **Fig 19d**. During the meeting at Horsham on the 8th of December 2009, apart from the laboratory cultivation of the identified Bryozoan species, the need to install a 'field growth laboratory' in 'the Pit' at Ouyen **Fig 19a** was raised by Andrew Barton The 'pit' would serve as an alternative means of propagating freshwater Bryozoans. John Orbell has incorporated the suggestion raised by Andrew Barton as one of the milestones (Milestone 3) in year 2 of the project (Refer to Milestone 5 report). However it is noteworthy, that prior to our designing the 'Field Rig' a publication related to the growth and development of biofouling freshwater Bryozoan by Smith (2005) was also consulted. Two sets of 'Field Rigs' were constructed, one of which would serve as a control and would be scraped clean at regular intervals and the other would serve as the experimental rig where the growth of the Bryozoans would be left uninterrupted to develop throughout the different seasons. The ultimate intention would be to estimate the percentage cover on the settling plates as exhibited by the growing colonies of Bryozoans at different times of the year. Other than the cement sheeting, the use of materials such as plastic mesh, white PVC and metal gauze are also on the plan. From a similar experiment carried out by Smith (2005) at the

Southern Reservoir water treatment station, Dunedin, New Zealand, it was apparent that *Plumatella repens* was found to grow on the underside of the plates while the other freshwater Bryozoan *Paludicella articulata* grew on the topside of the plate. As mentioned earlier members of the genus *Plumatella* and *Fredericella* tend to protect themselves from settling particles (Wood and Okamura 2005) and also avoid the accumulation of ammonia within the colonies thereby growing in a top-down orientation.

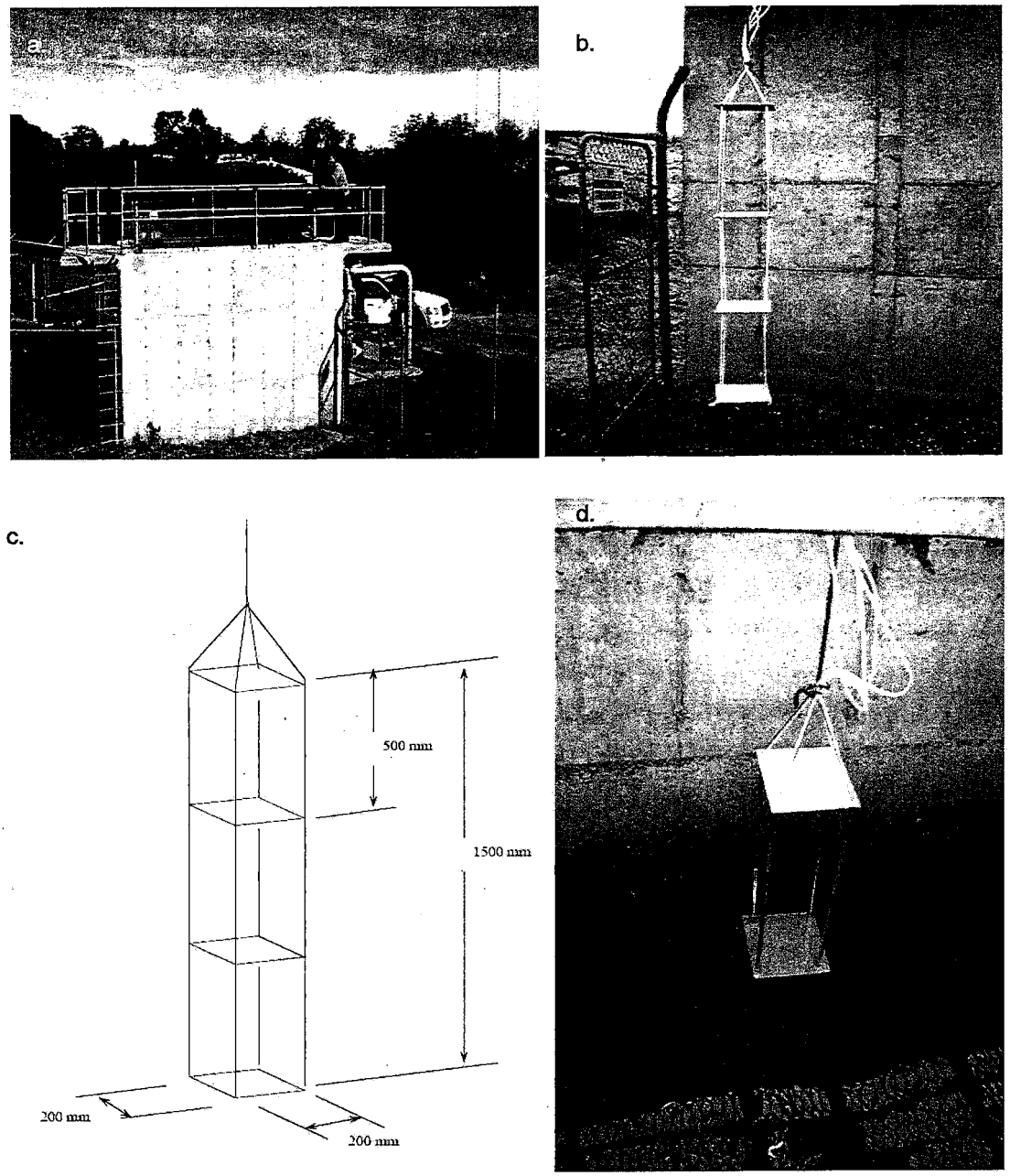


Fig 19. (a) The 'Pit' at Ouyen fed by open storages. (b) Completed 'Field Rig' (c) Schematic diagram of the 'Field Rig' designed by Andrew Barton and Steven Briggs (d) The 'Field Rig' being descended into 'the Pit'.

vi. Progress report in relation to Milestone 2 to be submitted with invoice for third instalment of $20,871.00 on Monday 1st March 2010.

3. Conclusions and recommendations

Currently SEM analysis is being carried out at Melbourne University using statoblasts that were isolated from the colonies collected during the December 2009 sampling tour. The February 2010 sampling tour has not been conducted as yet. With the completion of the SEM analysis which also encompasses the particle size analysis, it is anticipated that photomicrographs of statoblasts from all the five species of identified freshwater Bryozoa viz. (i) *Fredericella australiensis* (ii) *Fredericella sultana* (iii) *Plumatella emarginata* (iv) *Plumatella casmiana* and (v) *Plumatella vaihiriae* will be obtained for publication in any one of the journals listed below : -

(a) *Transactions of the American Microscopical Society*
(b) *Oecologia*
(c) *Freshwater Biology*
(d) *Hydrobiologia*
(e) *Limnologica - Ecology and Management of Inland Waters*
(f) *Limnetica*
(g) *Limnology and Oceanography*

Recommendation

Arrange a team meeting soon to discuss

(a) Review article on freshwater Bryozoans
(b) Methods to quantitatively enumerate the growth of Bryzoans in the settling plates of the 'Field Rig'
(c) water quality data
(d) methods pertaining to chemical control of freshwater Bryozoans

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APPENDICES

APPENDIX ONE

ASSESSOR REPORTS AND REJOINDER

Assessor 1

- **Investigator(s).**This project appears to be primarily focused on obtaining information on Australian freshwater bryozoan diversity, ecology and physiology in water distribution pipeline systems. Therefore it is surprising that only one member of the team has a biological background, and even then not in zoology, systematics, ecology or physiology. The engineering backgrounds of the other 3 CIs appear to be relevant primarily to the use of the flow system to assess the growth response of bryozoans in section C3.5. The CIs claim that they have developed skills in bryozoan systematics and culture through their current, related, ARC funded project. In my opinion CIs Orbell and Mitra appear to have comparatively poor recent research track records, even allowing for other responsibilities and the stage in their careers. The majority of the publications cited for CIs Orbell, Barton and Mitra appear to be conference presentations, rather than full-length refereed publications in edited conference proceedings. All of the publications listed for CI Mitra, and many of those of C I Orbell are from the journal "Asia Pacific Biotech News", which appears to be an industry magazine rather than a peer-reviewed scientific journal. CI Sargison appears to have a solid recent research track record, particular given the 3 periods of maternity leave in the past 6 years.
- **Significance and Innovation**The project is not particularly innovative in its methods or approach, which appear to be routine ecology. The fact that freshwater bryozoans are being studied in Australia at all IS innovative.
- **Approach and Training**The project methods as described are appropriate to the aims of the study. This study appears to be a classic ecological study, in that, it has a field sampling component involving species identifications and determining field distributions, and a laboratory component in which the organisms will be cultured and experiments will be performed on their growth under various conditions and treatments.
- **National Benefit**This project is an investigation into methods to control fouling due to freshwater Bryozoans in water pipeline systems in Australia. Given that our limited water resources are under increasing pressure from climate change, it is a very important field and the results will be important both in Australia and internationally.
- **Partner Organisation Commitment**The partner organisation appears to view this project as a priority and have contributed substantial cash (~ $120,000) and in kind support to the project.
- **Improvements (if not applicable, please enter n/a)**In the project description it is important to outline the experimental design, including null hypotheses, number of replicates, and the methods and approaches for the proposed statistical analysis.
- **Comments (if not applicable, please enter n/a)**n/a

Assessor 2

- **Investigator(s).**The investigators, including CIs and PI, are clearly well positioned to conduct the proposed work. They are already directly involved in addressing the overall challenge, i.e. that of better understanding and controlling the fouling by bryozoans in water pipe line systems, through an existing LP funded project (same overall objective and same investigators). Their expertise is highly appropriate for the proposed work, with the combined skill base and experience covering all aspects of the approach. The research output as measured by publications in peer reviewed international journals is not high across the applicants, but it is acknowledged that PI Barton is a practising senior water resources engineer, and that CI Sargison, whose publication track record is good has had three periods of maternity leave since 2003.
- **Significance and Innovation**It is clearly a significant advance scientifically, for management of water supply and distribution, and for environmental sustainability if the project arrives at an understanding of bryozoan fouling that enables the introduction of novel effective and environmentally friendly control measures. The approach follows the obvious route required to be able to be in a position to perform treatment experiments that are informative and predictive for upscale treatment procedures. It is unclear whether there is a strong element of innovation in the proposed work. Unfortunately, the section on Significance and Innovation in the application reads entirely as a research outline and a detailed breakdown of the experimental steps to take, and it does not speak to either Significance or Innovations. My understanding of their description of the proposed research though is that the statoblast-to-colony propagation is challenging and while they seem clearly reasonably advanced in delivering on this laboratory culturing of bryozoans in the existing LP project, this aspect of the work appears innovative.
- **Approach and Training**As for most of the text in the application, this section makes regular reference to the existing LP project. This reviewer found it difficult, for many of the parts, to distinguish between what has already been done, in full or in part, and exactly how the proposed work differs from what is already in place by the work conducted presently by this team. The proposed experimental sections are presented in a logical progression, with the overall objective being to arrive at a position where they can test a range of standard control agents in a manner that allows for reliable large scale procedures to be initiated. Subsections C3.1 and C3.2, which deal with obvious steps of the research activities do not require such detailed text. It would have been informative to explain why the use of the control agents proposed will prove successful, given that in the background (C1.2, page 7, last paragraph) it is made clear that the statoblasts have been found impervious to all the agents/treatments proposed to be tested again. Is it not likely that other measures may be required, and perhaps that the information acquired by studying the biology of the bryozoans may offer information that is of value in this regard? Related to an assessment of Approach and Training is the Timetable. Please explain why literature search is ongoing (just normal awareness of the state-of-the-art rather than guiding the experimental plan or delivering reports?), how a field manual can be in place before the project is completed, and how control agents can be tested under static and flow conditions already after the first three months of the project? The research training section is very general, and not provided in a context that highlights the uniqueness and specific opportunities for the RA and the student
- **National Benefit**While not presented in any detail, I agree that the proposed work with the ultimate aim to control bryozoan fouling in water distribution pipes is of very high national benefit. If successful, it will be a major advance for managing water supply and distribution, and make significant energy and financial savings, and may introduce environmentally friendly measures to control fouling. A successful outcome is a major practical contribution to a sustainable environment, in Australia and globally.
- **Partner Organisation Commitment**The commitment by GWMWater is first class. They are engaged in all stages of the proposed work, and they deliver expertise and knowhow that are unique and essential for the project. PI Barton appears to have a professional training profile and platform ideal to assist in driving the project forward. The financial contribution by GWMWater is adequate
- **Improvements (if not applicable, please enter n/a)**Areas requiring improvements are detailed in the preceding sections.
- **Comments (if not applicable, please enter n/a)**n/a

Rejoinder in response to the Assessors Reports. (prepared by Prof. John Orbell)

We appreciate Assessor 1's endorsement of the inherent innovation of studying freshwater Bryozoans in Australia. We also appreciate that that Assessor 2 recognizes the potential for significant scientific advancement with respect to management of water supply and distribution and for environmental sustainability. It is gratifying that both Assessors emphasize the importance of this project both to Australia and globally.

However, Assessor 1 is incorrect in suggesting that the primary focus of this work is ecological/zoological - leading her/him to question the backgrounds of the investigators. On the other hand, Assessor 2 has correctly recognized that the primary objective of the project is the investigation of different physical/chemical means for the prevention of biofouling of water pipeline systems (as reflected in the title itself and clearly delineated in the body of the application). Assessor 2 also recognizes the multidisciplinary nature of the project and confirms that the present team is well–positioned to conduct the proposed work – with the expertise stated to be "highly appropriate and with the combined skill base and experience covering all aspects of the approach".

Assessor 2 also has a more balanced view of the track records of the team members than does Assessor 1. Assessor 1 is of the opinion that CIs Orbell and Mitra have comparatively poor recent research track records. It has been clearly acknowledged in the application that CI Mitra's track record is not strong at this stage of his career, this being primarily due to his role in establishing teaching programs within the School of Arts & Sciences at the Monash University Sunway Campus in Malaysia (Feb 2001 to Sept 2008). The more experienced investigators on this proposal believe that his inclusion on this application as a CI will be of enormous benefit to his re-entry into research. In our view, his inclusion is also justified by his outstanding performance as a research fellow during the first year of the current collaborative project with GWMWater. Lead investigator CI Orbell cannot be said to have a comparatively poor recent research track record. In terms of the recent refereed journal articles listed in the application (since 2004), all of the publications are listed in the current ARC ERA database, including Asia Pacific Biotechnology News (APBN) – a respected regional publication, especially for review articles. Specifically, of the 14 referred journal articles listed since 2004, one is ranked A* in the ERA database, one is ranked A, five are ranked B and seven are ranked C. Furthermore, it should be pointed out that of CI Orbell's ten career best publications, four are ranked A*, one is ranked A, four are ranked B and one is ranked as C. The weighted average impact factor for these publications is 3.41 (ISI Web of Knowledge). CI Orbell is an experienced researcher and has a respectable weighted average impact factor for his career journal out put of ~ 2.74. His suitability to lead and manage this project is clearly evidenced in Section F13 of the application.

It should be pointed out that both Assessors wrongly identify the current (existing) collaborative project between Victoria University and GWMWater as an ARC/LP project. This is not the case and could give rise to a false perception of double-dipping. To clarify - the intention of the existing collaborative project, which started from scratch in October 2008, was to lay the groundwork for further research - given the potential scope of the work. In this regard, initial studies have focussed on the Northern Mallee Pipeline system, whereas subsequent investigations will also extend to the Wimmera Mallee Pipeline system. That real progress has been achieved in laying the foundation for this ARC Linkage proposal is evidenced by the fact that GWMWater has committed $120,000 cash and $225,000 in kind over three years in support of this ARC Linkage proposal. It is appreciated that both Assessors have acknowledged the substantial commitment of GWMWater to this project.

It is not clear what Assessor 2 means in relation to the literature search. The proposed field manual will be a work in progress as we gain more experience from the field trips and it is intended to place this on the web as a resource for comment and feedback by other researchers and water authorities. It is anticipated that control agents can be tested under static and flow conditions after as little as three months into the project due to the significant head start that has been provided by the existing project.

We feel that the research training opportunities for both the RA and the PhD student have been adequately described.

Assessor 1 has suggested improvements to the experimental design, such as a the inclusion of a null hypothesis, fails to recognize the inductive and exploratory nature of the project. Refining the

details of experimental design(s) that involve the testing of various control agents against cultivated Bryozoans under static and flow conditions will form part of the project itself – since there is no precedent for this.

APPENDIX TWO

LIST OF FRESHWATER BRYOZOANS ARTICLES FOR REVIEW

1. Allman, G. (1844). Synopsis of the genera and species of zoophytes inhabiting the fresh-waters of Ireland. *Annales of the magazine of natural history* 13: pp 328-331.

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17. Callaghan, T.P. and Karlson, R.H. (2002). Summer dormancy as a refuge from mortality in the freshwater bryozoan ***Plumatella emarginata.*** *Oecologia* **132**: pp 51-59.

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Bryo Technologies

Innovative Strategies for Biofouling Management

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AUSTRALIA

Hello Robin,

I have had a chance to examine the statoblasts that arrived a few days ago. Labels for the three containers read:

1. Ouyen Backwash
2. Piangil Membrane Filter
3. Nyah Membrane Filter

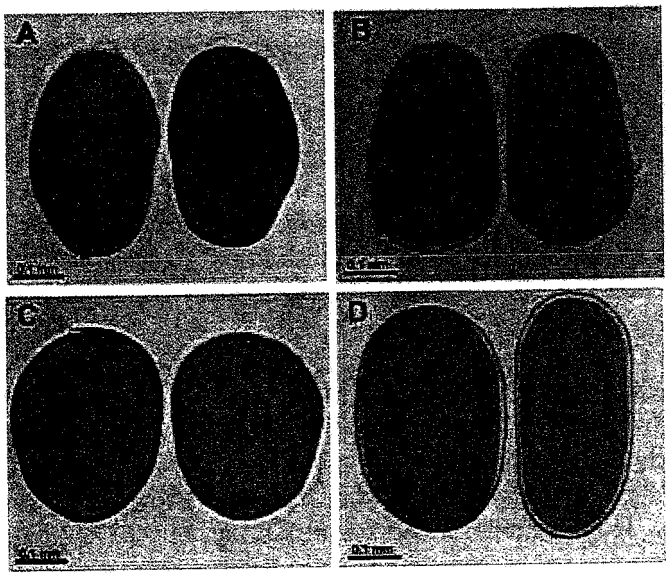


Figure 1. Fredericellid statoblast valves: A-C are from the Ouyen Backwash; D is *Fredericella sultana* from Bough Beech Reservoir in Kent, UK (for comparison).

The fredericellid statoblasts from the Ouyen Backwash pose an interesting dilemma. Figure 1C has the shape generally associated with *F. australiensis*; Fig. 1D is actually *F. sultana* from the UK. So what is the species in Figs. 1A and 1B? In all fredericellids the statoblast shape is strongly influenced by the tubule diameter. *F. australiensis* tends to have large tubules, hence broad statoblasts, but this may not always be the case. The only other known feature distinguishing *F. australiensis* is the frequency of its statoblasts (normally about one per zooid). Unfortunately, in this sample there are no strands long enough to make this determination with any confidence. Given these obstacles, and recognizing that *F. sultana* does exist in Australia, I would

venture a guess that this sample contains a mix of *Fredericella sultana* (Figures 1A and 1B) and *F. australiensis* (Figure 1C).

Two plumatellid species also occured in this sample: *Plumatella vaihiriae* and *Plumatella emarginata* (Figure 2). *Plumatella vaihiriae* (Figure 2A) is distinguished by a strong reticulation over the entire statoblasts surface, and a complete absence of tubercles. With light microscopy you can see only features of the statoblasts fenestra, and many people have trouble distinguishing tubercles from the net-like reticulation. The difference is this: tubercles act as little lenses, and by focusing up and down on the valve you can reduce tubercles to points of light. You cannot do this with a reticulated pattern.

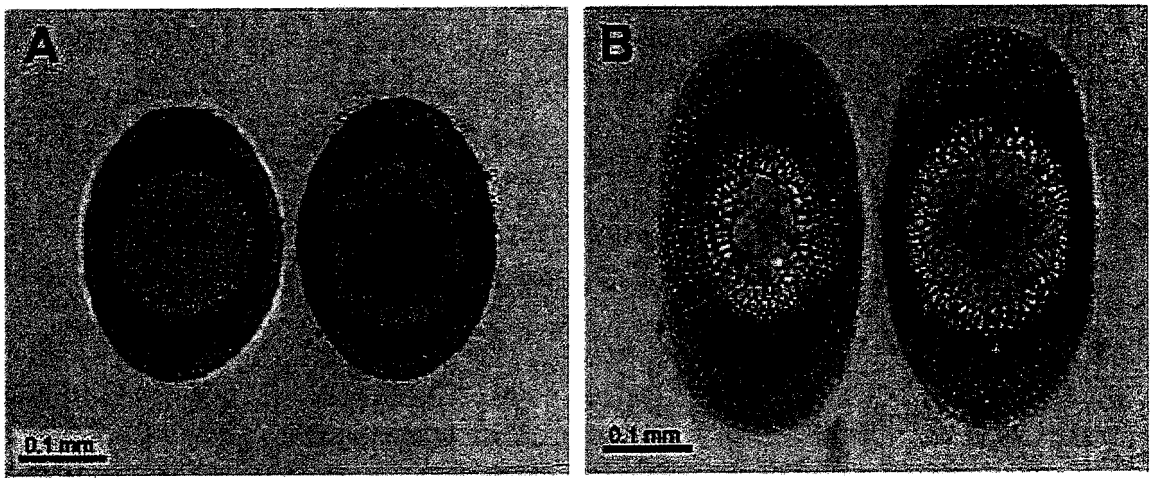


Figure 2. Plumatellid statoblast valves from Ouyen Backwash: (a) *Plumatella vaihiriae*; (B) *Plumatella emarginata.*

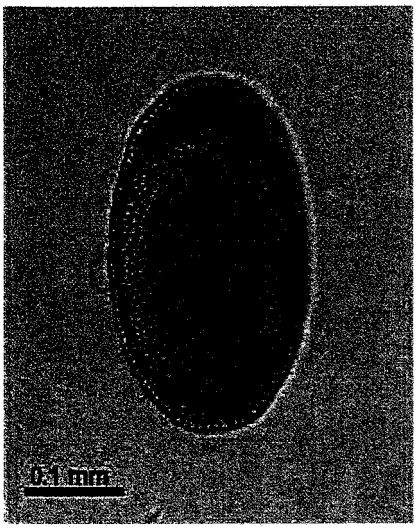
Another feature of *P. vaihiriae* is what appears to be an oversized capsule that causes the surrounding periblast to bulge out around it, especially on the dorsal side.

Plumatella vaihiriae is believed to be an Asian species, but its range is rapidly expanding worldwide, especially in North America and Europe. It is a serious biofouling species that causes expensive problems in irrigation, wastewater, and industrial cooling systems (Brisbane waterworks, for example!).

Plumatella emarginata is well known throughout Europe, North America, Australia, and New Zealand. A similar species is *P. mukaii*, which is somewhat smaller and bears a very fine surface wrinkling, best seen with reflected illumination. Also similar is *P. bombayensis* which is distinguished by a prominent reticulation across the ventral fenestra. And I should also mention *P. velata*, known only from Australia, in which the dimensions are similar, but the annulus completely obscures the dorsal fenestra.

The specimen from Piangil Membrane Filter was composed mostly of silt and the head capsules of dipteran larvae. The only bryozoan statoblasts I could find belonged to *Plumatella casmiana* (Figure 3). The distinguishing feature here is the elegantly shaped long-oval fenestra on both

dorsal and ventral valves, which follows the outer contours of the statoblast itself. *P. casmiana* also forms a second type of free statoblast, the thin-walled leptoblast which lacks a capsule and therefore has no obligatory dormancy.

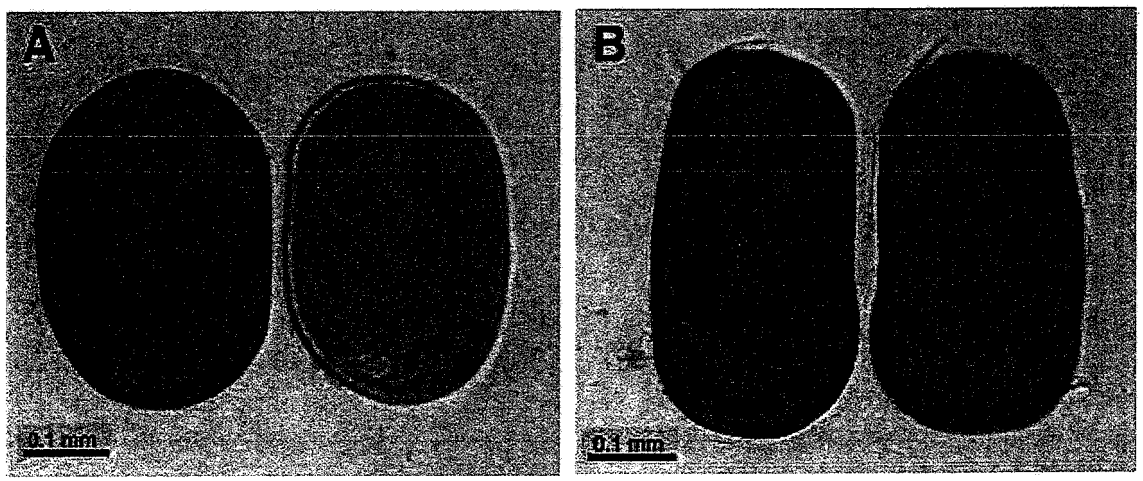


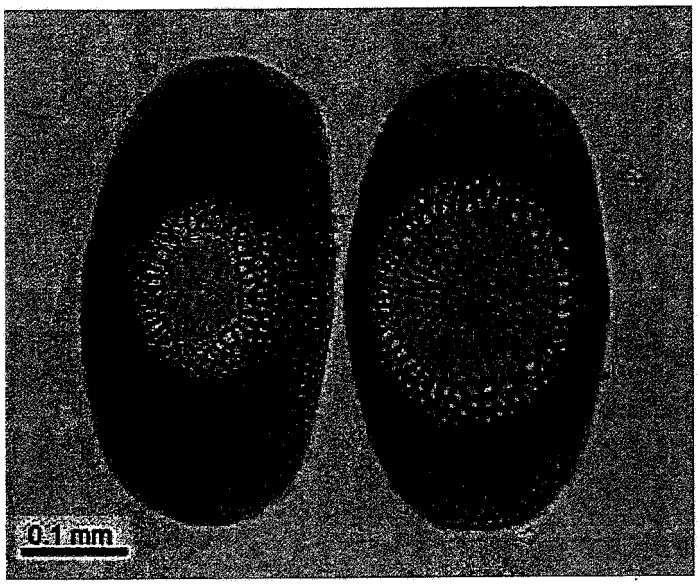
Neither the free (capsuled) statoblast nor sessile statoblast has any prominent surface features. Tubercles are usually low and faint, or else lacking altogether. The colony is extremely variable in its morphology – mostly flat with a prominent raphe, but not always. The zooid wall is usually heavily encrusted, but not always. When crowded the zooids grow perpendicular to the substratum, pressing together to form a solid mass. The lophophore bears fewer tentacles than any other plumatellid, usually less than 26, and this can be diagnostic for the species.

Figure 3. Statoblast of *Plumatella casmiana* from Piangil Membrane Filter.

From the Nyah Membrane Filter I found three species: *Fredericella australiensis* (Figure 4A), what we're calling *F. sultana* (Figure 4B) and *Plumatella emarginata* (Figure 5). In Figure 4A you can see a very faint pebbly texture which some people have interpreted as the pitting of *Fredericella indica*, but that is incorrect.

Figure 4. Statoblast valves of *Fredericella sutraliensis* from Nyah Membrane Filter.





Finally, *Plumatella emarginata* from Nyah Membrane Filter. The free statoblast is fairly consistent in its morphology – the ventral fenestra always with strong tubercles, the dorsal fenestra weakly tuberculated or sometimes even smooth. The ventral valve is always convex, the dorsal valve almost flat. The dorsal valve is slightly smaller than the ventral valve so the suture can be seen in dorsal view. Of course this feature is not apparent in Figure 5.

Figure 5. Valves of the free statoblast of *Plumatella emarginata* from Nyah Membrane Filter.

I hope all this is clear. Contact me with any questions about this report or if you want the original large versions of any of the photos.

Best regards,

Tim

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Bryo Technologies

Innovative Strategies for Biofouling Management

14 March 2010

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School of Engineering and Science
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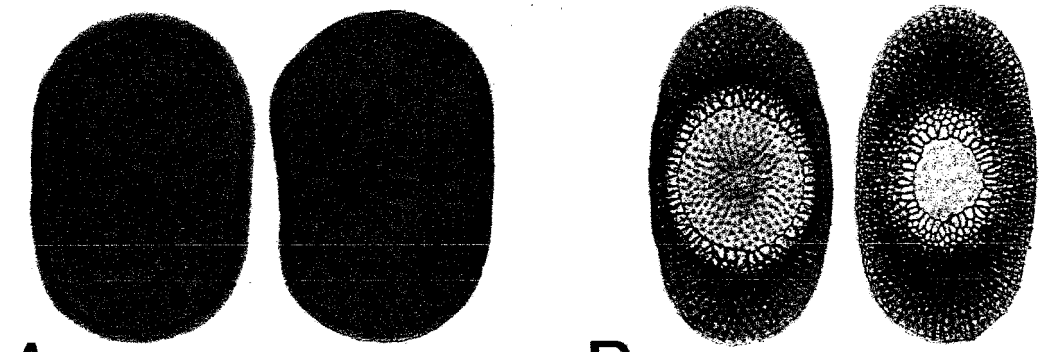
AUSTRALIA

Hello Robin,

The most recent specimens you sent were labeled "F" and "?," without date or provenance. Figures A and B below show representative statoblast valves from each sample. Based on critical dimensions and symmetry I have identified these as *Fredericella australiensis* (from "F") and *Plumatella emarginata* (from "?"). Each container also contained a few statoblasts from the other species. No other species were represented in the samples.



Statoblast valves. (A) *Fredericella australiensis;* (B) *Plumatella emarginata.*

Best regards,

Tim