Milestone 4: Report

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

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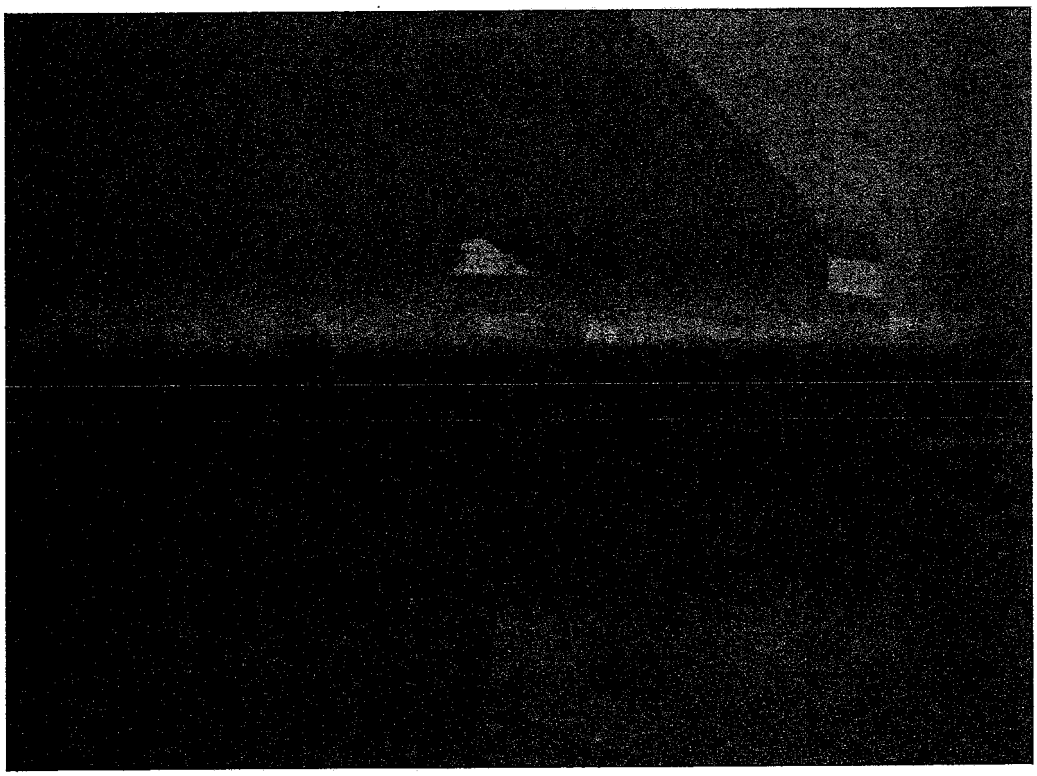


Bottom- most plate from the "Pit' with considerable growth on the bottom half.

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 control experiments with various chemical agents. Initial experiments will be carried out using chlorine as a benchmark. Other control agents, such as hypochlorite and nano-particulate silver will then be systematically tested.

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 3: salient points

i. Finalize and submit the review described in Milestone 2 no later than the end of March. The review is yet to be described and finalized.

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.

The above mentioned statement has been duly addressed with reference to particle size analysis using the Zeiss Axioplan 2 research-grade microscope available at Victoria University, St Albans campus and SEM related studies carried out at Melbourne University (Refer to Milestone 3 report May 2010 Sections 2.1 and 2.6). However the investigation on 'seasonality' is ongoing and in generic terms can be related to (a) the increase or decrease in the growth and proliferation of freshwater Bryozoan as observed in the field or in their natural habitat at certain periods of the year and (b) the ability to approximate these conditions as precisely as possible under laboratory conditions. The statoblast to colony propagation was successful through an artificial inducement of 'overwintering', however the colony-to-colony propagation is still being investigated in the laboratory.

iii. **Continue the statoblast-to-colony and colony-to-colony cultivation of the Bryozoans organisms in the laboratory.**

Currently the ongoing experiment is based on the colony-to-colony propagation using the growth material obtained from the 'Pit' at Ouyen and maintained both in the growth tanks at 26^{0}C and in six separate vessels incubated within a water bath at 30^{0}C.

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).**

As a result of a meeting held between Andrew Barton, Steven Briggs, John Orbell and Robin Mitra on Thursday the 11th of February 2010 the 'Pit apparatus also designated as the 'Field Rig' had been successfully suspended in the Ouyen Pit on the 4th of March 2010 **(Refer to Milestone 2 Report 2010, pp 31 – 32. Figs 19a, 19b, 19c and 19d).**

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 for carrying out static control experiments with various chemical agents. Initial experiments will be carried out using chlorine as a benchmark. Other control agents, such as hypochlorite and nano-particulate silver will then be systematically tested.**

Three growth plates with considerable growth in the bottom-most plate were duly handed to us by Steven Briggs and Paul Atherton after the Ballarat meeting on the 13th of July 2010. The plates were immediately placed on the growth tanks maintained at 26^{0}C at St Albans and immersed in a mixture of pit water and 'aged' water from the aquarium.

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**

A draft entitled 'Brief Technical Note on the preparation of freshwater Bryozoan statoblasts for Scanning Electron Microscopy (SEM) analyses has been duly disseminated to John Orbell, Andrew Barton and Steven Briggs for correction, the corrected draft will be handed to Simon Crawford from Melbourne University for the final comments. The following paper entitled "First findings of *Plumatella emarginata*, *Plumatella reticulata*, *Plumatella minuta*, *Fredericella australiensis* and *Fredericella sultana* from the Northern Mallee Pipeline system in Victoria' is in preparation.

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

Formal discussions pertaining to the extension of the investigations to the Wimmera-Mallee Pipeline system is yet to take place.

viii. **Progress report in relation to Milestone 4 to be submitted with invoice for fifth instalment of $ 20, 871 on Friday 30th July 2010.**

2. Addressing Milestone 4 (31st July 2010 to 30th September 2010)

2.1. Ballarat Meeting 13th July 2010.

The essence of the Milestone 4 report was based on the following salient points (i) to continue the static testing and acquire data in relation to the relative effects of various control agents (ii) to design, acquire and commission laboratory scale equipment whereby cultivated colonies may be systematically challenged with various control agents under flow conditions and finally (iii) to draft a technical paper based on our field sampling experience for publication in appropriate journal. The Ballarat meeting that was held at the Lake View Hotel, 22, Wendouree Parade was urgently called for two reasons that entailed (i) static testing, for which a planned program for trialling a number of control agents was to be made available along with a brief background information on the practicability and safety of using these agents in the potable water industry and (ii) a direct discussion with Mr Kain Chong and Miss Lorain Kong (Business Development Officer) from CC technologies Pty Ltd, on the feasibility of using their silver nano technology based product for the control of freshwater Bryozoans. The meeting was attended by John Orbell, Robin Mitra, Paul Artherton, Steven Briggs, Kain Chong and Lorain Kong. To address the first point, a brief outline of the experiments related to static testing in the form of a technical manual entitled 'Control Experiments' and a set of power point slides entitled 'Prevention of Bryozoan fouling of Water Pipeline Systems' **(refer to appendix one)** were disseminated among the participants present in the meeting. Steven Briggs brought along three growth plates from the 'Pit' together with approximately 100 litres of 'Pit' water. These plates were installed in the growth tanks at St Albans, on the same day for colony-to-colony propagation prior to the commencement of the control experiments. During the course of the meeting at Ballarat, Steven Briggs was also requested to provide us with some information on superchlorination and the manner in which the process was carried out at GWMWater. During the first field trip that was conducted in the summer of December 2008, we were informed by David Blandthorn that from Manangatang onwards, freshwater Bryozoan was much less of a problem and he attributed the cause to the use of chlorine. In this context, it was deemed that the information on superchlorination could prove very helpful in relation to the use of silver nanoparticles for the control experiments using chlorine as a benchmark. A set of queries that were sent to Steven Briggs in relation to superchlorination after the Ballarat meeting are provided below:-

(i) Owing to the fact, that the proliferation of the freshwater Bryozoans is seasonal, a request was made to Steven Briggs regarding the information on the time of the year the superchlorination is usually carried out by GWMWater.

(ii) A request for an opportunity to interact directly with the people who actually carry out the process of superchlorination in order to obtain first-hand details on the procedure.

(iii) The procedures and the criteria for selecting the section of the pipeline that is to be sealed off and the total length of time it is sealed up for.

(iv) Information on the volume of water in that sealed section of pipeline.

(v) The levels of chlorine used to treat that section of the pipeline and the length of time it is treated for? Request for any documented evidence for the effectiveness of chlorine so that the data could be used in relation to the use of silver nanoparticle as a control agent.

It was also emphasized that the Information on all of the above queries such as the volume of water, the levels of chlorine used to treat that particular section of pipeline etc would immensely help in the calculation of the amount of silver nanoparticles that could be used for the control experiments and concomitantly aid in comparing the effectiveness of silver on the freshwater bryozoans in relation to chlorine.

Another practical comment that was made by John Orbell during the course of the discussion at the Ballarat meeting was that the solution behind the control/eradication of freshwater Bryozoans lay not only in finding a way to obliterate the freshwater Bryozoan colonies but also in seeking a means that would concomitantly annihilate the dormant chitinous valves of the statoblasts. In this context, the benzoylphenylurea diflubenzuron may be worth looking at during the trialling experiments because this insecticide retains the capability to arrest chitin formation (**discussed in Section 2.4**). Refer to **appendix three** for the information on diflubenzuron that was kindly provided by Heather Bishop, Assistant Director, National Health and Medical Research Council in response to our request.

After the Ballarat meeting, Paul Atherton made available a draft of all the potential organizations that would be interested in coming on board for the current project (**Refer to appendix four for the list**). Andrew Barton made a general presentation to Coliban Region Water Corporation on the 29th of July 2010 entitled 'Biofouling Research' the presentation, besides biofilms, also addressed freshwater Bryozoan biofouling (**Refer to appendix two for the presentation slides**).

2.2. Installation of growth plates at St Albans from the 'Pit' at Ouyen

As a result of a meeting held between Andrew Barton, Steven Briggs, John Orbell and Robin Mitra on Thursday the 11th of February 2010 the 'Pit apparatus' also designated as the 'Field Rig' had been successfully suspended in the Ouyen Pit on the 4th of March 2010, **Figs 1a** and **1b.**

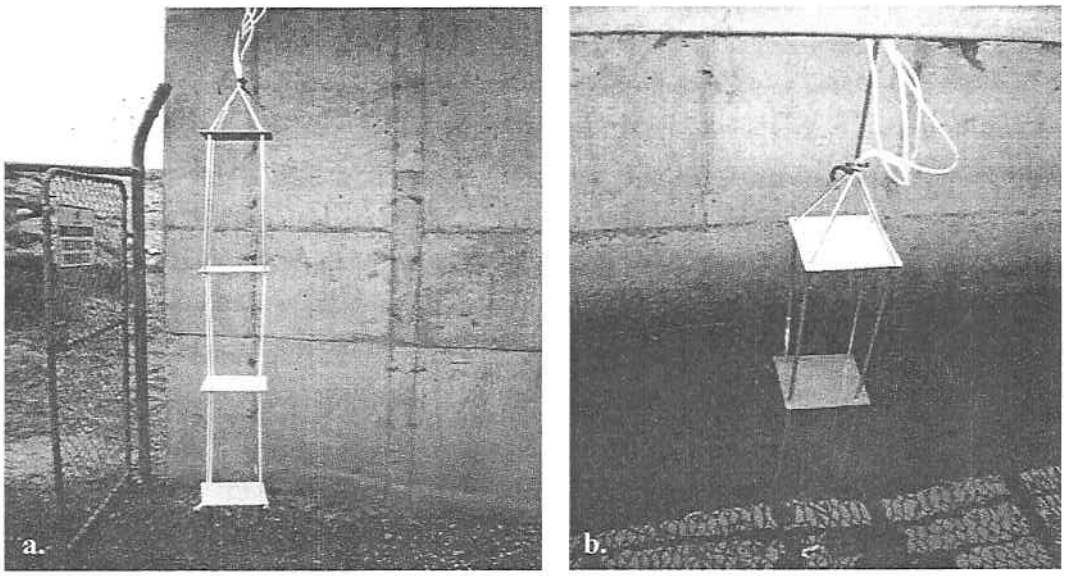
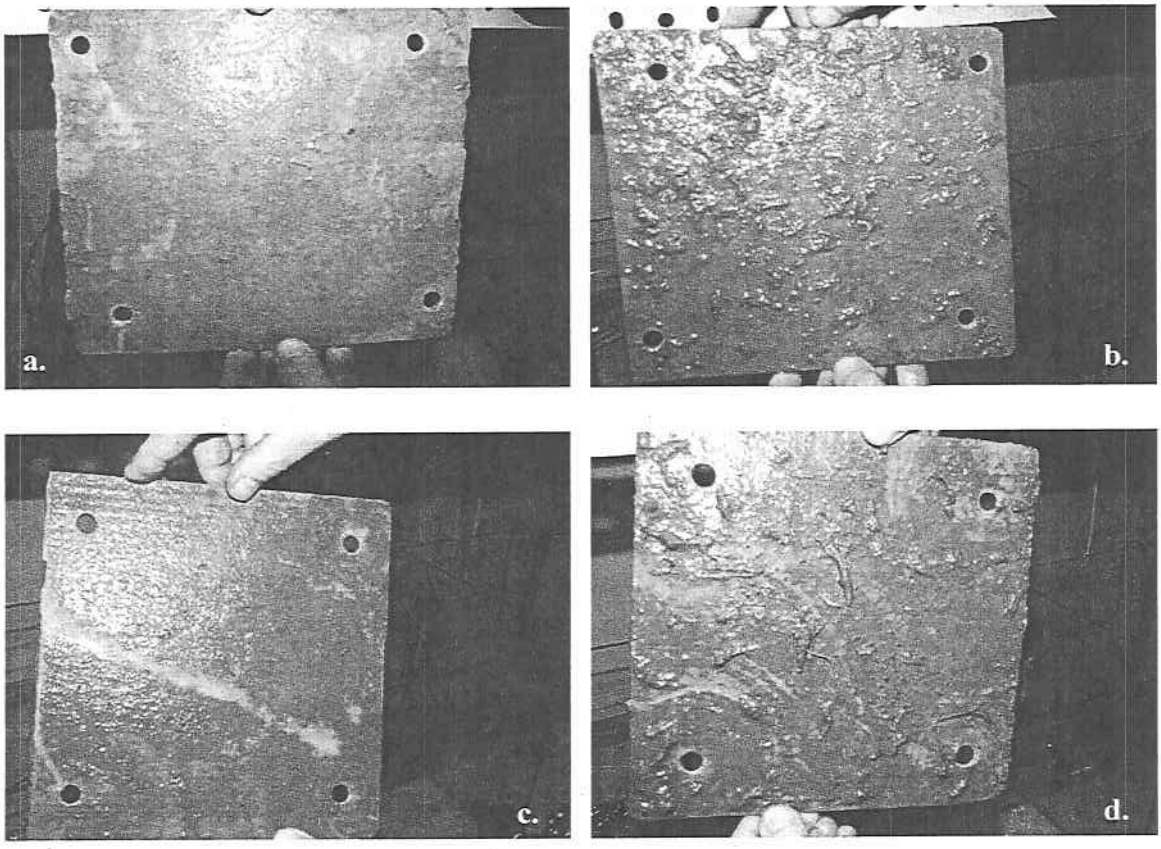


Fig 1. (a) Completed 'Field Rig' comprising of four 200 x 200 mm growth plates prepared from cement sheeting (b) The 'Field Rig' being suspended in the 'Pit' on the 4th of March 2010.

From reports obtained from Steven Briggs, it was apparent that for the first couple of months no growth was observed on the plates and the cause of the delay was attributed to the onset of the cold weather. However, the first sign of growth on the plates that were continuously kept suspended in the 'pit' was reported by Steven Briggs on the 18th of June 2010 after a lapse of about three months. Three of the four plates were duly handed to us by Steven Briggs and Paul Atherton along with four containers of 'Pit' water (approximately 100 litres) after the Ballarat meeting on the 13th of July 2010. These plates were immediately installed in the growth tanks stationed at St Albans. It was also noteworthy that the growth on the top two plates was meagre compared to the bottom-most plate on which a considerable amount of growth was displayed on both sides , **Figs 2a, 2b, 2c, 2d, 2e** and **2f.** A similar experiment was carried out by Smith (2005) at the Southern Reservoir water treatment station in Dunedin, New Zealand. From the experiment Smith (2005) reported that *Plumatella repens* was found to grow on the underside of the plates whilst a different species of freshwater Bryozoan *Paludicella articulata* was found to grow on the topside of the plates. According to Wood and Okamura (2005), under natural conditions most freshwater Bryozoans except for *Lophopus crystallinus*, tend to grow on the underside of rocks, logs, buoys and vegetation to protect themselves from the settling particles. Hence it was hypothesized that in the top material there was a possibility

that *Paludicella articulata* belonging to the family Paludicellidae could be present since this species is known to occur world wide, most often in flowing or turbulent water (Wood and Okamura 2005) and the bottom half would comprise of members belonging to the genus *Plumatella* and *Fredericella*. The presence of *Lophopus crystallinus* belonging to the family Lophopodidae, however was not expected in the top material because the distribution of this species is strictly holarctic (Rao 1992) meaning it is found to occur in the habitats of the Northern continents mostly throughout Europe especially in lotic habitats where the water is turbulent and swift flowing (Wood and Okamura 2005). The plates were contained within an esky and during the course of the transport from Ouyen to Ballarat and from Ballarat to St Albans some of the colonies were dislodged from the plates which occurred as debris at the bottom of the esky. The plates containing the growth were first photographed and then swiftly placed on the glass ledges that were built within the tanks at St Albans, **Fig 3.** The plates were then carefully submerged in approximately three quarters of 'Pit' water and one quarter of 'aged' water from the aquarium within the growth tanks. The temperature in the tanks was maintained at 26^{0}C. The upper-most plate from the rung of suspension obtained from Ouyen was placed within Tank 1, the middle plate in Tank 2, the lower-most plate in Tank 3, **Fig 4** whilst the nylon ropes and the mental snapper sinkers with some growth on them were placed in the fourth tank.

The filters were shut off in all tanks lest the turbulence of the water bubbles dislodge the colonies of growth from the bottom half of the plates. However after a week or so the outlets of the filters were fitted with valves to control the surge of water bubbles and introduced into the tanks 1, 2 and 3.



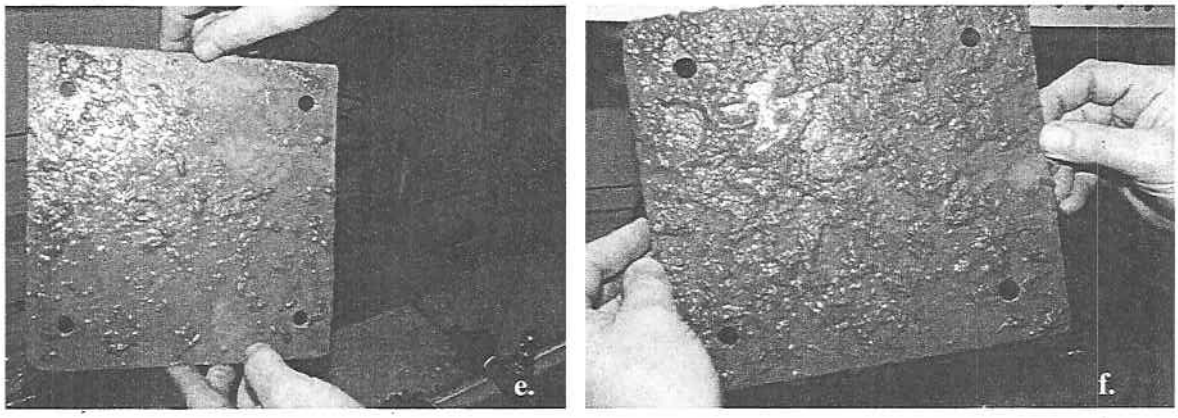


Fig 2. Uppermost plate (a) top half (b) bottom half. Middle plate (c) top half (b) bottom half. Bottom-most plate (e) top half and (f) bottom half.

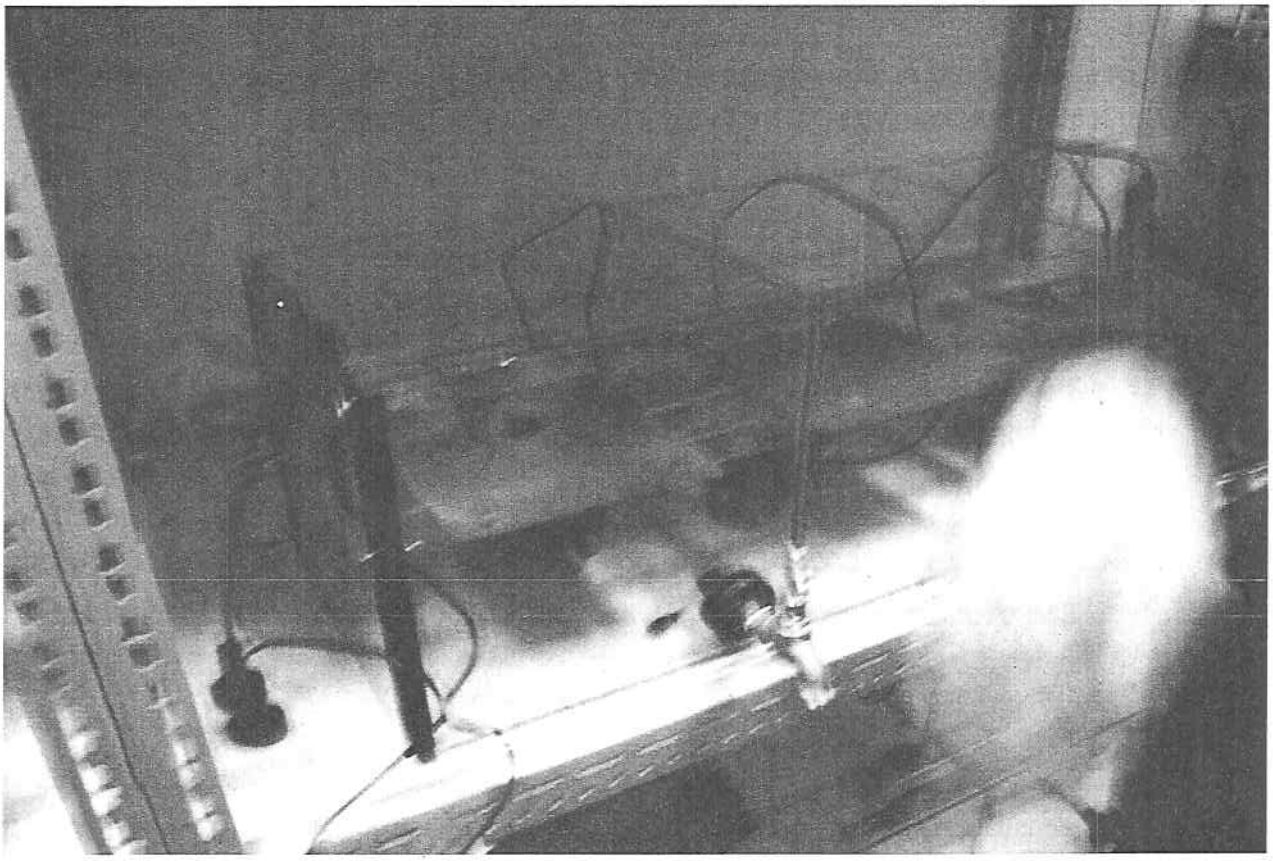


Fig 3 The growth plates were taken out of the esky, photographed swiftly and carefully placed on top of the glass ledges that were built within the tanks.

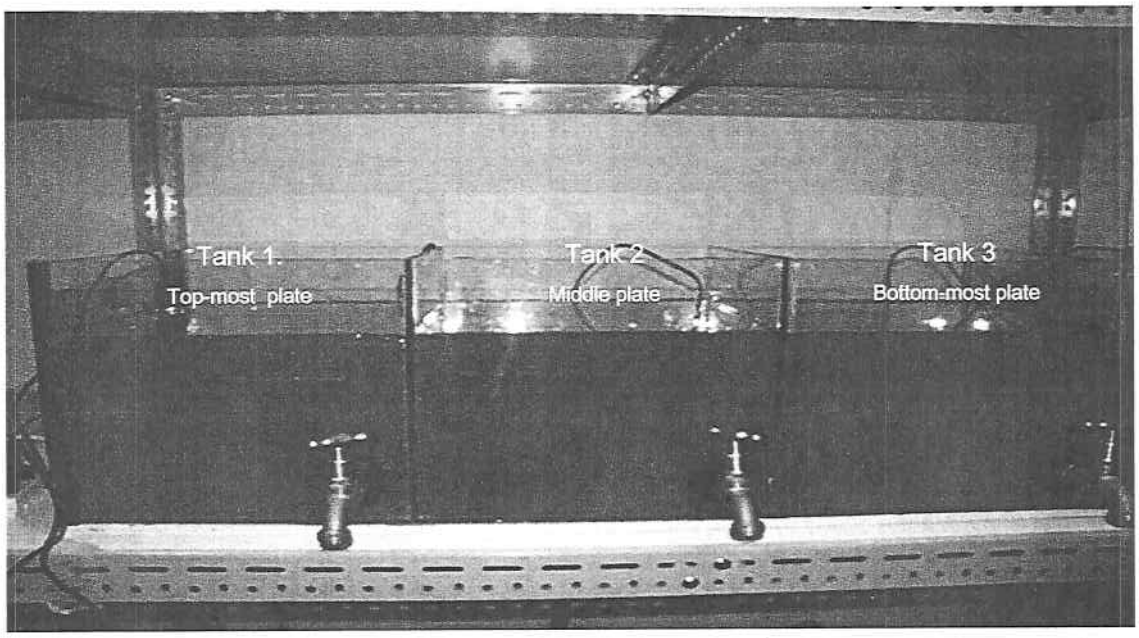
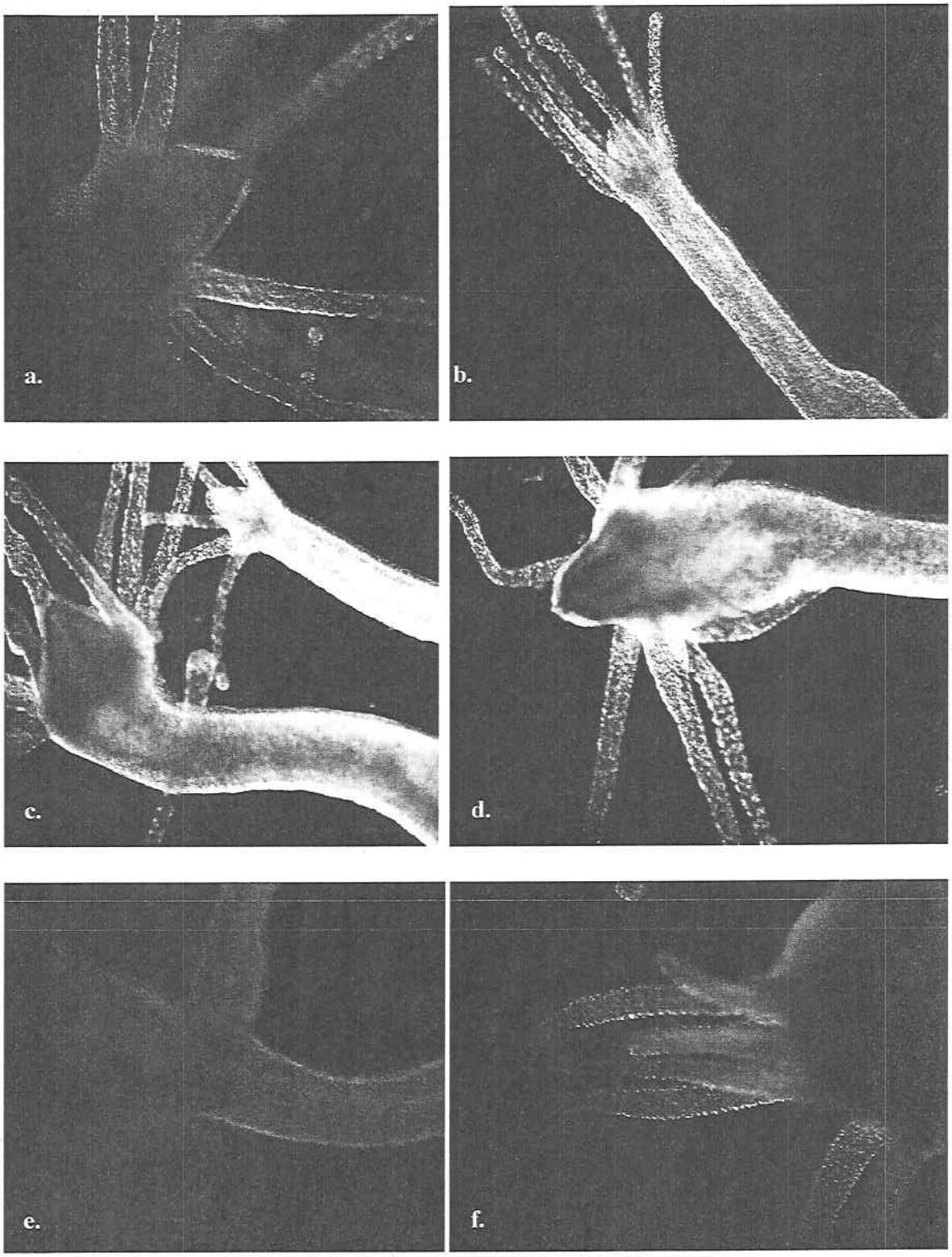


Fig 4 The tanks were then gradually filled with approximately three quarters of 'Pit' water and one quarter of 'aged' water from the aquarium.

The debris were carefully fished out of the esky and emptied into a 5 litre plastic beaker and allowed to settle overnight. Nothing was discarded. The following day, almost all of the intact colonies from the debris were isolated and subdivided into two plastic lids that served as petri dishes. The colonies in the lids were found to be dominated by the freshwater Cnidarian Hydra belonging to the class Hydrozoa, **Figs 5a, 5b, 5c, 5d, 5e** and **5f**. The Hydra is known to exist as solitary, sessile polyps within a colony in which all polyps are interconnected to each other by thin stems (Gooderham and Tsyrlin 2002). The interconnection of thin stems between the polyps was clearly observed within the lids. The presence of Hydra was later reconfirmed by Ana Miranda, a PhD student from RMIT Bundoora whose project involves the control of Hydra and a few of them were handed to her for rearing in her lab. Although a search was attempted however no literature was obtained highlighting any symbiotic relationship that may exist between the hydra and the freshwater Bryozoan.

Other than the abundant occurrence of Hydra, numerous dormant egg cases or ephippia of the planktonic crustacean Cladoceran (water-fleas) belonging to the class Branchiopoda were found to occur in the debris material, **Figs 6a, 6b, 6c** and **6d**. The ephippium somewhat resembles the statoblasts (floatoblasts) of *Lophopus crystallinus* in appearance except that they are larger in size and endowed with the characteristic flat side whilst the floatoblasts of the *L. crystallinus* are lemon shaped and pointed at both extremities (Rieradevall and Busquets 1990). Nevertheless, a second opinion was sought from Tim Wood who confirmed that the dormant structures that resembled the statoblasts of the freshwater Bryozoan *Lophopus crystallinus* were actually the ephippia of the Cladocerans.



F ig 5. (a), (b), (c), (d), (e) and (f) An abundant presence of Hydra were found in both the plastic lids in which most of the intact colonies were placed.

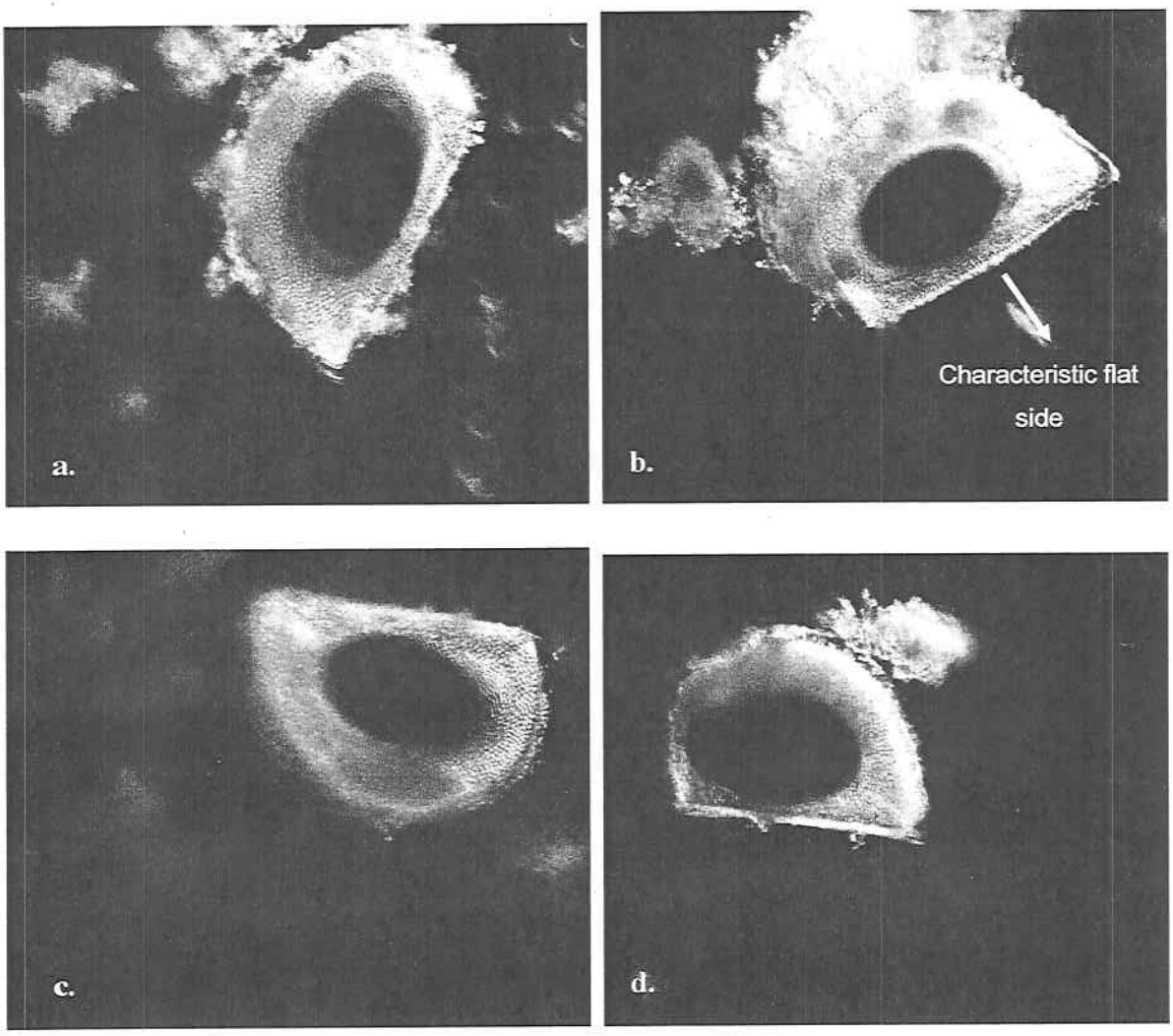


Fig 6. (a), (b), (c) and (d) The debris material was also abundant with numerous dormant egg cases or ephippia of the planktonic crustacean Cladoceran (water fleas) belonging to the class Branchiopoda.

However besides the presence of Hydra and the ephippia of the planktonic crustacean Cladoceran, colonies belonging to the genus *Fredericella*, most probably of the species *Fredericella australiensis* were found to occur in the debris material obtained from the 'Pit' water, **Figs 7a** and **7b**. Besides the colonies, isolated occurrences of statoblasts of the *Fredericella* type, **Figs 7c** and **7d**, was also observed in the debris material. Although no SEM analyses has been carried out on the statoblasts as yet but from the presence of the characteristic thickened rim around the statoblasts (Wood *et al.*, 1998) as observed under the light microscope it was surmised that the statoblasts may belong to the species *Fredericella australiensis*.

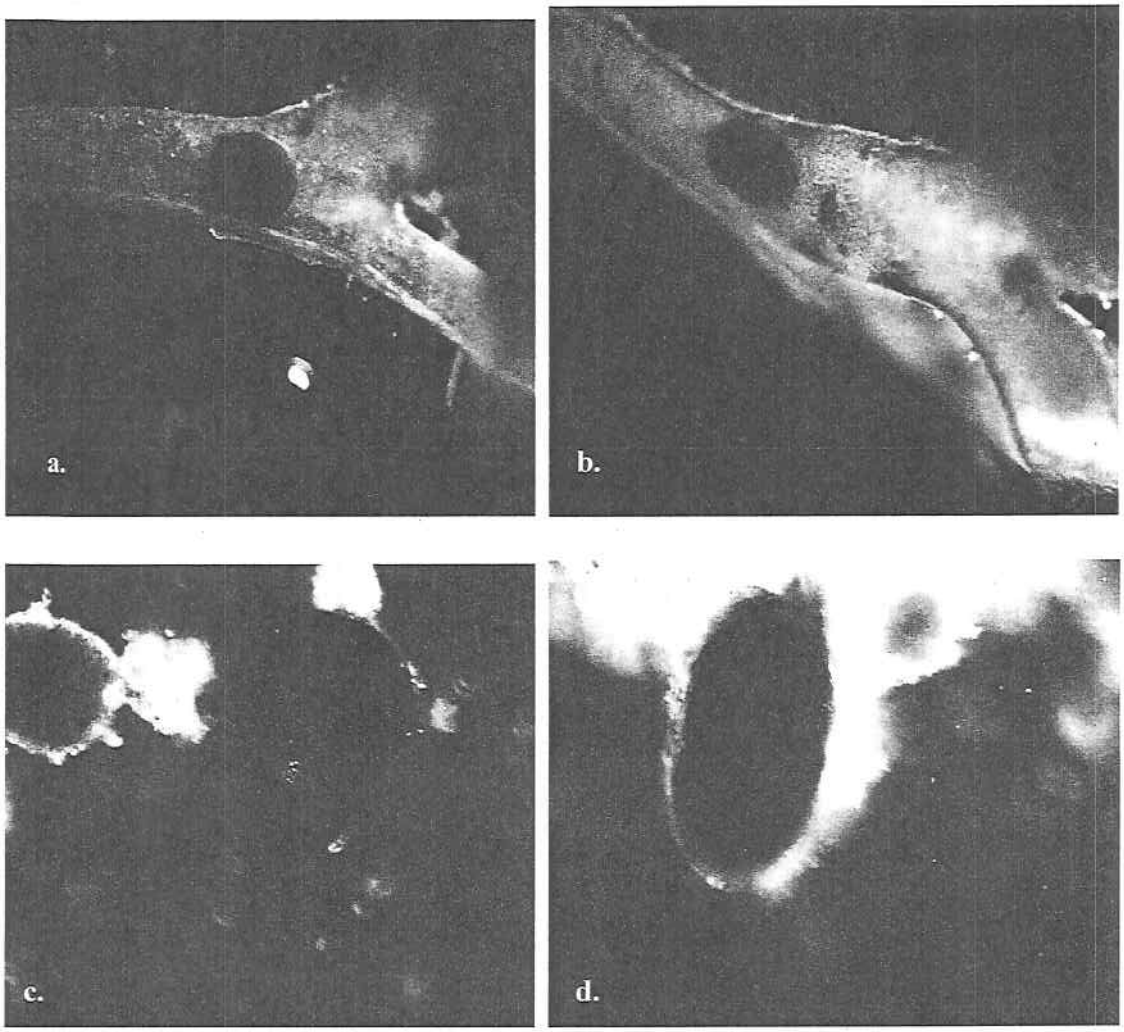


Fig 7. (a) and (b) Fragments of colonies of the genus *Fredericella* possibly belonging to the species *Fredericella australiensis* as inferred from the statoblasts visible under the light microscope. (c) and (d) isolated occurrences of statoblasts in the debris material. From the thickened rim observed around the statoblasts it was surmised that these statoblasts could belong to the species *Fredericella australiensis*.

Apart from the statoblasts of the Fredericellid type, floatoblasts that appeared somewhat similar to that of *Plumatella casmiana*, under the light microscope were also uncovered from the debris **Figs 8a, 8b, 8c and 8d.** The presence of *Plumatella casmiana* in the Northern Mallee Pipeline (NMP) system had been confirmed from a report submitted by Bryo Technologies dated the 16th of February 2010. Moreover, three floatoblasts of *Plumatella casmiana* were identified with the help of Tim Wood after the SEM work that was carried out in May 2010 **(Refer to the first three figures of floatoblasts Milestone 3 report May 2010 Fig 20: pp 32).** .

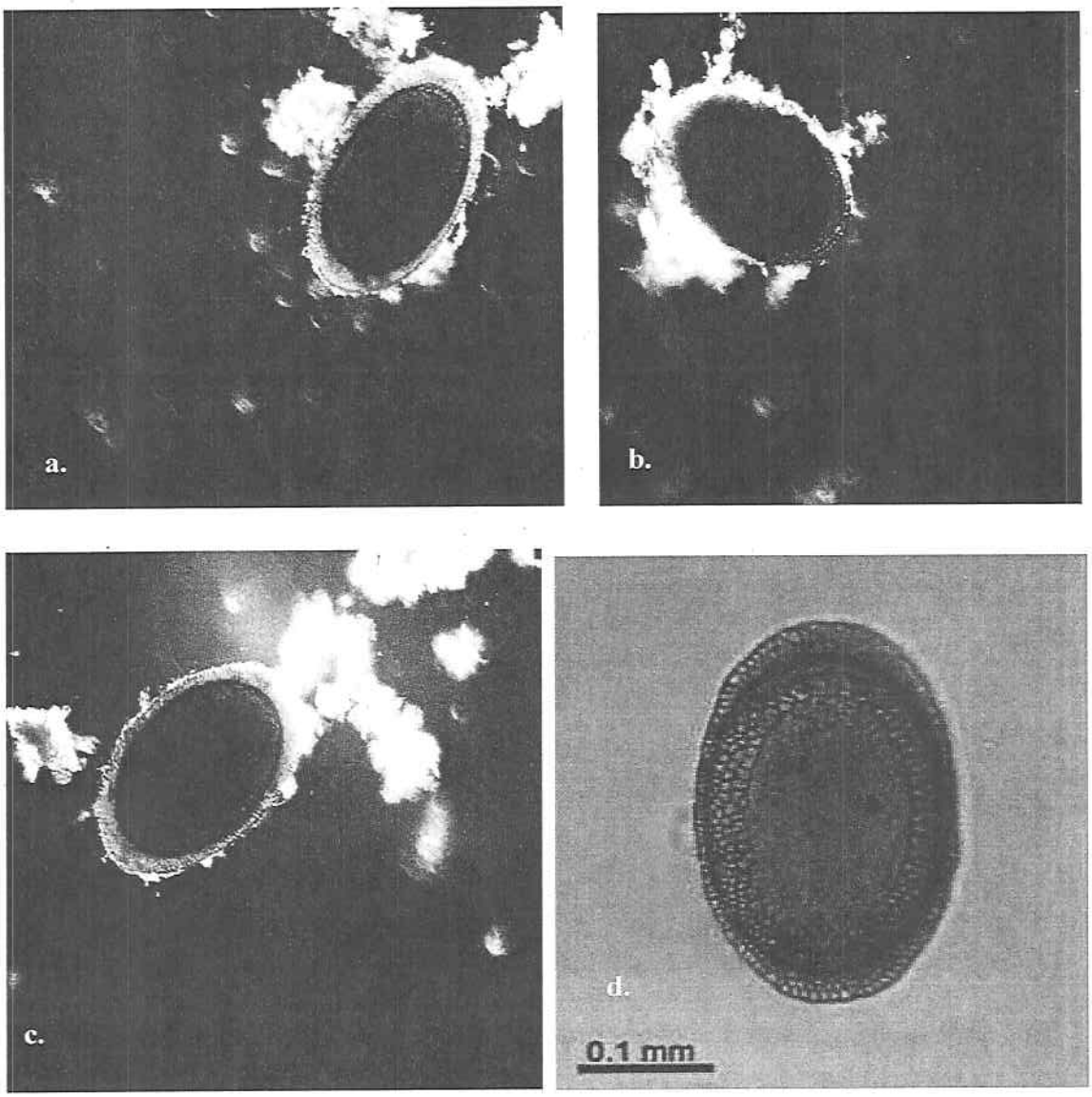


Fig 8. (a), (b) and (c) Floatoblasts uncovered from the debris that resembled that of *Plumatella casmiana* under the light microscope (d) Photograph of a floatoblast for comparative reasons provided by Bryo Technologies in a report dated the 16th of February 2010. The floatoblast was isolated from a membrane filter at the Piangil pump station.

As maintained earlier, intact colonies were isolated from the debris and maintained in two plastic lids that served as petri dishes. These lids were then tucked into a metal rack **Fig 9a** so that they remained perpetually immersed within the tank and did not float around. One of the problems encountered whilst cultivating colonies within plastic lids was that since these lids possess solid bottoms, the colonies are not in contact with the surrounding water in which the lids are floated. As a consequence, there is a need to monitor the floating lids containing the colonies on a regular basis lest the water evaporates and the colonies are exposed to the risk of dehydration. The metal rack containing the lids was placed in tank 4 that was maintained at 26^{0}C along with the nylon ropes and

the metal snapper sinkers from the 'Pit' that displayed some growth on them. Finally the entire row of shelves in which the aquariums were placed was totally covered with a black plastic sheet to mimic the darkness within the pipes, **Fig 9b.** The rest of the debris were later put into a lunch box and placed in the cool room at 5°C in order to investigate the type of statoblasts that the dying colonies would produce.

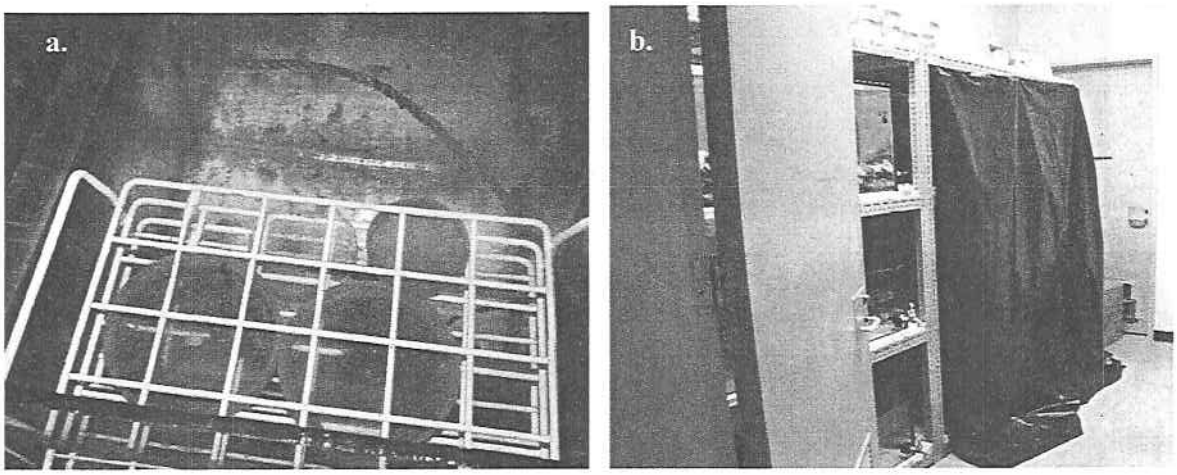


Fig 9. (a) Metal rack in which the plastic lids containing the colonies isolated from the debris were tucked in so as to prevent them from floating. The plastic lids are possessed with solid bottoms and hence the colonies are not in perpetual contact with the water. Therefore it necessitates a regular replenishment of water or the colonies would die of dehydration once all of the water has evaporated. (b) The shelves containing the growth tanks are usually covered by black plastic sheeting in order to mimic the darkness within the pipes.

On the 20th of July 2010, seven days after installing the plates in the growth tanks at St Albans some of the growth materials from both the top half and the bottom half of the plates were scraped off and examined under the light microscope. The growth materials not only revealed the presence of dormant egg cases or ephippia of the planktonic crustacean Cladoceran but also floatoblasts similar to that of *Plumatella casmiana* and a statoblast of the Fredericellid type, **Figs 10a, 10b, 10c** and **10d.**

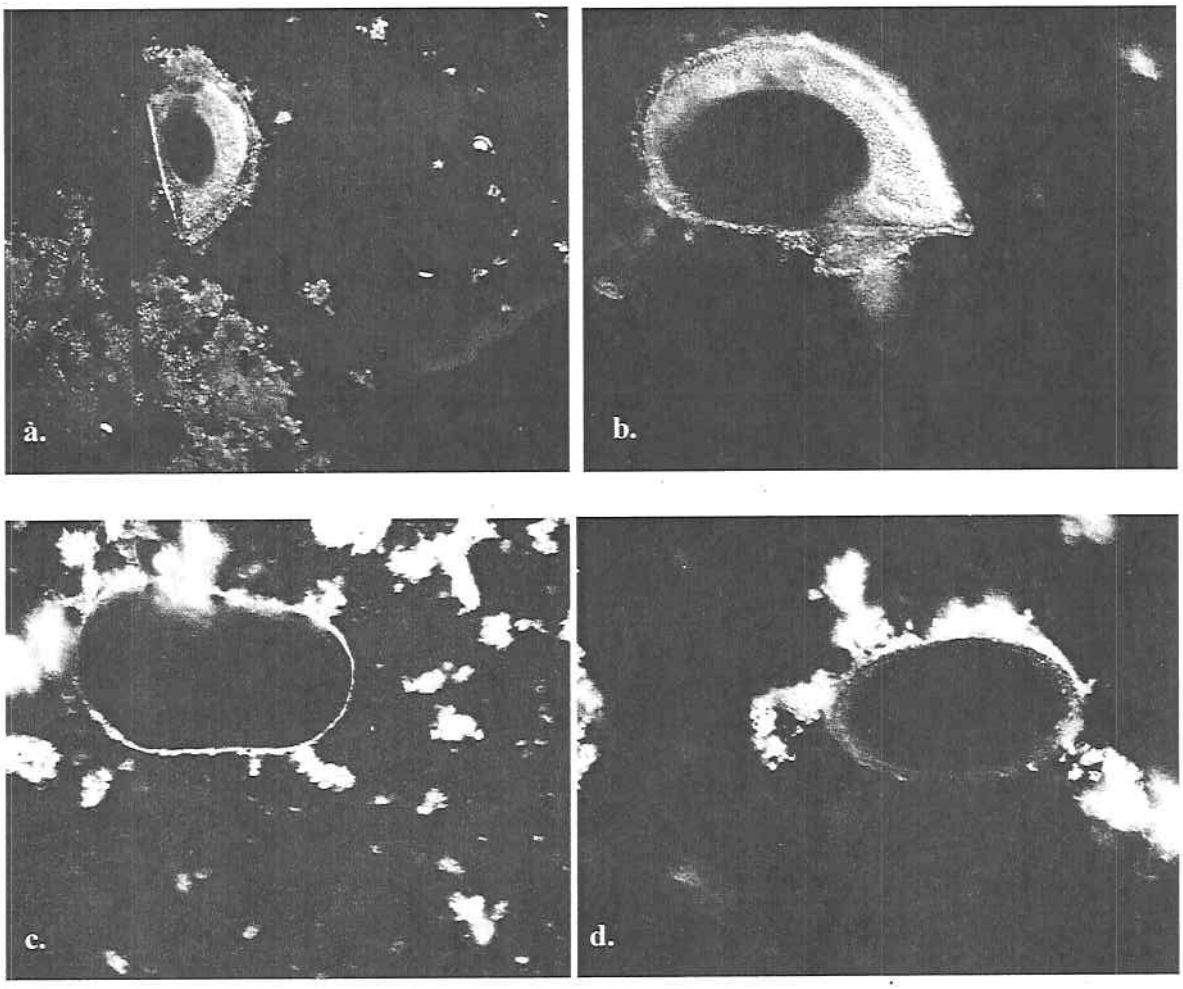


Fig 10. (a) and (b) dormant egg cases or ephippia of the planktonic crustacean Cladoceran (water fleas) belonging to the class Branchiopoda isolated from the growth scraped off the plates. (c) statoblast from the growth material scraped off the plates. Note the thickened rim around the statoblast indicating the presence of *Fredericella australiensis* (d) Presence of floatoblast in the growth materials from the plate that tend to resemble that of *Plumatella casmiana* under the light microscope.

2.3. Current colony-to-colony experiment at St Albans using the growth materials scraped off the plates from the 'Pit'.

Unlike previous attempts a radical change was introduced in the current colony-to-colony experiment that was initiated using the growth materials from the 'Pit' plates. First of all the solid bottoms of the plastic lids were hollowed out to create floats using tools from the ISI (Institute of Sustainability and Innovation, Victoria University) workshed located at the Werribee campus. The surrounding rims were left intact. Circular pieces of plastic nettings were then cut out and inserted to fit the hollow circumference in the inside of the rims. The nettings were then securely attached to the plastic floats using a masking tape. In this way the colonies would be in constant contact with the surrounding

water through the netting and hence the problem of replenishing the plastic lids with water in a regular manner to avoid desiccation of the colonies was resolved, Figs 11a and 11b.

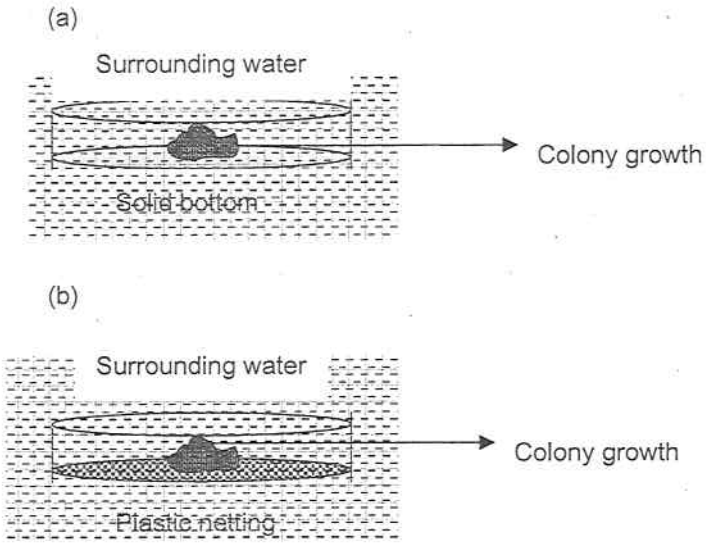


Fig 11. (a) Colony-to-colony propag ation experiments that were carried out in plastic lids with solid bottoms during the earlier attempts ran the risk of the colonies being desiccated once the water within the lids evapora ted and hence required periodic replenishment of water (b) Replacement of solid bottom s with plastic netting allowed the colonies to be in perpetual contact with the surrounding water.

The second modification that was introduced to the colony-to-colony propagation this time was that it was carried out within a water bath maintained at 30°C, Fig 12.

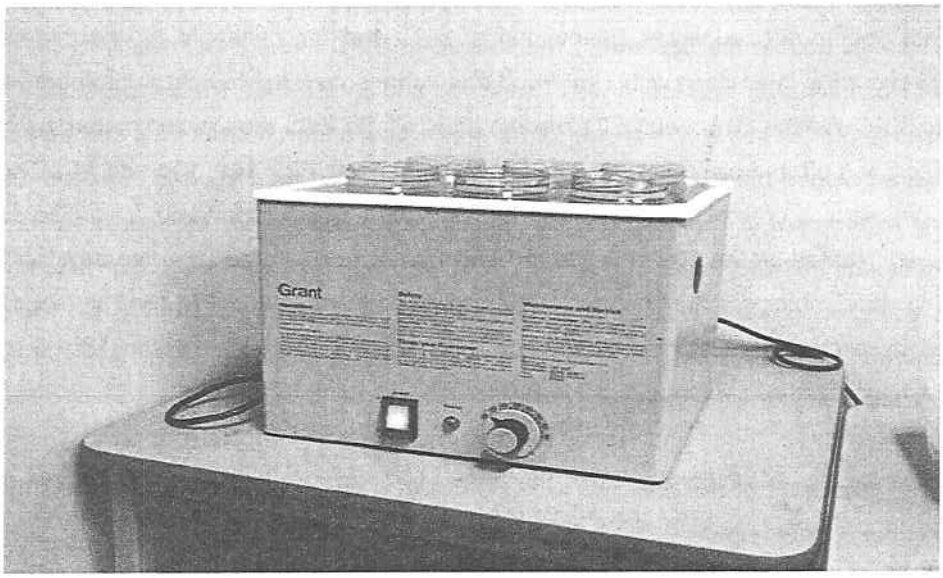


Fig 12. Ongoing Colony-to-colony propagation conducted within a water bath maintained at 30°C

First six floats were prepared by hollowing out the plastic lids and keeping the surrounding rims intact. The rims were then fitted with the plastic netting and floated in jars, **Fig 13** filled with a mixture of 'Pit' water and 'cured' water from the fish aquarium.

Fig 13. Experimental jars filled with a mixture of 'Pit' water and 'cured' water from the fish aquarium in which the colonies were floated and placed within a water bath maintained at 30^{0}C.

The six empty floats were then floated in six separate jars which in turn were placed within a water bath filled with double distilled water, maintained at 25^{0}C and left overnight for the temperature to stabilize. However after interaction with Tim Wood and John Orbell, the temperature in the water bath was raised to 30^{0}C. On the 30th of July 2010, some growth from both the top and bottom halves of the plates were scraped off and placed into the floats within the jars, **Figs 14a, 14b** and **14c.** The colony-to-colony experiment is currently ongoing and is being periodically monitored for proliferation. The experiment was initiated on the 30th of July 2010 and was last monitored after five days on the 4th of August, during which no significant changes in the growth were observed, **Fig 15.** The entire set-up is kept covered by a cardboard box punched with holes at all times **Figs 16** and **16b** to mimic the darkness within the pipeline systems.

With the proliferation of the colonies within the floats, the control experiments will commence. For details on the control experiments and the control agents refer to **appendix one.**

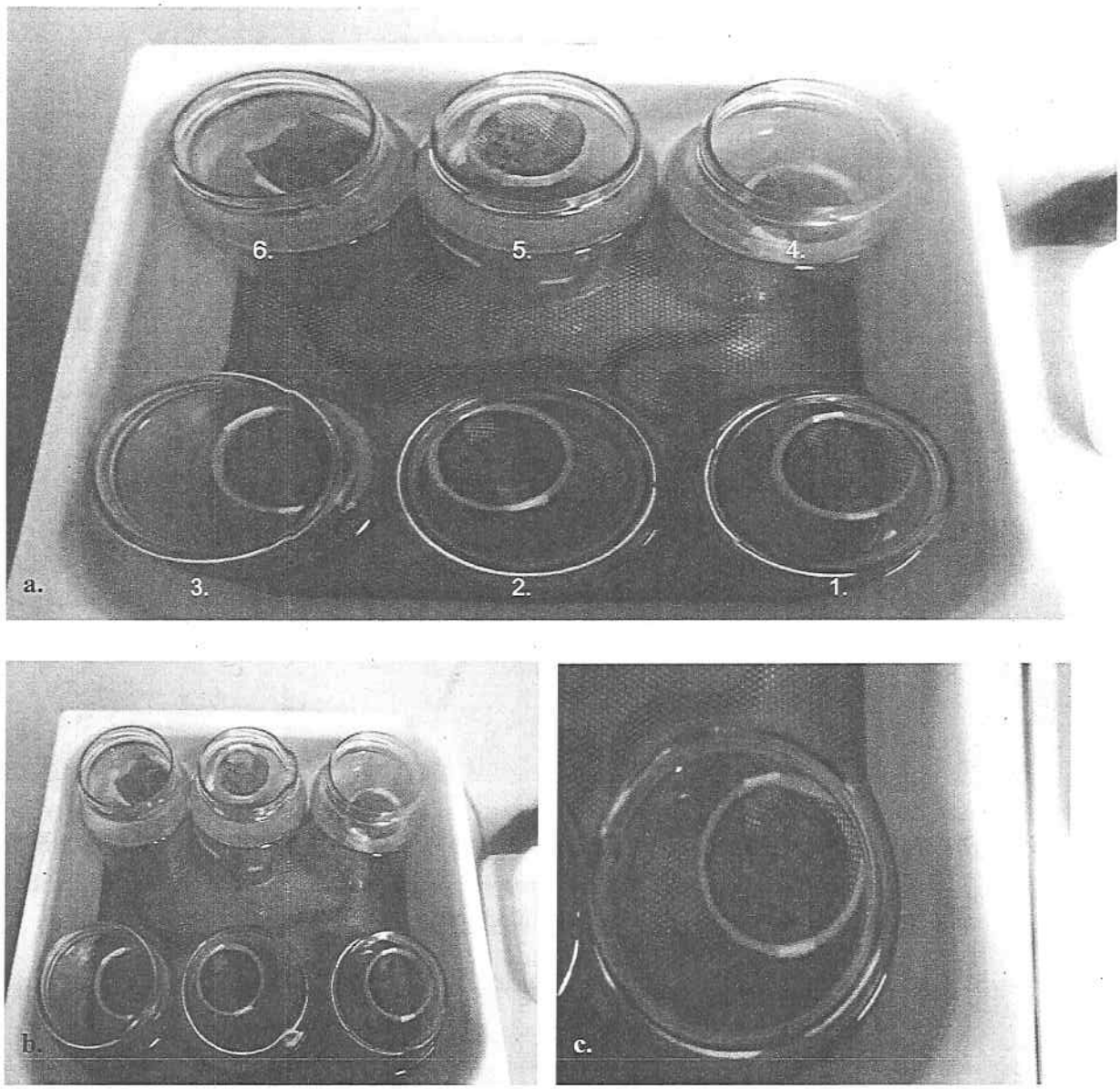


Fig 14. (a) and (b) Colony-to-colony propagation using growth scraped off from the plates obtained from the 'Pit' at Ouyen. The six experimental jars on which the floats with growth are immersed contain a mixture of 'Pit' water and 'aged' water from the fish aquarium. The water bath is maintained at 30°C. (c) A single float comprising of the plastic rim and the plastic netting taped to the rim which keeps the growth in perpetual contact with the surrounding water thereby curtailing the risk of desiccation of the colonies.

NB: From the next sampling trip onwards all colonies collected from the four prime sampling sites viz. Ouyen, Kiamal, Piangil and Nyah will be kept afloat in a esky using plastic strainers attached with plastic netting rather than in closed sampling containers.

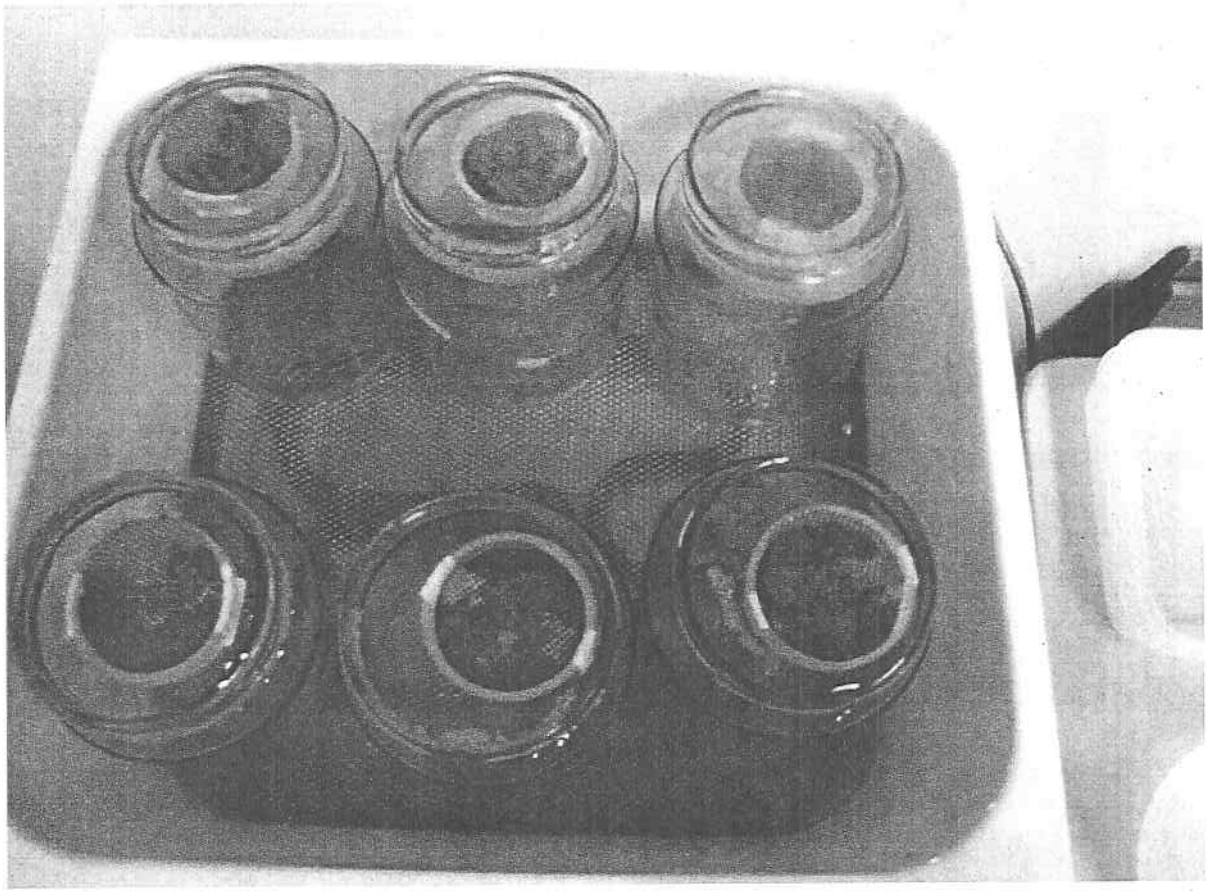


Fig 15. Status of the growth in the six experimental jars as on the 4th of August 2010, fives days after the commencement of the colony-to-colony propagation using 'Pit' material. No significant changes in the growth of the colonies was observed

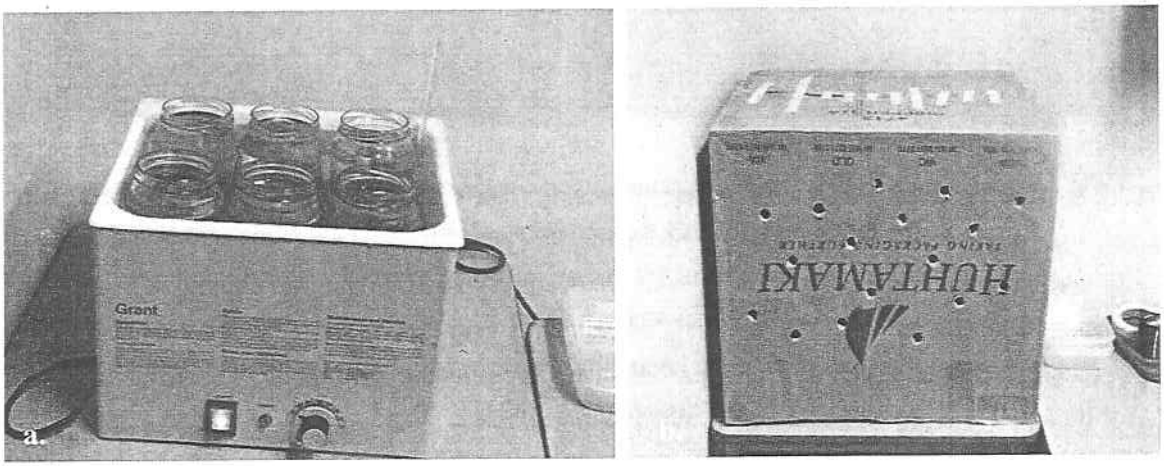


Fig 16. (a) The experimental set-up in a water bath (b) The set-up is kept covered by a cardboard box at all times to mimic the darkness within the pipeline system.

2.4. The chitinous valves of the statoblasts and the benzoylphenyl urea Diflubenzuron

Statoblasts are asexually produced internal buds that are unique to the phylactolaemates, they usually develop in the funiculus and detach from it when completed (Mukai 1982). The formation of statoblast occurs in stages commencing with the appearance of the yolk granules, followed by the formation of the chitinous layer and finally the development of the annulus (Mukai 1973). The valves of the statoblasts are made of sclerotized chitin, Fig 17 within which the yolky mass and the regeneration cells (Francis 2001) containing the genetic material for the next generation are enclosed. The statoblasts retain the capacity to germinate and produce a new colony when the conditions become favourable and thus function as reproductive, survival and dispersal agents helping the organism to survive freezing, desiccation and other detrimental conditions (Brown 1933; Bushnell 1974; Bushnell and Rao 1974, Francis 2001). When treated with chlorine or other control agents or when the conditions are unfavourable, the dying freshwater Bryozoan colonies release thousands of floatoblasts that can travel great distances and establish secondary colonies (Smith and Batson 2000). The statoblasts are known to survive acid exposure, alkaline exposure, complete desiccation, freezing, transport shock, animal digestion, X-rays and exposure to most noxious chemicals (Wood 1983; Hutchinson, 1993; Smith and Batson 2000; Wood and Marsh 1999) and hence according to Smith and Batson (2000) there is no known method of bringing about the complete extermination of the statoblasts. The statement was made ten years ago and till date no one has come up with any known method for the complete destruction of statoblasts but after the discussion at the Ballarat meeting it was evident that the remedy could lie in inhibiting the chtin formation in the statoblasts.

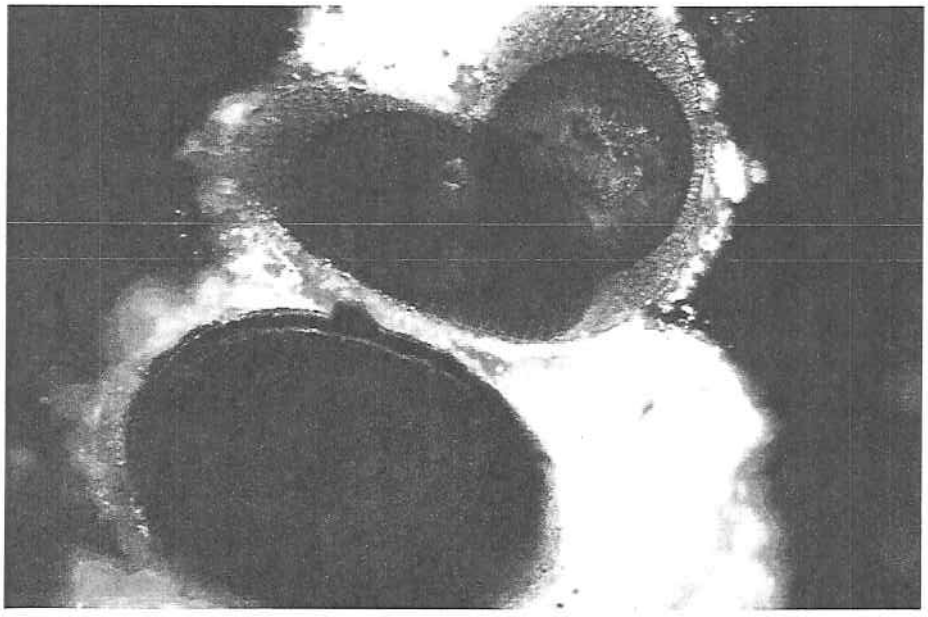


Fig 17. Sclerotized chitin valves of a sessoblast and floatoblasts belonging to the genus *Plumatella*.

Chitin is a polymer of *N*-acetylglucosamine (GlcNAc) and is known to contribute towards the formation of exoskeleton in many invertebrates (Gangishetti *et al.,* 2009). There is a class of potential insecticides known as the benzoylphenyl urea which includes the diflubenzuron and lufenuron (Cohen and Casida 1982; Nakagawa and Matsumura 1993; Nakagawa *et al.,* 1993) and these two retain the potential to reduce chitin in arthropods especially in insects belonging to the orders Lepidoptera and Orthoptera (Gangishetti *et al.,* 2009; Mauchamp and Perrineau 1987). The benzoylphenyl ureas are known to disrupt the regulatory mechanism associated with the polymerization step in chitin formation (Cohen and Casida 1984? citation missing from the book so I have included Cohen and Casida 1983 instead). Although Diflubenzuron is known to affect insect chitin synthesis but benzoylphenyl ureas as a whole are specific in their action and does not prevent the process of budding in yeast or fungal growth (Cohen and Casida 1980; Van Eck 1979) and hence will it affect the formation of chitin in statoblasts is still unknown. In this context, the benzoylphenyl urea diflubenzuron may be worth looking at during the trialling experiments Refer to **appendix three** for the information on diflubenzuron that was kindly provided by Heather Bishop, Assistant Director, National Health and Medical Research Council in response to our request.

3. Conclusions and recommendations

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

   A draft entitled 'Brief Technical Note on the preparation of freshwater Bryozoan statoblasts for Scanning Electron Microscopy (SEM) analyses has been duly disseminated to John Orbell, Andrew Barton and Steven Briggs for correction, the corrected draft will be handed to Simon Crawford from Melbourne University for the final comments. The following paper entitled "First findings of *Plumatella emarginata*, *Plumatella reticulata*, *Plumatella minuta*, *Fredericella australiensis* and *Fredericella sultana* from the Northern Mallee Pipeline system in Victoria' is in preparation.

2. 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.

   The field trip in June was not carried out. Three of the four plates from the field laboratory at Ouyen were duly handed to us by Steven Briggs and Paul Atherton along with four containers of 'Pit' water (approximately 100 litres) after the Ballarat meeting on the 13th of July 2010. Since then the plates have been installed in the growth tanks stationed at St Albans. Not much growth was observed on the top two plates compared to the bottom-most plate on which a considerable amount of growth was displayed.

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

The colony-to-colony experiment which commenced on the 30th of July 2010 is currently ongoing and is being periodically monitored for colony proliferation. The last observation was made on the 4th of August i.e. five days after the commencement of the experiment, during which no significant changes in the growth were observed. Static testing using chlorine and silver nanoparticles will only commence once the colonies begin to proliferate within the floats.

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

A draft entitled 'Brief Technical Note on the preparation of freshwater Bryozoan statoblasts for Scanning Electron Microscopy (SEM) analyses has been duly disseminated to John Orbell, Andrew Barton and Steven Briggs for correction. However it has been observed from the last sampling trips that Bryozoan colonies do not survive long within the sampling containers and hence all the four sampling sites viz. Ouyen, Kiamal, Piangil and Nyah has to be covered in a single day. From now onwards it is deemed that the colonies are kept afloat in a esky using plastic strainers attached with plastic netting rather than in sampling containers. The strainers containing the colonies can easily and swiftly be transferred directly into growth tanks at St Albans from the esky.

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

This is yet to be carried out.

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

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APPENDICES

APPENDIX ONE

CONTROL EXPERIMENTS

CONTENTS

INTRODUCTION

Fit The control experiments encompass two approaches. The first approach is based on the sampling of colonies carried out at the four sampling sites along the Northern Mallee pipeline (NMP) system viz. Ouyen, Kiamal, Piangil and Nyah. This approach is further subdivided into two strategies (a) percentage statoblast germination when exposed to various control agents such as chlorine (the concentration of which is established through sodium hypochlorite), chlorine dioxide, hydrogen peroxide, ozone and chloramines (b) Control experiments on Bryozoan colonies collected from the four prime sampling sites and exposed to chlorine dioxide, hydrogen peroxide, ozone and chloramines.

A. PERCENTAGE STATOBLAST GERMINATION : During the course of this project, it was observed and also evident from the studies carried out by Mukai (1977) and Wood and Marsh (1999), that the statoblasts be subjected to a period of cold and dark treatment in order to impose upon them the sense of 'overwintering' to enhance germination at room temperature. From previous experience, a

period of 5 – 6 weeks of cold treatment in the dark room at 5°C have been proposed in the current protocol prior to exposing the statoblast to a range of increasing concentrations of the various control agents.

B. CONTROL EXPERIMENTS ON BRYOZOAN COLONIES: The second strategy involves control experiments conducted on freshwater Bryozoan colonies mainly collected from the four prime sampling sites, and exposing them to various control agents reiterated from the statoblast germination experiments at an appropriate concentration that would prove detrimental to the survival of the colonies and concomitantly inhibit the proliferation of the colonies. The sample colonies are first cleaned off the adhered debris and sustained in the thermostat growth tanks maintained at 25°C in floating translucent plastic lids. A known fresh weight (FW) of these colonies are dished out in small petri dishes and transferred to small lunch boxes containing the appropriate concentration of the control agent. It is expected that the colonies exposed to a lethal concentration of 1.0 Mg/L of sodium hypochlorite (Wood and Marsh 1999; Wood 2005) or 1.0 Mg/L of chlorine dioxide (no previous study pertaining to the use of this control agent exist) would inhibit the proliferation of the colonies thus exhibiting a loss in total FW.

C. CONTROL EXPERIMENTS ASSOCIATED WITH THE PIT The third strategy involves control experiments associated with the pit at Ouyen - this study is partly based on a similar study carried out by Smith (2005). Three bottom plates from the 'Pit' at Ouyen would be brought to the growth tanks at St Albans and immersed in ¾ pit water and ¼ 'aged' water from the aquarium in three separate compartments and the plates would remain submerged until such times that the growth and proliferation of freshwater Bryozoans are stabilized in the laboratory. Once the growth and proliferation are stabilized, of the three compartments, one of them will be treated with 0.05 mg/L *n*Ag – given that the drinking water guideline for silver is 0.05 mg/L (Anon 1 2009) whilst the other compartment will be treated with 5mg/L of sodium hypochlorite. Anon 2 (1986), mentions that slug dosing of 10-20 mg/L of free chlorine (residual chlorine present as dissolved gas (Cl_2), hypochlorous acid (HOCl), and/or hypochlorite ion (OCl^-) in water has been recommended by the E. and W.S. Department to kill established Bryozoan growth (and mussels) at the Riverland Irrigation pipelines in South Australia. Folino-Ronen and Indelicato (2005) used sodium hypochlorite to establish chlorine concentrations ranging from 0.2 to 5.0 mg/L for the control of *Cordylophora caspia.* In December, GWMWater used 15- 20 ppm (14.98 - 19.97 mg/L) of chlorine for the control of Bryozoan growth at the Ouyen pump station (See Milestone 5 Report Dec 2009 pp.13). According to Anon 3 (2009) freshwater Bryozoans statoblasts can survive relatively high chlorine residuals up to 8 mg/ L. The third compartment will be used as a control and will contain pit water and aged water. With the 'Pit experiment' the weighing of the blotted FW cannot be carried out the outcome is purely based on visual estimation and description.

A. CONTROL EXPERIMENTS USING SAMPLES FROM THE FOUR PRIMING SITES: TWO STRATEGIES:-

(1) PERCENTAGE STATOBLAST GERMINATION

Part of the colonies placed in the cold room at 5^{0}C for a period of five to six weeks to produce statoblasts of mixed *Plumatella* species and mixed *Fredericella* species.

After five weeks place 25 – 50 statoblasts of *Fredericella* (both species) and 25 – 50 floatoblasts of *Plumatella* (mixed species) in the following petridishes and calculate % germination.

CONTROL

CHLORINE CONCENTRATIONS ESTABLISHED THROUGH SODIUM HYPOCHLORITE

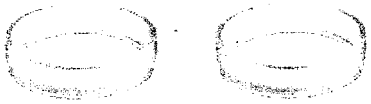
CHLORAMINES

HYDROGEN PEROXIDE

OZONE

CHLORINE DIOXIDE

Source water + Aged water (*Plumatella*)



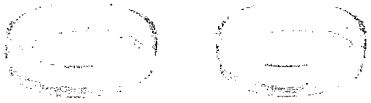
Source water + Aged water + 0.5 Mg/L sodium hypochlorite *Plumatella* and *Frdericella*

Source water + Aged water + 0.5 Mg/L chlorine dioxide *Plumatella* and *Frdericella*

Source water + Aged water (*Fredericella*)

Source water + Aged water + 1.0 Mg/L sodium hypochlorite *Plumatella* and *Fredericella*

Source water + Aged water + 1.0 Mg/L chlorine dioxide *Plumatella* and *Fredericella*



Source water + Aged water + 2.0 Mg/L sodium hypochlorite *Plumatella* and *Fredericella*

Source water + Aged water + 2.0 Mg/L chlorine diioxide *Plumatella* and *Fredericella*

(2) CONTROL EXPERIMENTS ON BRYOZOAN COLONIES

Sampling of Bryozoan colonies from the four sampling points

↓

Colonies rinsed off the adhering debris at St Albans and placed in translucent plastic lids because unlike the petri dishes they do not sink

↓

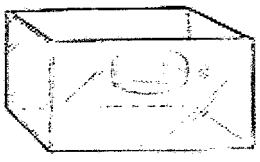
Colonies transported to the growth tanks maintained at 26^{0}C in a moist environment which serves as the repository

↓

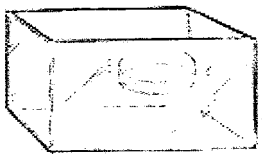
Take a known weight of living colonies in low water to attach to the bottom of the petri dish and float the dishes in a lunch box

Take a known weight of living colonies (Bryozoan) in low water to attach to the bottom of the petri dish and float the dishes in a lunch box for a period of six weeks and calculate change in FW

Source water + Aged water



Source water + Aged water + 1.0 Mg/L sodium hypochlorite



Source water + Aged water + 1.0 Mg/L chlorine dioxide

B. RATIONALE BEHIND THE USE OF CHEMICAL AGENTS

We will initiate the control experiments using (a) chlorine and (b) chlorine dioxide first and the use of ozone, hydrogen peroxide and chloramines are the other alternatives.

(a) CHLORINE

Usually sodium hypochlorite is used to establish chlorine concentrations and the colonies are exposed to an increasing concentrations of sodium hypochlorite for a fixed period of time (in our case six weeks) and such a study has been mentioned by Wood and Marsh (1999) and Wood (2005) where the Plumatellid colonies were killed by exposing them to 1mg/L sodium hypochlorite for 5 hours. The methodology and the results for the above study however remain unpublished.

(b) CHLORINE DIOXIDE

The purpose for the inclusion of chlorine dioxide in the control experiments is that it is deemed more beneficial than chlorine. The chlorine disinfectants are known to react with various substances present in the water through oxidation and electrophillic substitution whilst chlorine dioxide, on the other hand, reacts with water only via oxidation thereby mitigating the production of detrimental by products such as Trihalomethanes (Aieta and Berg 1986). For potable water treatment chlorine dioxide has been recommended both as a disinfectant and an oxidizing agent. Chlorine dioxide is a much stronger disinfectant in comparison to chlorine and chloramine. By far, chlorine dioxide has never been used in the control of freshwater Bryozoans but is a powerful disinfectant because of its capacity to produce free radicals.

(c) HYDROGEN PEROXIDE

Although a powerful oxidizer, in comparison to chlorine and ozone, hydrogen peroxide is not a very powerful disinfectant however it has the capacity to reduce chlorine to chloride thereby mitigating the chances of chlorine to react with the dissolved organic matter. This would reduce the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) (Batterman *et al.,* 2000). In their studies, Batterman et al. (2000), used a secondary disinfectant comprising of silver and hydrogen peroxide (Ag^+/H_2O_2). In the U.S. hydrogen peroxide is a popular means of treating water supplies to prevent the formation of colours, tastes, corrosion caused by both pollution (iron, manganese, sulphates) and microbial degradation. Hydrogen peroxide reacts rapidly and then disintegrates into hydrogen and water without the formation of any harmful by-products. Also, hydrogen peroxide has never been used in the control of freshwater Bryozoans.

(d) OZONE

Ozone is a very powerful disinfectant and has been known to inactivate more resistant pathaogenic microorganisms compared to chlorine dioxide however the required dosage is quite high. The use of high doses of ozone could lead to the formation of Bromate as a consequence of the oxidation of bromide through a combination of ozone and OH radical reactions (Gunten 2003). Bromate, has been deemed as a potential carcinogen (Gunten 2003) and hence ozone has been listed down in our choice of chemical agents as the fourth alternative in the current study. Ozone has never been used in the control of freshwater Bryozoans before.

(e) CHLORAMINES

Chloramines are the products of reaction between chlorine and ammonia and hence are amines with at least one chlorine atom which is directly bonded to the nitrogen atoms. When water or waste water is chlorinated, chlorine reacts readily with various nitrogenous compounds especially ammonia to form inorganic chloramines such as monochloramine, dichloramine or trichloramine (see below) depending on the pH of the water (Donnermair and Blatchley 2003). The chloramines are known for their germicidal effect.

$$HOCl + NH_3 \leftrightarrow NH_2Cl(\text{monochloramine}) + H_2O. \quad (1)$$

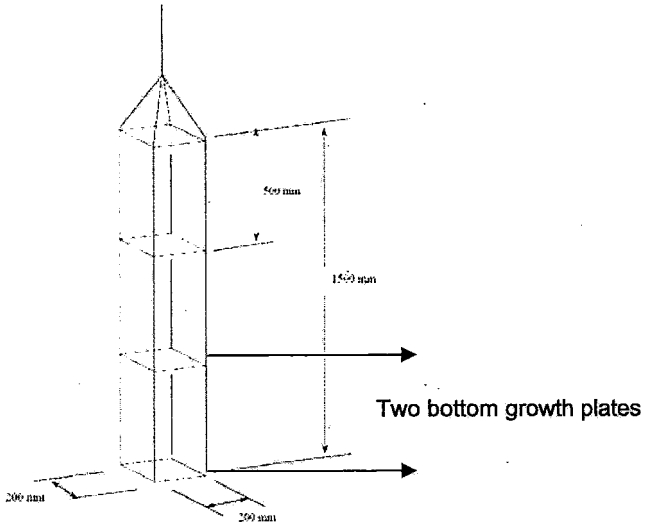
$$NH_2Cl + HOCl \leftrightarrow NHCl_2(\text{dichloramine}) + H_2O. \quad (2)$$

$$NHCl_2 + HOCl \leftrightarrow NCl_3(\text{trichloramine}) + H_2O. \quad (3)$$

From Donnermair and Blatchley (2003)

Monochloramine is the most abundant of all the chloramines and is considered as a very effective disinfectant, because it is known to react directly with the bacterial DNA. Conversely, compared to inorganic chloramines, organic chloramines have poor germicidal effect. In the US chloramines are more frequently used as an alternate means to chlorine for carrying out secondary disinfection of drinking water because chloramines are known to react with organic matter present in the drinking water less often than chlorine resulting in the formation of little to no trihalomethanes and other disinfection by products. Chloramines have never been used in the control of freshwater Bryozoans before.

C. CONTROL EXPERIMENTS ASSOCIATED WITH THE PIT



NANO SILVER TECHNOLOGY

The silver particle colloids exist mostly in the form of colloidal silver and are made up of tiny nanoparticles of metallic silver. In its ionic form, silver is highly reactive with other elements. Inside the human body, chloride is the most prevalent anion and silver ions immediately combine with chloride to form an insoluble compound of silver chloride. Silver chloride being an insoluble salt will not dissolve inside the body once formed and can be eliminated with ease by the kidneys through the urine. The drinking water guideline for silver is 0.05 mg/L.

The silver nanoparticles are endowed efficiently with antibacterial property which attach to the cell membrane of the bacteria and interact with the sulphur containing proteins as well as the phosphorous containing compounds like the DNA. The nanoparticles release silver ions in the bacterial cells enhancing their bactericidal activity (Rai *et al.,* 2009).

The silver colloids comprise of the silver particles and not silver ions and are unaffected by the hydrochloric acid produced by the stomach and can circulate in the blood stream killing pathogens and can be eliminated from the body.

Table 4. The germination rate (%) of statoblasts pretreated with silver, mercuric and cupric ions All the experiments were done with statoblasts from the same stock. (See text for morphogenesis)

Compounds	Concentration (M)							
	1	0.5	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
$AgNO_3$	0	0	0	0	0	0	3	100
$HgCl_2$	–	–	0	0	0	5	73	99
$CuCl_2$	75	82	8	45	63	74	98	98
$Cu(NO_3)_2$	75	85	50	50	61	68	98	100
$CuSO_4$	67	56	51	54	54	82	99	99

Mukai (1977) found the following ions Ag^+, Hg^+ and Cu^{++} very toxic for the germination of statoblasts belonging to the freshwater bryozoan *Pectinatella gelatinosa*.

D. REFERENCES

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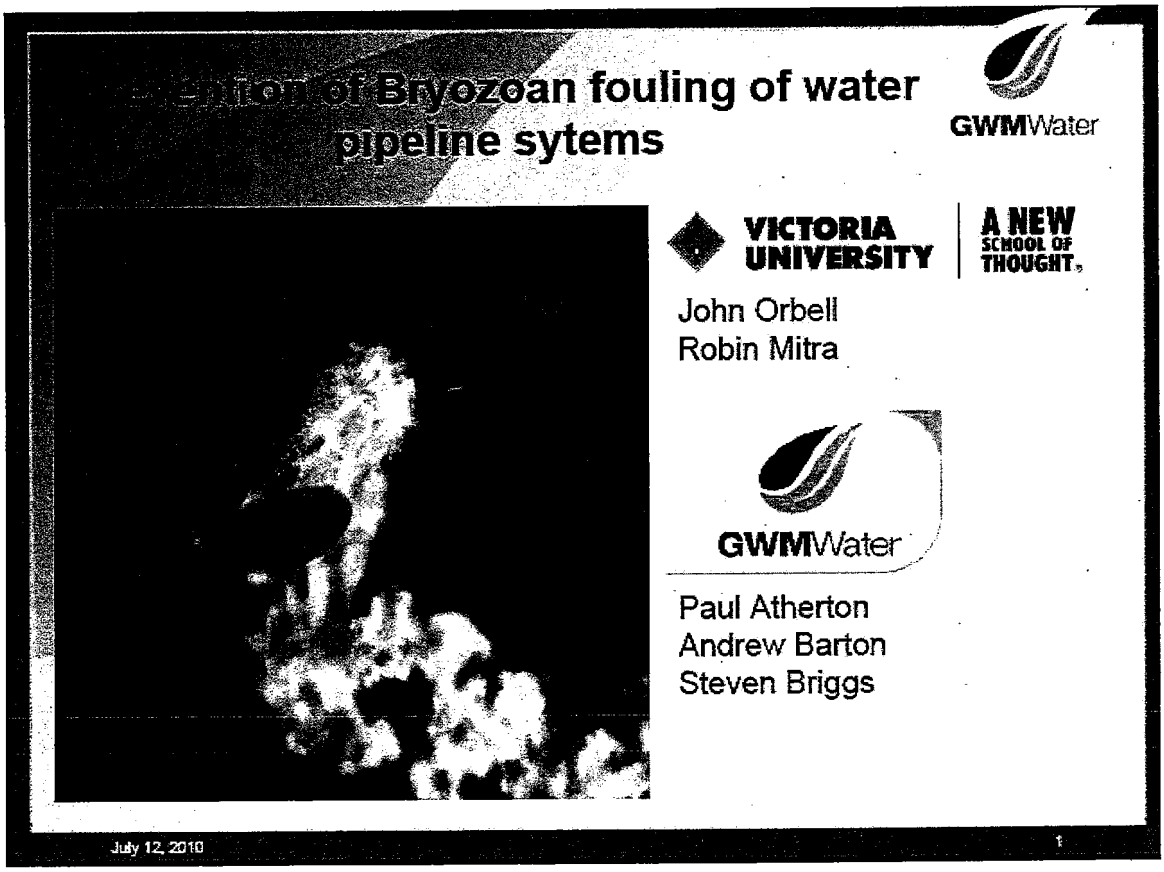
13. Wood, T.S. (2005). The pipeline menace of freshwater Bryozoans. *Denisia* **16** Neu Serie 28 pp 203 – 208.

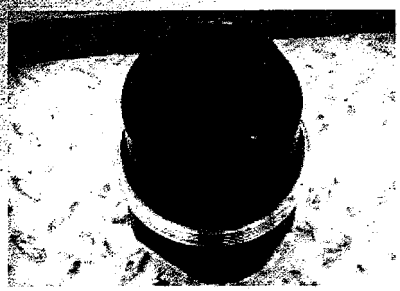
POWER POINT PRESENTATION SLIDES THAT WERE HANDED OUT TO ALL PARTICIPANTS DURING THE MEETING AT BALLARAT



Bryozoan biofouling within the NMP system

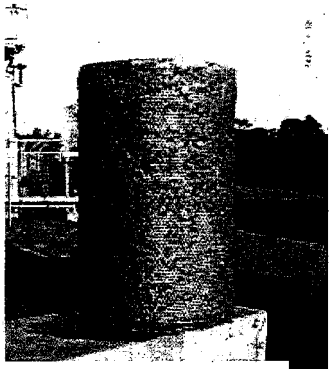
GWMWater

- Accumulation of biofouling material in filters and meters
- Increased pipe friction/flow resistance and therefore increased pumping costs
- Associated odour, taste and discoloration problems
- Ultimately higher operating costs



Second sampling trip March 2009

Fourth sampling trip Dec 2009



Third sampling trip May 2009

Bryozoan Biofouling problem in Victoria

GWMWater

Issue 6 - February 2009

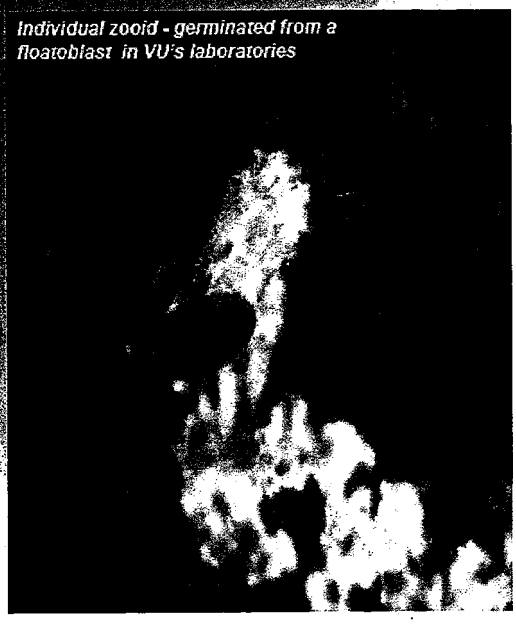
Water Quality Report - Current Water Quality Issues

Blocked meters in Lakes Entrance area:

Some customers in the Lakes Entrance area will have noticed reduced pressure. Naturally occurring, seasonal biological activity in open storage basins can sometimes lead to the growth of an organism in water mains called *Plumatella*. There are no health concerns associated with *Plumatella* but it can block water meters and thus reduce water pressure. A long term strategy to address the *Plumatella* problem is being developed along with a systematic water main cleaning program to clean or replace affected water meters.

Freshwater Bryozoans – what are they?

GWMWater



Individual zooid - germinated from a floatoblast in VU's laboratories

Sessile invertebrates - with ciliated tentacles for capturing suspended food particles.

Zooids give rise to colonies which proliferate into stringy brown masses that block water meters, irrigation nozzles and foul drinking water systems.

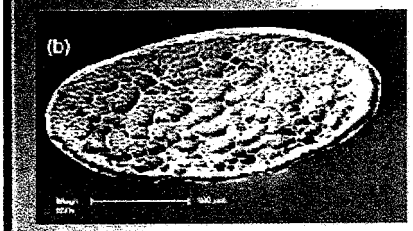
Colonies can appear as carpet-like deposits that resemble moss - hence known as 'moss animals' - also known as 'tobacco weed'.

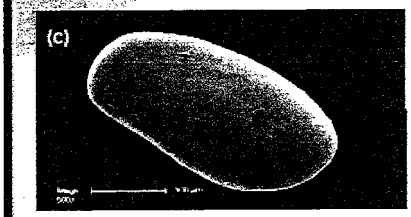
Reproduces both sexually (larvae) and asexually (statoblasts). Asexual reproduction is common.

Very pervasive!

Freshwater Bryozoans – many different species; difficult to eradicate – due mainly to asexual reproduction via statoblasts.

GWMWater





Identification of species via SEM

(VU /GWMWater Project)

(a) Floatoblast - usually bouyant and floating, responsible for dispersal – Genus *Plumatella*

(b) Sessoblast - usually sessile, aids in adhering the organism to the substrate – Genus *Plumatella*

(c) Statoblast non bouyant - usually dispersed through fragments of colonies – Genus *Fredericella*

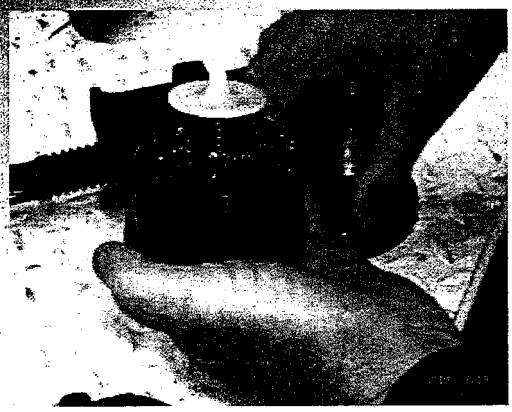
Statoblasts : - help to tide over unfavourable environmental conditions e.g. temperature extremes/harsh chemicals; protect the genetic and germinal material/yolky mass (nutrition) for the propagation of the next generation.

Victoria University/GWMWater Bryozoan Project – Progress to date (from October 2008)

GWMWater

Prime objectives: (a) To establish a research platform, as an Australian Resource, for the characterization and control of Bryozoan infestation in freshwater pipeline systems (b) To assess possible means for the control or eradication in terms of effectiveness, practicality and cost.

Note: Prior to this project, there have been no scientific investigations of Bryozoan biofouling in Australia. To date, the project has -



Established and consolidated field sampling protocols and performed extensive sampling across the NMP system.

Developed a protocol for species identification through Scanning Electron Microscopy (SEM).

Identified 10 species across the NMP system – no species had been previously identified.

Developed facilities and methods to germinate Bryozoans from statoblasts in the laboratory – for the purpose of conducting controlled eradication experiments.

Established a "field laboratory" for the cultivation of Bryozoan colonies – for colony-to-colony propagation in the lab.

Created an extensive literature database and established an international network (through the International Bryozoology Association).

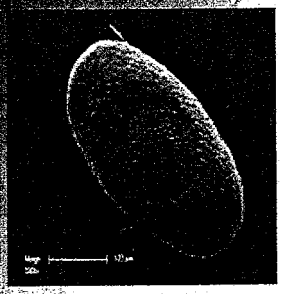
Species identified within the NMP System

GWMWater

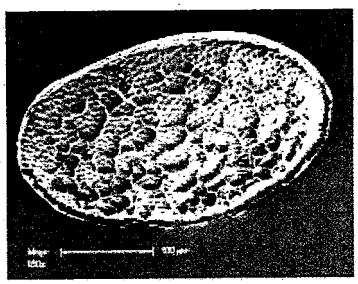
No	Species	Genus	Family	Order	Types of asexual buds produced	Location
1	*Fredericella australiensis*	*Fredericella*	Fredericellidae	Plumatellida	Sessile statoblast	Nyah and Piangil pump stations
2	*Fredericella sultana*	*Fredericella*	Fredericellidae	Plumatellida	Sessile statoblast	Nyah and Piangil pump stations
3	*Internectella bulgarica*	*Fredericella*	Fredericellidae	Plumatellida	Sessile statoblast	Nyah and Piangil pump stations
4	*Plumatella casmana*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast, free leptoblast	Nyah and Piangil pump stations
5	*Plumatella emarginata*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
6	*Plumatella minuta*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast still undetected, free floatoblast	Nyah and Piangil pump stations
7	*Plumatella repens*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
8	*Plumatella reticulata*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
9	*Plumatella vaihiae*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
10	*Plumatella javanica*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations

SEM species identification.....

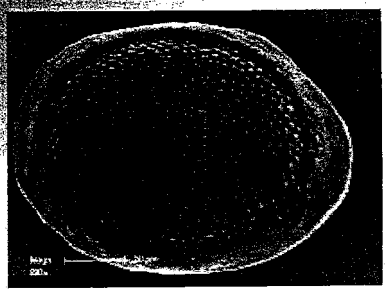
GWMWater



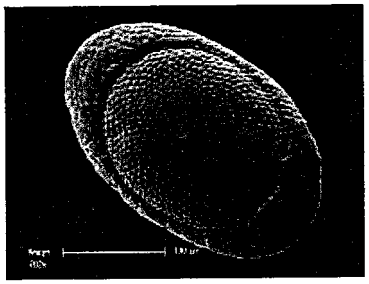
Plumatella emarginata



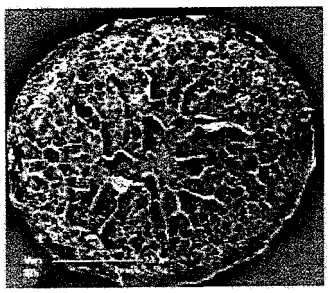
Plumatella reticulata



Plumatella minuta



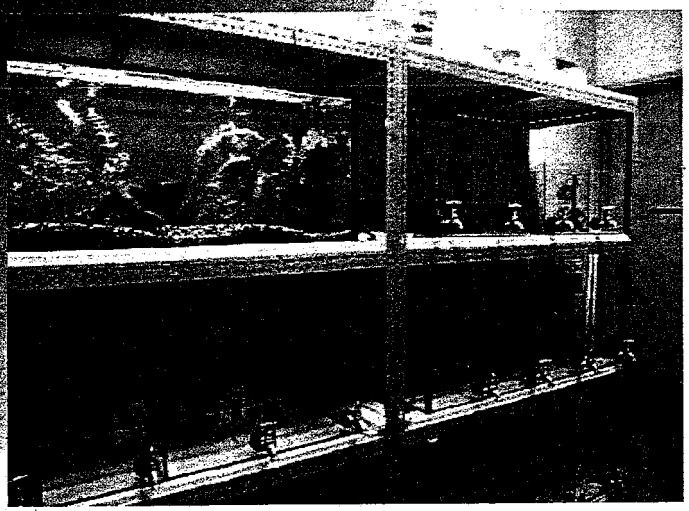
Plumatella javanica



Internectella bulgarica

Lab set-up and cultivation.....

GWMWater



Aquarium set up at the St Albans campus

Individual zooid germinating in a petri dish

Current control experiments, under development.....

GWMWater

CONTROL | CHLORINE | CHLORINE DIOXIDE | FUTURE CONTROL AGENTS

STATOBLASTS GERMINATION

HYDROGEN PEROXIDE
OZONE
CHLORAMINES
SILVER / COPPER

Source water + Aged water (*Plumatella*)

Source water + Aged water (*Fredericella*)

Source water + Aged water (*Fredericella* and *Plumatella*) 0.5, 1.0, 2.0 Mg/L Chlorine

Source water + Aged water (*Fredericella* and *Plumatella*) 0.5, 1.0, 2.0 Mg/L Chlorine dioxide

COLONIES LOSS IN FRESHWEIGHT (FW)

Petri dishes incubated in lunch boxes for a period of 6 weeks

Source water + Aged water

Source water + Aged water + 1.0 Mg/L chlorine

Source water + Aged water + 1.0 Mg/L chlorine dioxide

July 12, 2010 10

Field laboratory - the "Ouyen Pit".....

GWMWater

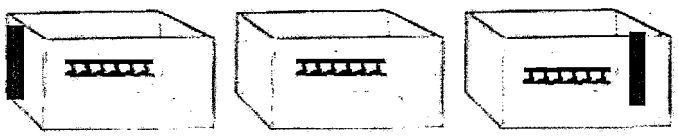
Growth plates with growing colonies are taken from the "Ouyen Pit".

The plates are transported to the VU lab and immersed in growth tanks containing ¾ pit water and ¼ 'aged' water for stabilization of colony growth.

Once stabilized the growth is challenged with 5mg/L chlorine and 0.05 mg/L *n*Ag (accepted level according to drinking water guidelines).

Heater set at 25°C

Heater set at 25°C



Pitwater + Aged water

Pitwater + Aged water + 5 mg/L Cl_2

Pitwater + Aged water + 0.05 mg/L nAg

APPENDIX II

VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

GWMWater

Biofouling Research

Victoria University
John Orbell
Robin Mitra

GWMWater
Paul Atherton
Steve Briggs
Andrew Barton

VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

GWMWater

Introduction

- GWMWater has a very active research program.
  - A dedicated R&D manager
  - Staff as university adjuncts
  - A number of staff undertaking PhD and Masters programs
- Projects include:
  - Climate change and headworks optimisation (ARC Linkage)
  - Environmental flows
  - Optimisation of pipeline operations (ARC Linkage)
  - Desalination/membrane research, brine disposal
  - Early detection of BGA
  - Point of Entry/Point of Use (Coliban a research partner)
  - Pipeline biofouling (subject for today)

VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

GWMWater

Biofouling - Introduction

- Conduits are susceptible to a deterioration in performance due to biological growths.
  - Increased wall friction
  - Increased headloss and reduced flow
- Biofouling can be attributed to any combination of bacteria, algae, fungi, and invertebrate organisms.
- The character of biofouling can vary widely.
  - Filamentous
  - Gelatinous

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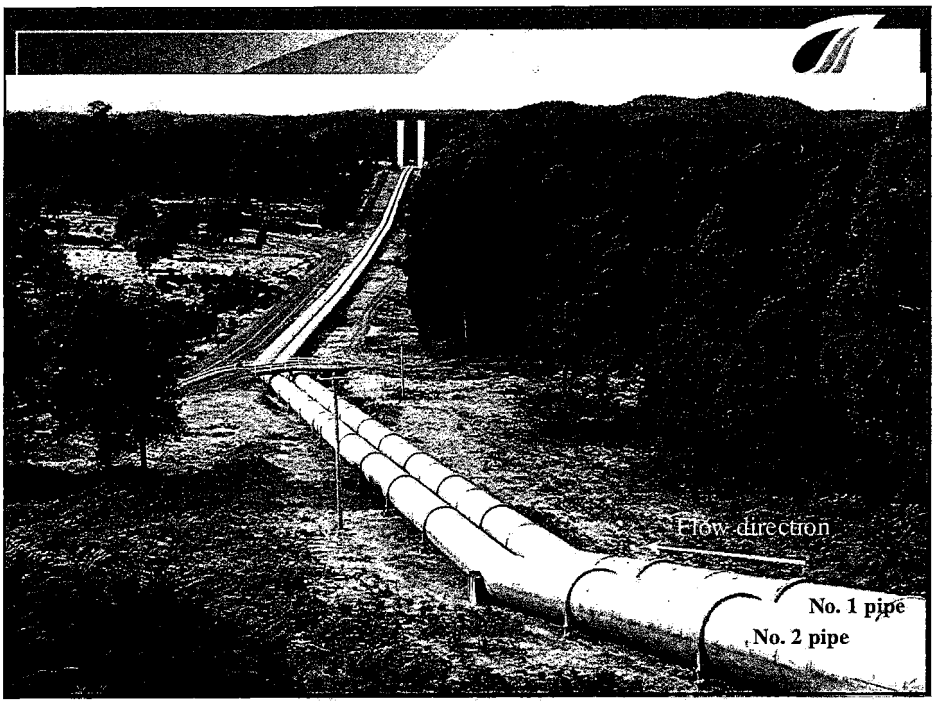
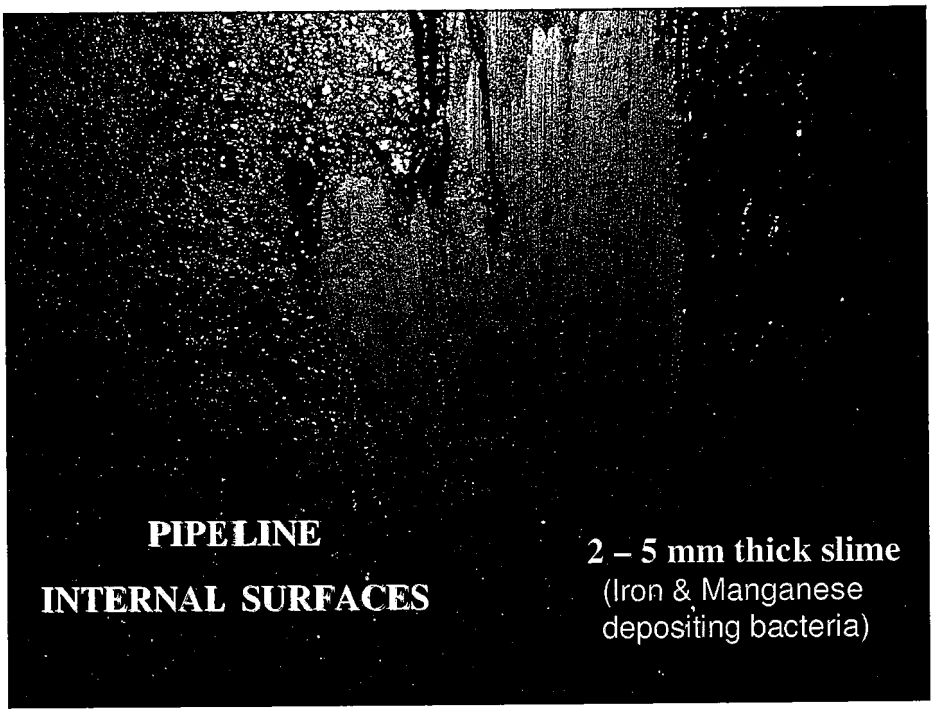
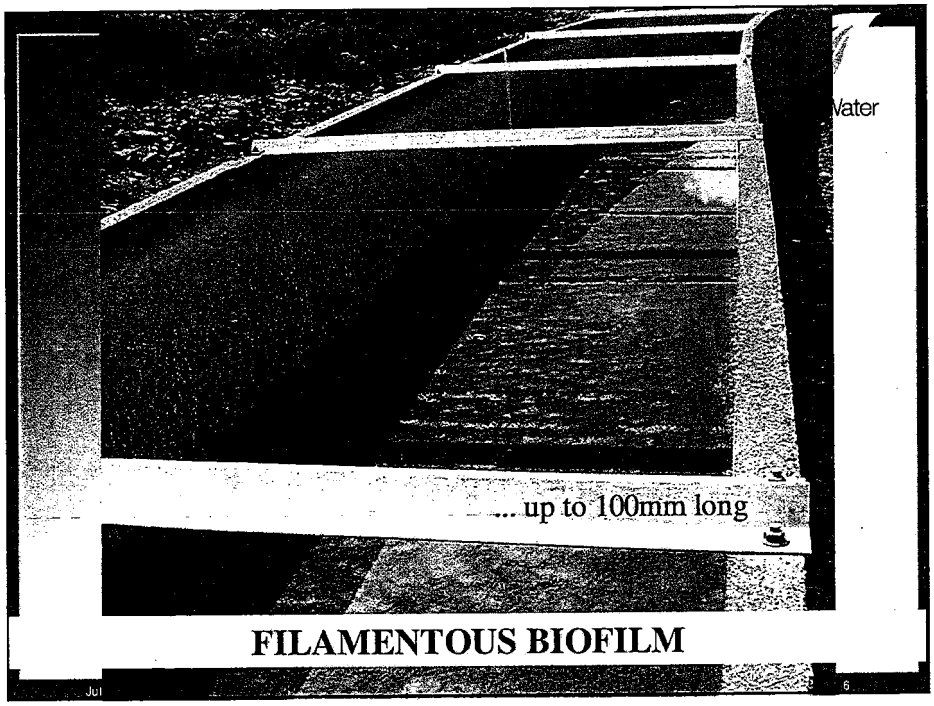
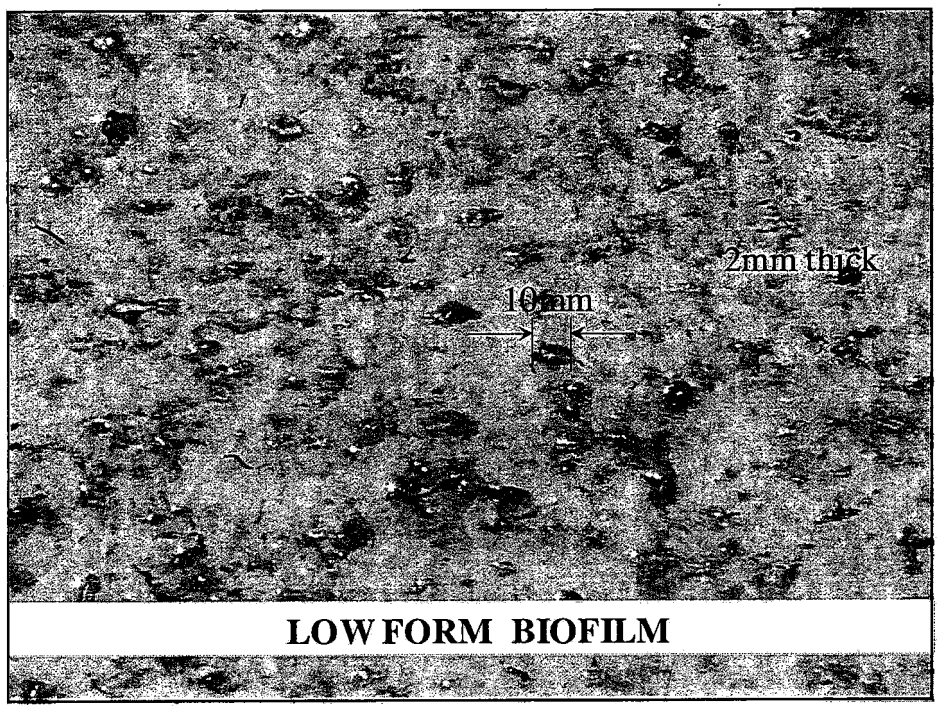



PIPELINE
INTERNAL SURFACES
2 – 5 mm thick slime
(Iron & Manganese
depositing bacteria)


Water
... up to 100mm long
FILAMENTOUS BIOFILM



LOW FORM BIOFILM

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GWMWater

Introduction

- GWMWater has put much effort into the piping of its open channel system.
  - Northern Mallee Pipeline (used in present study)
  - Wimmera Mallee Pipeline (completed in May this year)
- These pumped and pressurised systems provide a continuous supply of water to farms and townships.
- Much interest in reaping the full benefits (> $700 million spent).
  - More secure water supply
  - More efficient water use
  - Better quality water
  - A better service to the customer

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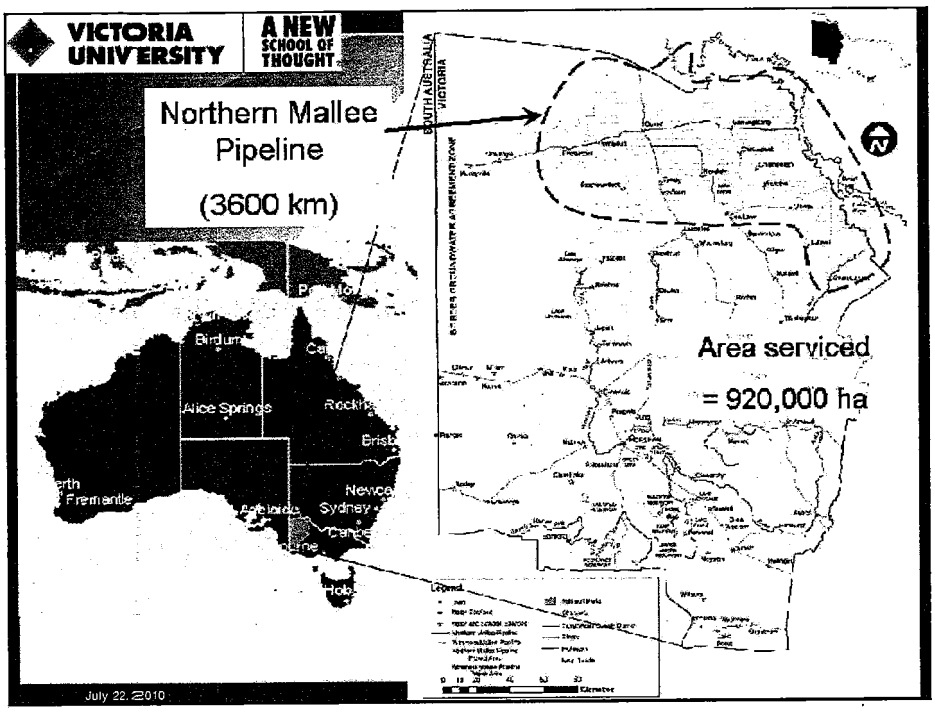
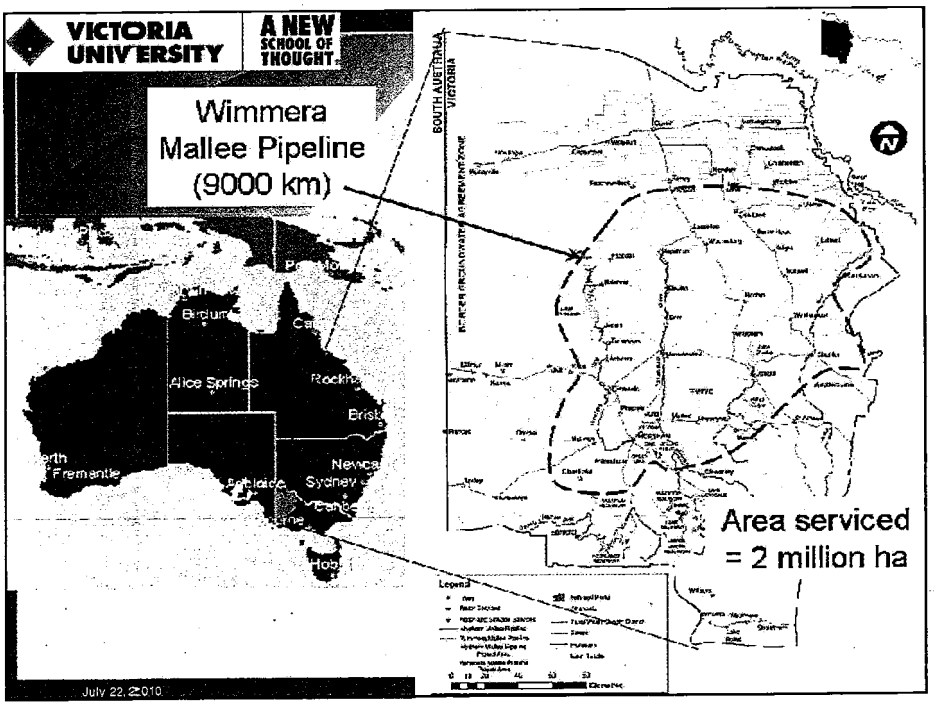

VICTORIA UNIVERSITY
A NEW SCHOOL OF THOUGHT
Northern Mallee Pipeline
(3600 km)
Area serviced
= 920,000 ha
July 22, 2010


VICTORIA UNIVERSITY
A NEW SCHOOL OF THOUGHT
Wimmera Mallee Pipeline
(9000 km)
Area serviced
= 2 million ha
July 22, 2010

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GWMWater

Biofouling

- Biofouling can impact on the distribution of water within these systems by:

1. The accumulation of biofouling material at filters and meters.
2. Increased pipe friction / flow resistance.
3. Odour, taste and discoloration problems.
4. Increased pumping and higher operating costs.

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GWMWater

Diversion from Murray River

Wemen Pump Station

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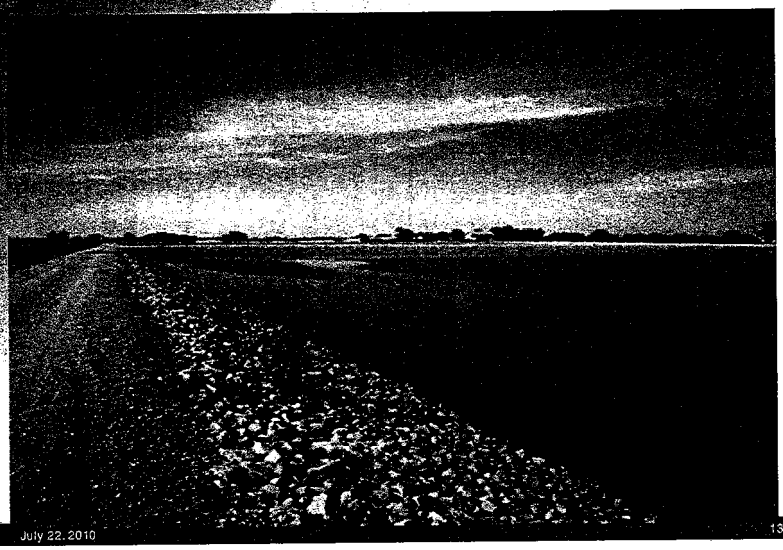
Ouyen Storages

GWMWater



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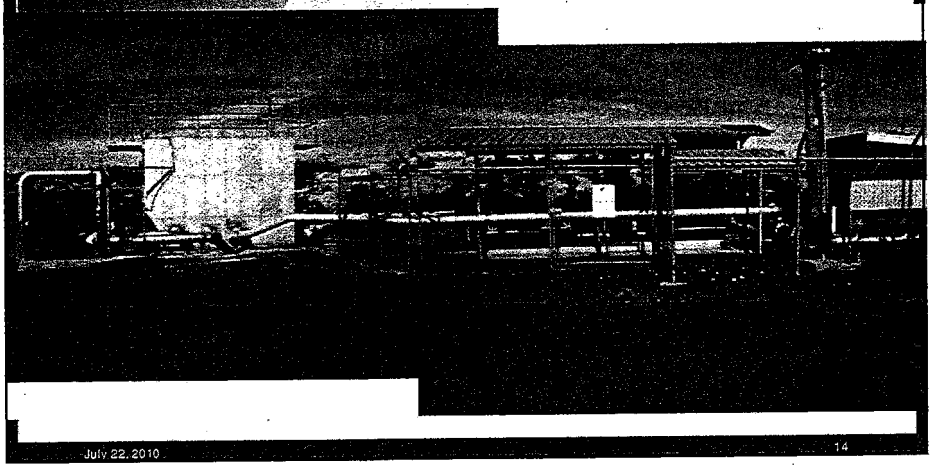
Ouyen Pumps

GWMWater

- Ouyen treatment plant
- NMP proper



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Booster Pump Station

Walpeup

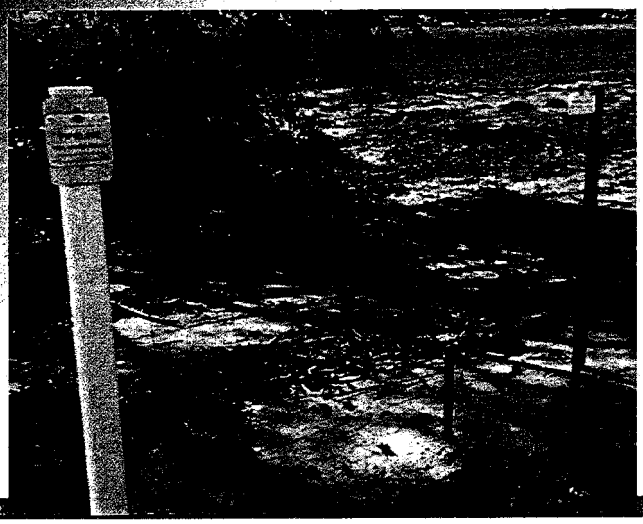
GWMWater

VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

Rural Supply

Typical metered user

GWMWater



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GWMWater

Introduction to Bryozoans

- Are actually animals.
- Mostly found in marine environments.
- Well documented, but dispersed evidence, of pipeline infestations.
- Many species found in NMP – including *Plumatella*
- A very resilient organism, difficult to eradicate.

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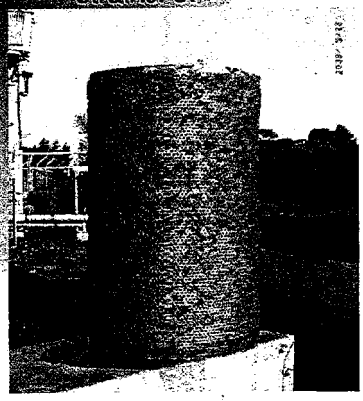
VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

GWMWater

Introduction to Bryozoans

- Tend to be most obvious where they accumulate on filters, strainers



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VICTORIA UNIVERSITY | A NEW SCHOOL OF THOUGHT

GWMWater

Bryozoans

No	Species	Genus	Family	Order	Types of asexual buds produced	Location
1	*Fredericella australiensis*	*Fredericella*	Fredericellidae	Plumatellida	Sessile statoblast	Nyah and Piangil pump stations
2	*Fredericella sultana*	*Fredericella*	Fredericellidae	Plumatellida	Sessile statoblast	Nyah and Piangil pump stations
3	*Internectella bulganca*	*Fredericella*	Fredericellidae	Plumatellida	Sessile statoblast	Nyah and Piangil pump stations
4	*Plumatella casmiana*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast, free leptoblast	Nyah and Piangil pump stations
5	*Plumatella emarginata*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
6	*Plumatella minuta*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast still undetected, free floatoblast	Nyah and Piangil pump stations
7	*Plumatella repens*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
8	*Plumatella reticulata*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
9	*Plumatella vaihiae*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations
10	*Plumatella javanica*	*Plumatella*	Plumatellidae	Plumatellida	Sessile sessoblast, free floatoblast	Nyah and Piangil pump stations

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GWMWater

Introduction to Bryozoans

- Resistant to control.
- Resting stage of lifecycle is by way of statoblasts.
- Primary means to evade eradication.
- Statoblasts have three main forms:
  1. Floatoblasts: the 'free type', which are buoyant and floating
  2. Sessoblasts: the 'adhered type' which stick to a submerged substrate
  3. Piptoblasts: unique type in that they are neither buoyant nor sessile

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GWMWater

Project Progress

- Objectives of the current research project

1. To establish a research platform, as an Australian Resource, for the characterization and control of Bryozoan infestation in freshwater pipeline systems – ***done***

2. To assess possible means for the control or eradication in terms of effectiveness, practicality and cost – ***underway***

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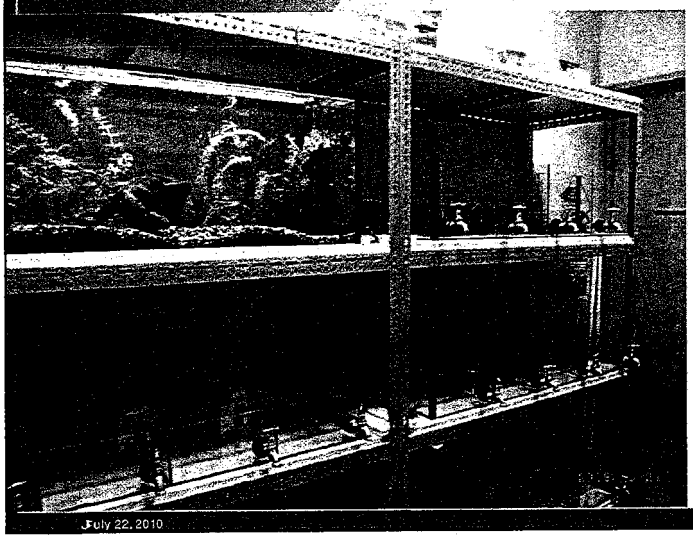
GWMWater

Research Platform



- Laboratory
- Temperature controlled.
- Nutrient controlled.
- Self sustaining population for experimentation.

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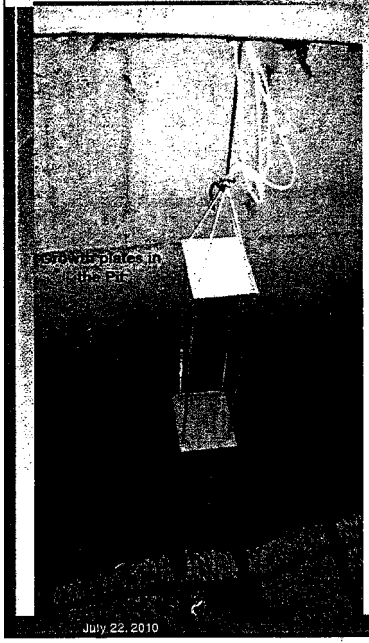
GWMWater



Research Platform

- Field laboratory
- Ouyen field site used to grow Bryozoans on plates.
- Idea is to produce "conditioned" and "ready to use" samples for immediate experimentation.

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GWMWater

Project Progress

- Prior to this project, there have been no scientific investigations of Bryozoan biofouling in Australia.
- To date, the project has:
  - Created an extensive literature database
  - Established laboratory facilities to germinate and sustain Bryozoan populations
  - Established a field laboratory to complement the formal Vic Uni laboratory
  - Performed extensive sampling across the NMP system
    - Have established field sampling protocols.

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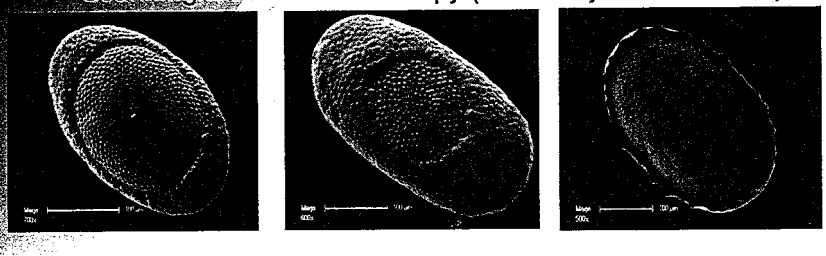
GWMWater

Project Progress

- Developed method for species identification
  - Scanning Electron Microscopy (University of Melbourne)



- Now embarking on a comprehensive treatment and control phase.
  - Silver nanoparticles
  - Superchlorination
  - Other....?

July 22, 2010 25

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GWMWater

Project Status – Way Forward

- GWMWater is seeking other industry partners.
- Project will benefit from other case studies – field sites.
- Chance to be involved with an established project.
- Project phase is now to conduct treatment and control trials.
  - We invite you to participate in this.
- Longer term, project will focus on leveraging funding through ARC and Smart Water funding (WQRA may facilitate).

July 22, 2010 26

APPENDIX III

WHO/HSE/AMR/08.03/6
English only

Diflubenzuron in Drinking-water:
Use for Vector Control in Drinking-water Sources and Containers

Background document for development of
WHO *Guidelines for Drinking-water Quality*

Diflubenzuron in Drinking-water: Use for Vector Control in Drinking-water Sources and Containers
Background document for development of WHO Guidelines for Drinking-water Quality

Printed by the WHO Document Production Services, Geneva, Switzerland

Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published on selected chemicals in 1998 and on microbial aspects in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2005, and the second addendum to the third edition was published in 2008.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America (USA) prepared the documents for the third edition and addenda.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health

Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meeting on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

Acknowledgements

The first draft of Diflubenzuron in Drinking-water: Use for Vector Control in Drinking-water Sources and Containers, Background document for development of WHO *Guidelines for Drinking-water Quality*, was prepared by Mr J.K. Fawell, United Kingdom, to whom special thanks are due.

The work of the following working group coordinators was crucial in the development of this document and others contributing to the second addendum to the third edition:

Dr J. Cotruvo, Joseph Cotruvo & Associates, USA (*Materials and chemicals*)
Mr J.K. Fawell, United Kingdom (*Naturally occurring and industrial contaminants*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Chemicals – practical aspects*)
Professor Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
Dr A.V. Festo Ngowi, Tropical Pesticides Research Institute, United Republic of Tanzania (*Pesticides*)
Dr E. Ohanian, Environmental Protection Agency, USA (*Disinfectants and disinfection by-products*)

The draft text was discussed at the Working Group Meeting for the second addendum to the third edition of the GDWQ, held on 15–19 May 2006. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinators were Dr J. Bartram and Mr B. Gordon, WHO Headquarters. Ms C. Vickers provided a liaison with the Programme on Chemical Safety, WHO Headquarters. Mr R. Bos, Assessing and Managing Environmental Risks to Health, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms Penny Ward provided invaluable administrative support at the Working Group Meeting and throughout the review and publication process. Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

ADI	acceptable daily intake
CAS	Chemical Abstracts Service
FAO	Food and Agriculture Organization of the United Nations
GDWQ	*Guidelines for Drinking-water Quality*
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_{ow}	octanol–water partition coefficient
LD_{50}	median lethal dose
NOAEL	no-observed-adverse-effect level
WHO	World Health Organization

Table of contents

This document is based on IPCS (1996), WHO/FAO (1996), and FAO/WHO (2002).

1. GENERAL DESCRIPTION

1.1 Identity

CAS No.: 35367-38-5

Molecular formula: $C_{14}H_9ClF_2N_2O_2$

The IUPAC name for diflubenzuron is 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea.

1.2 Physicochemical properties

Property	*Value*
Melting point	230–232 °C
Density	1.56
Water solubility (20 °C)	0.2 mg/l
Log octanol–water partition coefficient (log K_{ow})	3.89
Vapour pressure	0.000 12 mPa; virtually non-volatile from water

1.3 Major uses and sources in drinking-water

Diflubenzuron is a halogenated benzoylphenyl urea, an effective stomach and contact insecticide that acts by inhibiting chitin synthesis and so interfering with the formation of the cuticle. It is used in public health applications against mosquito and noxious fly larvae. WHO has assessed diflubenzuron as a mosquito larvicide suitable for application to containers of non-potable water (WHO, 2006a) and is considering it for use as a mosquito larvicide for drinking-water in containers, particularly to control dengue fever. The recommended dosage of diflubenzuron in potable water in containers should not exceed 0.25 mg/l (WHO Pesticides Evaluation Scheme, personal communication, 2006). Specific formulations for control of vectors are specified by WHO (WHO, 2006b).

1.4 Environmental fate

Diflubenzuron is a direct-acting insecticide normally applied directly to plants or water. It is rapidly adsorbed to soil and particles and is immobile in soil. It will also rapidly adsorb to sediments and the sides of vessels and pipes, but it may also partition into the surface film because of its low water solubility and high K_{ow}. In soils, over 90% is degraded by hydrolysis to 2,6-difluorobenzoic acid and 4-chlorophenylurea. In neutral and alkaline waters, diflubenzuron is rapidly hydrolysed. The parent compound and 4-chlorophenylurea may persist on sediment for more than 30 days (IPCS, 1996). Diflubenzuron is fairly unstable in water, with a half-life of approximately 0.5 day for solutions exposed to natural sunlight in the laboratory (Anton et al., 1993).

2. HUMAN EXPOSURE

It is reported that exposure of the public through either food or drinking-water is negligible (IPCS, 1996). However, there is a potential for direct exposure through drinking-water when diflubenzuron is directly applied to drinking-water storage containers.

3. TOXICOLOGICAL SUMMARY

Diflubenzuron is rapidly absorbed to a moderate extent (approximately 30%) from the gastrointestinal tract. Absorbed diflubenzuron is extensively metabolized, and >90% of the metabolites are excreted within 48 h, mostly in the urine, although some biliary excretion and enterohepatic circulation also occur.

Diflubenzuron is considered to be of very low acute toxicity, with oral LD_{50}s in mice and rats of >4500 mg/kg of body weight. The primary target for toxicity is the erythrocytes, although the mechanism of haematotoxicity is uncertain. High doses (10 000 mg/kg of body weight, 25% formulation) caused a small but significant increase in methaemoglobinaemia in mice and rats. The NOAELs for methaemoglobin and sulfhaemoglobin formation in mice, rats and dogs were 1.2, 2.0 and 2.0 mg/kg of body weight per day, respectively, after long-term exposure. Haematotoxicity showed both dose- and time-related trends, with the dose resulting in the detection of methaemoglobin decreasing with increasing duration of exposure. The NOAEL for pathological findings was the same as that for methaemoglobin formation in rats and dogs, but somewhat higher in mice. There were changes in liver spleen and bone marrow associated with haematotoxicity.

Diflubenzuron has been adequately tested for both genotoxicity and carcinogenicity, and there was no evidence that it is either genotoxic or carcinogenic.

Diflubenzuron was not fetotoxic or teratogenic and did not show significant signs of reproductive toxicity. There was evidence that young animals were not significantly more sensitive than adults to the effects of diflubenzuron.

In 2001, JMPR reconfirmed the previously established ADI of 0–0.02 mg/kg of body weight, based on the NOAEL for haematological effects of 2.0 mg/kg of body weight per day in the 2-year studies in rats and the 52-week study in dogs. However, the Committee also considered that an acute reference dose for diflubenzuron was unnecessary (FAO/WHO, 2002).

4. PRACTICAL ASPECTS

4.1 Analytical methods and analytical achievability

The concentration of diflubenzuron may be determined by high-performance liquid chromatography with ultraviolet detector (detection limit 0.05 mg/l) (Miliadis et al., 1999) or with fluorescence detector after on-line post-elution photoirradiation (detection limit 0.05 mg/l) (Martinez-Galera et al., 2001). The concentration of diflubenzuron may also be determined by gas chromatography with electron capture detection (detection limit 0.05 mg/l) (Mensah et al., 1997). It may also be determined

by liquid chromatography using negative-ion, selected-ion monitoring atmospheric pressure chemical ionization–mass spectrometry (detection limit 0.025 mg/l) (Barnes et al., 1995), using electrospray mass spectrometry (detection limit 0.002 µg/l), or using thermospray mass spectrometry (detection limit 0.002 µg/l) (Molina et al., 1995).

4.2 Use for vector control in drinking-water sources

Diflubenzuron is being considered for use as a larvicide for control of disease-carrying mosquitoes that breed in drinking-water containers. The maximum dose recommended by the WHO Pesticides Evaluation Scheme for this purpose is 0.25 mg/l. Users should carefully follow the recommendations for use.

Formulations of pesticides used for vector control in drinking-water should strictly follow the label recommendations and should only be those approved for such a use by national authorities, taking into consideration the ingredients and formulants used in making the final product.

5. CONCLUSIONS

It is not considered appropriate to set a formal guideline value for diflubenzuron used as a vector control agent in drinking-water. The ADI determined by JMPR in 2001 was 0.02 mg/kg of body weight (FAO/WHO, 2002). Young animals do not appear to be significantly more sensitive than adults. Where diflubenzuron is used for vector control in potable water, this will involve considerably less than lifetime exposure. The maximum dosage in drinking-water of 0.25 mg/l would be equivalent to approximately 40% of the ADI allocated to drinking-water for a 60-kg adult drinking 2 litres of water per day. For a 10-kg child drinking 1 litre of water, the exposure would be 0.25 mg, compared with an exposure of 0.2 mg at the ADI. For a 5-kg bottle-fed infant drinking 0.75 litre per day, the exposure would be 0.19 mg, compared with an exposure of 0.1 mg at the ADI. Exposure from food is considered to be negligible. However, the low solubility and the high log K_{ow} of diflubenzuron indicate that it is unlikely to remain in solution at the maximum recommended applied dose, and the actual levels of exposure are likely to be much lower than those calculated.

National authorities should note that this document refers only to the active ingredient and does not consider the additives in different formulations.

6. RECOMMENDATIONS

In setting local guidelines or standards, health authorities should take into consideration the potential for higher rates of water consumption in the area or region under consideration. Consideration should be given to using alternative sources of water for bottle-fed infants for a period after an application of diflubenzuron, where this is practical. However, exceeding the ADI will not necessarily result in adverse effects.

The diseases spread by vectors are significant causes of morbidity and mortality. It is therefore important to achieve an appropriate balance between the intake of the

pesticide from drinking-water and the control of disease-carrying insects. Better than establishing guideline values are the formulation and implementation of a comprehensive management plan for household water storage and peridomestic waste management that does not rely exclusively on larviciding by insecticides, but also includes other environmental management measures and social behavioural changes.

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Diflubenzuron

CAS 35367-38-5

HEALTH-BASED GUIDELINE

Based on human health concerns, diflubenzuron in drinking water should not exceed 0.07 mg/L.

RELATED CHEMICALS

Diflubenzuron belongs to the benzoylurea class of chemicals. Other pesticides in this class include chlorfluazuron (Tomlin 2006).

HUMAN RISK STATEMENT

With good water quality management practices, the exposure of the general population is expected to be well below levels that may cause health concerns.

If present in drinking water as a result of a spillage or through misuse, diflubenzuron would not be a health concern unless the concentration exceeded 0.07 mg/L. Minor excursions above this level would need to occur over a significant period to be of health concern, as the health-based guideline is based on long-term effects.

With good water quality management practices, pesticides should not be detected in source waters used for drinking water supplies. Persistent detection of pesticides may indicate inappropriate use or accidental spillage and investigation is required in line with established procedures in the risk management plan for the particular water source.

GENERAL DESCRIPTION

Uses: Diflubenzuron is an insecticide and a parasiticide for the control of blowfly and lice in cattle and sheep.

There are registered products that contain diflubenzuron in Australia. These products are intended for professional use and are available as topical solution/suspension formulations to be diluted and applied by dipping or hand jetting, or directly by pour-on along the midline of the back of sheep and cattle. Data on currently registered products are available from the Australian Pesticides and Veterinary Medicines Authority.

Exposure sources: The main source of public exposure to diflubenzuron is residues in food. Residue levels in food produced according to good agricultural practice are generally low.

The veterinary use of diflubenzuron provides some potential for contamination of drinking water through the washing of equipment near dams, streams or watercourses.

TYPICAL VALUES IN AUSTRALIAN DRINKING WATER

No published reports on diflubenzuron occurrence in Australian drinking water supplies were found. Exposure to diflubenzuron through drinking-water is expected to be negligible.

TREATMENT OF DRINKING WATER

No specific data on treatment for diflurobenzuron have been found. However, the low aqueous solubility (0.08 mg/L) and relatively high log K_{ow} of 3.7 suggest that it may be amenable to adsorption by activated carbon (WHO 2006). Reverse osmosis is also expected to be effective in removing diflurobenzuron, given its high molecular weight (310 g/mol).

MEASUREMENT

Diflubenzuron can be determined by high-performance liquid chromatography with ultraviolet detector. The method can achieve a limit of quantitation (LOQ) of 0.05 mg/L (Miliadis *et al* 1999). A high-performance liquid chromatography with atmospheric pressure chemical ionisation and mass spectrometry method can achieve a LOQ of 0.025 mg/L (Barnes *et al* 1995). Diflubenzuron in drinking water can also be determined by liquid chromatography with electrospray ionization and mass spectrometry, achieving a LOQ of 0.01 µg/L (Li *et al* 2006). Automated solid-phase extraction and high-performance liquid chromatography with diode-array detection method can achieve a LOQ of 0.1 µg/L for diflubenzuron (Nouri *et al* 1995). On-line pre-concentration method for the analysis of diflubenzuron in ground water samples using two C18 columns, and fluorescence detection after photochemical induced fluorescence post-column derivatization can achieve a LOQ of 0.01 µg/L (García *et al* 2006).

HISTORY OF THE HEALTH VALUES

The current acceptable daily intake (ADI) for diflubenzuron is 0.02 mg per kg of bodyweight (mg/kg bw), based on a no-observed-effect level (NOEL) of 2.0 mg/kg bw/day from long-term dietary studies in rats and dogs. The NOEL is based on haematotoxicity and liver damage. The ADI incorporates a safety factor of 100 and was established in 1985.

An *Australian Drinking Water Guidelines* health value has not been previously established.

HEALTH CONSIDERATIONS

Metabolism: Diflubenzuron is relatively well absorbed from the gastrointestinal tract in rats (up to 50%). The two major metabolic routes are via hydroxylation of the aromatic rings (80%) and via scission of the ureido bridge (20%). Diflubenzuron is rapidly excreted in the urine and faeces, almost completely within 72 hours. There is no accumulation in body tissues.

Acute effects: Diflubenzuron has low acute oral and dermal toxicity. Its skin sensitisation potential has not been tested.

Short-term effects: Medium-term dietary studies were conducted in rats and dogs. In rats, there was an increase in relative adrenal weights and an increase in the incidence of necrotic foci in the liver at 2.5 mg/kg bw/day and above. Haematological changes indicative of anaemia were observed in males at 10 mg/kg bw/day. In dogs, methaemoglobin, sulfaemoglobin and spleen weights were elevated and there was evidence of liver toxicity at 6.6 mg/kg bw/day.

Long-term effects: Long-term dietary studies were conducted in rats and dogs. The two-year rat study reported elevated methaemoglobin levels at 8 mg/kg bw/day. The one-year dog study reported increased methaemoglobin and sulfaemoglobin levels, and increased pigmentation in macrophages and Kupffer cells of the liver at 10 mg/kg bw/day. Liver and spleen weights were significantly elevated at 50 and 250 mg/kg bw/day, respectively. The NOEL was 2 mg/kg bw/day in both the rat and dog studies, and is the basis for the current ADI.

Carcinogenicity: Based on long-term studies in mice and rats, there is no evidence of carcinogenicity for diflubenzuron.

Genotoxicity: Diflubenzuron was positive in some *in vitro* short-term assays, but negative in all *in vivo* studies. Overall, it is not considered to be genotoxic.

Reproductive and developmental effects: One-, two- and three-generation reproduction studies in rats and developmental studies in rats and rabbits did not produce any evidence of effects on reproductive parameters or foetal development. Maternotoxicity occurred only at dose levels well in excess of the likely human exposure level.

Poisons Schedule

Diflubenzuron is included in Schedule 5 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) No.24 (2009). Current versions of the SUSDP should be consulted for further information. These are available from the website of the Therapeutic Goods Administration: http://www.tga.gov.au/ndpsc/susdp.htm.

DERIVATION OF THE HEALTH-BASED GUIDELINE

The health-based guideline of 0.07 mg/L for diflubenzuron was determined as follows:

$$0.07 \text{ mg/L} = \frac{2.0 \text{ mg/kg bodyweight/day} \times 70 \text{ kg} \times 0.1}{2 \text{ L/day} \times 100}$$

where:

- 2.0 mg/kg bw/day is the NOEL based on long-term dietary studies in rats (two-years) and dogs (one-year).
- 70 kg is taken as the average weight of an adult.
- 0.1 is a proportionality factor based on the assumption that 10% of the ADI will arise from the consumption of drinking water.
- 2 L/day is the estimated maximum amount of water consumed by an adult.
- 100 is the safety factor applied to the NOEL derived from animal studies. This safety factor incorporates a factor of 10 for interspecies extrapolation and 10 for intraspecies variation.

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NOTE: Unless otherwise cited, the toxicological information used in developing this fact sheet is from reports and data (listed below) held by the Office of Chemical Safety and Environmental Health (formerly the Office of Chemical Safety).

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APPENDIX IV

APPENDIX FOUR

Potential Partners	Contact
ACTEW	Chris Hepplewhite
Australian Water Quality Centre	Peter Baker
Barwon Region Water Corporation	Will Buchanan
Ben Lomond Water	Rick Kaminski
Central Highlands Water	Warren Jose
City West Water Ltd	Jacqueline Mekken
Coliban Region Water Corporation	Dharma Dharmabalan
Department of Water (WA)	
East Gippsland Water	Dean Boyd
Gippsland Water	
Goulburn Murray Regional Water Corporation	http://www.g-mwater.com.au/projects /researchanddevelopment/rd_eoi_projectfunding
Goulburn Valley Regional Water Corporation	Bruce Hammond
Hunter Water Corporation	Simon Groves
Lower Murray Water Corporation	Kevin Murphy
Melbourne Water Corporation	Melita Stevens
North East Water	Fiona Smith
Power & Water Corporation	David George
South Australian Water Corporation	Paul Dellaverde
South East Water Limited	Hamish Reid
Sydney Water	Peter Cox
United Water International Pty Ltd	
Wannon Region Water Corporation	Ian Bail
Water Corporation of WA	Cameron Gordon
Westernport Water	
Yarra Valley Water Ltd	Astrid Hartono