Fisheries Research Report Series: 7

Proceedings of the
Sydney Rock Oyster Hatchery Health Workshop
held on 8 and 9 August 2002
at Port Stephens, NSW

edited by

Dr Mike Heasman

Fisheries Research Report Series

This series presents scientific and technical information on general fisheries research and the documents in the series are intended to be progress reports on ongoing investigations. Titles in this series may be cited as publications, with the correct citation on the front cover.

Fisheries Research in New South Wales

Research at NSW Fisheries is based at a number of locations, including Cronulla, Port Stephens, Narrandera, Grafton and Coffs Harbour.

Studies cover Commercial fisheries, Recreational fisheries, Conservation issues and Aquaculture.

The major role of research conducted by NSW Fisheries is to provide information upon which relevant Fisheries Management policies and strategies are developed, monitored and assessed in terms of the Department's obligations under the NSW Fisheries Management Act, 1994.

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EXECUTIVE SUMMARY AND RECOMMENDATIONS

Introduction

This workshop, sponsored by NSW Fisheries and the Fisheries Research and Development Corporation, fulfilled a specific recommendation of the 'Benzie Review' *(Review of Hatchery Production Technology and Breeding Program for Sydney Rock Oysters, Benzie et al., FRDC 2001/213).* The workshop addressed recurrent mass larval and juvenile spat mortalities of Sydney rock oysters that to date have not been definitively diagnosed, nor prevented nor cured. Failure to overcome these problems is preventing commercialisation of a 10 year breeding program by NSW Fisheries that has developed fast growing, disease resistant strains for the benefit of the NSW Oyster Farming Industry. It brought together the best available Australian and International expertise to assess current information on these hatchery problems and to formulate appropriate strategies to overcome them.

The first day of the two-day workshop presented a systematic review of information of the diseases and of possible related health issues with other bivalves. The second day drew in additional information and practical experienced commercial hatchery operators and other key industry personnel, including all members NSW Oyster Research and Advisory Committee and representatives NSW Oyster Farming Associations, all of whom made significant contributions from a broad range of perspectives.

Key Findings, Conclusions and Recommendations

Although staff at the NSW Fisheries mollusc hatchery at the Port Stephens Fisheries Centre (PSFC) had successfully reared many species of molluscs, aspects of the hatchery infrastructure and procedures were considered less than optimal. An external review of the PSFC mollusc hatchery to better identify shortcomings was therefore recommended. However, the workshop recognised that new capital funds and the establishment of a small quarantine facility would be minimum outcomes of this process. Operation protocols recommended for immediate assessment included use of freshly collected rather than stored and settled seawater (currently constrained by the siting of the hatchery in an inner estuarine section of Port Stephens; away from good quality oceanic water); reconfiguration of seawater transfer systems and use of high-density flow-through larval rearing systems with continuous algal culture as opposed to static batch production of larvae and algae. Operation protocols recommended for longer term assessment included optimisation of temperature and salinity for growth and survival of larvae and spat.

Research into hatchery production of other edible oyster species has shown the importance of nutritional/biochemical factors in successful rearing of larvae and that large energy reserves are needed for successful larval metamorphosis. Accordingly, the nutritional/biochemical status of Sydney rock oyster eggs, larvae and spat needs to be evaluated.

To date, attempts to identify pathogenic agents responsible for larval or spat mortality have been unsuccessful. Discussions during the workshop suggested that SRO larval and spat mortality problems are consistent with those caused by bacteria in other oyster species. Scientific participants also suggested that apparently minor bacterial infestations previously dismissed as inconsequential might have played a role in mortality events and that some pathogenic bacteria have very specific temperature minima and maxima that may be easily combated by altering rearing temperatures more favourable to larvae but less so to the bacteria or by use of pro-biotic or bio-remedial bacteria.

Although consensus was reached that viral disease was unlikely to be a causative factor, routine sampling, archiving and examination of appropriately fixed larvae and spat was considered worthwhile and should be mandatory. In addition to infective agents, heavy metals and other pollutants could not be dismissed as contributing factors. As oyster larvae are extremely sensitive to heavy metals, such contamination of water and food from pumps, fittings, centrifuges and rearing vessels plastics pose a tangible threat to successful hatchery operations. Similarly, synthetic organic pollutants entering marine environments like pesticides, PCB's, halogenated compounds and petroleum hydrocarbons, and previously unidentified natural bio-toxins could be involved and need to be assessed initially using simple bio assay techniques with embryos and larvae.

It was acknowledged that hatchery problems encountered with Sydney rock oysters in several hatcheries in NSW but not in Albany, Western Australia may be husbandry related but might also be explained by genetic differences that need to be formally evaluated.

Outcomes

The following four complementary strategies for overcoming constraints to commercial hatchery production of SRO spat were developed, endorsed by industry and implemented:

1) Determine if in-house factors are responsible for variable hatchery success. Commission an external SRO seed production trial, using NSW SRO stock, to a reputable interstate/overseas hatchery.
2) Implement simple but potentially significant modifications to existing bivalve hatchery facilities, rearing equipment and operating protocols at PSFC facilities in accordance with recommendations of the Review of Hatchery Production Technology and Breeding program for Sydney Rock Oysters (Benzie et al., FRDC 2001/213) and of the Sydney Rock Oyster Hatchery and Nursery Health Workshop held in August 2002 at Nelson Bay.
3) Conduct a systematic 3-year program of R&D to overcome constraints to commercial hatchery production of SRO's (including rigorous investigation of rearing techniques, nutrition and potentially pathogenic or toxic factors).
4) In the event of success of the above 3 strategies, proceed with the establishment of a commercial bivalve hatchery in NSW in collaboration with a private sector partner. This in turn will enable the transfer of successful production technology and hence benefits of improved SRO genetic stock, already developed by NSW Fisheries, to industry.

The research recommended and discussed in this application pertains to the third of these strategies, the systematic assessment of constraints to production. The remaining strategies are being pursued with funding from NSW Fisheries and with the support of ORAC.

In the event of Strategies 1 and/or 2 being successful, and subject to the successful grant application to FRDC regarding Strategy 3, implementation of Strategy 3 will be reconsidered by ORAC.

AIMS OF THE WORKSHOP

To encourage/involve industry participation in an examination of:

1. To examine causes of mortality in oyster larvae and spat in hatcheries in NSW, other states and overseas.
2. To critically review procedures at the PSFC mollusc hatchery that might cause or contribute to Sydney rock oyster larval and spat mortality.
3. To assess the likelihood that strategic research can identify the problems causing mortality or develop processes to avoid it.
4. Depending on 3 above, to draft the objectives and methods for a three year research project aimed at solving hatchery mortality of Sydney rock oyster larvae and spat.

This workshop brought together a small number of the most experienced international and national experts in oyster hatchery technology. Unless new technology can be developed to overcome intractable problems with Sydney rock oyster larval and spat mortality, there is no future for Sydney rock oyster hatcheries in Australia. The workshop was a crucial first stage to thoroughly review past hatchery practice in the light of international scientific knowledge and to develop a structured, robust research proposal that has the best possible chance of overcoming hatchery mortality.

There are a number of reasons for the decline in this industry. Catastrophic and financially ruinous losses due to QX disease in northern NSW estuaries and the Georges River, south of Sydney, have reduced production in those estuaries by about 97%. Lack of profitability, brought about by increased costs and prices that have not kept pace are forcing many farmers, some of whose families have farmed oysters for generations, to abandon the industry. The increased costs are associated with maintaining a new quality assurance program (that includes compulsory purification), increased labour costs and increased costs for government approvals. On the other side of the ledger, increased competition from faster growing Pacific oysters from Tasmania, South Australia and New Zealand, have kept prices down. Sophisticated economic analyses (modeled as part of a NSW Fisheries/ORAC initiative) demonstrate that profitability will be greatly improved if farmers can grow single seed hatchery produced oysters selected for faster growth and resistance to disease.

In recognition of the long-term potential of genetically improved oysters, the Federal Government, through the Fisheries Research and Development Corporation (FRDC), and the NSW Government, have invested several million dollars over the last ten years to develop genetically improved *S. glomerata*. Selected lines have been shown to have significantly superior growth compared with non-selected control lines. Major advantages have also been demonstrated in relation to hatchery produced triploid Sydney rock oysters over diploid siblings. Advantages include significantly faster growth, allowing oysters to reach market-size up to 6 months earlier than unselected control groups, and enhanced resistance to disease. Recently the advantages conferred by triploidy and genetic selection have been shown to be additive allowing triploid, selected oysters to reach market-size up to 12 months earlier than controls. The selective breeding program has been extended to target resistance to the two most important intracellular parasites diseases, Winter Mortality and QX disease. A significant reduction in mortality following exposure to QX, as well as substantially faster growth, has been demonstrated for disease resistant lines.

Use of hatchery produced rather than wild caught *S. glomerata* spat is therefore becoming increasingly important to the NSW oyster industry and it is critically important that reliable, large-scale hatchery production of the *S. glomerata* single spat is developed. Unfortunately, although production of tens or hundreds of thousands of spat is easily achieved (allowing the mass selective

breeding program to proceed), major difficulties have been experienced over the past decade in routine mass hatchery and nursery rearing to produce the millions of spat required for commercial operation. These problems have been experienced at the NSW Fisheries, PSFC hatchery and also at several commercial hatcheries, all of which have been forced to cease operation because of their failure to overcome the mortality.

Foremost among these problems is an intermittent disease that has often caused mass (60 to 90%) mortalities of small (<2 mm) Sydney Rock oyster spat. Exacerbating this problem are chronically low hatchery yields compounded by intermittent catastrophic mortality of the larvae of *S. glomerata.*

WORKSHOP AGENDA

Day 1

8:00 am	*Bus transfer from Westbury's Marina Resort to Port Stephens Fisheries Centre for tour of hatchery*
8:30 am	**Welcome address** - Nick Rayns, Director of Aquaculture, NSW Fisheries
8:35 am	**Introduction and aims** - Mike Heasman, NSW Fisheries
8.40 am	**Progress report on SRO breeding R&D** – John Nell, NSW Fisheries
8:50 am	**Discussion tour of hatchery**
10:00 am	**Return to Westbury's Marina Resort**

10:30 am	Morning Tea

10:45 am **Brief global overview of hatchery and nursery health issues with bivalves with particular emphasis on oysters** – Ralph Elston)

Review of information on mass mortality syndrome of SRO larvae
- Hatchery methodology and operational constraints – Mike Heasman
- Epidemiology - Mike Heasman

12:30 pm	Lunch

1:00 pm **Review of information on mass mortality syndrome of SRO larvae** - *continued*
- Pathology - Dick Callinan
- Virology – Judith Handlinger and Mike Heasman
- Experimental investigations - Mike Heasman
   1. Challenge tests with vibrio Isolates
   2. Experiment to elucidate involvement of infective agent(s)

Discussion led by Ralph Elston, Dick Callinan, Judith Handlinger and Brian Jones

3:00 pm	Afternoon Tea

3:15 pm **Review of information on mass mortality syndrome of SRO spat**
- Hatchery methodology and operational constraints – Mike Heasman
- Epidemiology - Mike Heasman
- Pathology - Dick Callinan
- Experimental investigations - Mike Heasman

Discussion led by Ralph Elston, Dick Callinan, Judith Handlinger and Brian Jones

4:45 pm **Disease prevention**
- Optimisation of egg and larval quality through, nutrition of broodstock and larvae - Malcolm Brown
- Review of micro algae as a potential source of anti appetite factors and toxins, heavy metals - Ralph Elston

Discussion led by Ralph Elston and Malcolm Brown

6:15 pm	Drinks

Day 2

8:00 am   **Disease prevention** - *continued*
- Review and hygiene, equipment and protocols - Brian Jones
- Probiotics - Cheok Tan (UTS)
- HACCP & risk management of hatchery op' especially access to dedicated facilities and staff, quarantine and hygiene – Simon More

        Discussion of information in context of other related studies and information led by Simon More

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10:00 am   Morning Tea

Workshop Session

Development of a strategy and plan of action for overcoming problems
– involving Industry and ORAC delegates - led by Geoff Allan

10:15 am   **Welcome and Introduction** – Nick Rayns

10:25 am   **Summation of previous scientific sessions**
- Brief global overview of hatchery and nursery health issues with bivalves with particular emphasis on oysters – Ralph Elston
- Summary of info on mass mortality of SRO larvae – Mike Heasman
- Review of info on mass mortality of SRO spat - Mike Heasman
- Disease prevention options and issues (Nutrition, Hygiene, Algae issues Probiotics, HACCP and risk management) - Geoff Allan

11:45 pm   **General question and answer session** led by Geoff Allan

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12:15 pm   Lunch

12:45 pm   **R&D and other strategies** (out-sourcing production) led by Geoff Allan

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2:45 pm   Afternoon Tea

3:00 pm   **Summing up** - Nick Rayns

3.15 pm   **Close**

OYSTER HATCHERY AND NURSERY HEALTH ISSUES - OVERVIEW

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This is a very brief summary of the presentation that will be made. Handouts of the presentation slide will also be available at the workshop or upon request of the author.

Cultivation of oysters began in the 1950's with serious experimentation in Wales, the United States and perhaps other locations. The methods were commercialized on a broad scale starting in the 1970's and various species are now shipped worldwide. In spite of impressive technological advances there remains a tremendous scope for improved efficiency, consistency and quality through further technology development.

The cycle of intensive bivalve farming includes brood stock management and conditioning, larval production and juvenile cultivation.
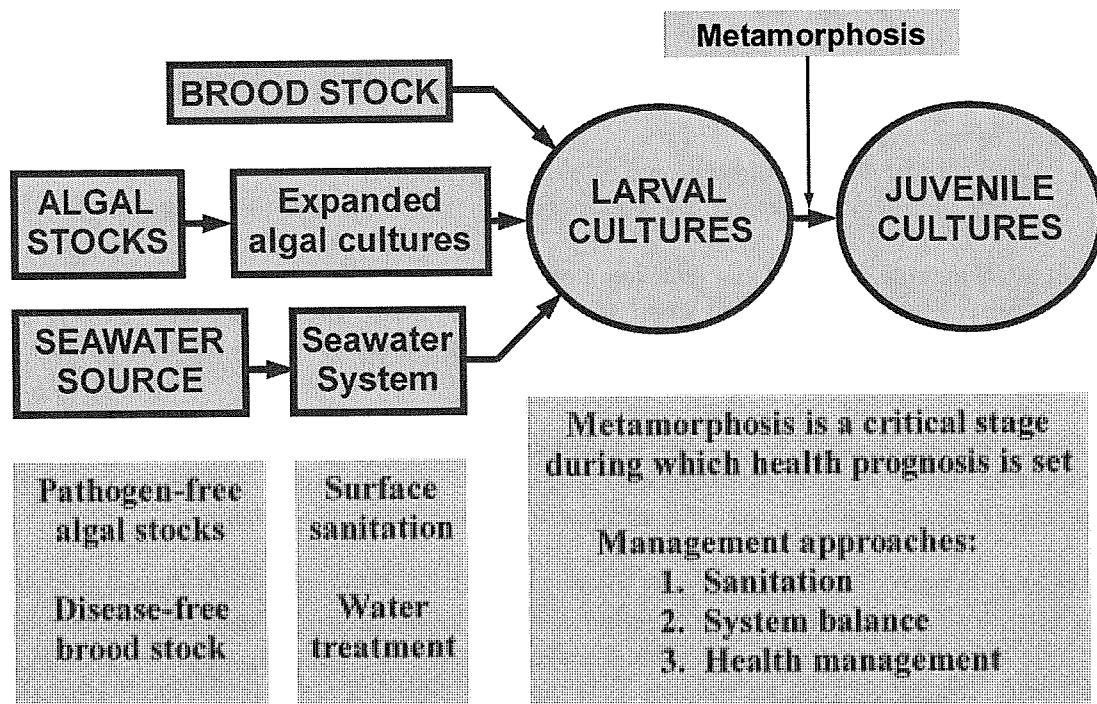
The following areas of hatchery health management need to be considered:
- Bacteriological management and pathogens.
- Other infectious diseases such as viral infections, parasites (e.g. invasive ciliates), fungi.
- Raising mixed stocks in multispecies hatcheries.
- Feed related issues: contamination, under or overfeeding.
- Water source factors (?); episodic toxic blooms.

A variety of species of *Vibrio*, *Pseuduoalteromonas*, *Pseudomonas* and others have been associated with diseases of bivalve larvae and shellfish. Most isolates of these genera are not pathogenic but perhaps 5 to 10% are moderately too highly pathogenic. Bacterial colonization in

the hatchery is inevitable and, in fact, may be considered to be essential. However, it is still an uncontrolled variable or a variable that is controlled in an indirect fashion.

Relationship of some known bacteria-associated lesions, etiological agents, and vibriosis in larval and juvenile oysters.

Morphological Disease	Etiological Agent
Acute pallial infections, larvae	*Vibrio* sp., *V. anguillarum* Possibly other species causative
Acute pallial infections, juveniles[2]	Undetermined
Chronic extrapallial infections, juveniles[3]	Undetermined
Toxin-induced velar damage	*Vibrio* sp., *Vibrio anguillarum*, *V. ordali*, *V. tubiashi*, *V. alginolyticus*
Visceral atrophy	*Vibrio* sp.
Undetermined; "bacillary necrosis" Bacterial swarms associated with velar and other organ damage	*Vibrio anguillarum*, *V. alginolyticus*, *V. tubiashi*, *V. splendidus*, *Pseudomonas* sp.

Other forms of bacterial disease:
- "Instant" larval dropout syndrome – *Pseudoalteromonas*.
- Velar degeneration syndrome – associated with bacterial toxins in some cases.

Bacterial attachment to the shell and invasion along the inner shell surface is the most commonly occurring form of "vibriosis" in both larval and juvenile oysters. The disease may be chronic in juveniles and may form extrapallial abscesses. This condition is often responsible for low recoveries in Pacific oyster (*Crassostrea gigas*) raised in hatcheries.

Pathogenic bacteria are often surface associated and display amplified growth rates over certain temperature ranges. They may establish residence in the culture system and show rapid regrowth after partial disinfection procedures. The temperature response is strain specific.

Hinge ligament disease is another bacterial disease caused by gliding bacteria ("*Cytophaga*-like") common to most if not all species of cultured bivalves. Erosion of the ligament impairs the ability of the shellfish to open its shell. The disease is easily overlooked, even in histological examination. The result of the disease may be complete dissolution of the ligament. N. American isolates are active in digesting ligaments at temperatures above 10°C.

An iridovirus and several herpes viruses have been described from larval oysters. Herpes viruses are also reported to affect juvenile oysters in France. Invasive ciliates are also observed occasionally in cultured oysters and other protozoa are invasive in other species of bivalves. Temperature is also an important factor in the development of viral diseases which may become productive (produce infectious viral particles) only within a specific temperature range.

Metamorphosis is a critical stage in the development of oysters and the nature of the bacterial substrate at metamorphosis may determine the development of chronic bacterial diseases or the success and rapid growth through the early juvenile stages. Clearly, benign or beneficial bacteria

present on metamorphic surfaces will facilitate normal development and retard the proliferation of pathogens on these surfaces.

The primary issue is how to intentionally manage bacteria in the culture system. Surfaces are a key niche that must be occupied by benign or beneficial bacteria. Pathogens may be rapid colonizers but beneficial bacteria may secrete BLIS (bacteriocin like inhibitory substances or natural antibiotics). Some bacterial strains likely have a positive nutritional value for oyster larvae and seed. Additional research is needed to identify benign bacteria or mixes of bacterial that can be stably incorporated into the culture system through co-cultivation with algal cultures or by some other method of introduction. Specific management of bacterial colonization in the hatchery should be regarded as the next phase of optimization and opportunity for increased production efficiency. There is also some evidence that some species of algae produce probiotic substances. Nonetheless, management of bacteria and algal food within the culture system must be approached in a holistic manner so that compatible and beneficial species of bacteria and algae can be maintained in the system.

Summary of discussion and additional comments

Q1   *What hygiene management procedures should be applied to broodstock?*

- Broodstock oysters should be introduced, conditioned, spawned and removed from hatcheries over the minimum practical timeframes. Ideally, they should be held in isolation from other hatchery rearing activities especially larval rearing operations. The practise of maintaining conditioned broodstock in hatcheries over protracted periods as a matter of ready accessibility is therefore strongly discouraged.
- Seawater used to maintain conditioning broodstock should be frequently changed and monitored for the type and quantity of bacteria especially presumptive *Vibrio* spp. Rearing vessels and equipment need to be cleaned daily and regularly disinfected.
- Prior to their introduction to the hatchery and immediately prior to induction of spawning, shells of broodstock should be scrubbed and disinfected.

Q2   *Should broodstock be subjected to depuration prior to being induced to spawn?*

Depuration may be useful but I am unaware of any hatchery that is routinely practising it.

Q3   *Do most hatcheries use closed or open systems to condition broodstock?*

Hatcheries generally use closed systems with regular replacement of seawater.

Q4   *What do you think of farmer complaints that hatchery produced seed are too "soft" and* having been reared under constant near ideal conditions, often succumb to more variable ambient conditions when transferred to field nurseries or farm leases?
There maybe some benefits to be gained by acclimatising hatchery seed to harsher field conditions but I suspect major losses are more as a consequence of some predisposing health issues such as poor nutritional status.

Q5   *Are any bivalve hatcheries using probiotic bacteria as a prophylactic to prevent bacterial related diseases?*

I am not aware of routine use of probiotic bacteria by any commercial hatchery possibly because of costs and difficulties of achieving and maintaining complex mixes of probiotic bacteria at targeted densities within rearing systems. Nevertheless I reiterate my view that achieving a healthy balanced micro-flora within seawater rearing systems would substantially improve average

performances of rock oyster hatcheries including prevention of occasional crashes that afflict most if not all of them.

Q6   *Referring to the critical role played by temperature in hatchery success highlighted in Dr Elston's key address and to an apparent discrepancy between the experimentally determined optimum temperature for rearing SRO larvae (see Fig13 of Appendix 2)– the question was asked "has any attempt been made to vary incubation and larval rearing temperature of SRO?*

Although a wide apparent discrepancy between routinely applied temperature of 24°C and an experimentally determined optimum growth temperature of 29°C was known continued adherence to 24°C as a preferred rearing temperature for SRO at PSRC occurred because of scepticism of some staff that results achieved in small experimental rearing trials may not be valid in relation to commercial scale cultures and to concern that higher rearing temperatures would advantage pathogenic bacteria ahead of SRO larvae.

On a number of occasions Dr Elston reiterated the critical importance of rearing temperature and need to determine and apply optimised rearing temperatures. He also repeatedly cited the spectacular case of a Pacific oyster hatchery that overcame chronically high larval mortality problems by simply raising rearing temperature from 24 to 26°C. This small temperature rise of 2°C was shown to significantly enhance growth and vigour of the larvae while at the same time suppressing the proliferation rate and virulence of a pathogenic strain of Vibrio sp. bacteria.

NB. The importance of maintaining constant temperature within a narrow range of about ±0.5°C for the health of larvae was remarked on by a number of hatchery operators present at the workshop.

Supplementary comments provided by Dr Elston on Day 2

Globally there is great scope for bivalve hatcheries to improve average yields, for reduce generally high variability between batches and for reducing the incidence of batch crashes that afflict almost if not all hatcheries including Pacific oyster hatcheries to some degree.

The reality is that all hatchery operations have to live with bacteria but we need better control over the micro-flora within seawater rearing systems and use of probiotics may be the key to such control.

What is clear from the beneficial effects of hatchery dry-outs and disinfection is that an infective agent, probably a bacterium or bacterial bio-toxins are implicated as a cause of the larval anorexia syndrome. It is also reasonable to conclude that classic invasive bacterial infections of oyster larvae are not applicable. With this in mind it may well be worthwhile to re-examine afflicted larvae imbedded in resins rather than wax to generate higher resolution sections using specialised bacterial stains.

Relatively minor increases in rearing temperature from 25 to 27°C were found to overcome chronic failures by a pacific oyster hatchery in the USA by promoting oyster larval growth and vigour and at the same time substantially suppressing replication rate of a particularly virulent strain of Vibrio bacteria. A wide discrepancy of 4-5°C exists between the rearing temperature of 24 to 25°C routinely applied to SRO larvae at the PSFC and the experimentally determined optimum of 29°C. In view of this, the efficacy of elevated rearing temperature in combating the larval anorexia syndrome, a simple exercise, should be formally evaluated at the earliest opportunity.

HATCHERY PROCEDURES FOR SYDNEY ROCK OYSTERS AT PSFC

In conjunction with Appendix 1
(Hatchery manual, Frankish et al., 1991)

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LARVAL CYCLE

Day -2

- Hatchery has been disinfected and dried (4 weeks) and water storage tanks filled and settled 1 to 3 weeks prior to enable shift in bacterial communities from a potentially pathogenic *Vibrio* dominated to a more benign flora (Douglas - Unpublished Report, 1996).
- If hatchery run occurs between June to September/(December for genetically improved stock) then broodstock will have been conditioned for 4-8 weeks @ 22 to 24°C – (see Section 5.2 of Frankish et al., 1991).

Day -1

- Incubation / larval tank filled with 1μm filtered seawater from settlement tanks and heated to 25°C.
- EDTA added @ 1g/1000L, low aeration.

Day 0

- Spawning induction using clean scrubbed mature broodstock (wild or conditioned) using thermal stimulation to 28°C and salinity reduction by up to 10g/L.
- Spawners immediately placed into individual containers (water from incubation tank) and spawned out.
- Selected eggs are washed through a 50μm screen with 1μm filtered and aged seawater, pooled into a 20L bucket, fertilised with selected pooled sperm (5-10 sperm/egg), homogenised and counted (replicated 1ml samples).
- Incubation tanks stocked at 3/mL.
- Larval sample examined at 100X to check development ("D" stage in 16 hrs is normal) and establish size (range and mean).
- Half daily feed ration (calculated from daily feed curve) of mixed algal species (*Pav, T. iso, C. cal*) is usually added in the afternoon.
- New larval tank filled as above and heated.

Day +1

- Larval sample used to establish size, gut content, development motility and general health.
- Feed ration (size x feed curve) of mixed algae divided into am. & pm. feed.
- New larval tank filled (as above) and heated.

Day +2

- Batch water change using wet screen of 63μm and 53μm backup.
- Washed larvae flushed into 20L bucket and sampled to establish total numbers, gut content, development, motility and general health.
- The known number of larvae are stocked into the new tank that has been fed with 50% of their daily feed ration.
- The balance of the feed ratio is added late afternoon.

Day +3
- Larval sample used to establish size, gut content, development, motility and general health.
- Feed ration (size x feed curve) of mixed algae divided into am. & pm. feed.

Day +4
- As for day 6. New larval tank filled (as above) and heated.

Day +5
- Batch water change using wet screen of 85μm and 63μm backup.
- Washed larvae flushed into 20L bucket and sampled to establish total number, gut content, development, motility and general health.
- The known number of larvae are stocked into the new tank which has been fed 50% of their daily feed ration.
- The balance of the feed is added late afternoon.

Days +6-15
- The above pattern is continued throughout the larval cycle until day 15.
- Screen size is increased at each water change, the screen selected depending on the growth of larvae and the number to be culled.
- Typical screen sizes – 100, 118, 150, 180, 212μm.

Days +16-18
- Batch water change using 212μm screen 180μm backup.
- Washed larvae flushed into 20L bucket and sampled to establish total number, gut content, development, motility and general health.
- If retained on 212μm screen, larvae transferred to set screens.
- If a significant number of larvae retained on 180μm screen, they are on-grown in new larval tank following above protocol for further 1-2 days, then screened on 212μm mesh and transferred to set system.

NURSERY CYCLE

Day -2
- Screens, tanks and equipment sterilised using sodium hypochlorite solution.

Day -1
- Tanks filled with 1μm filtered seawater from storage tanks, heated to 25 –26°C + aeration.

Day 0
- Larvae retained 212μm to set screens @ 200-250,000/screen – thin layer of shell (pass 350μm, retained on 200μm) cover over screen mesh. Overhead sprays deliver seawater flow (4-6ml/larvae/day).
- Daily feed ration (50-60,000 cells/larvae/day) of mixed algal species (*Pav, T. iso, C. cal*) is split over two feeds, am. & pm.
- Screens removed from system pm. and rinsed with ambient temp. seawater.

Day +1
- Screens removed from system and washed with saltwater am. & pm.
- Feed as per day 0, 50% exchange of set tank seawater.

Day +2
- Screens removed from system and washed with seawater am. & pm.
- Total change of seawater to set system.
- Tanks wiped clean and rinsed with freshwater.
- Hoses and spray pipes cleaned, fed am. & pm. as on previous days.

Day +3
- Screens rinsed am. & pm. with seawater.
- 50% seawater exchange, feed as per previous days.
- All set screens wet graded over 350μm screen.

- Retained spat moved to separate downweller system, fed as per previous days initially, then on demand, the frequency depending on the clearance rate of algae.
- Larvae and shell passing the 350μm screen returned to set system.

Day +4

- Shell set screens removed and washed with salt water am. & pm.
- Spat screens removed from system and washed with fresh water am. & pm.
- Total water exchange to shell set system, shell set systems fed as previous days.
- Spat systems fed on demand.

Day + 5

- Shell set screens washed am with salt water, spat screens am. & pm. with freshwater.
- Total seawater exchange to spat downweller systems.
- Systems fed as per previous days.
- All shell systems graded over 350μm screen.
- Retained 350μm spat moved to separate downweller system.
- Shell systems discarded – older spat graded over 500 screen.
- Retained 500 spat counted using volume method and transferred to field nursery upwellers.

Day +6

- Screens removed from system and spat washed with fresh water am. & pm.
- Fed as per previous days.
- Water change of 100% or 50% depending on previous sequence.

Day +7

- Spat screens removed from system and washed am. with freshwater
- Fed as per previous days.
- All spat graded over 500 screen.
- Retained spat counted and transferred to field nursery upwellers.

FIELD NURSERY OPERATIONAL PROTOCOL

1. 500μm screen retained spat transferred to field upwellers (350μm screen).
2. Approximately 400,000 spat per screen, flow rate approximately 6L/min (500μm spat) increasing to 12L/min. (3mm spat).
3. Upweller systems drained daily, screens rinsed with freshwater and tanks flushed with fresh water, refilled with unfiltered seawater, flows adjusted and spat spread over screen area. (Twice daily rinsing may occur if silt load in field nursery is high).
4. Spat graded at varying intervals according to growth rate (time of year and ambient temperature). First grading of nursery spat usually 7-14 days ex hatchery (temp.>20°C) then approx. 14 day intervals depending on growth, wet graded by hand using a series of screens, 670, 1000, 1250, 1400, 1800, 2000, and 3000μm.
5. Flow rates and densities for individual size groups not specific, flow rate for small spat (< 1.5mm) through upweller adjusted to maximum without major disturbance to spat layer.

OPERATIONAL CONTRAINTS TO HATCHERY PROCEEDURES-FOR SYDNEY ROCK OYSTERS AT PSFC

Seawater Source

- Limited access to ocean and outer bay beaches.
- Variable quality – seasonal factors, weather/sea conditions, (rainfall/run-off and drift seaweed; micro -algal blooms; Boulder Bay sewerage outfall. 3x marinas; large fishing fleet and general run-off into Port Stephens).
- Salinity 33 to 36.5g/kg; pH 7.9–8.4.

Seawater Conditioning

- 6 x 50,000 litres storages but variable high demand.
- Minimum of 1 week of storage sometimes compromised.

Constraints due to design and multi user demand on hatchery facilities

(see attached current operations schedule)
- Commercial spat production (PO, FO, Pearl O's).
- SRO Breeding program and associated R&D on SRO and other bivalves (Clams, Scallops and Pearl oysters and Abalone).
- Year-round micro-algae for fin-fish live food.
- Non modular with incomplete separation of micro-algae, larval, spat and broodstock conditioning areas and operations - hence little opportunity for comprehensive dry-out and disinfection.
- Batch micro-algal production imposes some hatchery options such as "flow through "Bayes" system.

PSFC Bivalve Hatchery Operations Plan   July 2002 – June 2003

		J	A	S	O	N	D	J	F	M	A	M	J
Algal Requirements (litres/day)													
Pearls				◄——— 400 ———►		◄——————— 700 ———————►						
Marine fish		◄———————————————— 1200 ————————————————►										
Pacific oysters				◄ 100 ►	◄— 500-1000 —►							
Flat oysters						◄— 500-1000 —►						
Sydney rock oysters								◄——— 500-1000/d ———►				
TOTAL		◄1600►	◄1700►	◄2100►◄2400►◄2400	►◄2900	►1900		———◄►◄—— 2400-2900 ——►◄►			1200	
Wet Floor Operations													
Pearls[1]	L												
	N												
Flat oysters	L												
	N												
Pacific oysters	L												
	N												
Sydney rock oysters	L												
	N												
Staffing													
Lynne Foulkes													
John Diemar													
Fish Tech[2] (Ian Diemar)													
Temp Fish Tech													

[1] Pearl oyster nacre quality research in controlled temperature room

L = larval cycle   N = nursery cycle   ▨ = known period   ▨ ▨ = possible extension

Summary of discussion session and additional comments

Q1   How are the SRO larvae fed and do feeding methods result in clear water periods?

The satiation level feeding regime used is administered as two equal batch additions of feed daily, once in the morning (9:00-10:00am) and around knock-off time (4:00-5:00pm). Accordingly, micro-algae cell numbers often do fall to very low levels especially in the morning some 16 –18 hours after the previous addition of algae.

NB. this issue generated lively discussion on advantages offered by continuous drip or trickle rather than batch feeding.

Q2   Why does your hatchery add feed on day 0 prior to the emergence of the first feeding stage "D" veliger larvae?

As described in the hatchery operations manual (Appendix 1) larvae are not fed until the emergence of "D" veligers on day1.

Q3   Have you tried complete changes of rearing vessels and water on day 1 to separate normal healthy "D" veligers from non-viable eggs, egg capsules, post hatch debris and associated metabolites and bacteria?

PSFC hatchery technician, Lindsay Goard responded, stating that while this practise had been imposed on some occasions it had not prevented anorexia in early larvae and was demanding on reserves of stockpiled and settled seawater.

NB. In general discussion it was conceded that day1 water changes should be adopted as a routine prophylactic practise.

Q4   How do you reconcile settlement and aging of seawater on the basis of views expressed by Dr Ralph Elston and supported by assembled oyster hatchery operators that simple filtration of new seawater provides better results?

Our general experience at the PSFC was that settlement and aging of local estuary water, introduced around 1985, improved hatchery performance with SRO's and that further improvements resulted with settled seawater collected and trucked from ocean beaches at Anna Bay or outer Port Stephens (Nelson Bay and Shoal Bay).

Further rationale for these practises was provided by published reports together with findings of our own in-house monitoring of seawater micro-flora. Results showed that over periods of 7 to 10 days of storage and settlement, bacterial densities fell and species assemblages shifted from those dominated by potentially pathogenic presumptive *Vibrio* spp (based on TCBS plate counts) to more benign types comprising more heterotrophic species (based in marine agar plate counts).

Accordingly, negative opinions on the merits of seawater settlement and ageing expressed by Dr Elston and others have come as a surprise.

Q5   Is it sensible and correct to use TCBS counts as either the only or most important index of quality of seawater used to hatchery rear oysters?

Other aspects of seawater flora and chemistry such as the presence of appetite suppressing bio-toxins could well be of critical importance but we have no easy means of identifying or quantifying such factors other than a bio-assay using SRO larvae!!

Q6   *Your experimental results suggest that higher rearing temperatures than 23 or 24°C may be beneficial. Have you or other hatcheries used higher temperatures for rearing SRO's and if so, what were the results?*

Our adherence to conservative rearing temperatures of 23-24°C have been based on a concern that higher temperatures might promote more rapid proliferation of pathogenic bacteria. However systematic in depth investigation of effects of temperature on larvae and potentially pathogenic bacteria as described by Dr Elston in relation to pacific oyster operations in one USA hatchery, has not been attempted for SRO's to date.

NB Jonathan Bilton, former manager of the Ocean Foods International hatchery at Albany, WA, commented that they routinely incubated and reared *S. glomerata* ("Western rock oyster") larvae at 27°C not 24 °C and had not experienced larval anorexia nor spat mortality problems over the past 5 annual hatchery seasons.

Q7   *Are there any recognised analytical methods of testing for possible appetite suppressing toxins in seawater?*

This question was referred to Dr Elston who commented that toxins may have biological activity at concentrations below the limits of analytical detection but it was likely that such toxins would be present in raw seawater. He also cautioned that it is probably wrong to concentrate on single factors because clinical manifestations of the larval disease may be multi-factorial in origin arising on different occasions from alternative combinations of several predisposing factors.

Q8   *Are there any recognised practical methods for pre-treating seawater, such as pre-filtration though activated charcoal, that provide more stable micro-biological and biochemical environments conducive to good health of developing larvae?*

This question was referred to the floor promoting comments to the effect that the performance of well managed bivalve hatcheries with continuous access to good quality seawater, was more due to the inherent quality of local seawater source than any other factor. Depth of source water was identified as a critical issue for some hatcheries dealing with more difficult to rear bivalve species. There was general consensus that simple physical filtration of seawater in the broad range 1 to 20 μm using drum, or depth filters was all that was required. Opinions on the merit of UV filtration in relation to bivalves was divided although UV is widely accepted as being critical to larval rearing success with abalone and other gastropods.

REVIEW OF INFORMATION ON MASS MORTALITY SYNDROME OF SYDNEY ROCK OYSTER LARVAE EPIDEMIOLOGY

(See Appendix 2, Sections 1.1.1, 5.1 and 5.2)

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Summary of discussion session and additional comments

Q1   Is the 'anorexia syndrome' in SRO larvae a product of suppressed feeding, ingestion or digestion/assimilation of food (micro-algae).

The problem appears to one of a failure to ingest food. Afflicted larvae exhibit normal velar swimming and feeding activity or even hyper-activity.

Q2   Is anorexia universal or does it occur in some but not other larvae?

If anorexia strikes early in the cycle (days 2 to 4), it afflicts all larvae, the digestive tracts of which become and remain uniformly transparent. In episodes in which onset of anorexia is delayed to days 5 to 8, there is often variable effects on feeding with small larvae generally the worst afflicted. In such cases it is not uncommon for a small cohort of "front-runners" to eventually overcome anorexia and successfully undergo settlement and metamorphosis.

Q3   Do survival data presented for successful and failed SRO hatchery runs relate only to all surviving larvae or do they exclude those cull graded at each water change?

These data apply only to surviving larvae retained on primary grading screens and effectively combine dead larvae (morts) and culled larvae (those retained on smaller mesh back-up screens).

NB. Assuming that the average culling rate applied at each water change at PSFC is 20% and that 7 water changes are applied per successful larval production cycle, culling alone would restrict cumulative yield of ready to set (competent) to only 20%. Bearing in mind that the mean survival rate actually achieved at the PSFC with successful large scale batches of SRO larvae is about 15%, an important implication is that background mortality rates are low.

Q4   Have you tried increasing the feeding rate with anorexic larvae?

Yes we have tried on many occasions even when food is clearly abundant but no change in feeding status has been observed.

NB. John Mercer commented that he had commonly encountered periods of anorexia when rearing other species of oysters and scallops but this was generally reversible after several days over which time one or a succession of water changes were applied. His conclusion was that short-term fluctuations in water quality/chemistry were responsible. While supplementary water changes have been commonly used at PSFC in an attempt to combat anorexia in SRO larvae, such attempts have rarely resulted in tangible improvement. A possible explanation for these

contrasting observations is that at PSFC, the same stockpile of aged seawater is often used for successive water changes.

Q5   How long can starved larvae live?

Larvae used in unfed control treatments in 7 to 9 day dietary trials consistently exhibit high survival rates and little apparent reduction in activity levels. These observations suggest that elevated mortality rates observed from the beginning of anorexia episodes are not due to starvation alone.

Q6   Have you transferred anorexic larvae into alternative sources of seawater environments or alternative rearing vessels etc. to see if the condition can be reversed?

No we haven't but, we have attempted to rear sibling day 1 stock in seawater sourced or pre-treated differently on several occasions including a formal experiment described in Section 5.4 of Appendix 1.

NB. This was a very good question. Its practical implication, that experiments could be used to test if anorexia can be reversed by such factors as improved water quality, will be included in strategic planning.

Q7   What proportion of SRO hatchery runs over the past 5 years failed as a consequence of the anorexia syndrome?

In the 5 year period 1995 to 1999, more than half (14 out of 22 or 64%) hatchery runs were successful the remaining 36% succumbed to anorexia syndrome.

NB. Only 29% of large-scale hatchery runs produced in 20,000L tanks subsequently escaped the mass spat mortality syndrome. This was considerably lower than that of 46% associated with smaller batches of spat produced in 1000L tanks and suggest that the larger the batch of spat being reared the higher the probability of mass mortalities.

Indeed over the fourteen years 1988 to 2001,only two very large batches of larvae put to set subsequently escaped spat mortality to produced commercial scale (>10million spat) batches of spat. The first of these was the inaugural batch produced in the "new hatchery" in July 1988. The second, in Feb 2000, followed a protracted heat wave during which powerful air blowers were used to thoroughly dry out the hatchery over a two week period.

During ancillary discussions most hatchery managers agreed that:
- Regular dismantling, cleaning, disinfection of commercial bivalve hatcheries is standard practice and should be considered mandatory to good husbandry. It was also agreed that the hygiene protocols should be augmented by annual decommissioning and dry-out periods of several months during which staff could rest and take annual leave prior to the next hatchery season that usually entail highly extended working hours including weekends and nights.
- In common with the experience with SRO's at the PSFC, best hatchery results achieved elsewhere in Australia and New Zealand are generally achieved through late Winter and Spring and worst results in through and Autumn. John Mercer commented that dry-outs were unnecessary for multi-species bivalve hatchery operations in Canada. However the consensus was that this degree of latitude in hatchery management was possible only in the absence of problematic species like the SRO.

Q8   Have anorexia problems ever been encountered with pacific oyster larvae reared at the PSFC?

No significant problems larval or spat mortality problems have been encountered over the past 5 years of producing commercial scale (5 to 7 million) batches of pacific oysters.

Q9   Have batches of SRO larvae affected by the anorexia syndrome been reared side by side reared in Pacific oysters and was there any evidence of the same problem in the Pacific oysters?

In several instances successful production of pacific oysters occurred at the same time that batches of SRO larvae were succumbing to the anorexia syndrome.

NB. The same comments were made by Wayne O'Connor in relation to about 40 batches or Pearl oysters (*Pinctada imbricata*) and by Mike Heasman in relation to many commercial scale batches of scallops (*Pecten fumatus*) and flat oysters (*Ostrea angasi*) and produced at PSFC over the past decade.

Q10   Can you and do you regularly dismantle "Mexican style" PVC ball drainage valves on the 20,000 L larval rearing tanks?

These valves and similar valves fitted to smaller 1000L larval tanks cannot be easily dismantled but are routinely disinfected with chlorine solution and left open between uses to dry out.

NB. John Mercer commented that an inaccessible cavity surrounding the ball valve spigot was a trap for organic material and settlement stage larvae and harboured several mLs of anoxic and potentially toxic fluids. He further advised that all such valves should be replaced and that all valves be dismantled, cleaned and disinfected after each use.

Q11   Do you consider that existing facilities and hygiene protocols at the PSFC are sufficient to prevent build up of pathogens and toxins?

Areas identified as in need of replacement or modification include underground seawater supply pipelines from pumps to the hatchery building. These cannot be completely drained or dried and will be replaced with over-head mounted pipelines.

NB. The hatchery managers present agreed that for effective cleaning and disinfection of plumbing, acid /alkali solutions should be used to digest and remove bacterial slimes and other accumulated organic matter.

Q12   Has anorexia in SRO larvae problems occurred at other hatcheries other than the PSFC?

Yes, anorexia has been encountered at all sites at which large scale hatchery production of SRO's has been attempted on multiple occasions. These sites included a private hatchery at Broom's Head (where water storage did improve overall results) and a makeshift hatchery at the Cronulla Fisheries Centre. It is reasonable to state that overall success rates achieved elsewhere to date have been lower rather than higher than at PSFC.

Q13   Have all these SRO hatchery rearing attempts involved static culture techniques?

Yes.

Q14   Have you attempted flow through rearing techniques?

Yes, but results have been mixed with an initial success followed by subsequent anorexia linked failures. As stored seawater used in routine static culture was also used in these attempts, the success or failure of flow-through hatchery runs with SRO larvae probably reflected variability in the quality of the settled and aged seawater.

It is therefore apparent that any future meaningful evaluation of flow-through seawater techniques must be conducted at oceanic sites using continuously pumped and filtered seawater.

PATHOLOGICAL CHANGES ASSOCIATED WITH RECURRENT MORTALITY SYNDROMES IN HATCHERY-REARED LARVAE AND SPAT OF THE SYDNEY ROCK OYSTER SACCOSTREA GLOMERATA

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During the past 15 years, samples of Sydney rock oyster larvae and spat collected prior to, during and after various mortality events at PSFC hatchery and Brooms Head hatcheries were examined at Regional Veterinary Laboratory Wollongbar. The descriptions below are based on results of histopathological examinations of samples from those mortality events which were clinically consistent with the syndromes under consideration. Note, therefore, that more than one disease, each having characteristic causal factors, may be grouped within each of these loosely defined syndromes.

Larval mortality syndrome

Mortalities in affected larval batches typically began at 2-3 days of larval culture. Affected larvae stop feeding and die.

Only non-specific, relatively minor lesions were seen in larvae from affected batches. There was vacuolation of digestive gland epithelium and progressive low grade to moderator accumulation of haemocytes in tissues and spaces in some individuals (Fig 1).

There was no convincing evidence of an infectious cause. No viral inclusion bodies were recognised. Small numbers of bacteria were occasionally present on the external shell surfaces of larvae from affected batches but were rarely seen in extrapallial space, pallial cavity or tissues.

These findings suggest the larval mortality syndrome is probably not due to:
- viral infection.
- bacterial infection in or on larvae.

Causal factors could include:
- biotoxin (e.g. microbial toxin build-up in culture system).
- nutritional deficiency or imbalance.
- multifactorial, involving one or more of these causes plus other unrecognised factors.

Spat mortality syndrome

Typically, mortalities occurred in spat batches at approximately 7 to 30 days post settlement. Affected spat (350-900um) stopped feeding, gaped and appeared unable to retract their mantles. Mortalities over 80% usually occurred simultaneously within subgroups of original batches regardless of location post settlement.

A spectrum of similar lesions was present across batches. Mantle lesions appeared to predominate in some batches, and muscle lesions were more prominent in others, although examples of both lesion types could usually be found in spat from any outbreak. Spat showed moderate to severe

proliferation and/or degeneration and necrosis of mantle epithelium, with infiltration of inflammatory cells into underlying connective tissue (Figs 2). In many cases there was mild to severe adductor muscle myopathy (Fig 3) and, less often or less easily seen, mantle muscle myopathy. There was no convincing evidence of an infectious cause. No viral inclusion bodies were recognised. Bacteria were occasionally seen in small numbers on the external shell surface of moribund spat with minor to severe lesions but were rarely seen in internal spaces or tissues. In most samples collected during outbreaks, however, some spat had larger numbers of usually morphologically diverse bacteria in tissues without host response, consistent with opportunistic *perimortem* invasion. Similarly, invasive, apparently opportunist ciliates were present in tissues of affected spat in some batches. It is possible that invasion by bacteria or ciliates is facilitated by adductor and, if present, mantle myopathy.

Taken together, pathological findings suggest the spat mortality syndrome is probably not due to:
- viral infection.
- bacterial infection in or on spat.
- physiological factors (case records suggest the condition affects spat settled on both culch and slats but this needs to be confirmed).

Causal factors could include:
- biotoxin (e.g. microbial toxin build-up in culture system).
- nutritional deficiency or imbalance.
- multifactorial, involving one or more of these causes plus other unrecognised factors.

Systematic, detailed pathological studies of the larval and spat mortality syndromes, linked with epidemiological studies to identify risk factors, are essential the causes are to be found and effective control and prevention measures put in place.
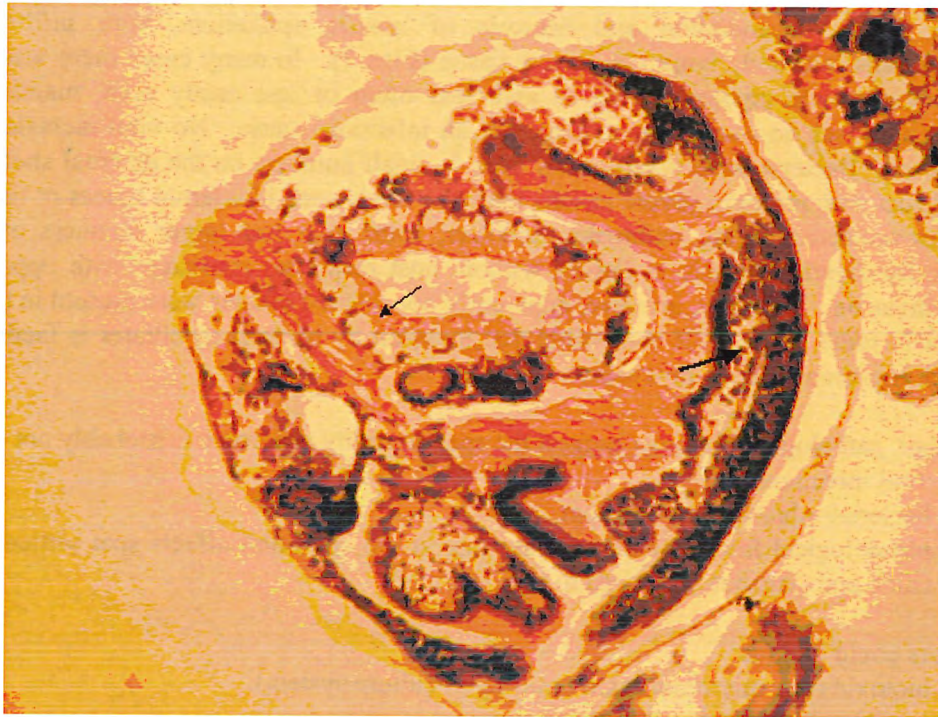
Figure 1.   Section of Sydney rock oyster larva from batch showing larval mortality syndrome. There is vacuolation of digestive gland epithelium (thin arrow) and accumulation of haemocytes in and adjacent to mantle (thick arrow).

Figure 2.   Section of Sydney rock oyster spat from batch showing spat mortality syndrome. There is necrosis of mantle epithelial cells with sloughing into the extrapallial space (thin arrow) and accumulation of haemocytes in mantle connective tissues (thick arrow).
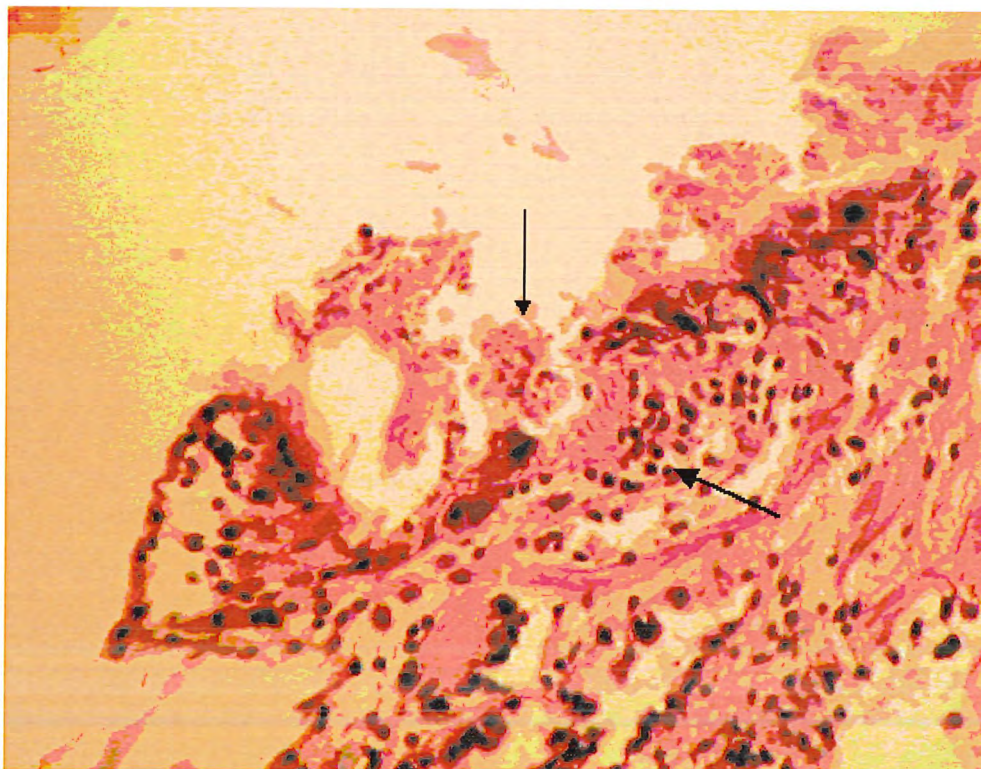
Figure 3. Section of Sydney rock oyster spat from batch showing spat mortality syndrome. There is severe degeneration and necrosis of adductor muscle fibres (arrow).

Summary of discussion session and additional comments

Discussing pathological changes evident in H&E stained larval sections, Ralph Elston expressed the view that moderate aggregations of haemocytes around the mantle were suggestive of bacterial infection in spite of the fact that no focal infection (clear aggregations of bacteria or cellular necrosis) was apparent.

Ralph Elston in response to the question posed by Dick Callinan "does infection precede anorexia or vice versa?" stated that infection would logically precede anorexia and therefore infection must occur within 24 hours of fertilization.

Patrick Hone commented that ATPase and enzyme activity in the absorptive region of the gut, recently used by Robert Kemp (SA Dept. of Agriculture) to investigate quality of formulated diets developed for abalone, and in particular anti appetite factors associated with some major ingredients, could be used to investigate the nature and hence possible cause of anorexia in SRO larvae.

Q1 *Have you prepared sections of spat embedded in plastic resin rather than wax as this enables thinner sections to be cut and results in enhanced resolution of host tissues, cells (of both the host and pathogens) and of the ultra structure of cells, during microscopic examination?*

Dick was unaware if plastic embedding was available in Australia. Judith Handlinger confirmed that it was but was not widely used.

Q2   Dick Callinan asked Ralph Elston.... what is your opinion of the distinct myopathy and apparent lack of invasive bacteria observed in the adductor muscle of afflicted spat?

Infection by bacteria may be serious without being invasive particularly if bacterial biotoxins are involved.

Q3   Are you satisfied that nutrition of the SRO spat is adequate?

We have generally used the same combination of three species of micro-algae diet as used for feeding larvae namely equal amounts (on a dry cell-weight basis) of *Pavlova lutheri, Isochrysis* sp. Tahitian and *Chaetoceros calcitrans*. This is in spite of the fact that our own research has identified some alternative diets (O'Connor et al., 1992, Heasman et al., 1999) as supporting better growth and survival. There is therefore considerable scope to improve the diet of spat to promote fastest possible growth of spat beyond 2mm (the size beyond which juvenile SRO achieve apparent immunity to the mass mortality syndrome).

Q4   Is nursery rearing of SRO spat conducted in the light or in the dark?

From the time of set until spat are moved to a field upweller nursery at Wanda Head they are reared in low light conditions in the hatchery under ambient photoperiods. However at Wanda Head the spat are maintained in continuous darkness within fully enclosed tamper and vandal proof cabinets. There is however no reason to believe that lighting regimes have any influence whatsoever on the mass mortality syndrome that has and has not occurred over a wide range of lighting conditions.

Additional comments

Discussing pathological changes evident in H&E stained larval sections, Ralph Elston expressed the view that moderate aggregations of haemocytes around the mantle were suggestive of bacterial infection in spite of the fact that no focal infection (clear aggregations of bacteria or cellular necrosis) was apparent.

Ralph Elston in response to the question posed by Dick Callinan "does infection precede anorexia or vice versa?" stated that infection would logically precede anorexia and therefore infection must occur within 24 hours of fertilization.

SUMMARY OF VIROLOGY UNDERTAKEN FOR LARVAL & JUVENILE SYDNEY ROCK OYSTERS

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Virology examinations undertaken (generally within the framework of the CRC for Aquaculture Project A.2.1: Improved Early Survival of Molluscs) on Sydney Rock Oysters comprise histological assessment at the light microscopy level (presented elsewhere by R. Callinan), and a limited number of electron examinations undertaken at Tasmania's Department of Primary Industries, Water and Environment, (DPIWE) Mt Pleasant Laboratories, examined by J Handlinger, at the Australian Animal Health Laboratory (AAHL), examined by Alex Hyatt, and at the New Zealand National Institute of Water and Atmospheric Research (NIWA), examined by Mike Hine. In Mike Hine's absence, J Handlinger was asked to collate this data (not all of it available until the meeting).

The summary includes examinations of both the larval mortality syndrome, characterised by anorexia typically commencing between days 3-5 with 100% mortality by day 14, and the settled spat (juvenile) mortality syndrome commencing 7-43 days post settlement.

For larvae

Clinical findings of anorexia could be consistent with virological disease, as these may affect the velum, resulting in cessation of feeding due to the mechanical effect of loss of velar ciliary cells. However this is also likely to result in loss of swimming ability, which was not a feature. No consistent ciliary loss was seen.

The involvement of similar losses in multiple mollusc species including clams and scallops in one outbreak examined, is also suggestive of a non-viral cause as most viruses show relatively narrow species specificity. However other species were generally successfully raised through the larval period, so this outbreak may not be typical of the syndrome as a whole.

Histological examination gave no indication of virological involvement, though viruses could not be fully excluded by this process. Repeated examination showed no intranuclear or intracytoplasmic inclusions indicative of the major mollusc larval virological diseases so far recognised (herpes virus, which typically show large numbers of intranuclear inclusions, and oyster velar virus disease, which typically shows large intracytoplasmic inclusions). A variable number of cells with disturbed nuclear chromatin patterns were occasionally detected in larvae. These were regarded by the pathologists as typical of degenerating cells, though electron microscopy was needed to ensure the degeneration was not due viruses.

Bacteria were variably detected in affected batches of larvae, but as secondary bacterial involvement is a virtual inevitability in batches with larvae dying from any cause, this cannot be taken as conclusive evidence of a bacterial cause.

Electron microscopy

Although the project summary indicates that numerous samples of symptomatic larvae were submitted for light microscopy, relatively few samples were examined by electron microscopy, and

those air-freighted frozen to Tristan Renault in France did not arrive in a condition to allow the PCR probes to be used.

1. SRO Larval examinations at DPIWE Mt Pleasant Laboratories (J Handlinger) comprised two batches in 1996.
   - One batch showed a typical food intake decline by day 4, mortality between day 7-10.
   - The second batch was submitted at a time when mortalities were also being experienced in this hatchery in clams and scallops. Some velum loss and occasional degenerate cells were seen, but no definite viral inclusions.

In both groups the electron micrograph quality was limited by the relatively low resolution of this microscope (Hitachi H300 transmission electron microscope), and sample preparation factors. No viruses (herpes or iridovirus like) were seen, though some degenerating and separating cells were present in velum and anterior gut.

2. SRO Larval examination at the Australian Animal Health Laboratory was undertaken on two batches of larvae in 1997. These consisted of two consecutive crashes, showing anorexia n day 4, mortality by day 8. They were examined using both transmission and scanning electron microscopy and were found to be negative for viruses, and with no detectable histological variations which where not also present in control, unaffected oyster larvae (I do not have details of the source or species of control animals).

3. Two E-mails from Mike Hine in May 1999, indicated several sub-batches of SRO larvae were examined (I do not have all batch details available.) Although no abnormalities were detected by light microscopic examination, two types of virus like particles were detected or suspected in some samples.
   - One group of samples (MSD1) reportedly looked "rough", with cytoplasmic membranes bearing suspected small putative virus particles as published previously as small RNA viruses in dying adult molluscs of several species in NZ. Due to the small size, there was doubt if these were virus particles or artefacts.
   - Other groups (CD on 25/3, MSD1 fixed 24/3 and MSD fixed on 26/3) showed replication of herpes type viruses, with one groups (MSD1 of 24/3 showed mature virions, nearly all with tails, present extracellularly. Other samples showed apoptotic dense cells containing nuclear and cytoplasmic arrested stages of herpes virus development.

Findings were interpreted by Mike Hine as suggesting that initial mortalities were due to some other cause, followed by a herpes virus event on days 6-8, the normal window for these viruses.

Overall virogenesis was low, in comparison with herpes virus induced Pacific oyster larval mortalities.

For post settlement spat (juvenile) mortalities

Electron microscopic examination has apparently been carried out only once with post-settlement mortality, a mass mortality episode in 1994, in which mortality occurred 1-3 days post grading which had involved exposure to bore water. Results were negative for viruses by negative contrast microscopy, scanning electron microscopy, and transmission electron microscopy of ultra thin sections.

(1999 samples submitted for light microscopic examination are still being held, and could probably be processed for electron microscopy if required. However this outbreak was not typical in that ciliate invasion, not previously seen in spat, was evident in this outbreak.)

Histological findings on light microscopic examination are not regarded as typical of viral involvement.

Summary of discussion session and additional comments

Discussion included whether sufficient electron microscopic examination had been undertaken to exclude virus infection as a cause of either larval or spat losses, especially given that some outbreaks so examined were atypical. In summary the comments were:

Light microscopic examinations (which were extensive) would have been expected to detect evidence of herpes viral infection if this was a significance and primary factor. B. Jones observed that many molluscs may harbour low levels of herpes virus, and their presence without evidence of significant multiplication may be questioned.

Doubt was cast on the putative finding in some samples by M Hine of small RNA virus-like particles. The basis of this doubt was:
- Mike Hine's suggestion that these could be artefacts induced by fixation,
- pathology typical of infection in older molluscs was not present (B. Jones),
- uncertainty of the exact identity of described particles from older NZ molluscs.

However because of the more limited resolution of the Mt Pleasant electron microscope compared to those available at AAHL and NIWA, there was the possibility that if these had been present in the 1996 samples, they would probably have been missed.

It was concluded that viral disease was unlikely, but that there may be value in re-examination of stored or future samples to ensure exclusion, and possibly of re-submission of samples for PCR work, using appropriately fixed (alcohol) rather than fresh samples.

The following additional advice on future viral investigations was provided by Dr Serge Corbeil (CSIRO/AAHL)

After considering the available E.M. results as well as the overall data accumulated, I tend to agree with Ralph Elston and Judith Handlinger that it does not look like a virus is the major culprit for the larvae and spat production failures. I would recommend, however, that the additional virological examinations be undertaken be completed, such as use of the PCR herpes virus probe developed by IFREMER as well as additional the E.M examinations, to confirm the presence or absence of small RNA virus particles.

In the event that involvement of an infective virus cannot be confirmed using these techniques but that a virus is still suspected as causing significant stress on the larvae and/or spat, I believe that the subtractive DNA hybridisation assay (Clonetec PCR Select) would be useful for identifying such a virus or any other type of undetected pathogen such as bacteria etc.

Contrary to Judith Handlinger's opinion, if control (uninfected) specimens are reared under the same conditions as infected ones, both uninfected and infected specimens will contain DNA or RNA from unrelated innocuous organisms such as algae, benign bacteria etc. that happen to be present with the oysters. I do nevertheless concede that use of DNA hybridisation assay which has been developed to "find needles in haystacks" would certainly be difficult and time consuming to implement assay in the context of your hatchery.

EXPERIMENTAL INVESTIGATIONS OF LARVAL ANOREXIA

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1. **Challenge tests with Vibrio Isolates (see Appendix 2, Section 5.4.1)**
2. **Experiment to elucidate involvement of infective agent(s) (see Appendix 2, Sections 5.4.2 to 5.4.4)**

Summary of discussion session and additional comments

Q1   *What measures have you put in place to prevent movement of people and equipment between different parts of the hatchery?*

The high and varied demand on the hatchery for commercial production of Sydney rock oysters spat of and associated experimentation largely precludes separation let alone quarantining of particular operations. Various operations include mass selection breeding and ploidy research operations involving SRO's, year-round pearl spat production and associated research, production of large commercial batches pacific and flat oyster spat and year round production of algae. Approximately half of algae is used to produce rotifers used as live food for the production of marine finfish.

Having said this a number of modifications to hatchery buildings, equipment and operations have been introduced over recent years. Dedicated equipment is retained within specific areas of the hatchery. The main larval rearing tanks were physically isolated from the rest of the hatchery with the erection of a full floor to ceiling dividing wall in 1996. A chemical footbath to the larval rearing area is maintained during the course of hatchery rearing operations and access to the room is strictly limited to hatchery staff.

Q2   *When you sample larvae do you use new pipettes and screens on each occasion?*

Pipettes are not used and miniature sampling screens are sterilized between uses to minimise risks of transmitting infective agents.

Q3   *How confident are you that 1 micron depth filters remove all bacteria from rearing water?*

Depth filters are not designed nor intended to remove all bacteria absolute using membrane filters. To the contrary, 1 micron filtration is intended only to reduce total bacteria to acceptably low overall levels of 10^2 to 10^3 /mL by removing suspended matter with which surface attaching bacteria are associated. Indeed removal of all bacteria that requires filtration to 0.2micron absolute has a serious well documented negative influence on the ability of seawater to support good health and normal growth of marine bivalve larvae.

NB. In the course of ensuing general discussions the possibility of non biological sources of toxins was raised. Detected sources of such toxins were in one case traced to a length of non food grade hose (cited by Jonathan Bilton in relation to SRO larval rearing operations in Albany WA)

and non food grade O rings (cited by John Mercer as preventing scallop larvae from developing to first feeding "D" veligers).

Q6   *Referring to the critical role played by temperature in hatchery success highlighted in Dr Ralph Elston's key address and to an apparent discrepancy between the experimentally determined optimum temperature for rearing SRO larvae (see Fig13 of Appendix 1) –the question was posed - have you attempted to vary incubation and larval rearing temperature of SRO?*

We were aware of the wide discrepancy between routinely applied temperature of 24°C and an experimentally determined optimum growth temperature of 29°C. However continued adherence to 24°C as our preferred rearing temperature for SRO at PSRC has occurred because of scepticism among our technical staff that results achieved in small experimental rearing trials may not be valid in relation to commercial scale cultures and to concern that higher rearing temperatures would advantage pathogenic bacteria ahead of SRO larvae.

At this point Dr Ralph Elston reiterated the critical importance of rearing temperature and need to determine and apply optimised rearing temperatures. He again cited the spectacular case of a Pacific oyster hatchery that overcame chronically high larval mortality problems by simply raising rearing temperature from 24 to 26°C. This small temperature rise of 2°C was shown to significantly enhance growth and vigour of the larvae while at the same time suppressing the proliferation rate and virulence of a pathogenic strain of Vibrio sp. bacteria.

NB. The importance of maintaining constant temperature i.e. within about ±0.5°C for the health of larvae was stressed by a number of hatchery operators.

REVIEW OF INFORMATION ON MASS MORTALITY SYNDROME OF SYDNEY ROCK OYSTER SPAT

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1. **Epidemiology (see Section 6.2 of Appendix 2)**
2. **Experimental investigations (see Section 6.3 of Appendix 2)**

Summary of discussion session and additional comments

Q1   Are you concerned about possible adverse effects of physical agitation applied during grading on the health of spat?

Although grading has been shown to trigger some episodes of spat mass mortality, this is not always the case. It is nevertheless reasonable to assume that trauma and other forms of stress can precipitate outbreaks of the disease.

This in turn prompts the question, should use of scallop shell and periodic grading be abandoned as means of reducing stress? As discussed in my presentation, a large scale experiment comparing use of epinephrine and of graded scallop shell fragments for the production of single seed SRO spat, (see Section 6.3 of Appendix 1) demonstrated that there are no significant advantages of scallop shell over culchless settlement in terms of mean growth, growth variability nor yield, both systems yielding about 66% spat from competent larvae.

The same experiment demonstrated that culchless settlement considerably reduces labour requirements. On the other hand, results of epinephrine induced settlement with SRO have been inconsistent and raise the need for dedicated experiments to optimise epinephrine dose and exposure times. As no particular benefit could be demonstrated experimentally for grading spat below 2mm (the maximum size at which spat are susceptible to mass mortality), the labour intensive practice of grading probably should be abandoned.

NB. The pathology of adductor muscle associated myopathy and gaping, lack of invasive bacteria, normal uninterrupted growth of survivors suggests that the mass spat mortalities are caused by one or a combination of traumatic stress, bacterial toxins and other predisposing factors compromising the general health and vigour of the spat.

Q2   Have you tried using fluidised bed upwellers or spat bubblers used by many Pacific oyster hatcheries as an alternative to conventional downweller and upweller screens?

We have undertaken preliminary steps but have not implemented this technology that usually involves single pass seawater flow through technology best suited to sites with continuous access to good quality seawater as opposed to use or re-use of imported seawater, that is stockpiled, settled and aged. We therefore acknowledge that this alternative nursery technology should be evaluated as a means of preventing mass spat mortalities in SRO.

Q3   *Are their any major differences between nursery techniques applied at PSFC and those used for producing SRO spat at the Albany hatchery in WA?*

Mike Heasman referred this question to Jonathan Bilton who provided the following response.

The only notable difference in nursery technology applied to SRO's at Albany is the application of a single daily wash and rinse of spat using freshwater rather than twice daily (am and pm) washes with seawater. Adherence to use of scallop shell rather than epinephrine for production of single spat has been prompted by the occurrence of heavy mortalities in culchless spat late in the nursery cycle.

MICROALGAE AND THEIR ROLE IN BIVALVE HATCHERY NUTRITION

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Over the last four decades, several hundred microalgae species have been tested as food for bivalve molluscs, but probably less than twenty have gained widespread use in aquaculture. Microalgae must possess a number of attributes to be useful aquaculture species. They must be of an appropriate size for ingestion, e.g. from 1 to 10 μm for larvae and spat (Webb and Chu 1983; Jeffrey et al 1992) and readily digested. They must have rapid growth rates, be amenable to mass culture, and also be stable in culture to any fluctuations in temperature, light and nutrients. Finally, they must have a good nutrient composition, including an absence of toxins that might be transferred up the food chain.

Strains identified by Persoone and Claus in 1980 as being successful for bivalve culture included *Isochrysis galbana, Isochrysis* sp. (T.ISO), *Pavlova lutheri, Tetraselmis suecica, Pseudoisochrysis paradoxa, Chaetoceros calcitrans* and *Skeletonema costatum*. It is noteworthy that now, over 20 years later, mollusc hatcheries are still using essentially the same strains. Culture request data from the CSIRO Collection for Living Microalgae (C. Johnston, pers. comm.) shows that *Isochrysis* sp. (T.ISO), *Pavlova lutheri, Chaetoceros calcitrans* and *C. muelleri* are the most common species used in Australian hatcheries, with *Thalassiosira pseudonana, Skeletonema costatum* and *Tetraselmis suecica* also popular. For larvae, the most popular diet is a mixture of *Isochrysis* sp. (T.ISO), *P. lutheri* and *C. calcitrans;* this diet has been successfully applied for larval culture of Sydney rock oyster *Saccostrea glomerata*, Pacific oyster *Crassostrea gigas* and scallop *Pecten fumatus* (O'Connor and Heasman 1997; Richard Pugh, Shellfish Culture Ltd., pers. comm.).

Microalgae can vary significantly in their nutritional value, and this may also change under different culture conditions (Enright et al 1986a; Brown et al. 1997). Several factors can contribute to the nutritional value of a microalgae, including its size and shape, digestibility (related to cell wall structure and composition), biochemical composition (e.g. nutrients, enzymes, toxins if present) and the requirements of the animal feeding on the alga. In the context of the current workshop, much knowledge has been obtained by NSW Fisheries on the requirements of larval and juvenile *S. glomerata* from experiments comparing different algal and alternative diets (Nell and O'Connor, 1991; Numaguchi and Nell, 1991; O'Connor et al., 1992; Nell et al., 1996; Heasman et al., 2000). Many of the algal diets found to be successful for *S. glomerata* have also proven successful for other molluscs, suggesting similarities in nutritional requirements.

Since the early reports that demonstrated biochemical differences in gross composition between microalgae (Parsons et al., 1963) and fatty acids (Webb and Chu 1983), many studies have attempted to correlate the nutritional value of microalgae with their biochemical profile. However, results from feeding experiments that have tested microalgae differing in a specific nutrient are often difficult to interpret because of the confounding effects of other nutrients. Nevertheless, from examining all the literature data, including experiments where algal diets have been supplemented with compounded diets or emulsions, some general conclusions can be reached (Knauer and Southgate 1999).

Microalgae grown to late-logarithmic growth phase typically contain 30 to 40% protein, 10 to 20% lipid and 5 to 15% carbohydrate (Brown et al. 1997; Renaud et al., 1999). When cultured through to stationary phase, the proximate composition of microalgae can change significantly; for example when nitrate is limiting, carbohydrate levels can double at the expense of protein (Harrison et al., 1990; Brown et al., 1993b). There have been few reports that have established a strong correlation between the gross composition of microalgae and their nutritional value. However, diets with high levels of carbohydrate produced the best growth for juvenile oysters (*Ostrea edulis*) provided polyunsaturated fatty acids (PUFAs) were also present in adequate proportions (Enright et al. 1986b). Similarly, larval scallops (*Patinopecten yessoensis*) fed carbohydrate-rich diets had the best nutritional condition (as assessed by their energy content) (Whyte et al., 1989).

PUFAs derived from microalgae, i.e. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are known to be essential for various larvae (Langdon and Waldock 1981; Sergeant et al., 1997). A summary of the proportion of these important PUFAs in 46 strains of microalgae are shown in Figure 1 (data from Volkman et al., 1989 and other papers from CSIRO Laboratories). The fatty acid content showed systematic differences according to taxonomic group, although there were examples of significant differences between microalgae from the same class.

Most microalgal species have moderate to high percentages of EPA (7 to 34%; Fig 1). Prymnesiophytes (e.g. *Pavlova* spp. and *Isochrysis* sp. (T.ISO)) and cryptomonads are relatively rich in DHA (0.2 to 11%), whereas eustigmatophytes (*Nannochloropsis* spp.) and diatoms have the highest percentages of AA (0 to 4%). Chlorophytes (*Dunaliella* spp. and *Chlorella* spp.) are deficient in both C20 and C22 PUFAs, although some species have small amounts of EPA (up to 3.2%). Because of this PUFA deficiency, chlorophytes generally have low nutritional value and are not suitable as a single species diet (Brown et al. 1997). Prasinophyte species contain significant proportions of C20 (*Tetraselmis* spp.) or C22 (*Micromonas* spp.) - but rarely both.
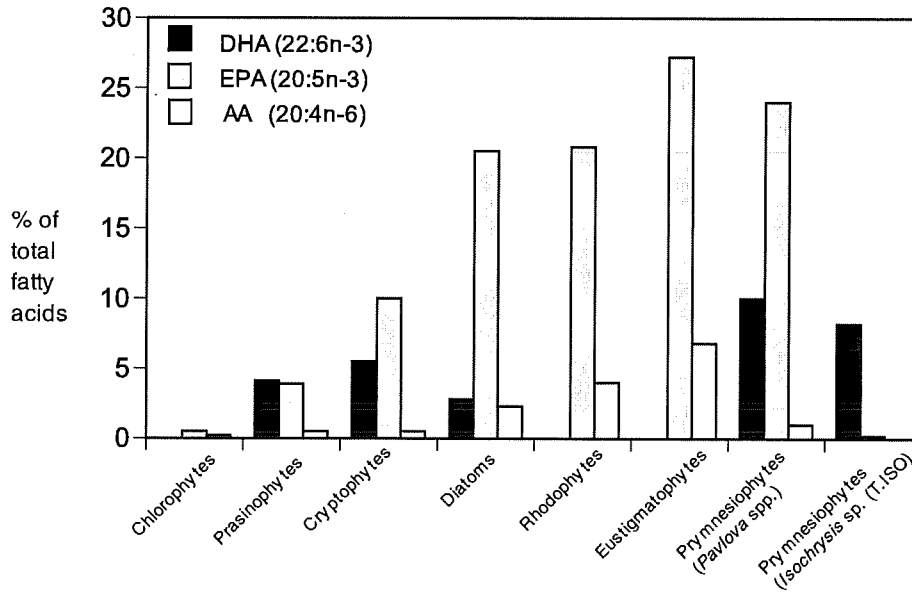
Figure 1. Average percentage compositions of the long-chain PUFAs docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (20:4n-6) of microalgae commonly used in aquaculture. Data compiled from over 40 species from the laboratory of CSIRO Marine Research.

While the importance of PUFAs is recognized, the quantitative requirements of larval or juvenile animals feeding directly on microalgae is not well established (Knauer and Southgate 1999). Thompson et al. (1993) found that the growth of Pacific oyster *C. gigas* larvae was not improved by feeding them microalgae containing higher than 2% (total fatty acids) of DHA; moreover the percentage of dietary EPA was negatively correlated to larval growth. However, the authors found a correlation between the percentage composition of the short chain fatty acids 14:0 + 16:0 in microalgae, and larval growth rates. They reasoned that diets with higher percentages of the saturated fats were more beneficial for the rapidly growing larvae, because energy is released more efficiently from saturated fats than unsaturated fats. In late-logarithmic phase, prymnesiophytes, on average, contain the highest percentages of saturated fats (33% of total fatty acids), followed by diatoms and eustigmatophytes (27%), prasinophytes and chlorophytes (23%) and cryptomonads (18%) (Brown et al. 1997). The content of saturated fats in microalgae can also be improved by culturing under high light conditions (Thompson et al. 1993).

The amino acid composition of the protein of microalgae is very similar between species (Brown 1991) and relatively unaffected by the growth phase and light conditions (Brown et al. 1993a, b). Further, the composition of essential amino acids in microalgae is very similar to that of protein from Sydney rock oyster larvae (*S. glomerata*; see Fig.2). This indicates that it is unlikely the protein quality is a factor contributing to the differences in nutritional value of microalgal species.
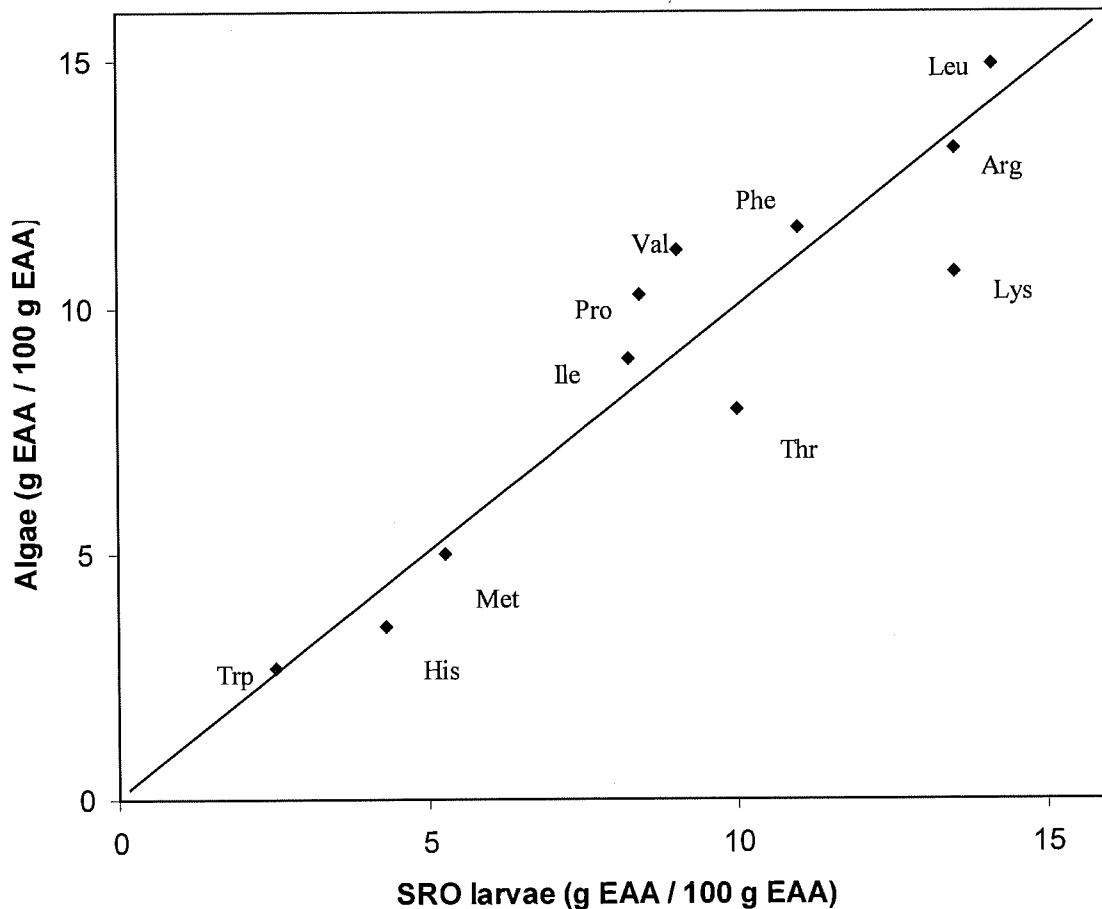
Figure 2. Comparison of the % composition of essential amino acids (EAA) in Sydney rock oyster larvae, compared to a mix (1:1:1; dry weight) of *Isochrysis* sp. (T.ISO) / *P. lutheri* / *C. calcitrans*. SRO data from Brown et al. (1996).

Sterols (Knauer et al., 1999), minerals (Fabregas and Herrero 1986) and pigments also may contribute to nutritional differences of microalgae.

The content of vitamins can vary between microalgae. Ascorbic acid shows the greatest variation, i.e. 16-fold (1 to 16 mg g^{-1} dry weight; Brown and Miller 1992). Concentrations of other vitamins typically show a two- to four-fold difference between species, i.e. $\square$-carotene 0.5 to 1.1 mg g^{-1}, niacin 0.11 to 0.47 mg g^{-1}, $\square$-tocopherol 0.07 to 0.38 mg g^{-1}, thiamin 29 to 109 µg g^{-1}, riboflavin 25 to 50 µg g^{-1}, pantothenic acid 14 to 38 µg g^{-1}, folates 17 to 24 µg g^{-1}, pyridoxine 3.6 to 17 µg g^{-1}, cobalamin 1.8 to 7.4 µg g^{-1}, biotin 1.1 to 1.9 µg g^{-1}, retinol $\leq$ 2.2 µg g^{-1} and vitamin D < 0.45 µg g^{-1} (Seguineau et al., 1996; Brown et al., 1999). To put the vitamin content of the microalgae into context, data should be compared with the nutritional requirements of the consuming animal. Unfortunately, nutritional requirements bivalves are, at best, poorly understood. However, the requirements of the aquacultured species (e.g. marine fish and prawns; Tacon 1991; Conklin 1997) are better known and in the absence of other information, can serve as a guide for bivalves. These data suggest that in general, a carefully selected, mixed-algal diet should provide adequate concentrations of the vitamins for aquaculture food chains.

There is a recognition that egg quality and ensuing larval vigour are influenced by the broodstock diet, but there are few examples where this has been documented experimentally on a biochemical basis. In a recent case, Caers et al. (1999) compared the composition of eggs from broodstock scallops (*Argopecten purpuratus*) fed either a standard algal mix (*Isochrysis* sp. (T.ISO), *P. lutheri, C. gracilis, T. suecica*) or the same diet supplemented with emulsions containing PUFAs. Supplementation with a DHA-rich emulsion significantly increased both the content and percentage of DHA in eggs, and also the fecundity of the broodstock.

In summary, carefully selected mixtures of microalgae can provide an excellent nutritional source for bivalves. Most microalgae have adequate levels of the essential fatty acids (i.e. EPA and/or DHA) to support animal growth, but information on absolute requirements are still poorly defined. There is evidence that the nutritional value of algae can be improved by increasing their percentages of saturated fatty acids and carbohydrate. This can be achieved by culturing algae under high light conditions and/or harvesting during stationary phase. The protein quality of algal species appears to be high. Vitamin concentrations in algae are variable, and can be manipulated by culture conditions – but algal mixtures are likely to fulfil animals' requirements. More research is needed to assess whether egg quality and larval vigour can be improved by manipulation of broodstock diets.

References

Brown, M. R., 1991. The amino acid and sugar composition of 16 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology, 145: 79-99.

Brown, M. R. and Miller, K. A., 1992. The ascorbic acid content of eleven species of microalgae used in mariculture. Journal of Applied Phycology, 4: 205-215.

Brown, M. R., Barrett, S., Volkman, J. K., Nearhos, S., Nell, J. and Allan, G., 1996. Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. Aquaculture, 143: 341-360.

Brown, M. R., Dunstan, G. A., Jeffrey, S. W., Volkman, J. K., Barrett, S. M. and LeRoi, J. M., 1993a. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis* sp. (clone T-ISO). Journal of Phycology, 29: 601-612.

Brown, M. R., Garland, C. D., Jeffrey, S. W., Jameson, I. D. and Leroi, J. M., 1993b. The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T.ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. Journal of Applied Phycology, 5: 285-296.

Brown, M. R., Jeffrey, S. W., Volkman, J. K. and Dunstan, G. A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture, 151: 315-331.

Brown, M. R., Mular, M., Miller, I., Trenerry, C. and Farmer, C., 1999. The vitamin content of microalgae used in aquaculture. Journal of Appliedfiscus Phycology, 11: 247-255.

Caers, M., Coutteau, P., Cure, K., Morales, V., Gajardo, G. and Sorgeloos, P., 1999. The Chilean scallop *Argopecten purpuratus* (Lamarck, 1819): II. Manipulation of the fatty acid composition and lipid content of the eggs via lipid supplementation of the broodstock diet. Comp. Biochem. Physiol., B., 123: 97-103.

Conklin, D. E., 1997. Vitamins. In: Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, D'Abramo LR, Conkin DE and Akiyama DM (eds), World Aquaculture Society, 1997, pp. 123–149.

Enright, C. T., Newkirk, G. F., Craigie, J. S. and Castell, J. D., 1986a. Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros calcitrans* Schütt of varied chemical composition. Journal of Experimental Marine Biology and Ecology, 96: 15–26.

Enright, C. T., Newkirk, G. F. Craigie, J. S. and Castell, J. D., 1986b. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. Journal of Experimental Marine Biology and Ecology, 96: 1-13.

Fabregas, J. and Herrero, C., 1986. Marine microalgae as a potential source of minerals in fish diets. Aquaculture, 51: 237-243.

Harrison, P. J., Thompson, P. A. and Calderwood, G. S., 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. Journal of Applied Phycology, 2: 45–56.

Heasman, M., Diemar, J., O'Connor, W., Sushames, T. and Foulkes, L., 2000. Development of extended shelf-life micro-algae concentrate diets harvested by centrifugation for bivalve molluscs – a summary. Aquaculture Research, 31: 637-659.

Jeffrey, S. W., LeRoi, J.M. and Brown, M. R., 1992. Characteristics of microalgal species for Australian mariculture. In: G. L. Allan and W. Dall (Editors), Proceedings of the National Aquaculture Workshops, Pt. Stephens, NSW Australia, April 1991, pp. 164-173.

Knauer, J., Barrett, S. M., Volkman, J. K. and Southgate, P. C., 1999. Assimilation of dietary phytosterols by Pacific oyster *Crassostrea gigas* spat. Aquaculture Nutrition, 5: 257-266.

Knauer, J. and Southgate, P. C., 1999. A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. Reviews in Fisheries Science, 7: 241-280.

Nell, J. A. and O'Connor, W. A., 1991. The evaluation of fresh algae and stored algal concentrates as a food source for Sydney rock oyster *Saccostrea commercialis* spat. Aquaculture, 99: 277-284.

Nell, J.A., Diemar, J.A. and Heasman, M.P., 1996. Food value of live yeasts and dry yeast-based diets fed to Sydney rock oyster *Saccostrea commercialis* spat. Aquaculture, 145: 235-243.

Numaguchi, K. and Nell, J. A., 1991. Effects of gelatin-acacia microcapsule and algal meal supplementation of algal diets on growth rates of Sydney rock oyster *Saccostrea commercialis* (Iredale & Roughley) larvae. Aquaculture, 94: 65-78.

O'Connor, W. A. and Heasman, M.P., 1997. Diet and feeding regimens for larval doughboy scallops, *Mimachlamys asperrima*. Aquaculture,158: 289-303.

O'Connor, W. A., Nell, J. A. and Diemar, J. A., 1992. The evaluation of twelve algal species as food for juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). Aquaculture, 108: 277-283.

Parsons, T. R., Stephens, K. and Strickland, J. D. H., 1961. On the chemical composition of eleven species of marine phytoplankters. Journal of the Fisheries Research Board Canada, 18: 1001–1016.

Renaud, S. M., Thinh, L.V. and Parry, D. L., 1999. The gross composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture, 170: 147-159.

Sargent, J. R., McEvoy, L. A. and Bell., J. G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture, 155: 117-127.

Seguineau, C., Laschi-Loquerie, A., Moal, J. and Samain, J. F., 1996. Vitamin requirements in great scallop larvae. Aquaculture International, 4: 315–324.

Tacon, A. G. J., 1991. Vitamin nutrition in shrimp and fish. In: Akiyama DM, Tan RKH (eds), Proc. of the Aquaculture Feed Processing and Nutrition Workshop, Thailand and Indonesia, September 1991, American Soybean Association, Singapore, pp. 10-41.

Thompson, P. A., Guo, M.X. and Harrison, P. J., 1993. The influence of irradiance on the biochemical composition of three phytoplankton species and their nutritional value for larvae of the Pacific oyster (*Crassostrea gigas*). Marine Biology, 117: 259-268.

Volkman J. K., Jeffrey S. W., Nichols, P. D., Rogers, G. I. and Garland, C. D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology, 128: 219-240.

Webb, K. L. and Chu, F. E., 1983. Phytoplankton as a food source for bivalve larvae. In: G. D. Pruder, C. J. Langdon and D. E. Conklin (Editors), Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition, Louisiana State University, Baton Rouge, LA, pp. 272–291.

Whyte, J. N. C., Bourne, N. and Hodgson, C. A., 1989. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. Aquaculture, 78: 333-347.

Summary of discussion session and additional comments

Q1   What are the most critical algae nutrient constituents?

As discussed in my address vitamins, protein, essential amino acids and HUFA's (EPA or DHA) are not likely to be important limiting constituents within micro-algae diets for oyster larvae. Key factor influencing larval growth are the simple easily digested and assimilated saturated fats in particular C14:0 and 16:0 that are promoted by high ambient light conditions).

Q2   To what extent have effects of SRO broodstock nutrition and health on the quality and viability of their eggs and larvae been explored?

This line of research was planned and initiated in 1995 in collaboration with Dr Paul Southgate (James Cook University) and again in 1998 as a collaborative project with Drs Kevin Williams and Frances de Sousa (CSIRO) in conjunction with the SRO mass selection breeding operations. However on several occasions during 1995 and again in 1998 and 1999, sampling of larvae for biochemical analysis was abandoned. This occurred when batches were either wiped out entirely by the anorexia syndrome or decimated to the extent that sampling was viewed as a threat to producing minimum numbers of spat required to perpetuate each of six genetic lines being propagated.

Q3   Could there be some adverse factors associated with seawater be retained in micro-algae cultures and transferred to larvae?

This could well be the case but we have no practical method of detecting or quantifying, let alone removing, such factors.

NB. Use of artificial seawater to culture algae or use of algae concentrates rather raw algae cultures, constitute simple ways of reducing or even eliminating risks of natural toxins in seawater being conveyed to larvae via food. Use of artificial seawater to propagate algae would also have the advantage that it eliminates the need to chlorinate seawater, a process that does generate chloramines and bromoforms byproducts that are potentially toxic to larvae.

Q4   Can micro-algae themselves become toxic under certain culture conditions?

Yes this can occur as illustrated by variable toxicity of red tides but more importantly *Pavlova lutheri* has been shown to develop anti appetite factors at certain stages within bloom cycles.

Q5   *What are the most critical nutrient reserves of first feeding stage D veliger and for pediveligers needed to ensure successful settlement and metamorphosis?*

According to research on other bivalves, lipid reserves of eggs are critical indicators of viability for both early first feeding stage larvae and for competent pediveligers (larval quality).

NB. Simple neutral red staining techniques and relative density criteria successfully developed to evaluate egg quality in scallops in Europe were tried but could not be readily adapted when developing improved protocols for larvae-culture of the Tasmanian scallop, *Pecten fumatus*. However as discussed in consultation with Dr Peter Thompson, such techniques warrant further investigation in concert with comprehensive quantitative and qualitative biochemical assays including analyses of lipid, carbohydrate, protein/amino acids and PE ratios. This entire topic needs to be pursued beginning with a comprehensive review of the literature.

Q6   *As CSIRO are carrying collections of around 600 species of micro-algae, why do you only use six for larval and spat rearing?*

Results of our own extensive R&D on SRO larval and spat diets are that a ternary diet of equal amounts of *Chaetoceros calcitrans, Pavlova lutheri* and Tahitian *Isochrysis* optimises growth and survival of the larvae of SRO. These results have been corroborated by similar findings for the larvae of other bivalves including clams, mussels and scallops. On the other hand, best diets for juvenile SRO's and scallops (*Pecten fumatus and Chlamys asperrima*) comprise binary or ternary diets containing one or two species of diatom, especially *Skeletonema costatum, Thalassiosira psuedonana* or *Chaetoceros calcitrans,* in combination with a *Tetraselmis* spp. Having said this, the ternary diet of *Chaetoceros calcitrans, Pavlova lutheri* and Tahitian *Isochrysis* is often fed to SRO post larvae at PSFC and may be considerably less than optimal.

NB. Another important issue is that different species of algae produced at hatcheries are grown using common rearing equipment, lighting levels, growth media and temperature, (23°C in the case of PSFC) that promote reasonable rates of production for all species. Very few if any commercial hatcheries can afford to routinely propagate more than 2 to 6 species of micro-algae to satisfy all requirements.

Q7   *What is the natural diet and relative nutritional status of wild planktonic SRO larvae?*

This work simply has not been done in relation to SRO's, so the short answer is we don't know. What is most important to know is the relative biochemical composition and nutrient reserves of comparable wild and hatchery reared larvae. Lipid profiles including those of fatty acid would be the most useful in deducing whether or there were major discrepancies between the diets of wild and cultured larvae and, if thought necessary, how these differences might be reduced by changing hatchery diets. Gut content analyses can also be used as an indicator of diet although these are extremely difficult, costly and results are equivocal in that gut residues are often dominated by poorly digestible less important components of the diet.

NB. In subsequent general discussion it was noted considerable research on the season and location of SRO larvae in Port Stephens by the late Dr John Holliday and by Stephen McOrrie (NSWF) could serve as a very useful platform for such studies.

Q8   *Could adductor muscle myopathy associated with gaping and death in mass spat mortality episodes be induced by selenium deficiency within micro-algae diets or is selenium acquired directly from seawater?*

I don't know the answer but possible dietary deficiency is very easily overcome by boosting selenium levels within algae culture media?

Q9   *Why are saturated fats especially 14:0 and 16:0 a better dietary lipid source than unsaturated fats?*

According to Dr Peter Thompson, this is because breakdown of unsaturated fats especially HUFA's, including EPA, is much more complicated and protracted. Moreover, HUFA's yield lower amounts of available energy than do saturated fats. In other words, once relatively small amounts of essential HUFA's needed to satisfy dietary requirements are met, digestible energy yield becomes the most important aspect of quality of dietary lipids.

REVIEW OF HYGIENE, EQUIPMENT AND PROTOCOLS

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The successful production of quality oyster larvae requires four main elements. These are good quality fertilized eggs, an abundance of high quality water, abundant food followed by a suitable settlement substrate.

The acquisition of good quality fertilized eggs is outside the scope of this paper but experience has shown that many hatchery "crashes" can be attributed to the quality of the fertilised eggs rather than the equipment used or any subsequent handling.

Inlet water quality and equipment composition (plastics in particular) can adversely affect production in ways that are very difficult to detect since mollusc larvae are highly sensitive to pollutants and contaminants usually at levels far below the detectable level. High quality oceanic water is highly desirable and careful attention to the composition and 'weathering' of new or replacement equipment, including wash-down hoses, is essential.

Some form of hatchery hygiene is also essential, whatever the level of production and sophistication of the operation, to achieve quality spat and cost-effective production. This requires a high level of filtration of incoming water to remove other larvae and most bacteria; anyone who has had to clean barnacles or sabellid tube worms out of hatchery pipework will appreciate that point! There is a linear relationship between the number of particles in water and the number of bacteria in the water (Kobler & Boller 1997). This is brought about by the adhesion of bacteria and viruses to particle surfaces. Thus the fewer the number of particles, the fewer the number of pathogenic microorganisms and thus the lower the risk of a successful infection being established.

Increasingly, environmental concerns are also driving requirements to monitor or filter discharge water as well –from concern over a variety of issues including release of disease agents, genetic pollution and antibiotic residues. Discharge water filtration is a complex issue and will not be covered further in this paper.

A separate set of hygiene protocols surrounding the propagation of axenic algal culture has evolved in the industry and these are routinely practiced whatever the mollusc species under cultivation.

A major issue in hatcheries is the management of the bacterial flora. The influence of the bacterial flora in both the main hatchery water supply, the tanks and in the algal culture facility occurs in three ways:– by direct bacterial invasion of larvae and spat; by the secretion of bacterial exotoxins, including proteinases and ciliostatic toxins which are pathogenic to larvae (Nottage & Birkbeck 1987, 1990; Nottage et al. 1989, Riquelme et al. 1996); and by a complex interaction between the bacterial species comprising the flora itself (Yamamoto et al. 1982a,b). Storage of seawater also has a major impact on the composition of the bacterial flora.

Management of bacteria is achieved by a combination of filtration, a high level of internal cleanliness in the hatchery to avoid introduction of biofilms and introduction of pathogenic bacterial strains, "drying out" periods (Sorgeloos 1995) and more recently through use of probiotics (the subject of a separate paper at this workshop). The use of antibiotics is both unnecessary and is being increasingly proscribed by legislation.

References

Kobler, D., Boller, M., 1997. Particle removal in different filtration systems for tertiary wastewater treatment - a comparison. Water Sci. Technol., 36: 259-267.

Nottage, A.S., Birkbeck, T.H., 1987. The role of toxins in *Vibrio* infections of bivalve mollusca. Aquaculture, 67: 244-246.

Nottage, A.S., Birkbeck, T.H., 1990. Interactions between different strains of *Vibrio alginolyticus* and haemolymph fractions from adult *Mytilus edulis*. J. Invert. Pathol., 56: 15-19.

Nottage, A.S., Sinclair, P.D., Birkbeck, T.H., 1989. Role of low molecular weight ciliostatic toxins in vibriosis of bivalve molluscs. J. Aquat. Anim. Health, 1: 180-186.

Riquelme, C., Toranzo, A.E., Barja, J.L., Vergara, N., Araya, R., 1996. Association of *Aeromonas hydrophila* and *Vibrio alginolyticus* with larval mortalities of scallop (*Argopecten purpuratus*). J. Invert. Pathol., 67: 213-218.

Sorgeloos, P., 1995. Bioengineering of hatcheries for marine fish and shellfish. J. Mar. Biotec., 3:42-45.

Yamamoto, H., Ezura, Y., Kimura, T., 1982. Effects of antibacterial action of seawater on the viability of some bacterial species. Bull. Jap. Soc. Sci. Fish., 48: 1427-1431.

Yamamoto, H., Ezura, Y., Kimura, T., 1982. Evaluation of biological agents affecting on the survival of *Vibrio parahaemolyticus* in seawater. Bull. Jap. Soc. Sci. Fish., 48: 1433-1439.

Summary of discussion session and additional comments

Q1   What measures have you put in place to prevent movement of people and equipment between different parts of the hatchery?

The high and varied demand on the hatchery for commercial production of Sydney rock oysters spat of and associated experimentation largely precludes separation let alone quarantining of particular operations. Various operations include mass selection breeding and ploidy research operations involving SRO's, year-round pearl spat production and associated research, production of large commercial batches pacific and flat oyster spat and year round production of algae. Approximately half of algae is used to produce rotifers used as live food for the production of marine finfish.

Having said this, a number of modifications to hatchery buildings, equipment and operations have been introduced over recent years. Dedicated equipment is retained within specific areas of the hatchery. The main larval rearing tanks were physically isolated from the rest of the hatchery with the erection of a full floor to ceiling dividing wall in 1996. A chemical footbath to the larval rearing area is maintained during the course of hatchery rearing operations and access to the room is strictly limited to hatchery staff.

Q2   When you sample larvae do you use new pipettes and screens on each occasion?

Pipettes are not used and miniature sampling screens are sterilized between uses to minimise risks of transmitting infective agents.

Q3   *What additional observations and sampling protocols should be made documented in the future?*

Routine collection and appropriate preservation and archiving of eggs, larvae and spat, is very cheap and easy to implement> It is therefore sensible to impose mandatory collection and preservation of samples coincident to water changes every 2 to 4 days for all future all future hatchery runs. Such material could prove invaluable for confirming or denying involvement of infective agents, especially viruses and for better identifying subtle pathological changes at the tissue and cellular level.

NB. Cryo-preserved samples initially frozen in liquid nitrogen and stored in a –80C freezer can be used 'a postiori' to characterise and contrast nutritional profiles of successful and unsuccessful batches while absolute ethanol preserved fixed larva are suitable for the application of PCR and ELISA probes to demonstrate or dismiss the involvement of viruses.

Q4   *How confident are you that 1 micron depth filters remove all bacteria from rearing water?*

Depth filters are not designed nor intended to remove all bacteria absolute using membrane filters. To the contrary, 1 micron filtration is intended only to reduce total bacteria to acceptably low overall levels 0f 10^2 to 10^3 /mL by removing suspended matter with which surface attaching bacteria are associated. Indeed removal of all bacteria that requires filtration to 0.2micron absolute has a serious well documented negative influence on the ability of seawater to support good health and normal growth of marine bivalve larvae.

NB. In the course of ensuing general discussions the possibility of non biological sources of toxins was raised. In one case cited by Jonathan Bilton in relation to SRO larval rearing operations in Albany WA, the source of such toxins were in one case traced to a length of non food grade hose. In another case cited by John Mercer, compounds released by non food grade O rings prevented scallop larvae from developing to first feeding "D" veligers.

Q5   *How often is the hatchery completely dried out?*

Continuous year round demand on the hatchery for algae for finfish live food production prevents dry out of the algae area that has only been shut down once in 14 years. The rest of the hatchery is generally subject to an annual dry out that has been over Christmas /New year in recent years.

Q6   *What procedures do you follow to flush and steriliser your seawater lines?*

Seawater lines are thoroughly flushed each morning to ensure that anaerobic or stale seawater is fully purged. However complete sterilisation of the plumbing system from the storage tanks generally occurs only in conjunction with dry outs. Sodium hypochlorite solution is used for this process and it is recognised that to remove accumulated organic slimes that acid/alkaline digestion should be used in conjunction with dismantling and physical cleaning of valves and filter housings. An acknowledged and important limitation of the plumbing system is the subterranean pipe-work between the external pump-house and the hatchery.

Q7   *What procedures do you follow in annual dry-out and disinfections?*

In most years the entire lower floor but not upper algae production floor of the hatchery is cleaned, chemically disinfected and dried out for a minimum period of two weeks. Over the past 3 years pearl or abalone stock have been maintained in the constant temperature room on the lower floor to cater for temperature related experimentation or brood-stock conditioning. As algae production as a source of food for rotifers used in turn as live food for marine fin-fish (snapper, mulloway and

Australian Bass) continues year round the main storage tanks, pumps and plumbing delivery system are not subject to dry-out. Indeed these facilities have been dried out on only one occasion since 1988.

It is also pertinent that a successful hatchery runs in the absence of larval anorexia and the generation of multimillion batches of post-larvae are almost invariably followed by spat mass mortality episodes. Indeed since the commissioning of the bivalve hatchery at PSFC, only two commercial scale batches of spat have been produced as a result of the absence of both larval anorexia and spat mass mortality. The first was the inaugural large-scale batch in July to September 1988 and the second in Jan to March 2001. The latter coincided with a particularly thorough dry-out over the Christmas-New Year period that was aided by low humidity heat wave conditions and use of industrial extractor fans.

NB. These issues raise the need for separate controlled temperature experimental facilities for mollusc research and or a separate hatchery module(s) either at PSFC or elsewhere such as the Tomaree Point abalone facility that has direct and continuous access to high quality coastal seawater.

Q8   What are the characteristics of stored seawater?

Results of our own investigations (and of others) on the micro-flora of stored seawater indicates that over a period of 7 to 10 days there is a shift in total bacteria counts from 10^3 to 10^4/mL to much lower numbers of 10^1 to 10^3 /mL. Over the same period relatively high (10^1 to 10^3 mL) counts of presumptive *Vibrio* spp. fall to low or non detectable levels (<10^0 mL). Over the same period the huge bulk of suspended matter is settled out thereby greatly reducing loads applied to serially arranged depth filters in the hatchery. However as raised by Ralph Elston, bacteria may generate toxins when in decline and the act of storage could inadvertently be promoting such events.

NB. As discussed earlier there is no sure way of evaluating the quality of seawater for rearing larvae SRO other than performance of larvae themselves using some other sources of seawater as a control treatment. Nor is there any simple inexpensive process to remove a massive spectrum of potentially harmful compounds such as bacterial or algal generated toxins, heavy metals, etc.

Q9   John Mercer posed the question "the data you presented suggests that probability of the SRO larval disease increases with successive runs between dry outs. This contrasts with 7 years experience in Canada that showed that many consecutive batches of scallops clams and oysters could be generated between annual 2 month dry outs with no apparent detrimental effects. How do you account for this disparity?

There are at least two issues here. The first is that regular alternation between several species could act as a type of crop rotation thereby preventing accumulation or increased virulence of pathogens that are commonly species specific. The second is that successive batches of pearl oysters, Pacific oysters, flat oysters as well as SRO's are also commonly produced at PSFC free of problems i.e.; our problems are entirely restricted to SRO's.

NB. All other commercial hatchery operators present at the workshop agreed that performance of consecutive batches of the same species generally declines hence the use of regular planned hatchery dry-out and disinfection is regarded as a fundamental hatchery management tool. Likewise, consecutive batches of pearl oyster larvae produced at PSFC are often observed to exhibit diminished growth following a succession of hatchery production runs especially if combined with use of brood-stock that been held in conditioning systems over protracted periods.

Q10   Can the absence of anorexia in small scale experimental vessels be explained by the fact they are conducted in different remote areas or following dry outs?

Small-scale experimental rearing operations have been conducted in the main in the upstairs algae production area or within temperature controlled shaker incubators located remote to the hatchery building.   However experiments are initiated with first feeding stage D veligers spawned, fertilised, incubated and on-reared for 24hours collectively with other larvae in the lower hatchery. Moreover the same source of food and seawater is generally used for experimental and large scale rearing operations. It is possible that the larvae reared in large scale (1000 to 20000L) vessels being heated via direct contact submersible titanium heaters electrical circuits subject to peak demand power shedding may be subject to greater temperature fluctuations and low temperature spikes but other differences are difficult to pinpoint.

NB. There is an interesting parallel between SRO and mud crab (*Scylla serrata*) larvae in that in both can be routinely reared without significant disease problems in small but not large vessels. In the case of mud crabs, this is clearly related to Vibrio bacterial factors that can be nullified with use of appropriate antibiotics.   From this perspective, an alternative larviculture technology developed for commercial hatchery production of pacific oysters needs to be evaluated in relation to SRO larval anorexia.   These so called "Bayes'systems" employ very high larval stocking densities of 100-400 larvae/mL and small volume (100- 300L) cylindro-conical rearing vessels. While both the Bayes System and the large (1000 and 20000L) static flat bottom tank systems used at PSFC operate on a similar overall seawater budgets, they contrast in that Bayes systems are continuously supplied with high flow rate temperature equilibrated seawater injected with algae to maintain food at minimum concentrations required for satiation feeding. Moreover microalgae used in Bayes Systems is produced semi-continuously and uses pasteurised rather than chlorinated/de-chlorinated seawater. Plans to trial the Bayes System at PSFC with SRO larvae in 1996 were developed with two hatchery staff being sent to SABDEV at Port Lincoln in South Australia for training in its set-up and operation. Unfortunately however practical evaluation of the system for rearing SRO larvae was not implemented.

PROBIOTICS

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Introduction

Disease outbreaks have become a significant constraint on mollusc production, affecting the economic development of this sector in many countries. Conventional treatments have been in the use of antibiotics and disinfectants, but the abuse of antibiotics for disease control in aquaculture has led to the development of resistance. Today, the use of antibiotics is less frequent in mollusc culture, and alternatives such as the use of probiotics have become more favourable.

Probiotics are beneficial bacteria that improve the survival of the host organism by modifying and improving the microbial community; enhancing immune response; or providing nutritional value.

This current investigation deals with two organisms, *Aeromonas media* and *Vibrio alginolyticus*. The former is a brackish water species, shown by Gibson *et al* 1998, to protect the survival of *Crassostrea gigas* larvae when challenged with *Vibrio tubiashii*. *In vitro*, A. *media* produces Bacteriocin-Like-Inhibitory-Substance (BLIS), which inhibits the growth of a number of aquatic bacteria including vibrios.

Vibrio alginolyticus was used as a pathogen challenge in the initial trial, but it was found to have some beneficial properties, and hence, lead to further investigations.

Results

Trial 1.   (Refer to Figure 1)

The aim of this trial was to investigate the survival and growth of *S. glomerata* larvae over 10 days. The conditions tested were: sterile versus 1 μm filtered seawater; the presence or absence of the following: (i) *V. alginolyticus* (challenge); (ii) *Derxia* sp. (food bacteria) and (iii) *A. media*.

The results showed the average percent survival and growth of *Saccostrea glomerata* larvae were highest in treatments reared in sterilised (autoclaved) seawater inoculated with *V. alginolyticus*. The mean +/-S.E range was between 53% to 68% for survival and 104μm to 114μm for growth (Figure 1).

In contrast, larvae reared in conventionally treated seawater (stored and filtered to 1μm) without *V. alginolyticus* showed a mean survival and size between 4% and 23% and 92μm to 93μm respectively.

In the absence of *V. alginolyticus,* there was a significant difference in mean survival between those reared in sterile seawater (22%) to those reared in 1μm-filtered seawater (12%). Furthermore, the size of *S. glomerata* larvae was also significantly higher in sterilised seawater treatments (between 106μm to 112μm) than non-sterilised seawater (97μm to 99μm).

The results suggest that the effect of sterilising seawater (autoclaving) and the addition of *V. alginolyticus* may enhance *S. glomerata* larval survival and growth.

In vitro (diametric streak) tests of *V. alginolyticus* showed no evidence for any zones of clearing, but showed signs of competition as it overgrow the normal flora despite it having lower initial counts.

Trial 2.   *(Refer to Figure 2)*

The aim of this trial was to investigate the survival and growth of *S. glomerata* larvae in differentially treated seawater over 10 days. The treatments included: autoclaved, 1 um filtered; UV-treated; 0.2 um filtered; pasteurised at 65'C and 85'C; chlorinated; carbon activated; and finally chlorinated and carbon activated seawater.

In contrast to trial 1, highest *S. glomerata* larval survival and growth for the pre-water treatment trial was reported from the 1μm filtered seawater and without *V. alginolyticus* with mean survival and growth of 60% and 59μm respectively. In contrast, autoclaved seawater and the presence of *V. alginolyticus* had 23% survival and growth of 44μm over 10 days.

The mean *S. glomerata* larval survival for treatments with *V. alginolyticus* was significantly lower in all but 3 treatments (0.2μm, U.V and pasteurization at 85°C).

Chlorinated treatment and activated carbon and chlorinated treatment showed high mean larval survival, but growth was compromised. This may be due to chlorine residues in the water.

The results from the pre-water treatment trial showed the control treatment in the absence of *V. alginolyticus* were more favourable for *S. glomerata* larvae survival and growth.

Trial 3.   *(Refer to Figure 3)*

The aim of this trial was to determine if *V. alginolyticus* (Val1) and *A. media* has the ability to provide protection to *S. glomerata* larvae when challenged with the pathogen *Vibrio tubiashii*. A second strain of *V. alginolyticus* (Val 2) was used as a control.

The results showed the presence of *A. media* and *V. alginolyticus* (Val 1) significantly improved survival of *S. glomerata* larvae when challenged with *Vibrio tubiashii*. In the presence of the Val 1 and *A. media*, survival of *S. glomerata* larval improved by 34% and 29% respectively.

The second strain of *Vibrio alginolyticus* (Val 2) showed similar trend as the control

Trial 4.   *(Refer to Figure 4.1 and 4.2)*

To determine the survival of *S. glomerata* larvae when challenged with *V. tubiashii*. The larvae were treated with *V. alginolyticus* and *A. media* at three different concentrations (10^2, 10^3, 10^4).

The optimum density of *A. media* and *V. alginolyticus*, which produced highest mean survival for *S. glomerata* larvae when challenged with *V. tubiashii* were between 10^3 and 10^4 cfu/mL. Survival of larvae treated with 10^3 and 10^4 cfu/mL of *A. media* was 48% and 50% respectively, while *V. alginolyticus* had 47% and 42%.

The mean larval survival for *A. media* at 10^2 cfu/mL was 21%, and *V. alginolyticus* at the same concentration was 30%.

Trial 5.   (Refer to Figure 5.1 and 5.2)

The aim was to determine the effects of *A. media* and *V. alginolyticus* on *Pavlova lutheri* survival over 5 days.

It is critical that the probiotics of interest does not have any adverse effect on the host's food source. Three species of algae were tested, *P. lutheri, T. iso,* and *Isoschrysis galbana,* on the two probiotics, *A. media* and *V. alginolyticus.*

The results for the algal species *P. lutheri* will be discussed below (the results for the remaining two algal species showed a similar trend to *P. lutheri*).

Aeromonas media's density decreased during the first 24 hours, but increased from day 2 to day 5 by 10 folds. Similarly, *P. lutheri*'s density also increased over the 5 days from 4.54×10^6 to 4.65×10^6. The decrease in algal density on day 2 may have been the result of sampling error.

Vibrio alginolyticus density increased by 100 folds over the 5 days from 2.5×10^4 to 1.8×10^6, while the algal density increased by 1.9% from 4.54×10^6 to 4.63×10^6.

The results showed that *V. alginolyticus* and *A. media* does not have any effect on the growth or survival of the algae species, nor did the algae had any effect on the growth of the bacteria.

Trial 6.   (Refer to Figure 6.)

The aim of this experiment was to determine the potential of the probiotics *A. media* and *V. alginolyticus* in a large-scale trial.

The results from this trial found that both probiotics when administered separately and in combination did not improve the growth of *S. glomerata* larvae, with the symptoms of anorexia appearing on day 4. The mean growth rate over the first 4 days was 13, 12, 11 and 8μm for the control, *A. media, V. alginolyticus* and combination treatment respectively. The mean growth of larvae from day 4 to 7 was 2, 3, 1 and 3μm for control, *A. media, V. alginolyticus* and combination treatment respectively.

Results from previous successful runs showed the mean shell height at day 7 to be approximately 115μm (results taken from Heasman *et al.,* (2000) - Improved early survival of Molluscs: Sydney Rock Oyster (*Saccostrea glomerata*) page 12, Figure 5).

Several microbial species including *Psuedomonas* sp, *Aeromonas* sp and *Vibrio* sp. was isolated from the control water sample, however the bacterial counts were too low (less than 10^2) to suggest any of them could have been the causative agent.
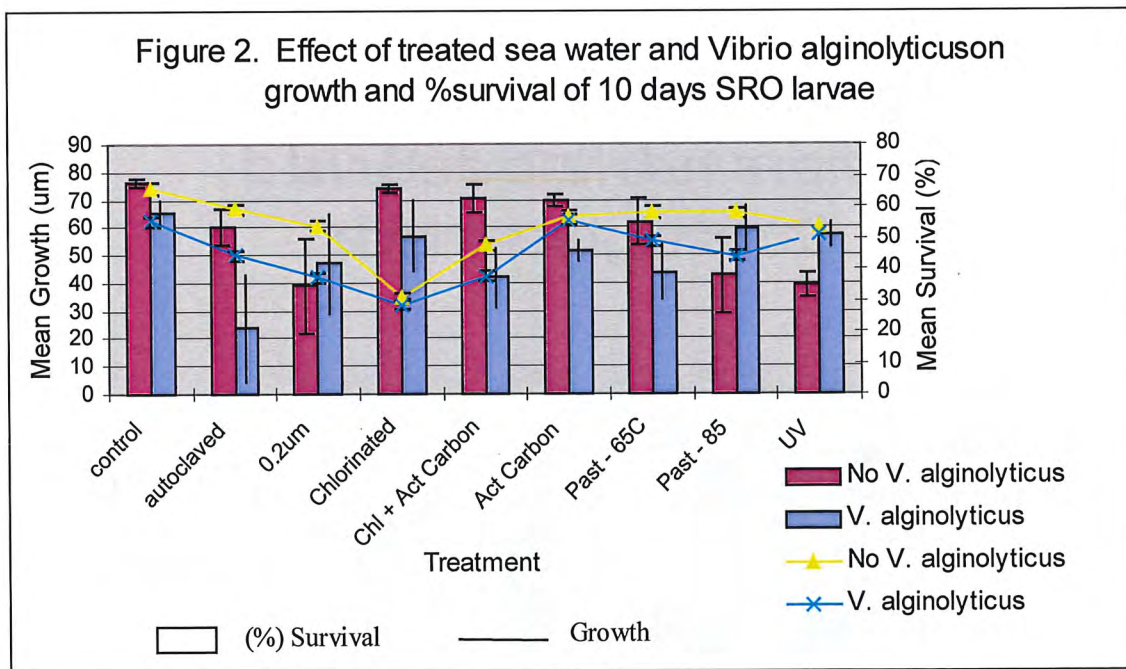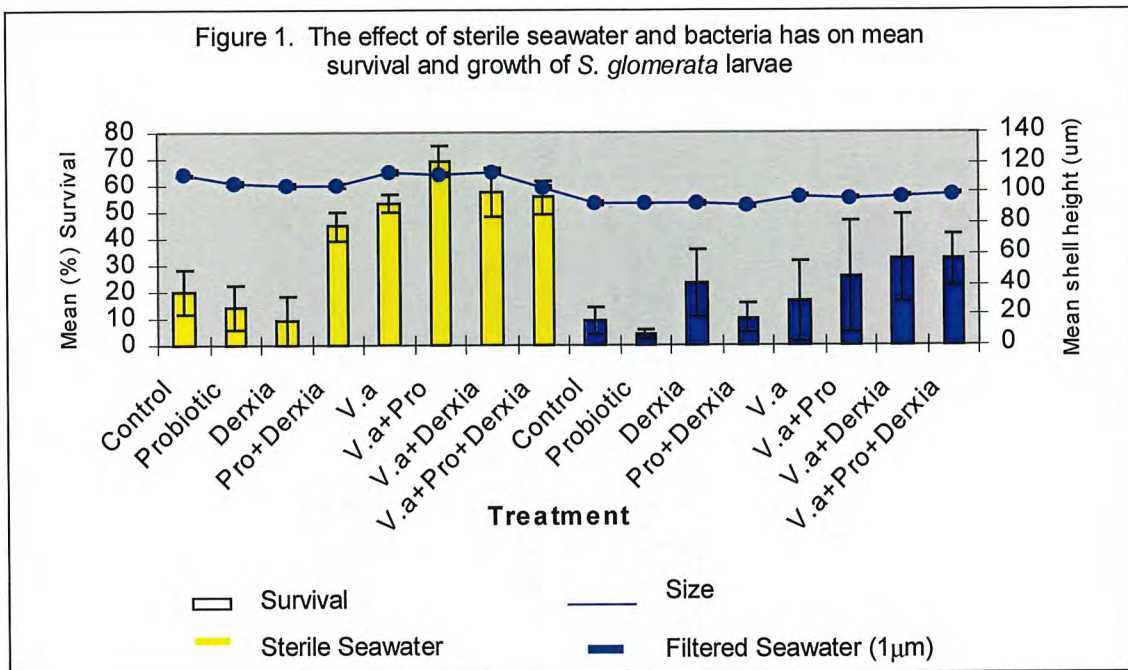
Figure 1. The effect of sterile seawater and bacteria has on mean survival and growth of *S. glomerata* larvae

Figure 2. Effect of treated sea water and Vibrio alginolyticuson growth and %survival of 10 days SRO larvae

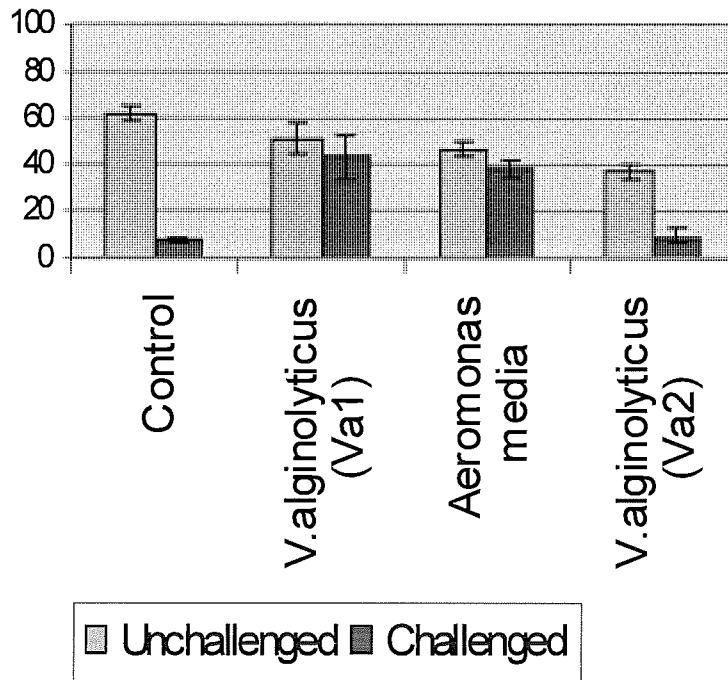Figure 3. Effect of Vibrio alginolyticus and Aeromomas media has on the survival of S.R.O larvae challenged with Vibrio tubiashii

Figure 4.1 Effect of 3 different density of *Aeromonas media* has on the Survival of *Saccostrea glomerata* larvae
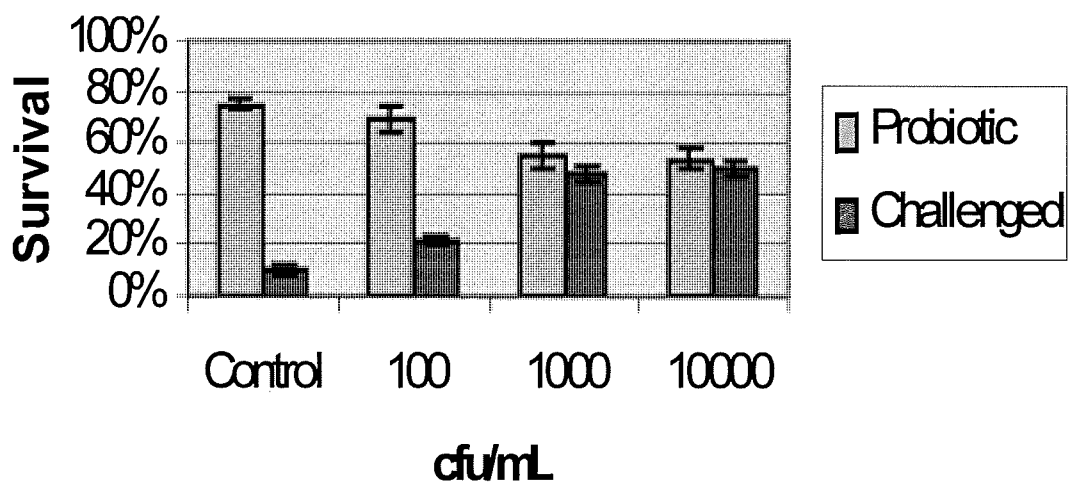
Figure 4.2 Effect of 3 different density of *Vibrio alginolyticus* has on survival of *Saccostrea glomerata*
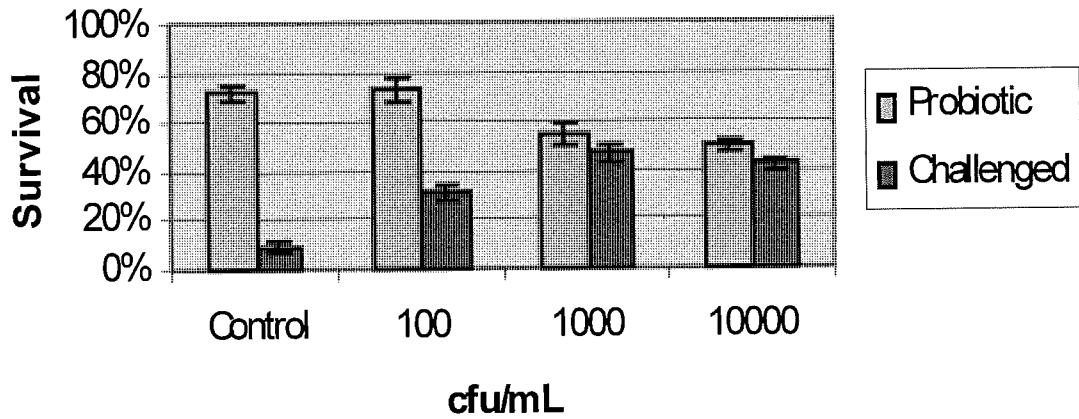

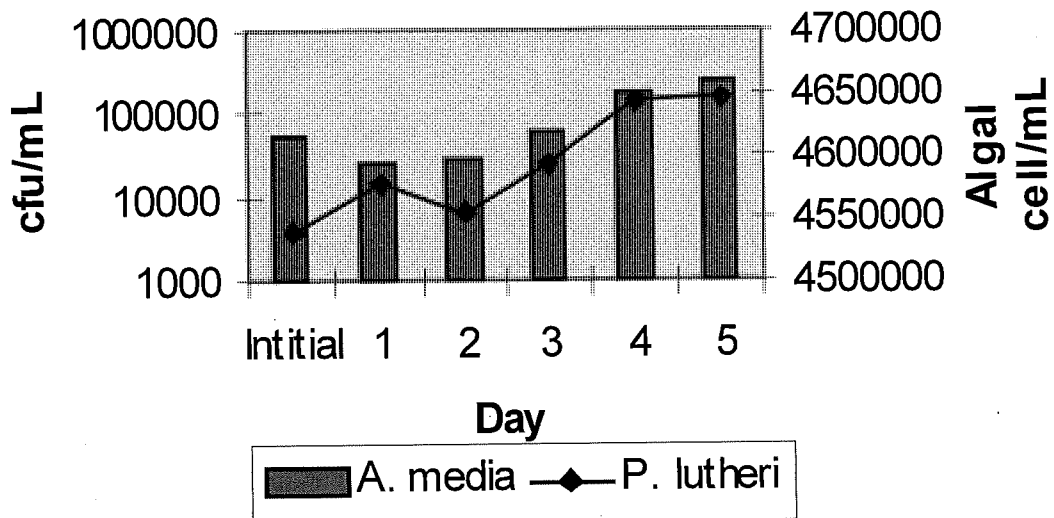Figure 5.1. Effect of *A.media* has on *P.lutheri* density over 5 days.

Figure 5.2 Effect of *V.alginolyticus* has on *P. lutheri* density over 5 days.
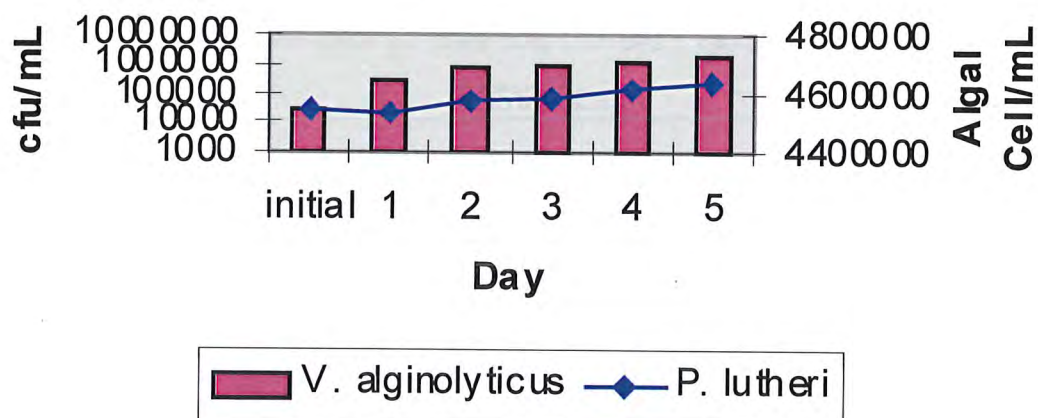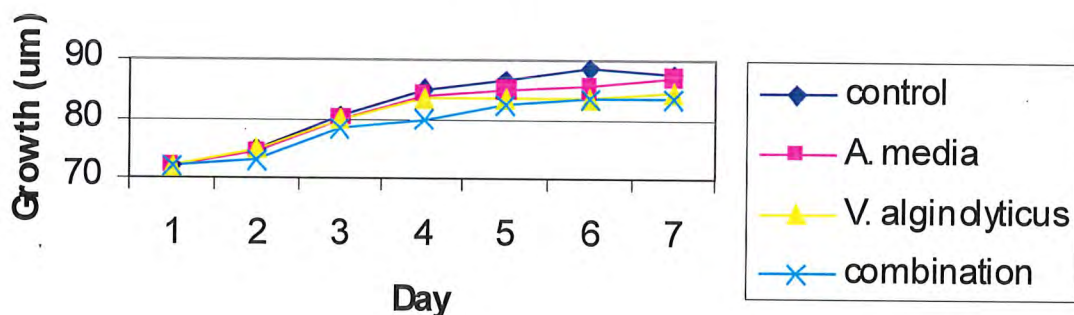

Figure 6. Effect of potential probiotics on growth of S. glomerata larvae affected by anorexic like problem

Summary of discussion session and additional comments

Q1   Do the two strains of bacteria act probiotics or are they simply providing supplementary nutrition?

Probiotic properties of single or multiple strains of bacteria embrace any and actual or potential negative effects or other organisms on the host organism in this case SRO larvae. Such actions thus include competitive exclusion of other organisms such as competition for surfaces and substrates through to release of antibacterials properties.

MASS MORTALITY SYNDROME IN LARVAL AND JUVENILE SYDNEY ROCK OYSTERS:

# AN EPIDEMIOLOGICAL PERSPECTIVE

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The larval and juvenile mass mortality syndromes have been a major and ongoing problem in an otherwise successful 10 year breeding program to produce fast-growing, disease-resistant Sydney Rock oysters *(Saccostrea glomerata)* (Heasman et al, 2000). Similar problems have not been experienced in other major oyster species, including the Pacific oyster. A range of strategies, including appraisal of epidemiological information, has been used with the aim to address and overcome both of these diseases.

Larval mortalities

Key epidemiological features of this syndrome include:
- Presentation, with signs suggestive of anorexia, at day 2-8 (and most frequently 3-5) of culture, and complete mortality by d14.
- A marked seasonal variation in incidence, with the syndrome being more common in the first half of the year.
- A close association between dry-out and disinfection, with incidence increasing with time (and number of batches) since previous dry-out and disinfection.
- A strong association with tank size, with the syndrome occurring more frequently in 1,000L as compared to 20,000L tanks.
- Association with Sydney Rock oysters and a related native rock oyster, but not with many other bivalves commercially-produced at the PSFC hatchery.
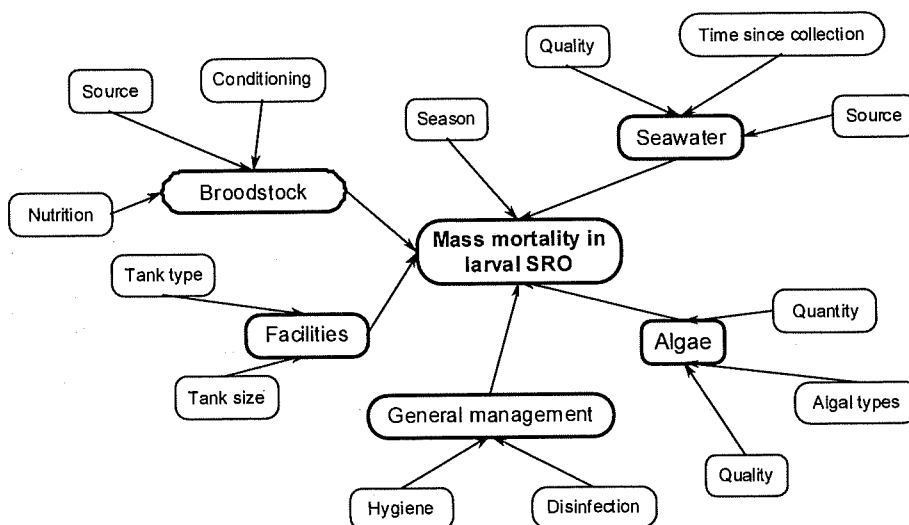
In common with most disease syndromes relating to modern agri- and aquaculture, it is likely that the syndrome is multifactorial, and to involve a range of factors relating to different phases of oyster production. Indeed, on the basis of information presented at the workshop, the following factors may all be linked with the syndrome:

There is some evidence in support of infectious involvement in this syndrome, particularly the association with time since dry-out and disinfection. However, to ensure that this association is not spurious, it is important that available data are re-analysed to test that the association between run failure and time since dry-out/disinfection is not confounded by the effect of season. Although histopathology is equivocal, and despite clear evidence against the possibility of vertical transmission, it is still possible based on expert opinion that key aetiological agents may have been overlooked. A range of methodologies from experimental epidemiology (including transmission trails, field trials and laboratory studies) are available which may assist in testing potential causes from the above causal web. The views of industry as expressed during the workshop, and particularly concerns relating to seawater quality and the general hatchery environment, need to be addressed in association with any such work.

In theory, additional information could be obtained by conducting a risk factor study to identify difference between failed and successful runs with respect to components of the putative causal web shown previously. A 'run' would be considered the unit of interest in any analysis. In practice, however, there are only a very limited number of runs each year. Consequently, there may be insufficient runs (units of interest) to provide the analysis with sufficient statistical power to detect differences if in fact they are present.

Spat mortalities

Key epidemiological features of this syndrome include:
- Wide variation in the time of presentation, with first signs of disease being seen from 7 to 43 days after settlement.
- Conflicting information about the spectrum of signs in affected batches, including an all-or-none presentation [with affected spat dying rapidly, whereas surviving spat (in the same tray) are apparently-unaffected (and do not exhibit a growth check)] and histopathological results suggesting varying pathology in both clinically and apparently-unaffected individuals.
- Confinement to spat below 2mm shell-height.
- Mortality events associated with recent management changes.
- Mortality relating to the location of spat in the settling trays.
- No seasonal trend.

Again, the syndrome is probably multifactorial. Furthermore, given the association with recent management events, it is likely, as illustrated below, that a non-infectious "trigger" is an important contributor to these mortalities.
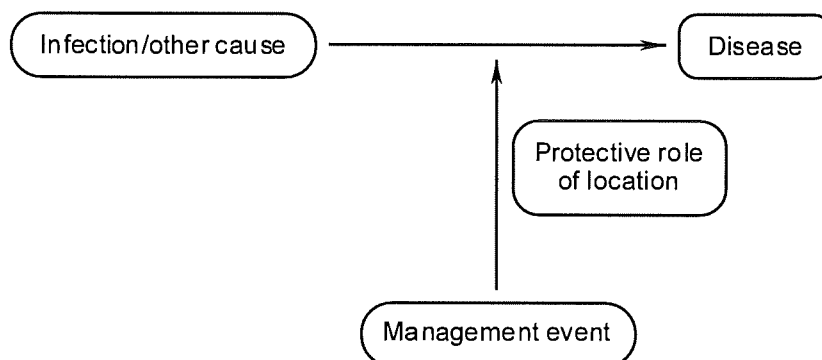
A critical evaluation of the association of mortality with location is also likely to provide a clue with respect to causation. For reasons not readily apparent, spat located on slats and the sides of each tray appear at much lower risk of mortality than spat in other locations.

Although a defined aetiological agent has not yet been identified, it is important to note that there may be opportunities for effective control of this syndrome by modifying or removing the non-infectious trigger(s) that appear related to management events and with location. Experimental studies, possibly relating to permutations of a proposed 'low trauma' system, would enable defined hypotheses to be rigorously tested. In common with previous comments, risk factor studies about spat mortalities may be of limited value if there are only a limited number of runs with available data.

References

Heasman, M.P., Goard, L., Diemar, J. & Callinan, R.B., 2000. Improved early survival of molluscs: Sydney Rock Oyster (*Saccrostrea glomerata*). Aquaculture CRC Project A.2.1, December 2000. NSW Fisheries Final Report Series No. 29, pp. 63. NSW Fisheries, Nelson Bay.

DIGESTIVE HEALTH OF OYSTER SEED

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This is a very brief summary of the presentation that will be made. Handouts of the presentation slide will also be available at the workshop or upon request of the author.

Oyster seed going off feed may be a sign of the presence of noxious or toxic bacterial or algal products in the water. The digestive gland of very young oyster seed is visible through the shell (at least for *Crassostrea* spp. and *Ostrea* spp.) and should be darkly pigmented from the consumption of algal cells. Rating of the digestive gland condition, based on histological analysis can be useful in assessing seed condition:

> The height of the digestive gland epithelium is rated as (1) **high** (indicating normal active metabolism and ingestion), (2) **medium** (indicating a condition at the lower end of the normal range and an animal at risk from insufficient nutrition), (3) **low** (a pathological condition indicating insufficient nutrition or a toxic dietary effect, but a recoverable condition) and (4) **very low** (a distinctly pathological condition indicating insufficient nutritional intake or a toxic dietary effect, that may be unrecoverable in some cases).

Exfoliation of the digestive gland epithelial cells in oyster seed appears, in some cases, to be associated with the presence of toxic algal cells. Limited experimental work in this area suggests that some species of *Prorocentrum* may cause such an effect but more extensive work on a variety of toxic algal species is needed. Severe exfoliation of digestive gland epithelium results in a metaplastic change (alteration in cell type or morphology) in the digestive gland and is probably an irreversible condition.

An example of oyster seed deployed to Tomales Bay in California shows that the digestive gland exfoliation can occur very rapidly (over a period of one week) and lead to nearly 100% mortality in seed oysters.

Bacterial exotoxins may also cause digestive gland lesions. Ciliostatic toxins from bacteria would be expected to cause a reduction or cessation in feeding and ingestion as well as other motility functions of the digestive tract. In several cases, we have found that ground oyster shell used as cultch material was a substrate for and harboured a variety of microorganisms that appeared to be ingested by the oyster. Oyster seed in this condition did not normally ingest available algal feeds and failed to grow normally.

Gymnodinium splendens (*sanguineum*) and *Ceratium fusus* were associated with larval Pacific oyster mortalities in bioassays. Using predominant but mixed species colonies of these species from field collections, we were able to reproduce the larval oyster mortalities. However, bacteria isolated from the field collections of algae also produced similar mortality rates in Pacific oyster larvae.

Gymnodinium aureolum produced a loss of digestive gland cell height in 1 of 8 species tested (*Argopecten irradians*).

Prorocentrum minimum is reported to caused digestive gland effects and poor growth in *Mercenaria mercenaria* (hard shell clam) as well as a mortality response in bay scallops (*Argopecten irradians*).

Alexandrium sp. and *Gymnodinium* sp. are reported to produce a differential effect on clearance rate in different species of oysters.

Heterocapsa circularisquama has been associated with mass mortalities of pearl oysters and manila clams (*Venerupis (=Tapes) japonica*) in Hiroshima Bay and other locations around Japan.

It is clear that additional and definitive studies are needed to identify bivalve toxin strains of algae and to link the algal strains to specific pathologic effects.

Summary of discussion session and additional comments

Q1   Is there any information on the occurrence of toxic alge blooms in the Port Stephens region that could have a bearing on the larval anorexia syndrome?

The only toxic algae bloom information we are aware of is shellfish poisoning in pippies on Stockton Beach. How such blooms or associated toxins released into the seawater might affect SRO larvae is unknown.

Q2   Are there any recognised cheap, easy and universally effective precautionary methods of removing suspected toxins and heavy metals from coastal seawater?

Ralph Elston stated that he was unaware of any hatcheries that routinely processed seawater to remove potential toxins. Mike Heasman reported that passage of incident seawater through activated charcoal filters on several occasions but without tangible effect on the occurrence or severity of anorexia episodes.

NB. According to Prof. Gustaaf Hallegraeff (University of Tasmania) possible involvement of algal toxins may be complex and very difficult to prove. An example he quotes is " the dreaded Pfiesteria", where algal cells never have been found to contain endocellular toxins, but these fractions only appear in the seawater and very complex chemistry is needed to adsorb them onto charcoal and elute them off. Commenting in relation to a mass kill of farmed SRO's in Wonboyn Lake in February and March 2002, Prof. Hallegraeff commented, "The possibility that culture strains only produce toxins under special (stressed) conditions can also not be excluded. We are pursuing this with sensitive bioassay experiments co-culturing *Prorocentrum minimum* and a very sensitive alga Chattonella which is readily destroyed by *P. minimum* allelochemicals."

Q3   If naturally occurring toxins in collected seawater are involved in the anorexia syndrome then either simple, cheap methods will need to be devised to routinely rid seawater clean of such compounds, or alternatively, readily available chemical free sources of seawater will need to used to rear larvae. Three possible sources are:

- artificial seawater,
- chemically suitable inland saline water such as potassium supplemented oceanic salinity seawater available from Wakool,
- subterranean coastal saline water pumped from wells or spear points on the land-ward sides of coastal dunes,

Q4   *What about surfaces of algae providing added substrate for bacteria especially Vibrio spp that are usually algae associated?*

Bacteria on surfaces of micro-algae food probably play a large part in determining the composition of enteric micro-flora during the first few days of feeding.

NB. This certainly true in the case of rotifer fed marine fish larvae and successful commercial hatchery production of turbot and other difficult to rear northern hemisphere species has occurred through better control of enteric bacteria. This draws attention back to the probiotic research of Cheok Tan that has demonstrated potential use of probiotic bacteria laden micro-algae food that in enabling SRO larvae to be successfully reared in sterilised seawater. This in turn may open the way for use of artificial and inland saline seawater thereby avoiding complex and difficult to detect appetite-suppressing toxins that may sometimes be present in coastal seawater.

STRATEGIES FROM A COMMERCIAL PERSPECTIVE

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Increase priority of SRO program.
Remove conflicts and compromises with other research needs.

Suggested Commercial Hatchery Changes

Algal system

- Change to continuous flow production system.
- Removes chlorination in bags.
- Continuous flow feeding (trickle feeding), improved digestion.
- Increase diatom level to 50%.
- Increase second diatom (3H)?
- Have period of no feeding?
- Examine nutrient levels for certain species, toxic residues.
- Never feed concentrates except spat with flow through.
- Improve natural light availability in combination with fluorescent.

Note: No commercial operator would build an algal system like the one use at Port Stephens

Broodstock system

- Reduces egg quality variability.
- Enables continuous production runs.
- Domestication reduces stress and mortality.
- Hold in field for regeneration to improve nutrient composition.
- Feed broodstock multiple species.

Spawning

- No obvious changes.

Larval Culture (Ensure procedural repetition)

- Drop tank as soon as D stage reached.
- New tanks to reduce chance of bacteria in cracks and blisters.
- Tanks size, small with high flow through 200 l.
- Tanks size, large with low flow through 20,000 l +.
- Flow through daily. Trial and error rates.
- Temperature increase?
- Constant temperature.

- Remove stainless screw.
- Remove metal supports.
- Ensure effective cleaning of all seawater components.

Seawater system

- Continuous seawater flow.
- No static storage of water.
- Instantaneous heating system (elec, gas).
- No sand filters some other alternative (disc, drum, etc) 20u.
- Filter @ larval tank 1-5u????
- Carbon trials site specific.

Settlement

- Epinephrine use, multiple treatments if necessary.
- Trickle feeding during post set.
- Flow through during settlement.

Spat grow out (500u)

- Management hire commercial operator to refine system.

> **Requires lots of little trials whilst running standard hatchery protocol**
> **Resource the ability to carry out trials**

Commercial Options for Trial

- New site with good water.
- Deep-water intake 10-20m, good quality bore, offshore site.
- Trial at other existing site which have not been trialled (Sea AGG).
- Business plan for potential hatchery re demand for spat (Is it worth it for a commercial operator).
- Staff on production incentives.

Summary of discussion session and additional comments

Q1   *Have any other hatcheries in been able to produce SRO and avoid or overcome the larval and spat mortality diseases?*

The Albany hatchery in WA has been successfully producing commercial batches of spat over the past 3 or 4 years without having apparently encountered either disease. However there are a number of confounding issues as follows:
- The hatchery is located about 500km south of the nearest natural SRO stocks and therefore remote to diseases that might be present in the wild.
- Broodstock are sourced from these remote stocks to the north.
- The genetic relationship between SRO in NSW and Western Australian stocks is uncertain.
- The Albany hatchery is only operated for a limited period and over a small number of cycles involving only SRO. Prior to decommissioning the algae and hatchery rearing systems are dismantled, physically cleaned, chemically disinfected and left to dry over the entire off-season each year.

Q2 *Your experimental results suggest that higher rearing temperatures than 23 or 24 ℃ may be beneficial. Have you or other hatcheries used higher temperatures for rearing SRO's and if so, what were the results?*

Our adherence to conservative rearing temperatures of 23-24°C have been based on a concern that higher temperatures might promote more rapid proliferation of pathogenic bacteria. However systematic in depth investigation of effects of temperature on larvae and potentially pathogenic bacteria as described by Dr Elston in relation to pacific oyster operations in one USA hatchery, has not been attempted for SRO's to date.

NB. Jonathan Bilton, former manager of the Ocean Foods International hatchery at Albany, WA, commented that they routinely incubated and reared "Western rock oyster" larvae at 27°C not 24 °C and had not experienced larval anorexia nor spat mortality problems over the past 5 annual hatchery seasons.

WHERE TO FROM HERE (STRATEGIES)

G. Allan

NSW Fisheries, Port Stephens Fisheries Centre
Taylors Beach, NSW, 2316, Australia

1   Document and record meeting
- ORAC.
- Key Issues R&D.
- Strategy.

2   Working group to consider strategy, e.g.
- Best guess solution at PSFC.
- Best guess solution at Tomaree.
- Research application.
- Histopathology from existing samples.
- *Bonamia* (winter mortality) for broodstock translocation.
- Larval biosecurity protocols.
- Routine sampling and archiving for pathology.
- Farmer participation in settlement/nursery.
- Broodstock conditioning – nutrition.
- Water storages.
- Temperature (larval rearing).
- Import to NSW – SRO from Western Australia.
- Genetics of SRO population – VIC / NSW / WA.
- Biochemical nutritional profiles.

    - Broodstock   Hatchery
    - Larvae   vs
    - Spat   Wild

    _____

    + Algae

D Strategies
- Other hatcheries EOI.
- Import WA stock to NSW.
- New hatchery in NSW.
- Change to facilities at PSFC and protocols → Commercial recommendations.
- New operators at PSFC.

R Strategies
- Bacterial (assoc) problems with larvae and spat.
- Trials to generate observational data – to generate hypotheses.
- Broodstock nutrition.
- Continuous water – new hatchery.
- Continuous examination of old data to generate hypotheses.

Simple possible problems
- Water storage.
- Too much organic material – where from?

- Pumps, pipes (dead spots, anaerobic pockets).
- Tanks.
- Temperature.

To help EOI
- Separate broodstock and eggs.
- Biosecure hatchery.
- Tasmania
  - Risk of larval problems
  - Risk of broodstock
    - QX (PCR)
    - Perkinsis
    - Winter Mortality (PCR)
  - Biosecurity – water etc
  - Contaminant/treatment/disposal

Action
Brian Jones and Judith Handlinger to 'draft' conditions likely to make importation of SRO possible.

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APPENDICES

Appendix 1.   **Frankish, K. R., Goard, L. J. and O'Connor, W. A. (1991). The development of hatchery rearing techniques of the Sydney rock oyster (*Saccostrea commercialis*).**

THE DEVELOPMENT OF HATCHERY REARING TECHNIQUES OF THE SYDNEY ROCK OYSTER (*SACCOSTREA COMMERCIALIS*)

**at the Brackish Water Fish Culture Research
Station, Salamander Bay, NSW, 2301
K. R. FRANKISH, L J. GOARD and
W.A. O'CONNOR
1991**

CONTENTS

1 SUMMARY

This report reviews the development of hatchery techniques for the Sydney rock oyster (*Saccostrea commercialis*) at the BWFCRS between 1970 and 1990, and outlines current hatchery practices and areas where future research is required. Technical information is based on the personal observations of the authors, their fellow researchers at the BWFCRS and the available literature.

Broodstock were selected from mature wild stock when available or conditioned out of season using a closed recirculating system. Oysters were held at 22-24°C and reached sexual maturity in 4-8 weeks on a diet of 3×10^9 algal cells per oyster per day. Spawning was induced through thermal stimulation (max 28°C) combined with a salinity reduction of up to 10g/kg.

Optimal larval rearing temperature was 24-25°C, and salinity of 30-35 g/kg. Egg stocking density varied from 2-15/mL, with the highest larval retention of 50% at 2/ml. Eggs developed to D-stage larvae in 16 h, eyed larvae in 13 days and pediveligers in 16-18 days. Mean pediveliger retention of the best 5 batches was 46.14 ± 9.15%

Larvae were fed a mixed algal diet of Tahitian *Isochrysis* aff. *galbana, Pavlova lutheri* and *Chaetoceros calcitrans.* Each larvae was fed according to size, with the optimum feed rate rising from 7,500 to 32,000 cells/larvae/day by the time larvae were ready to set. Pediveliger larvae were transferred to a setting unit utilizing scallop shell chips as a setting substrate. Setting larvae were held at 24-260 and fed a mixed algal diet at a rate of $50\text{-}60\times10^3$ cells/larvae/day. Spat were separated from the scallop shell and held in a closed recirculating system at 22-24°C until 700 μm in size. Final grow out to sale size (4-6mm) was achieved in passive outside upwellers.

Spat survival has been as high 77% but was often poor. Severe mortality (80-90%) of juvenile spat (<1 mm) in nursery systems is a recurring problem and is the main factor limiting commercial production in NSW. Despite these problems commercial quantities of spat have been produced. Between November 1988 and January 1989, 11.3×10^6 Spat were sold to 34 farmers from 16 NSW estuaries.

The hatchery at the BWFCRS ceased commercial Sydney rock oyster spat production at the end of 1989 and is now solely a research facility.

2 INTRODUCTION

The Sydney rock oyster (*Saccostrea commercialis*) is farmed using the traditional stick and tray system from eastern Victoria through most estuaries and rivers in New South Wales to Moreton Bay in Queensland. By far the majority of production comes from New South Wales, where the industry is currently worth $A28 million, employs 3,000 people and produces around 8 m dozen annually (Nell, 1992 unpublished data).

Seed stock for the Sydney rock oyster industry is available through natural spatfall and is commonly caught on tarred sticks. Until recently 70% of all sticks used for this purpose were deployed in Port Stephens (Malcolm, 1987). However, the advent of new catching substrates (Holliday et at., 1988) and governmental regulations, to contain the spread of the Pacific oyster (*Crassostrea gigas*) (Holliday and Nell, 1990), has encouraged farmers to use other estuaries to obtain their replacement stock.

The traditional methods of on-growing the catch has involved a manual grading or 'culling' process that significantly contributes to the overall cost of production (Marshall & Espinas, 1987, Espinas et al, 1988). This cost may be avoided by producing single seed oysters from a hatchery or by using 'scrape off' technology from natural spatfall (Holliday et al., 1988). Single seed production, which enables the use of mechanical graders, is gaining acceptance with many farmers and provides a product attractive to processors.

Attempts to rear the Sydney rock oyster began at the BWFCRS in the early 1970's (Wisely, 1974), with the aims of supplying an alternative source of spat and to selectively breed faster growing disease resistant oysters. Techniques were adapted from those used in other countries, but progress was slow until the construction of a pilot scale oyster hatchery in 1981. In 1986 a commercial scale hatchery was designed (referred to here as the 'hew hatchery') and was commissioned in 1988.

Bivalve rearing technology at the BWFCRS has been successful in rearing commercial quantities of Sydney rock oysters, the commercial scallop (*Pecten fumatus*), and the blue mussel, (*Mytilis edulis planulatus*), as well as small quantities of the native flat oyster (*Ostrea angasi*) and the Pacific oyster (*Crassostrea gigas*).
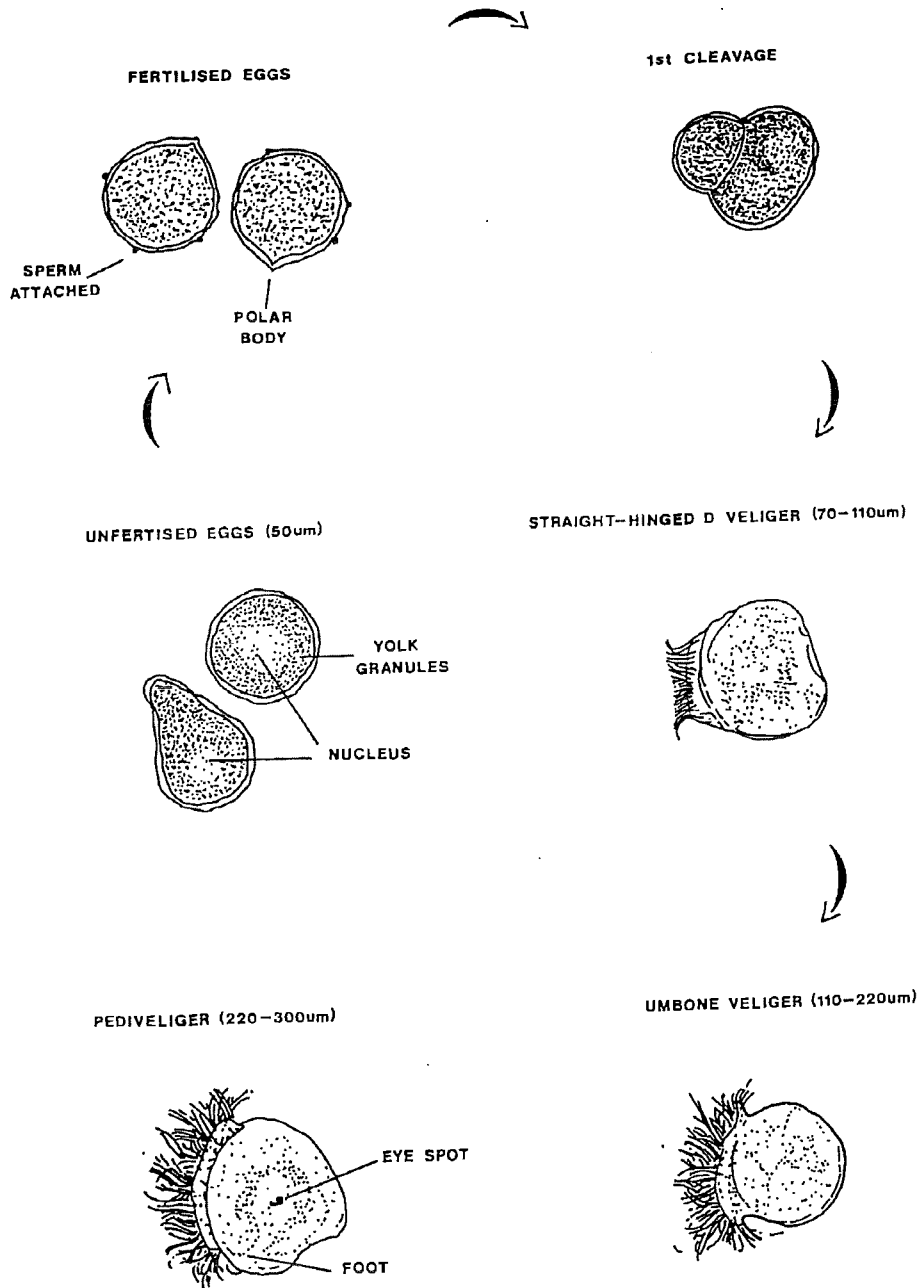
3 BIOLOGY

Sydney rock oysters are bivalve molluscs, hermaphrodites, and filter feeders. After a resting phase during winter sexual development is triggered by increasing water temperatures. Fecundity in the Sydney rock oyster is high, with the female capable of producing in excess of 20 million eggs per spawning, while the male produces many times this number of spermatozoa. Gametes from both males and females exit the gonad via genital pores and empty into the exhalant chamber above the gills. In males, sperm leaves the oyster in a steady stream with the exhalant water. The females spawning mechanism is more complex, with eggs passing from exhalant to the inhalant chamber, where contraction of the adductor muscle ejects the eggs approximately every 12 sec (Walne, 1974; Quayle and Newkirk, 1989). Upon release mature eggs have a characteristic pear shape due to crowding in the ovarian follicle (Fig 1), however after a short time in seawater these eggs take on a rounder shape. Light microscopy reveals the nucleus as a large transparent area surrounded by densely packed yolk granules. Many sperm may attach to the egg membrane but usually only one penetrates to complete fertilization (Fig 1). The egg then contracts; assuming a perfectly spherical shape, and the cytoplasm becomes so dense that the nucleus is no longer visible. The appearance of polar bodies depends on water temperature and the quality of the eggs, but at 25°0 the first should appear within 20-30 min and the second after 45-60 min post fertilization (C.J. Mason - pers. comm., 1989). At this temperature embryonic development continues to the stage of a free swimming trochophore in about 6 h, at which time the polar bodies are shed. The hinged D-veliger appears 16 h post fertilization, denoted by two equal prodissiconch shells.

In the hatchery healthy larvae show signs of food consumption by day 2 (shell length 78µm) and by day 5 (108 µm) the gut should be dark brown in colour. Young larvae consume particles smaller than 10 µm in size, but the maximum size that can be eaten increases slightly as the larvae grow (Wisely and Reid, 1978). Early umbone development; the protuberances of the shell on the straight hinge line (Quayle and Newkirk, 1989), is apparent by day 6 (120 µm) and as development continues become more prominent. Eyespots are apparent at around day 12 or 13 (220 µm) and soon after the foot can be seen through the transparent larval shell (Fig 1). As early as day 16 (280 µm) larvae may be seen extending a foot from the shell while swimming. The presence of these pediveligers indicates that setting and metamorphosis will soon occur. The length

of the larval cycle depends on environmental factors (Calabrese and Davis, 1970; Walne, 1974), but by day 18-20 (300 µm) the majority of pediveligers will be exploring the substratum preparatory to settlement.

When a suitable substratum is found the pediveliger exudes a secretion from the byssus gland and then turns so that the left shell valve is pressed against the secretion which hardens rapidly (Green, 1968). The newly settled spat undergo some major changes during metamorphosis, including the resorption of the velum and foot, degeneration of the anterior adductor muscle, absorption of the eye spot, some relocation of major organs and the commencement of more rapid shell growth of the dissoconch (Galstoff, 1964). Favorable environmental conditions will allow the Sydney rock oyster to reach market size (50 g) in three to four years.

Figure 1   Development of the Sydney rock oyster, *Saccostrea commercialis,*
            from egg to mature pediveliger.

FERTILISED EGGS

1st CLEAVAGE

SPERM
ATTACHED

POLAR
BODY

UNFERTISED EGGS (50um)

STRAIGHT-HINGED D VELIGER (70-110um)

YOLK
GRANULES

NUCLEUS

PEDIVELIGER (220-300um)

UMBONE VELIGER (110-220um)

EYE SPOT

FOOT

4   ALGAE

Attempts to culture algae at the BWFCRS began in the early 1970's, with the development of a system using 20 L glass carboys and 250 L fiberglass drums. It was not until 1979 that the forerunner of the current algal system was introduced. Using a 200 L vessel similar to that developed at Conwy (Helm et al., 1979) a system was designed incorporating sixteen 200 L vessels and ten 1000 L polyethythene bins. Although constantly evolving, this system remained in use until late 1987 when a number of changes were made in preparation for the transition to the new oyster hatchery (Fig 2).
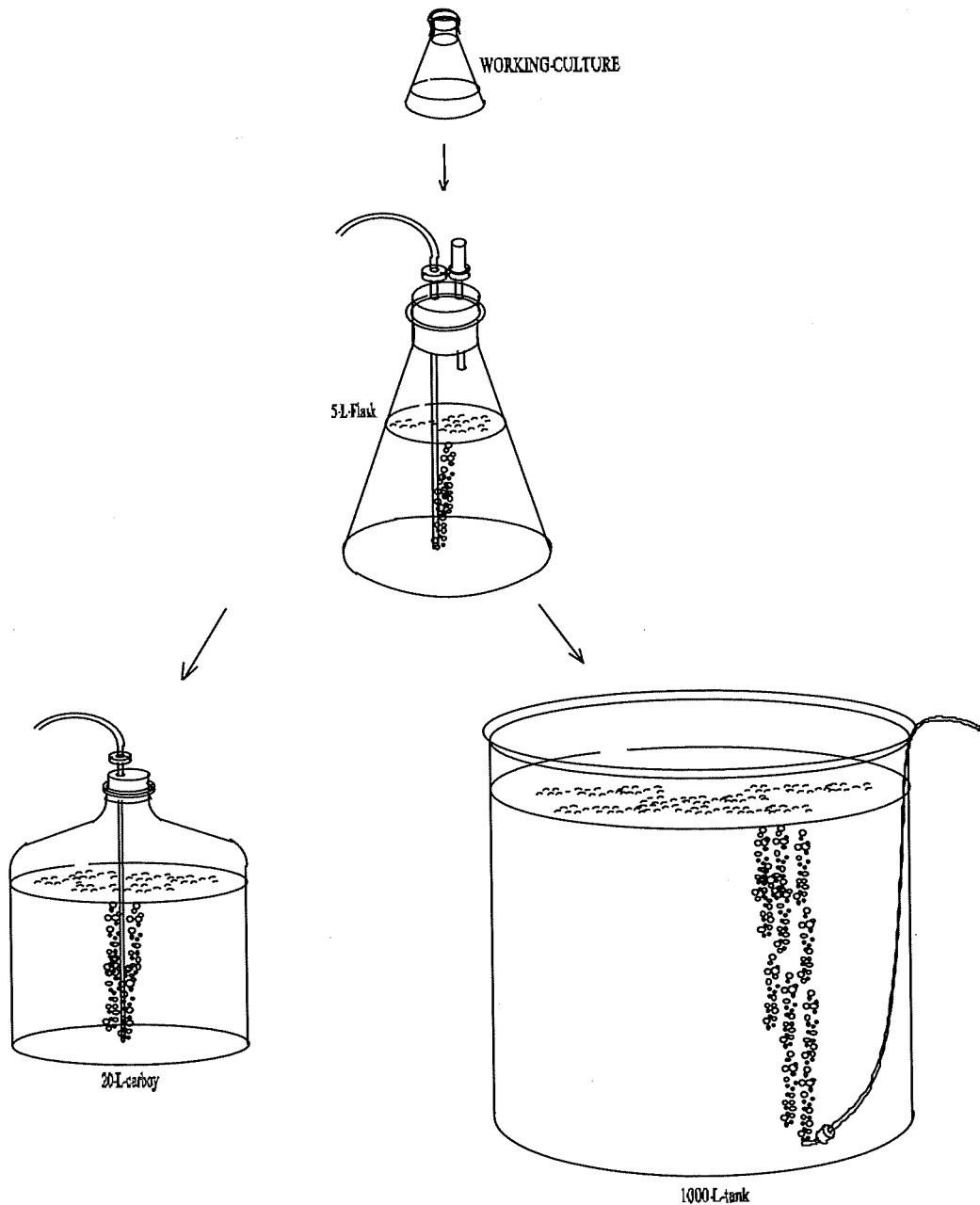
Figure 2   New hatchery algal production process

4.1 Seawater treatment

Estuarine water was collected at high tide and passed through a high pressure sand filter followed by 10 and 1 μm nominal cartridge filters. Water to be used in the stock and working cultures was passed through a further 0.45 μm cellulose acetate membrane filter prior to autoclaving.

To reduce bacterial loads in 20 L, 200 L and 1000 L cultures, sufficient sodium hypochlorite was added to maintain a residual chlorine reading of 10 mg/kg for a period of at least 4 h. Prior to the addition of any algal inoculum the chlorine was neutralised with a 1 M sodium thiosulphate solution.

With the move to the new hatchery, water filtration has been simplified using only high pressure sand filters and 1 μm cartridge filters. The introduction of increased water storage capacity has allowed coastal seawater to be trucked in, overcoming problems associated with the variability of local estuarine water quality. Chlorination still remains the primary means of bacterial reduction but this can now be supplemented by a 0.22 μm microfiltration unit. The use of the 0.22 μm microfiltration unit has made continuous culture techniques much simpler, but has not replaced chlorination due to the high cost of filters.

4.2 Media

Although a variety of nutrient media have been tested in the algal unit, (including Erdschrieber, Mod F, Medium G, and M.A.P medium), Guillards F/2 beta (Guillard, 1983) remains the medium used in routine production. Although not the best growth medium in all cases, F/2 is useful across the range of species kept at the BWFCRS and has performed as well as all others on the commonly grown species.

4.3 Culture conditions

Originally the algal unit was divided into two areas, the laboratory area, where all stock and working cultures were held, and the pilot hatchery where 20 L, 200 L and 1000 L cultures were housed. With the use of air conditioners all cultures, with the exception of those in 1000 L tanks were maintained between 21 and 23°C. 1000 L tanks were located outside, under the hatchery eaves, where heaters and shade were used to maintain temperatures between 20 and 28°C.

Continuous illumination to cultures was provided by fluorescent lamps at an intensity of approximately 4000 lux at the container surface.

With the move to the new hatchery it became possible to house the entire unit in one large, wet laboratory. This allowed significant reductions in the time taken to perform most routine tasks. Stock, working, 5 L and 20 L cultures are kept in a separate air conditioned room at a constant 21 ± 1°C. Mass cultures are housed in an adjacent air conditioned wet area at 24°C. The slightly higher temperature is used to accommodate *Brachionus plicatilis*, a food organism used for fish culture, which requires a higher water temperature. All cultures with the exception of 1000 L tanks are still illuminated with fluorescent lamps at an intensity of 3500 to 4500 lux, but time clocks have been introduced to deliver a 16 to 8 h light/dark cycle. 1000 L tanks are lit with 400 W metal halide lamps suspended above the tanks.

Experimental CO_2 enrichment was tested in the pilot hatchery and introduced permanently in the new facility. Through the use of a gas regulator and a 'floating Brooke's tube flow meter, the air supply is enriched to 2% CO_2 to maintain pH levels between 7.5 and 8. All

air supplied to cultures is passed through in-line 0.45 µm filters fitted to each individual culture vessel.

4.4 Stock cultures

In the early 1980's the culture collection comprised sixteen of the most commonly used algal species in Australian mariculture. Each of these stocks were maintained in triplicate in 250 mL cotton wool stoppered Erlenmeyer flasks and sub cultured on a three weekly basis. Stock cultures were tested for bacterial contamination once a month and were used only when new working cultures were required.

Since 1987 the C.S.I.R.O. Marine Laboratories, Hobart, have been able to supply axenic stock cultures within 72 h. This has permitted the algal unit to keep just one parent flask when sub culturing, and to immediately replace any stock line that fails to perform or is suspected of contamination. Commercially available reautoclavable compressed fibre bungs with an aluminum foil skirt have been found better than the covered cotton wool stoppers used at first. The three week cycle for subculture has been retained.

4.5 Working cultures

Prior to 1989 those algal species that were needed to be produced en masse for particular experiments were held in a 500 mL and a 2 L flask. The 2 L flask was used as an inoculum for the next step in the production process, the 20 L bag, whilst the 500 mL flask was divided to provide inocula for new 500 mL and 2 L working cultures. Under normal circumstances a pair of working cultures were allowed seven days growth.

In the new hatchery, with the increase in production of *Chaetoceros calcitrans* and other species it was found that a 2 L flask was often a larger inoculum than necessary. A single 500 mL flask was found to be sufficient and was divided to provide an inoculum for a larger culture as well as the inoculum to begin another working culture.

4.6 Intermediate cultures

Within the pilot hatchery an inoculum of approximately 10% of the total volume to be cultured was used, i.e. 2 L into 20 L and 20 L into 200 L. The 20 L bag system involved the use of a double skinned transparent wine cask bladder supported within a plastic cage. Filled with chlorinated seawater a combination air inlet, outlet and harvest line was lowered into the bag through its cap. When ready for inoculation this bag was transported to the laboratory where an algal inoculum and growth medium were added in a sterile laminar flow cabinet. The bag was then returned to the hatchery and the culture allowed seven to 10 days growth before it in turn was used as an inoculum for 200 L bags.

With the move to the new hatchery the 20 L bag system was replaced by a 5 L flask and a 20 L carboy (Fig 2). Both the containers and the air inlet and outlet bungs for these containers are autoclaved to ensure complete sterility. Inoculation and installation of the sterile bung take place in a laminar flow cabinet to decrease the chance of bacterial contamination. After seven days growth the 5 L flask is used as an inoculum for either a 20 L carboy, a 500 L bag or a 1000 L tank.

With a 5 L inoculum, 20 L carboy cultures achieve much greater densities in four days than previously attained in 20 L bags after ten days. High densities, and short turn over time, have seen the carboy cultures used extensively as larval food, particularly for the production of *Ch. calcitrans*.

4.7 Mass cultures

Originally larvae were fed algae grown in 200 L plastic bags of similar design to the 20 L units. When shortages occurred this could be supplemented with food from 1000 L outdoor tanks. The combination of 200 L bags and 1000 L tanks gave the pilot hatchery a total algal capacity of up to 13,200 L, approximately a third the capacity of the new hatchery algal unit. However, increases in algal culture density achieved across all species has meant the extra space in the new hatchery has been devoted more toward the diversification of production and experimental culture than increases in output volume. In the final year of its operation the mass culture output of the pilot hatchery algal unit was 97% *P. lutheri* and *T. Iso* In 1989 only 62% of the output of the new algal unit was devoted to these two species, with the remaining output comprising *Ch. calcitrans, Ch. gracilis, Tetraselmis suecica* and *Nannochloris atomus*. In 1990, *P. lutheri* and *T. iso.* together formed less than 40% of total production.

A 500 L bag has replaced the 200 L unit, and the internal air and harvest lines have been discarded. An air inlet and harvest tap are now sterilized and pushed through the wall of the bag resulting in reductions in the level of bacterial contamination at the time of harvest.

The 1000 L tanks used in the pilot hatchery are still in operation, although they are no longer sited outside and dependent upon sunlight. The significant increase in cell densities and the low labour input has meant these tanks provide the bulk of the food for mollusc broodstock and Brachionus.

5 OYSTER BREEDING

5.1 Water Treatment

Estuarine water is pumped to the hatchery through an 800 m line, using two positive displacement rubber diaphragm pumps. Although this water often carries a high silt load, salinity rarely drops below 25 g/kg. During periods of low salinity a large water storage facility allows production to continue. In the early 1980's this water was rigorously filtered and sterilized. Filtration, which included sand filters and 10, 5, and 1 µm cartridge filters (Holliday, 1985), was followed by ultraviolet irradiation or the use of an antibiotic such as neomycin sulphate. Routine sterilization is no longer practiced and is only advocated if there is evidence that oysters are being affected by pathogens. While bacterial numbers are initially reduced by sterilization, a rapid escalation in numbers may subsequently occur due to lack of competition from a mixed biota, resulting in total culture collapse (Garland et al., 1986).

Currently, water is settled for 6-7 days to remove suspended materials, and if necessary filtered through graded layers of gravel (12-2 mm) supporting a 100 mm layer of fine beach sand before entering the hatchery. Further filtration is usually not required, although recently 45 µm filters have been installed to avoid contamination of culture water with Pacific oyster larvae and to remove the occasionally troublesome levels of copepods.

To reduce the possibility of heavy metals interfering with growth and development of embryos and larvae, all larval rearing water is dosed with the chelating agent NaEDTA (ethelenediaminetetraacetic acid, Utting and Helm, 1985) at the rate of 1 mg/L.

5.2 Broodstock and Conditioning

The abundance of estuaries along the NSW coast usually enabled the collection of sexually mature (ripe) oysters (Wisely, 1983), however, when ripe oysters are unavailable, particularly between the months of June to September, broodstock conditioning is required. A closed recirculating oyster conditioning system was installed in 1987. Approximately 10-15 L of seawater was available to each oyster with complete water changes every three days, temperature varied between 22 and 24°C depending on gonad condition. A feeding rate of 3×10^6 cells of *Tahitian isochrysis* aff. *galbana* (*T. iso.*) equivalents/oyster/day was used. The maturity of oysters was determined using a condition index and by visual observations. The condition index is a measure of the size of the oyster meat relative to the shell cavity, as the gonad comprises a considerable portion of the body tissue a high value indicates a large gonad, in the Sydney rock oyster, values range from 4.0-17.0. However, ripeness is ultimately determined from visual observations. The presence of vein like tubules on the surface of the gonad, in conjunction with eggs which readily become round in seawater, is indicative of a sexually mature oyster. Current hatchery practice involves selection of oysters of uniform gonadal development, followed by a 4-8 week conditioning period prior to spawning. Oysters with little or no development are avoided as conditioning period becomes excessive. Fig. 3 shows the development of three groups of oysters that spawned naturally in the conditioning systems after 9, in and 6 weeks.

Figure 3. Relationship between condition index (C.I.) and time for three batches of Sydney rock oysters conditioned at 22° C; (batch 1 spawned naturally after nine weeks; batch 2 spawned naturally after ten weeks: batch 3 spawned naturally after six weeks).
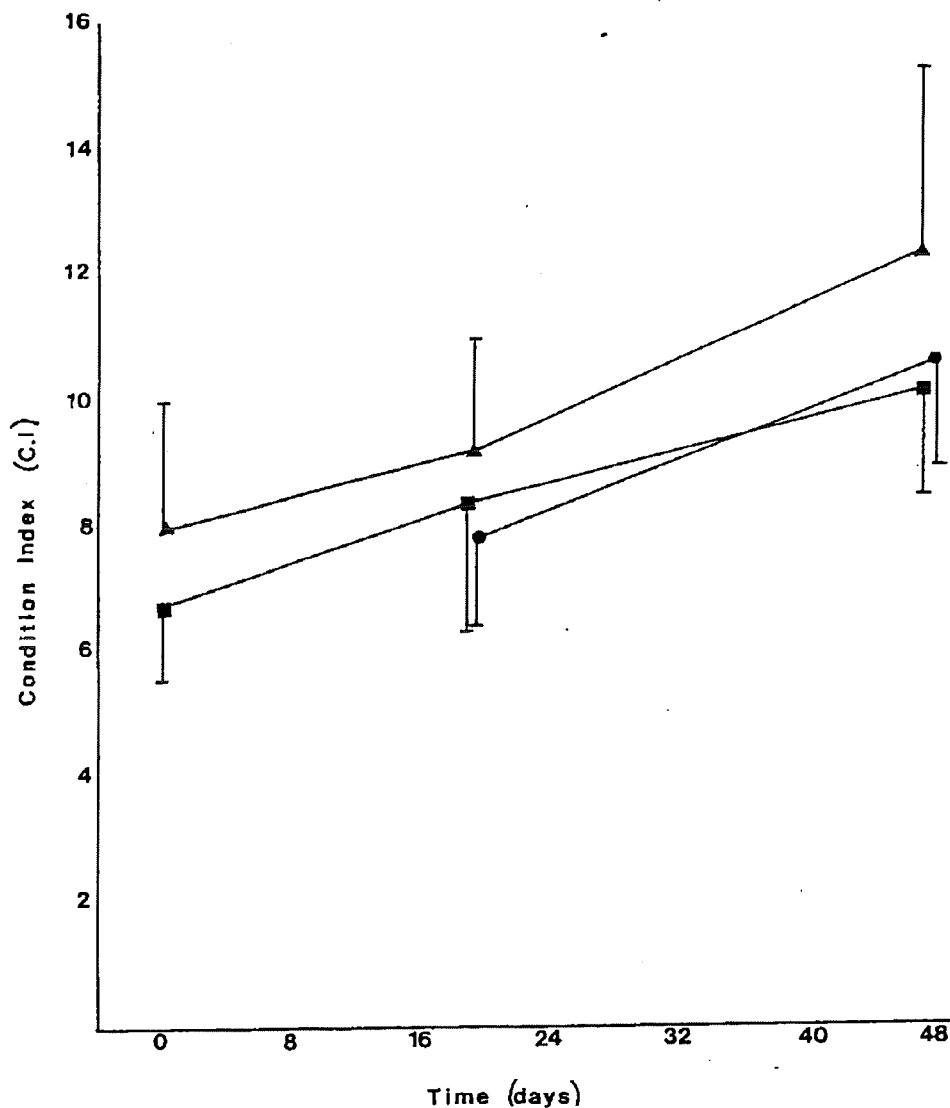
5.3   Spawning and Fertilization

Spawning induction using thermal stimulation combined with a salinity reduction has been successful since the early 70's (Wisely, 1974). Sexually mature oysters were placed in ambient seawater and the temperature was slowly increased to 28°C over a period of 1-1.5 h. If spawning had not commenced soon after the temperature had reached 28°C the salinity (30-35 g/kg) was reduced by up to 10 g/kg. Spawning usually commenced within 15 min of the addition of fresh water, if not, the whole procedure was repeated. When spawning began, males and females were in placed individual jars containing seawater at full salinity. Originally the eggs were pooled and two sperm per egg were added (Wisely, 1983; Holliday, 1985), however, in mass spawnings sperm from the spawning table has access to the inhalant chamber where eggs collect prior to release (Mason, 1986), up to 90% of eggs may be fertilized before oysters are removed from the spawning table (Frankish, pers. obs., 1989). A general rule for fertilization has been to supply enough

sperm so that no more than five sperm can be seen attached to the periphery of each egg (Wilson, 1980). Initially, fertilized eggs were held overnight in aquaria (Wisely, 1983) and the resulting D-veligers were stocked into larval tanks. The fertilized eggs are now washed through an 80 μm screen to remove debris produced during spawning then stocked directly into larval tanks.
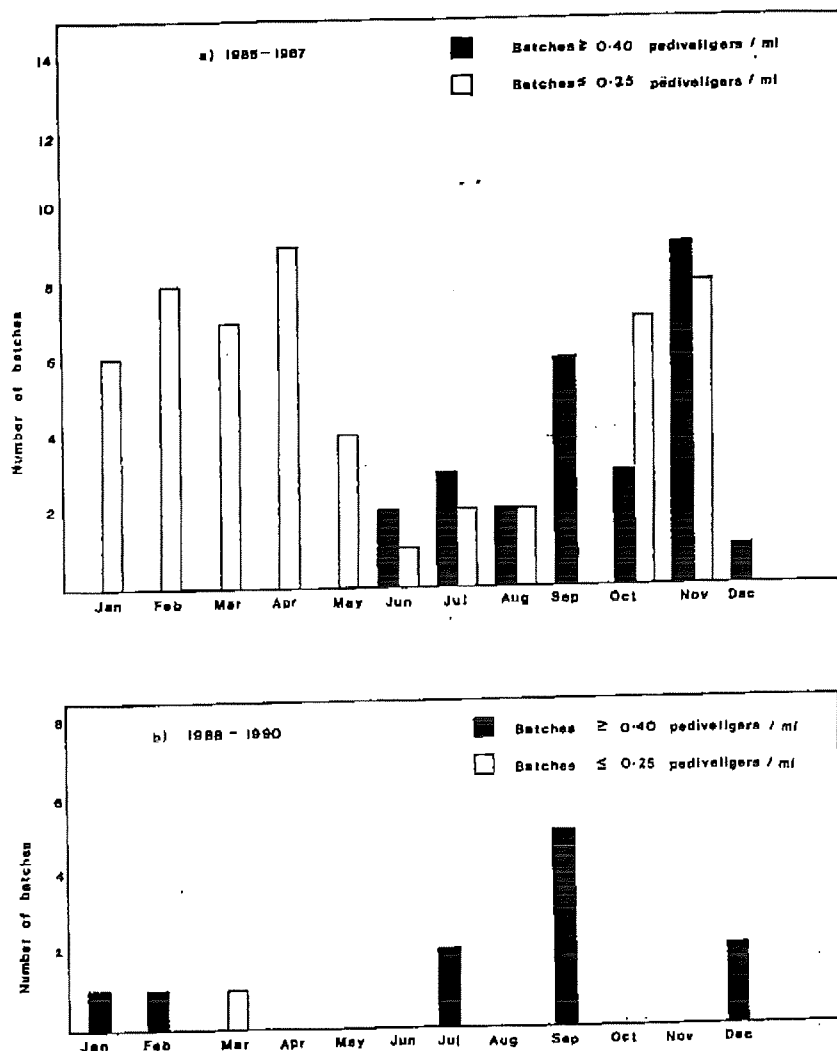
5.4   Larval rearing

Research into the rearing of the Sydney rock oyster began in the mid 1970's and in April 1981 larvae were successfully reared beyond metamorphosis to yield spat. Many larval batches followed, with success, measured in terms of densities of pediveligers produced, varying from 0.05 to 0.25/ml. Failures were thought to be due to disease caused by a Vibrio spp. and were countered with UV irradiated water and the antibiotic, neomycin sulphate. The larval feed rate, approximately 100,000 cells/mL of larval culture water, was similar to that used by some overseas hatcheries (Curtin, 1979). In the latter half of 1985, feed rates were cut by up to 50% and UV irradiated water and antibiotics were abandoned, coinciding with the first commercially successful larval batch producing approximately 1.0 pediveliger larvae/ml of culture water.

From late 1985 larval culture involved stocking tanks with D-veligers at densities of 6-15/ml. Water was maintained at 250C and changed every other day. The predominant algal species fed were, *T. iso.* and *Pavlova lutheri* at between 30,000 and 50,000 cells/mL culture water. Feed rates were further reduced in mid-1986 to, 15,000 cells/mi until day 6, then 30,000 cells/mL until day 12 and 50,000 cells/mL until the pediveliger stage. With this reduction two batches were produced in spring 1986 with larval retention rates of approx. 35% to the pediveliger stage.

In early 1987 batch after batch failed, with food consumption poor, growth negligible and development halted prior to early umbo stage. It was found that treating the water with up to 10 mg/kg chlorine followed by carbon filtration enabled the larvae to feed and metamorphose but growth was still slow and
survival poor.

Despite the spasmodic occurrence of successful cultures an annual pattern was emerging. There was a far greater chance of success for cultures spawned in the latter half of the year (Fig 4). The average growth rate of Sydney rock oyster larvae from the best five batches is shown in Fig 5. All batches exhibit a reduction in growth around day 13 corresponding with the development of the eye spot. In 1987 the daily measuring of a larval sample was adopted, as growth rate is a good indicator of larval health.

Figure 4. Relationship between season and pediveliger yield in batches of Sydney rock oysters 1985-1990.

The completion of the new hatchery saw further changes to the larval rearing strategy. Eggs were stocked at densities of approximately 3/ml. Water changes were made every three days and culture water was settled for 6-7 days and then sand filtered before entering the larval tanks (20,000 L). Feed rates were further reduced to between 7500 and 32000 cells/mL.

These changes subsequently produced the best growth and larval retention rates since the program's inception, although, at the end of 1988 there still had not been a successful batch of Sydney rock oyster larvae in the months of January to June (Fig 4). Collation of data from successful batches in the months June to December provided some valuable information with regards to stocking density, larval survival (Table 1), and food consumption (Fig 6).

Since moving to the new hatchery and the further development of hatchery techniques, larval retention rates have seldom dropped below 25% and batches have been reared in the early months of the year (Fig 4).

Figure 5   Average larval growth rate and retention for the best five batches of

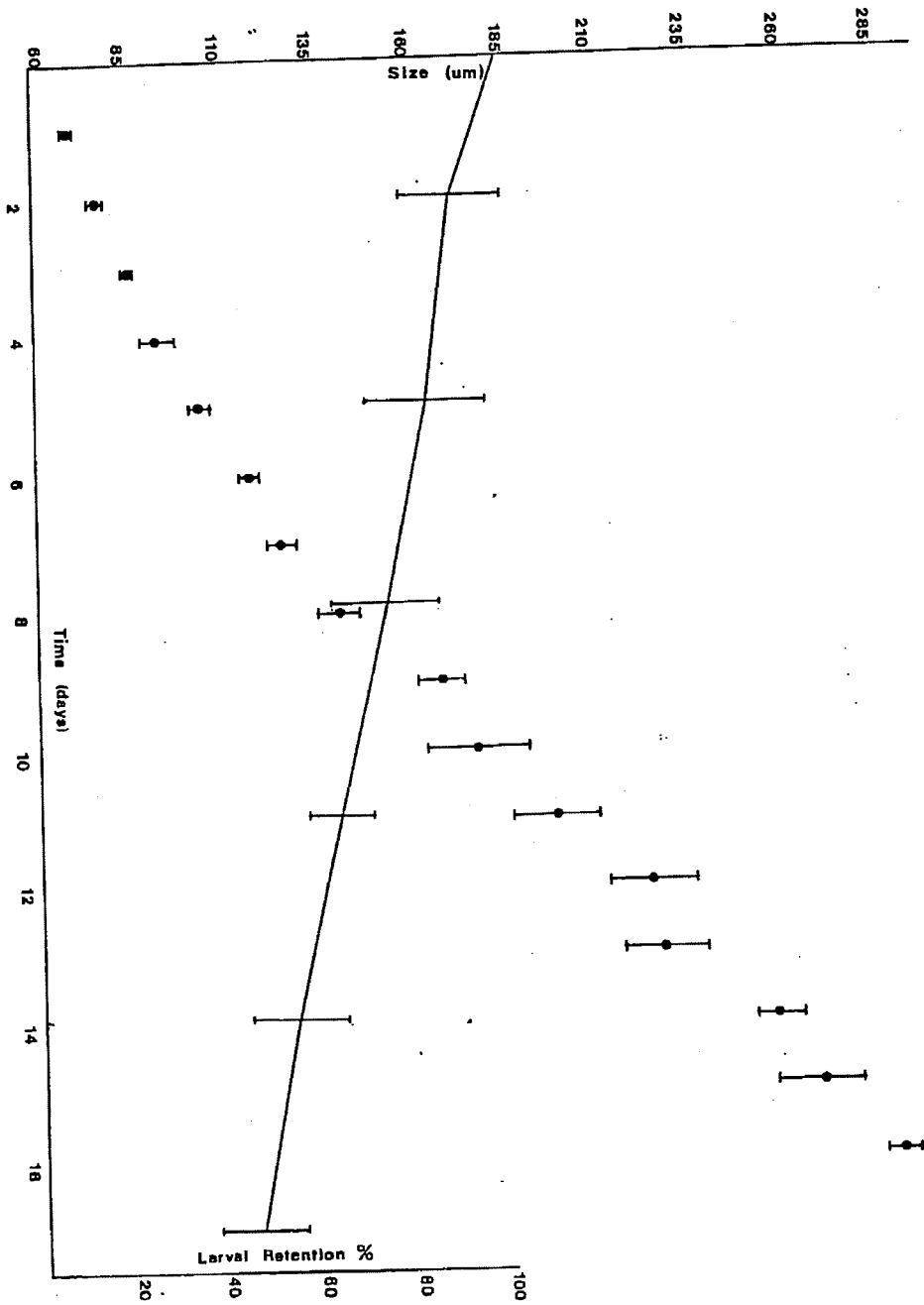Sydney rock oysters, *Saccostrea commercialis*, at the BWFCRS.

Figure 6   Relationship between shell diameter and food consumption for larvae of the Sydney rock oyster, *Saccostrea commercialis*.
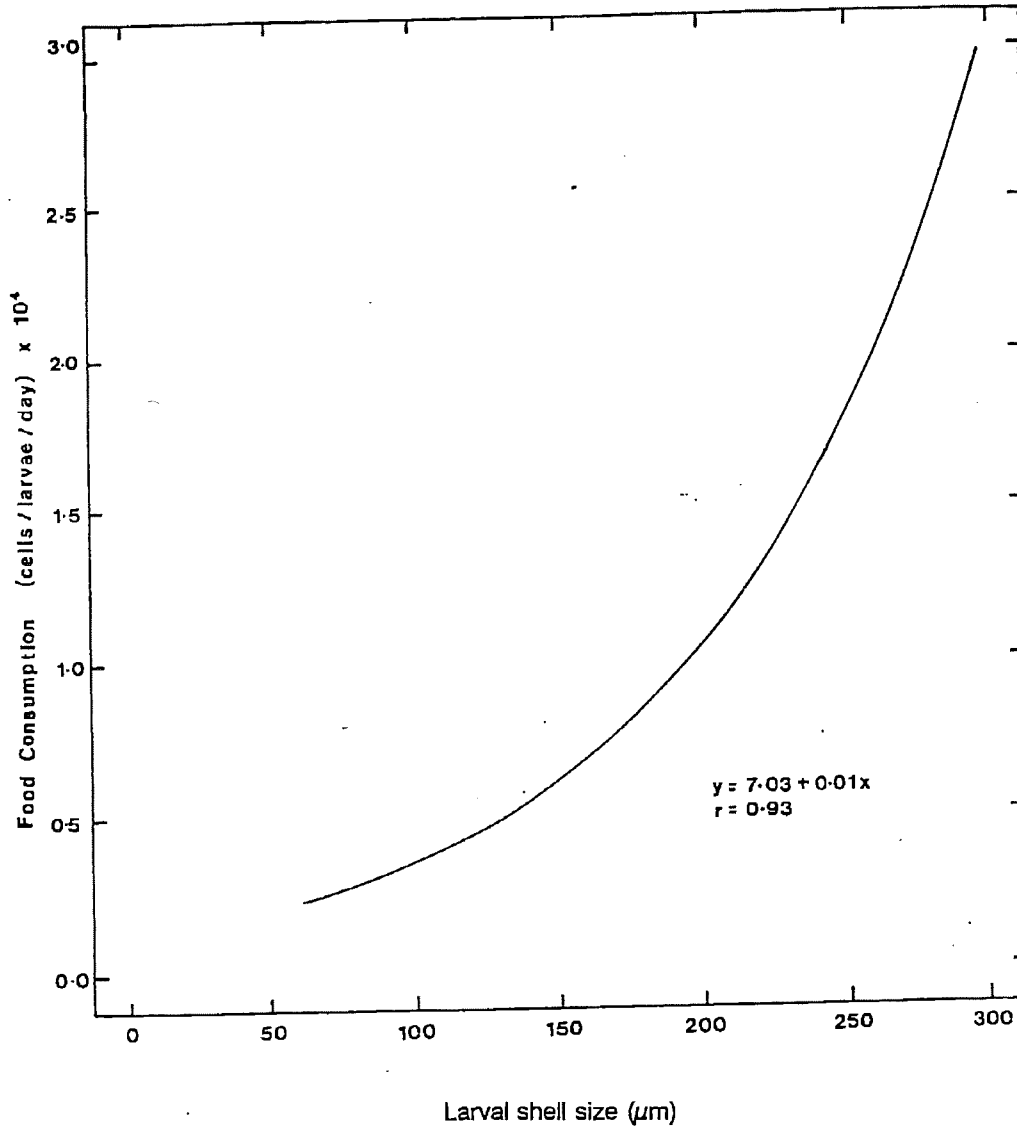

$$y = 7.03 + 0.01x$$
$$r = 0.93$$

Larval shell size (μm)

Table 1. Relationship between stocking rate of eggs and retention of pediveliger larvae for successful batches run from June-December 1985-1989.

Stocking rate[1] (eggs/mL)	Density of Pediveligers (N°/mL)	Larval Retention[2] (% of initial stocking)
15	1.1	7.33
15	1.2	8.0
15	0.6	4.0
13.5	0.4	2.96
10	1.0	10.0
8.8	1.1	12.5
7.0	2.5	35.7
3.0	0.9	30.0
2.75	1.1	40.0
2.5	0.9	36.0
2.2	1.1	50.0

[1]Batches whose initial density was recorded.
[2]Larval Retention = Density of Pediveligers x 100 Stocking Rate.

5.5 Intermediate set and early nursery

A variety of collecting surfaces have been tested to produce single seed spat, including glass, PVC, scallop shells and ground scallop shell (250-500 µm size range). From 1982 to early 1985, the highest recorded set was 260,000 spat from a 2,000 L tank. It was not until 1986, when a downweller set system (Bayes, 1981) was introduced, that large numbers of single seed spat were produced, however, severe mortality regularly caused losses in excess of 80%. In 1988 a modified downweller was introduced to grow seed to approximately 700 µm for transfer to outside upwellers.

This setting unit (Fig 7) holds ten round PVC sieves (area=1590 cm-2, 200µm mesh) which each have 80-100 g of scallop shell spread across their base. This shell has been passed through a 350 µm mesh and retained on 200 Clm mesh. Each PVC screen holds 250,000 pediveliger larvae. A spray unit supplies flow to the larvae by way of two submersible pumps located in the reservoir tank. This tank is aerated and a titanium 4 kW immersion heater controls water temperature at 25°C. Although oyster larvae do not feed during metamorphosis, algae is added to the reservoir tank twice daily to provide food for those larvae still swimming and metamorphosed spat. The feeding rate starts with 50-60,000 cells/larvae/day and is adjusted depending upon the rate of grazing (Table 2). The system is given a total water change every two days and a partial change every other day. All tank surfaces, pumps, pipes and aerators are cleaned with a solution of sodium hypochlorite and then rinsed with fresh water. Screens are removed and rinsed twice daily, larvae with seawater and spat with fresh water.

To maintain single seed production and prevent doubles or triples etc, spat are separated from the larvae using a 350 Clm screen. Approximately 90% of spat are removed within 5 days of settlement commencing. After approximately two weeks spat are large enough to be retained on a 500 µm mesh and are removed from this system and placed in outside upwellers. Spat retained on a 350 µm mesh have also been successfully on grown in upwellers, and if located in areas of favorable growing conditions could be removed from the hatchery within 3-4 days of introduction to the settlement system.

Large spat mortalities (up to 90% in 36 h) have occurred between 8 to 30 days after the larvae were introduced to the settlement system. Apparently healthy spat have been

moved from hatchery systems to upwellers in different estuaries and have experienced sudden and severe mortalities after 5-7 days. When this type of mortality occurs it has always coincided with mortality of spat remaining at the hatchery. However, on one occasion spat, some of which were moved out of hatchery after 3-4 days (size 350 μm), survived in upwellers and died in the hatchery. These mortalities may be hatchery or batch related, however, it is interesting to note that similar mortalities have not occurred in experimental batches of Pacific oysters reared, although these have been run parallel to *S. commercialis* batches which have suffered significant mortalities.

Table 2.   Important parameters in the setting and early nursery systems.

Per screen	Larvae	Spat
Density	250,000	100,000
Scallop shell	80-100 g	-
Flow rate	4.6 mL/larvae/day	9.2 mL/spat/day
Overall System		
Density	1.4 larvae/mL	0.7 spat/mL
Feed Rate	50-60,000 cells/larvae/day	140-180,000 cells/spat/day
Temperature	24-26°C	22-24°C

Figure 7   Setting system as used in the new hatchery at the BWFCRS.
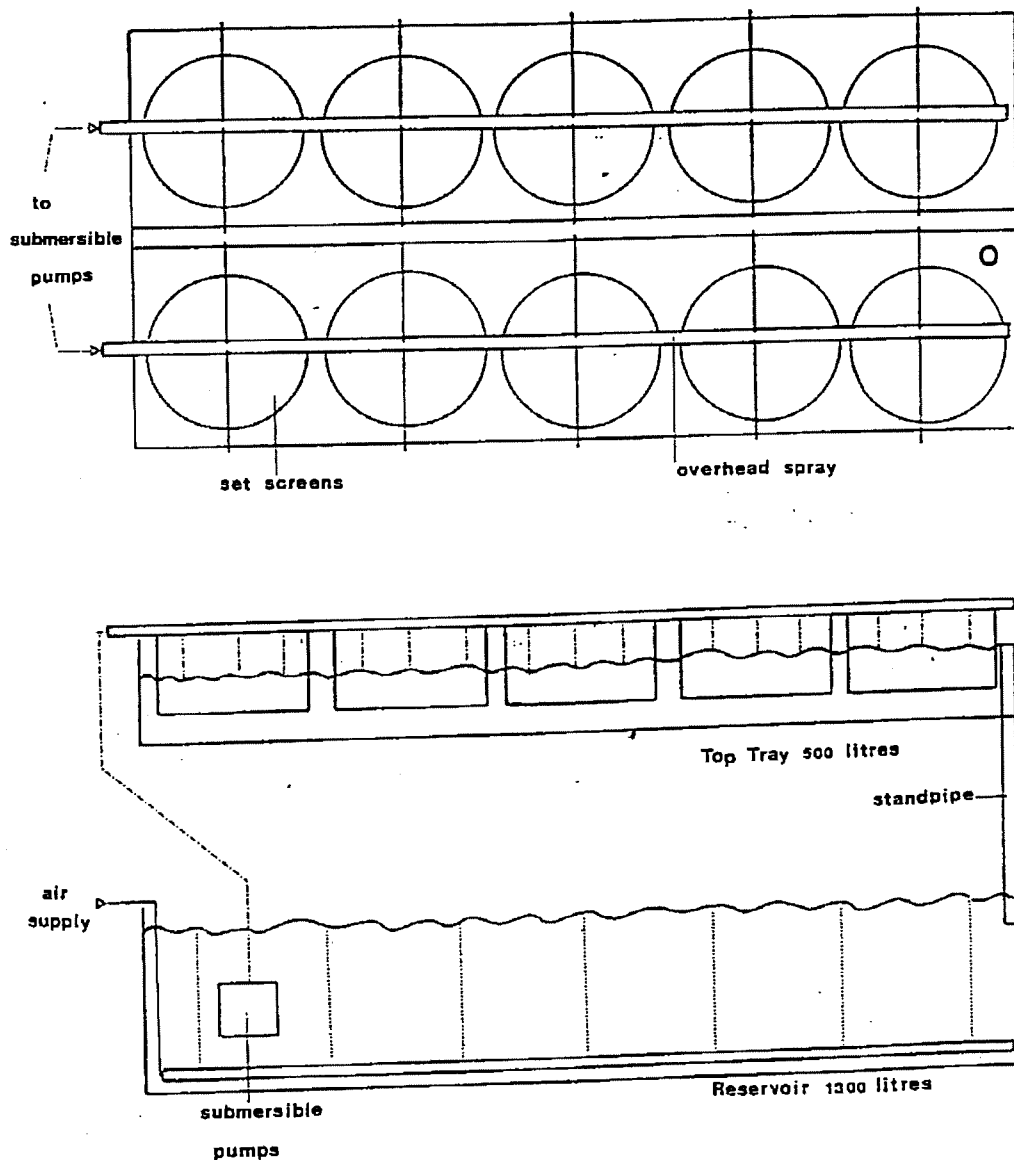
5.6   Outdoor Nursery

Early nursery attempts were made using floating trays in outside tanks with an air lift pump to provide flow. Grow out on a variety of cultch was tested, and although larvae set well on most substrates, survival was low and settlement density difficult to control. Generally these methods showed limited success as they failed to capitalize on the potential yield available from hatchery production.

In 1983 a battery of vertical forced upwellers (Bayes, 1981; Holliday, 1985) was installed between two tidally flushed ponds from which water could be drawn. The spat were held on mesh in long tubes, which were drained daily and washed with freshwater. This system was difficult to monitor and the washing was too harsh, small spat (approx. 0.7 mm) showed poor survival, although better results were achieved with spat larger than 2

mm. Sales from these upwellers were 0.8 million spat from 1984-86 and a further 1.0 million in the 86/87 season. The latter coinciding with the introduction of the scallop shell setting/early nursery system.

For the 1987/88 season forced tube upwellers were abandoned and a passive upweller system installed (Fig 8). These upwellers are shallower (250 mm) with fine mesh (350 μm - 1 mm) capable of holding very small spat (> 500 μm). Unfiltered water is pumped into the unit and allowed to flow through mesh at the bottom of each upweller, past the bed of spat and through a meshed outlet to waste. Flow rates to individual upwellers ranged from 6-12 L/min depending on spat size and density.

The ponds at the BWFCRS have been suitable as a nursery site for juvenile Pacific oysters but have not reliably supported growth and survival in juvenile Sydney rock oysters. In 1985/86 experimental work to evaluate the inlet and outlet effluent from the Vales Point power station, Lake Macquarie for the nursery culture of Sydney rock oysters found the inlet to be a favorable site (Holliday et al., 1991). In 1987/88 nursery operations commenced at the inlet canal, which had uniform salinity (30-35 g/kg), low silt levels and warmer winter temperatures (approximately 3°C higher than Port Stephens). The lack of Pacific oysters in Lake Macquarie was also an advantage as this eliminated the possibility of contamination of Sydney rock oyster seed.

While the first introduction of spat to the Vales Point Nursery proved very successful (77% survival to sale size (4-6 mm)) subsequent batches were plagued with problems, such as chlorine and oil leaks and vandalism. Nevertheless 2.5 million spat were produced for sale from this site. The nursery site was then expanded and positioned further down the inlet canal where survival and growth were satisfactory. Stocking densities (Table 3) may have been conservative but higher densities are as yet untested.

In a week period from November to January 1989 a total of 11.3 million spat were sold to farmers from various estuaries in NSW.

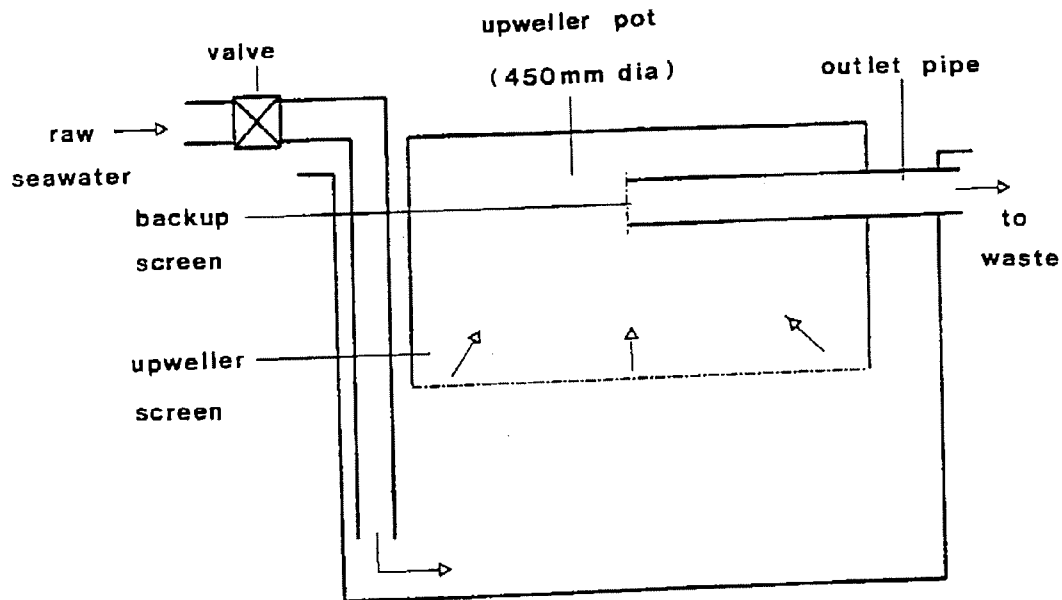Figure 8   Nursery upweller unit used at the BWFCRS.

Table 3.   Stocking densities of Sydney rock oyster seed in passive upwellers at Vales Point Nursery 1988

Size of screen (µm)	Vol. of seed (mL)	Est. of numbers (10^4)
500	190	60
670	250	50
1000	500	40
1250	750	33
1400	1000	30
1800	1500	26
2240	2000	16
3000	3000	10

6 CONCLUSIONS

The BWFCRS oyster breeding program was Australia's first attempt at hatchery production of the Sydney rock oyster. It has proved a difficult species to produce and to date the program has been unable to overcome all of the remaining production problems. The four other bivalve species cultured at BWFCRS presented few problems, which in part may be due to the refinement of rearing techniques evolved during Sydney rock oyster research.

The first hatchery reared Sydney rock oyster spat were produced at BWFCRS in 1981. Although seasonal influences have on occasion limited production, the larval rearing techniques for the Sydney rock oyster are now well established and commercial quantities of larvae have been produced since 1985. The major factor limiting the commercial production of hatchery spat is the high mortality of juvenile spat in the nursery systems. The downweller system and passive upwellers that have been used to set and grow spat have worked successfully on occasions, however, spat survival in general has been poor. It is thought the mortality is most likely linked to the hatchery setting process and not the nursery system itself. Research into this problem has been limited, due mainly to staffing restrictions and other research commitments. Researchers are confident the remaining problems can be overcome and recent investigations have narrowed the field for further research. It has been established that if larvae are set on solid collectors rather than scallop shell, spat survival is increased, although the reason for this is unclear. Settlement on collectors may be a commercially viable alternative to the scallop shell system if settlement density can be controlled and spat recovery from collectors provides sufficient yield to support a hatchery operation.

The future of hatchery production of diploid Sydney rock oysters is limited due to high competition from natural spatfall. If markets are to be established, researchers and hatchery operators must address the remaining production problems and guarantee a consistent supply of their product. The economic viability of NSW hatcheries may lie with the production of seed that cannot be reliably obtained from the wild. This may include the production of triploid Sydney rock and Pacific oysters, mussels, scallops and flat oysters.

7 ACKNOWLEDGMENTS

8   REFERENCES

Bayes, J.C., 1981. Forced upwelling systems for oysters and clams using impounded water systems. Proc. Int. Workshop on Nursery Culturing of Bivalve Molluscs, Spec. Pub. Eur. Maricult. Sec., 7: 73-83.

Calabrese, A., Davis, H. C., 1970. Tolerance and requirements of embryos and larvae of bivalve molluscs, Bureau of Commercial Fisheries, Biological Laboratory, Milford, Conn., U.S.A. Helgolander wiss, Meeresunters, 20: 553-564.

Curtin, L., 1979. Oyster hatchery pilot scheme, setting up, operation, and future role. Technical Report No. 155, MAF, N.Z., 16 pp.

Espinas, V., Marshall, G., Rish, B., 1988. Economic survey of the New South Wales oyster farming industry; 1981-1982 to 1983-1984; Part 2 - Physical Aspects. Division of marketing and economic services, Miscellaneous Bull., No. 54. NSW Agriculture & Fisheries, Sydney, 48 pp.

Galstoff, P. S., 1964. The American oyster, *Crassostrea virginica* (Gmelin). Fishery Bull., No. 64. United States Department of the interior, Washington, 480 pp.

Garland, C.D., Cooke, S.L., McMeekin, T.A. and Valentine, J.E., 1986. Effects of 0.2 µm membrane-filtered seawater as a culture medium on fertilised eggs and larvae of the Pacific oyster (*Crassostrea gigas*). Aust. J. Mar. Freshwater Res., 37: pp. 713-720.

Green, J., 1968. The Biology of Estuarine Animals. Sidgwick and Jackson, London,401 pp.

Guillard, R. L., 1983. Culture of phytoplankton for feeding marine invertebrates. In: C.J. Berg, Jr. (Editor). Culture of Marine Invertebrates. Hutchinson Ross Publishing Company, Stroudberg, PA, pp. 108-132.

Helm, M. M., Laing, I. and Jones, E., 1979. The development of a 200L algal culture vessel at Conwy. Fisheries Research Technical Report No. 53, pp 1-7.

Holliday, J.E. and Nell, J.A., 1990. Pacific oysters in New South Wales, Agfact F2.1.3, Second edition, NSW Agriculture and Fisheries, 4pp.

Holliday, J. E., 1985. International Developments in Oyster Hatchery Technology. Misc. Bull. No. 1, Department of Agriculture NSW, Sydney, Brackish Water Fish Culture Research Station, Salamander Bay, NSW, 101 pp.

Holliday, J. E., Maguire, G. B. and Nell, J. A., 1988. Alternative cultivation methods for the Sydney rock oyster (*Saccostrea commercialis*). In L.H. Evans and D. O'Sullivan (Editors), Proceedings of the First National Shellfish Aquaculture Conference, Curtin University of Technology, Perth, WA, pp. 234-265.

Holliday, J. E., Maguire, G. B., Nell, J. A., 1991. Evaluation of sites for nursery culture of single Sydney rock oysters (*Saccostrea commercialis*), Fish. Bull. No.5, NSW Agriculture & Fisheries.

Malcolm, W. B., 1987. The Sydney rock oyster, Agfact, F3.1.1, Department of Agriculture NSW, 12 pp.

Marshall, G., Espinas, V., 1987. Economic survey of the NSW Oyster Farming Industry - I. Financial Miscellaneous Bulletin, No. 48. NSW Agriculture & Fisheries, NSW, Sydney, 59 pp.

Mason, C. J., 1986. Sydney rock oyster genetics programme, Mortality of the Sydney rock oyster in northern New South Wales and Southern Queensland. Final report to
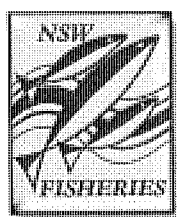
FIRTA, Brackish Water Fish Culture Research Station, Department of Agriculture, Salamander Bay, NSW, 55pp.

Quayle, D. B., Newkirk, G. K., 1989. Farming Bivalve Molluscs: Methods for Study and Development. The World Aquaculture Society, Baton Rouge, L.A., 294pp.

Utting, S. D., Helm, M. M., 1985. Improvement of seawater quality by physical and chemical pre-treatment in a bivalve hatchery. Aquaculture, 44: 133-144.

Walne, P. R., 1974. Culture of Bivalve Molluscs, 50 Years of Experience at Conwy, The Buckland Foundation, London, pp. 87-89.

Wilson, J., 1980. Hatchery rearing of *Ostrea edulis* and *Crassostrea gigas*. Aquaculture Tech. Bull. Ireland, 4: 1-34.

Wisely, B., 1974. Progress of the Oyster Culture Programs. In: collected Open Day Booklets (1973-1988) of the Brackish Water Fish Culture Research Station, Salamander Bay, NSW, pp. 28-38.

Wisely, B., and Reid, B. L., 1978. Experimental feeding of Sydney rock oysters (*Saccostrea commercialis* = *Saccostrea culcuilata*). I. 'Optimum particle sizes and concentrations. Aquaculture, 15: 319-331.

Wisely, B., 1983. Hatchery spat production. In: collected Open Day Booklets (1973-1988) of the Brackish Water Fish Culture Research Station, Salamander Bay, NSW, pp. 218-224.

Appendix 2. Heasman, M. P., Goard, L., Diemar, J. and Callinan, R. B., 2000. Improved early survival of molluscs: Sydney rock oyster (*Saccostrea glomerata*).

Improved Early Survival of Molluscs:
Sydney Rock Oyster (*Saccostrea glomerata*)

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Aquaculture CRC Project A.2.1

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ACKNOWLEDGEMENTS

1.0   SUMMARY

Continuation of an otherwise very successful 10 year breeding program to produce fast growing disease resistant Sydney rock oysters *Saccostrea glomerata* depends on whether diseases of larval and juvenile *S. glomerata* below about 2 mm can be overcome. The objective of this project has been to systematically address and overcome both of these diseases. Five complimentary strategies have been adopted to investigate mass mortality diseases of larval and juvenile *S glomerata.*

1. Appraisal of all existing epidemiological information including in-house hatchery records and reports of previous epizootics and monitoring and documentation of ongoing hatchery and nursery operations at the PSFC.
2. Collection and preservation of larvae and spat for histopathological examination and possible diagnosis in of infective agents linked to disease episodes.
3. Experimental investigations of:
   - possible causes
   - predisposing and ameliorating factors to disease outbreaks
   - the effectiveness of alternative methods of disease control and prevention.
4. Determination and adoption of optimal physio-chemical rearing conditions for *S. glomerata* larvae.
5. Use of probiotic agents to provide more favourable and stable micro-flora environments within hatchery rearing system - (this strategy is now being addressed separately in a collaborative project initiated in September 1999).

1.1 Results - Larval Disease

1.1.1 Epidemiological investigations

The following important information on the clinical manifestation of the larval mortality syndrome was gained from analyses of hatchery records for the period 1990 to 2000.
1. There has been a continuing trend of improving hatchery performance with *S. glomerata* over the past decade beyond that achieved at the PSFC up to 1990.
2. First non specific clinical signs of the disease, anorexia, occurs most commonly (about 75% of the time) on days 3 to 5 but also occurs as early as day 2 and as late as day 8.
3. Sub-lethal effects on growth rate of diseased larvae coincident with anorexia are evident from about day 4.
4. The larval disease is characterised by elevated mortality culminating in complete mortality on average by day 14.
5. Whether or not the disease occurs and what proportion of larvae survive to settlement stage are not related to apparent egg quality as indicated by yield of D veligers from eggs. Like-wise, survival rate of D veliger larvae is not correlated to subsequent yield of spat.

Useful epidemiological information gained from the same analyses of hatchery records was as follows:
1. There is a marked seasonal variation in the incidence of mass larval mortality. Indeed the calendar year can be divided into halves of contrasting hatchery fortunes for *S. glomerata.* The first half of the year (January to June), is characterised by chronically poor survival with successful hatchery runs being achieved only once in every two to six attempts i.e. a success rate of 17 to 40%. By contrast chances of success during the second half of year (July to December), are much improved, ranging from 40 to 100%.

2. Dry-out and disinfection of the hatchery markedly reduced the occurrence of larval disease. Following a minimum hatchery disinfection and dry-out period of four weeks, the probability of a successful hatchery operation decreases over successive runs from about 60% on the first run to 40% on the second and 25% on the third. These findings highlighted the critical need of regular programmed dry-out and disinfection of hatcheries intending to produce *S. glomerata* and the desirability of a modular design for any such hatchery.

3. Use of commercial scale 20000L larval rearing tanks substantially improves chances of success.

4. The mass mortality disease appears virulent only to larvae of *S.glomerata* and a related native rock oyster, *S. cuculata*. Many other bivalves including two species of clam, two species of scallop, two species of pearl oysters, blue mussels and the Australian flat oyster and the pacific oyster, are regularly produced in multi-million batches at the PSFC hatchery without significant problems. This is true even when the larvae of such species are hatchery reared in company with afflicted batches of *S. glomerata* larvae.

1.1.2 Pathology

Numerous samples of symptomatic larvae have been submitted to standard light microscope histological examination by specialist pathologists including Dr Judith Handlinger (Mt Pleasant Laboratories, Launceston), Dick Callinan (Wollongba Veterinary Laboratory, NSW, DPI) and Dr Ralph Elston (USA). Many samples of larvae and rearing water have also been submitted to Dr Peter Hanna and colleagues (Deakin University, Vic) for bacterial examination using monoclonal antibodies specific for particular species of *Vibrio* bacteria and to Dr Jeremy Carsons (Mt Pleasant Laboratories, TASDPIF, Launceston). Samples have been submitted for electron microscopy (TEM and SEM) examination to Dr Mike Hine (NIWA- New Zealand) and to Dr Alex Hyatt (AAHL/(CSIRO), Geelong,Vic.). Four lots frozen larvae have similarly been air-freighted to La Tremblade, France for specialist examination by Dr Tristan Renault (IFREMER) using PCR probes recently developed for detection of a herpes virus that causes a very similar anorexia disease of hatchery reared pacific oyster larvae and spat in western Europe.

1.1.3 Experimental investigations

Results of an experiment in December 1997 showed that disease was transferred when larvae were reared in a separate facility but in contact with 1.0 micron filtered seawater previously exposed to diseased larvae. Histopathological and microbiological evidence supported the conclusion that the disease is probably bacterial in origin and probably associated with bacteria that can persist for long periods between successive production cycles on wet surfaces within the bivalve hatchery or associated seawater storage and plumbing systems.

Preliminary research to evaluate probiotics in combating larval disease was conducted in collaboration with the Department of Cell Biology, UTS. Several strains of potentially pathogenic bacteria were isolated from surfaces of vessels containing diseased larvae and identified as either *Vibrio* or *Aeromonas* species. All but one strain were found sensitive to tetracycline and all were sensitive to strains of probiotic bacteria. Results of a trial to test prophylactic use of probiotic bacteria on *S. glomerata* larvae were very encouraging and have served as the basis of a collaborative 3 year SPIRT funded project.

1.1.4 Optimisation of rearing conditions

Results of the first of three experiments showed that sperm should be stored at or below 15°C and preferably used within an hour of collection. In the second experiment, storage periods over which eggs retained high rates (>90%) of fertilisation were found to decrease from 2h at 15°C to less than 30 mins at 30°C. Results of the third experiment showed optimum larval rearing temperature to be about 29°C which is 5°C higher than a temperature of 24°C routinely used to rear *S glomerata* larvae at the PSFC.

1.1.5 Experimental use of probiotics

In December 1999, a joint 3 year R&D project was initiated by UTS and NSW Fisheries to further evaluate use of probiotics for combating mass mortality diseases of larval and early juvenile *S. glomerata*. Although only one experiment has been conducted at the time of this final report, results showed that sterilization of sea water followed by re inoculation with one or a combination of 3 different selected species of "friendly" bacteria raised survival of 8 day old *S, glomerata* larvae from a base level of only 9% (typical of disease episodes) to a range of 45 to 68%. The latter survival rates are among the highest achieved with *S. glomerata* larvae. Benefits conferred by sterilization and re-inoculation of seawater with " friendly" bacteria were equally dramatic in relation to enhanced larval growth.

1.2 Results- Juvenile (Spat) Disease

1.2.1 Epidemiological investigations

A review of hatchery records for the period 1988 to showed that first manifestation of the disease has continued to range widely (from 7 to 43 days after settlement) and that the disease is confined to spat below 2 mm shell-height. In contrast to the larval disease, no seasonal trends were apparent in the relative occurrence of mass mortality of juvenile *S. glomerata*.

1.2.2 Pathology

As with the larval disease, numerous samples of diseased spat have been submitted to the same array of specialist pathologists using a similar array of histopathological techniques. All resultant pathology reports contained all or some of the following elements.
1. Histopathological examination revealed a range of conditions from asymptomatic to severely diseased individuals among mass mortality spat.
2. Lesions seen in all obviously diseased individuals comprised some or all of the following:
   - mild to severe focal to diffuse inflammation or necrosis of connective tissue sometimes associated with rod shaped bacteria
   - diffuse mild to severe degeneration of retractor muscles associated with infiltration by large numbers of haemocytes.
   - parietal mantle surface often severely effected with exudation of proteinacious fluid and cell debris.
   - partial dissolution of the hinge ligament associated with moderate to many bacteria of various morphological forms including filamentous.

Although no other potential causative agents were seen associated with the above lesions, similar but less common and less severe lesions to the above were often seen among the batches of

apparently healthy spat. High numbers of bacteria have often been detected on external surfaces of spat reared at high densities of 100 000 to 200 000 per 450 mm diameter nursery screens. However reports of invasive bacterial infections have been confined to dead or moribund gaping spat. In this sense the conclusion of Dick Callinan in 1990 that histopathology was more indicative of proteolytic bacterio-toxins than of any other potential agent capable of inducing this type of histopathology, must still be regarded as the most plausible albeit unproven hypothesis put forward to date. The only exception to the above was a major mass mortality event in 1999 that was ascribed by each of four pathologists consulted to an aggressive and invasive ciliate (*Euronema sp.*) infection. Re-examination of archived samples of diseased spat however failed to detect evidence of involvement of these ciliates in other disease episodes.

1.2.3 Experimental investigations

An experiment was run to test hypotheses that:
- the primary cause of the disease is the development of large concentrations of virulent strains of bacteria and exotoxins thereof associated with the use of ground scallop shell for producing single seed spat.
- manifestation of mass mortalities (as opposed to sub-clinical disease in apparently healthy stock) depends on a triggering factor especially trauma or other stressors associated with grading and counting of small spat.
- mass mortalities, if caused directly or indirectly by bacteria, could be prevented or mitigated against with prophylactic use of antibiotics.

Absence of the disease from all eight experimental treatments precluded definitive findings on the cause(s), control or prevention of the disease. Nevertheless an important inadvertent finding was that cultchless settlement using epinephrine induction of metamorphosis and omission of periodic grading of small *S.glomerata* spat, drastically reduces hatchery labour requirements without adversely effecting the rate or uniformity of growth.

2.0   BACKGROUND AND JUSTIFICATION

The 120 year old Sydney Rock Oyster industry employs more people (about 1200) than any other form of aquaculture in Australia. However, it has suffered a 40% decline from peak production levels of about 130 000 bags per annum maintained during the 1970's, representing lost gross annual revenue of about $20 million and hundreds of jobs in regional NSW from Tweed Heads to Eden. Also as a consequence of this decline in production, domestic market share has been lost to the faster growing pacific oyster produced predominantly from single-seed hatchery stock in Tasmania, South Australia and New Zealand. Significant contributing factors to the decline include:

- Escalating costs associated with inherently slow growth and hence protracted turn-off time (3 to 4 years) of the native Sydney rock oyster (*Saccostrea glomerata*).
- High susceptibility of *S. glomerata* to fatal diseases, especially Winter Mortality and QX disease caused by intracellular parasites.

Over the past 10 years the Federal Government, through the Fishing Industry Research and Development Corporation (FRDC) grants and the NSW Government, has invested in excess of 1.0 million dollars in research to develop genetically improved *S. glomerata*. Third generation selected lines have been shown to confer significantly superior weight for age (in the order of 20%) over control non-selected lines (Nell, et al., 1999). Major advantages have also been demonstrated in relation to hatchery produced triploid Sydney rock oysters over diploid siblings. These advantages which have been shown to hold over a representative range of sites and farming techniques, include significantly faster weight for age (in the order of 30 to 40%), much more protracted retention of market condition and considerably enhanced resistance to disease especially winter mortality (Hand et al., 1998). Recent revision of this R& D includes an evaluation of whether the advantages conferred by triploidy can be combined and enhanced by using the progeny of selected line breeding stock. The selective breeding program has also been extended and expanded to target single and dual resistance to two important intracellular parasite diseases - Winter Mortality (haplosporidiasis) and QX disease (marteiliasis). The latter commonly causes annual mortalities above 10% on oyster farms in Northern NSW and on occasions such as oyster farms on the lower Georges River since 1995, financially ruinous losses above 80%.

Increased productivity using improved inter-tidal and deep-water suspended culture in conjunction with stocks genetically selected for rapid growth and disease resistance thus offers the best prospects for arresting the decline of the industry. Use of hatchery produced rather than wild caught *S. glomerata* spat is therefore becoming increasingly important to the NSW oyster industry. Hence it is essential that reliable hatchery production of the *S. glomerata* single spat is developed.

The successful transfer (widespread commercial adoption) of this technology is however subject to successful demonstration of reliable cost effective hatchery and nursery production technology for supply of juveniles (seed) to the industry. NSW has an absence of private sector mollusc hatcheries but also has an indefinite prohibition on the importation on seed oysters from existing interstate hatcheries. Under these circumstances NSW Fisheries has been attempting to supply commercial quantities of genetically improved seed oysters to the ailing industry out of its research hatchery at the Port Stephens Fisheries Centre (PSFC) since 1996. Lack of a private sector bivalve hatcheries has been due to insufficient demand for hatchery spat, due in turn to high cost compared with that of wild caught spat (<Aus$0.001ea.). High costs of hatchery produced spat can be ascribed to very high costs of establishing (=Aus$1 million) and operating (=$0.3million pa) conventional batch production oyster hatcheries.

Thus a supply and demand impasse ("*a Catch 22*") exists for hatchery production of single seed *S. glomerata* spat. To break this impasse, demand at current market prices of $15 to $20 per thousand

for 6 mm spat will need to reach or exceed an estimated 25 million per annum. This minimum production level would generate sufficient gross revenue ($375 000 to $500 000) to cover all costs and yield adequate internal rates of return (>15%pa) to justify the level of risk attached to this type of investment. While real demand does appear to be growing for genetically improved hatchery produced single seed oysters, major difficulties have been experienced over the past decade in routine hatchery and nursery rearing of *S. glomerata* at the PSFC.

Foremost among these problems is an intermittent disease that has often caused mass (60 to 90%) mortalities of small (<2 mm) Sydney Rock oyster spat since 1987 (Goard, 1990 and Nell et al., 1991). Exacerbating this problem are chronically low hatchery yields further compounded by intermittent catastrophic mortality, especially since 1996, of the larvae of *S. glomerata* and very occasional and far less severe disease outbreaks in several other bivalves for which aquaculture technology has also been developed at the PSFC over recent years. The latter include the Tasmanian scallop, *Pecten fumatus* (Heasman et al., 1998) and the clams, *Katylesia rhytiphora* (Nell et al., 1995) and *Tapes dorsatus,* (Patterson and Nell, 1997).

3.0 OBJECTIVES OF THE RESEARCH PROJECT

The objectives of this project were to improve the cost and reliability of hatchery producing *S. glomerata* by combating mass mortality diseases of both larvae and early juveniles.

4.0 OVERALL STRATEGY

Five complimentary strategies were adopted to investigate mass mortality diseases of both larva and juvenile *S glomerata.*

1. Appraisal of all existing epidemiological information including in-house hatchery records and reports of previous epizootics and monitoring and documentation of ongoing hatchery and nursery operations at the PSFC.
2. Collection and preservation of larvae and spat for histopathological examination and possible diagnosis of infective agents linked to disease episodes.
3. Experimental investigations of:
    -   possible causes
    -   predisposing and ameliorating factors to disease outbreaks
    -   the effectiveness of alternative methods of disease control and prevention.
4. Determination and adoption of optimal physio-chemical rearing conditions for *S. glomerata* larvae and juveniles.
5. Use of probiotic agents to provide more favourable and stable micro-flora environments within hatchery and nursery rearing systems. (This strategy is now being addressed separately in a collaborative project started in September 1999.)

5.0 INVESTIGATION OF MASS MORTALITY SYNDROME OF LARVAL *S. GLOMERATA*

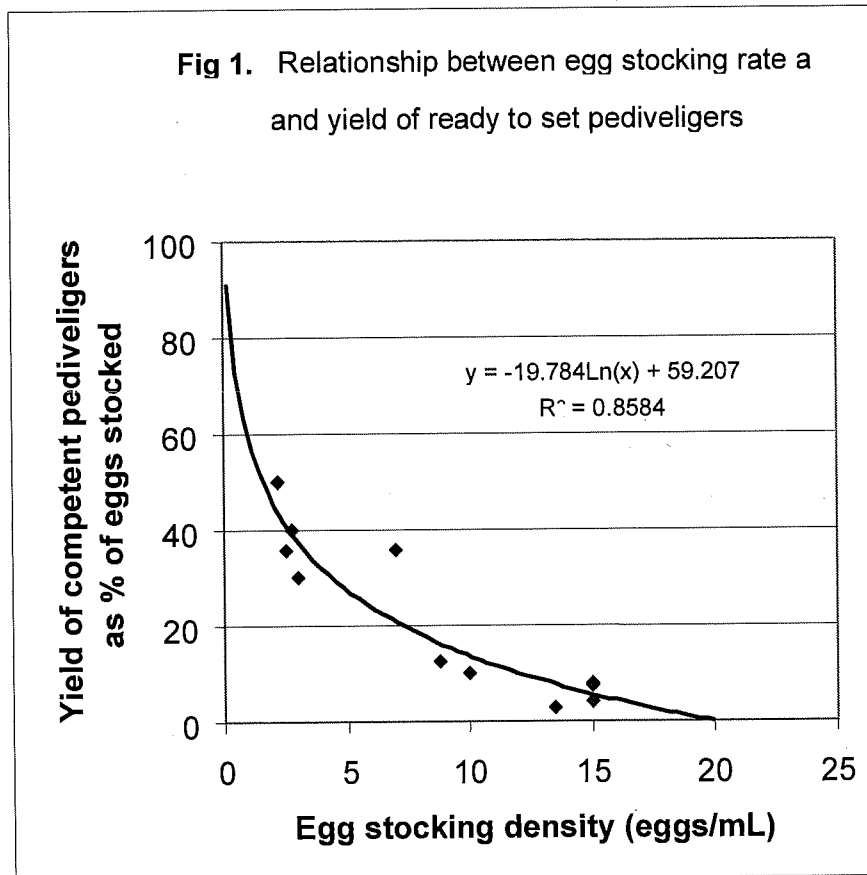## 5.1 Epidemiological Investigations

5.1.1 Methods

All available oyster hatchery performance information was extracted from diary records dating from September 1985. Reports compiled by Goard and Nell (1990), Nell et al. (1991) and by Frankish et al., 1991 were also reviewed in the light of more recent experience and information gathered. A search of published literature was also conducted in an attempt to uncover disease case-histories and research findings bearing close apparent resemblance to those afflicting larvae of *S. glomerata*.

5.2 Results and Conclusions

5.2.1 Re-evaluation of hatchery records and reports for the period 1985 to 1989

Frankish et al., 1991 in their report, *The Development of Hatchery Rearing of the Sydney Rock Oyster,* drew particular attention to the link between remarkable improvements in larval rearing success achieved from the mid 1970's. Improved forms of husbandry introduced over this period included:

- Use of coastal rather than estuarine water and its pre-settlement (6-7 days) and filtration (to 1 μm nominal).
- Reduction in stocking rates from 10 to 15 eggs /ml down to 3 to 5 eggs/ml. (Fig 1)
- Progressive refinement of larval nutrition, including the species composition, microbial quality and feeding levels of micro-algae diets. Feeding levels originally set at 100 000 micro-algae cells/ml/day on the basis of overseas information are now adjusted daily to closely match demand (consumption rate). This involves a progressive increase from 7500 to 32000 cells/ml/day over the full duration of the larval rearing cycle.
- Use of much larger (20 000L) commercial scale rearing tanks providing far lower surface area to volume ratios and more stable physio-chemical conditions.

Fig 1. Relationship between egg stocking rate a and yield of ready to set pediveligers

$y = -19.784\text{Ln}(x) + 59.207$

$R^\wedge = 0.8584$

Another important observation made by Frankish et al. (1991), from their review of hatchery records for the period 1985 to 1989, was a marked seasonal variation in hatchery success (Fig 2). While hatchery operations conducted during the first half of the calender year (January to June) almost invariably produced poor results (yields of ≤ 0.25 competent pediveligers/ml), those conducted during the second half of the year (July to December) often produced good results (yields of ≥ 0.40 competent pediveligers/ml).

Fig 2. Seasonal variation in hatchery success (as indicated by pediveliger yield/ml) in batches of *S. glomerata* 1985-1989 (from Frankish et al., 1991)
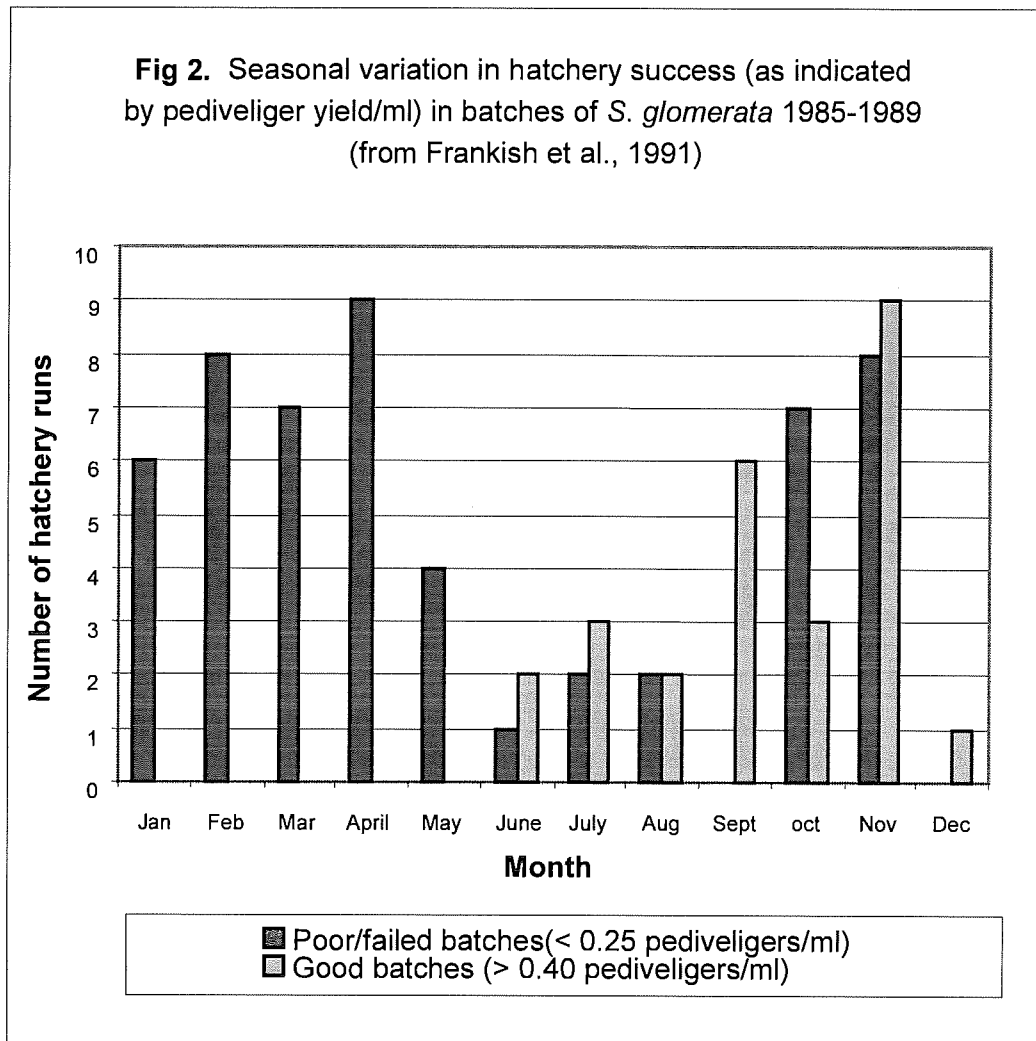
5.2.2 Analyses of hatchery records and reports for the period 1990-2000

Summarised hatchery performance data for the period 1985 to 1989 are compared with that of the subsequent period of 1990 to 2000 in Appendix 1. These data relate to a total of 70 hatchery runs.

Some salient information on the clinical manifestation of the larval mortality syndrome derived from these data are as follows:

1. A continuing trend of improving hatchery performance with *S. glomerata* over the past decade beyond that achieved by Frankish et al. 1991 is evident from a plot of successful and failed hatchery runs depicted in Fig. 3.

2. First non specific clinical signs of the disease, anorexia (Fig 4) occurred most commonly (about 75% of the time) on days 3, 4 or 5 but also occurred as early as day 2 and as late as day 8.

3. Sub-lethal effects on growth rate of diseased larvae coincident with anorexia were evident from about day 3 or 4 on (Fig 5).

4. The larval disease is characterised by elevated rates of mortality (Fig 6) culminating in complete mortality on average by day 14.

5. As indicated in Fig 7, whether or not the disease occurred and what proportion of larvae survived to settlement stage were not related to apparent egg quality as indicated by yield of D veligers from eggs. Like-wise survival rate of D veliger larvae was not strongly correlated to subsequent yield of spat.(Fig 8)

Fig 3. Successes and failures of *S. glomerata* larval runs 1985/86 to 1999/00

Fig 4. Day of manifestation of anorexia over the period 1985 to 1998 (n=51)

Fig 5. Mean +/- s.d. growth for successful (n=14) and failed (n=11) *S.glomerata* larval runs

Fig 6. Mean +/- s.e. % survival of *S. glomerata* larvae associated with successful and failed hatchery runs in small rearers

Fig 7. Apparent egg quality (% of eggs yielding normal D veligers) vs % surviving to set (F=failed run due to the mass larval mortality disease)

$$y = -0.0293x + 8.3751$$
$$R^2 = 0.0038$$

Fig 8. % yield of D veliger larvae surviving to set
Vs % of larvae put to set that yielded spat

y= 0.7461x + 28.629
R = 0.0232

% yield of D larvae surviving to set

Some very useful additional information on the epidemiology of the disease was also derived from analysis of these 15 years of hatchery records.

1. The marked seasonal effect on the incidence of mass larval mortality syndrome recorded by Frankish et al., 1991(Fig 2) has continued over the past decade (Fig 9). As over the previous decade, the calendar year can be divided into halves of contrasting hatchery fortunes for *S. glomerata*, viz.:

    -   The first half of the year (January to June) based on records of 37 attempted hatchery runs, is characterised by chronically poor survival with successful hatchery runs only being achieved once in every two to six attempts i.e. a success rate each month ranging from 17 to 40%.
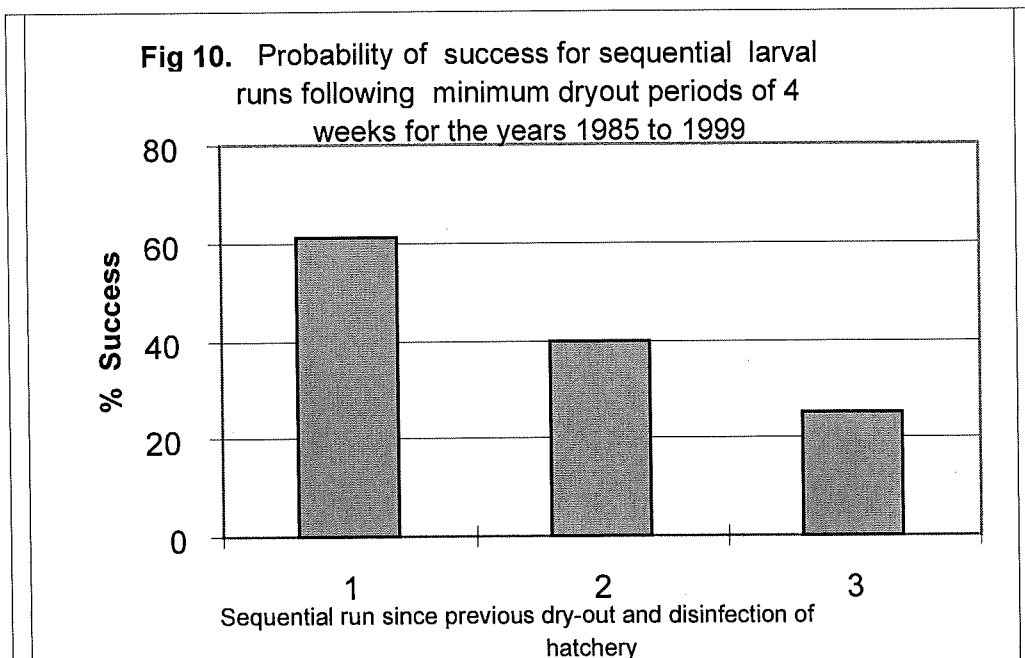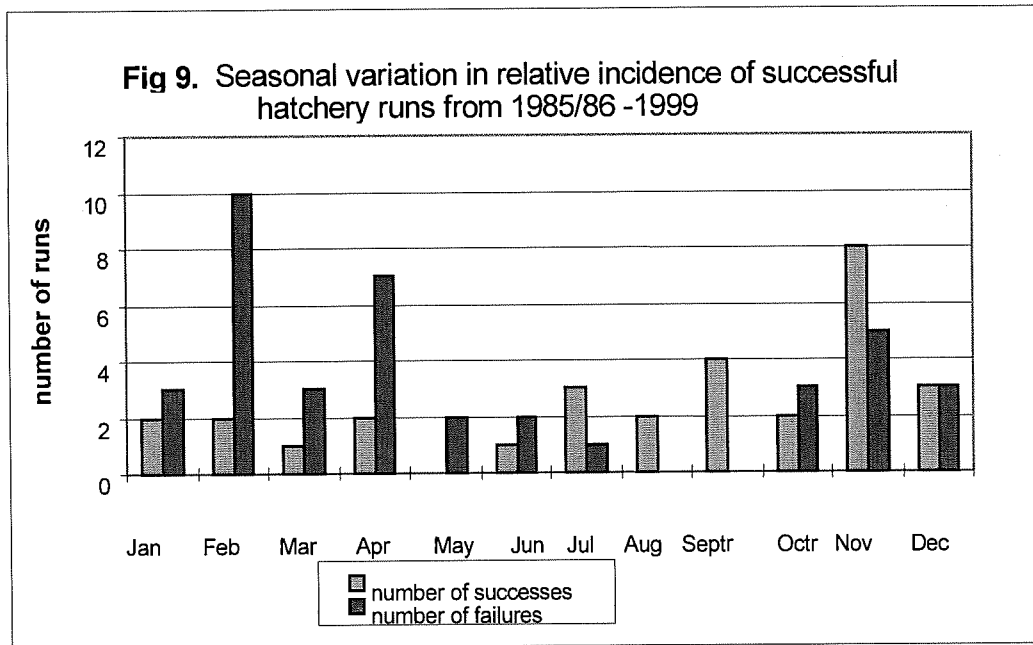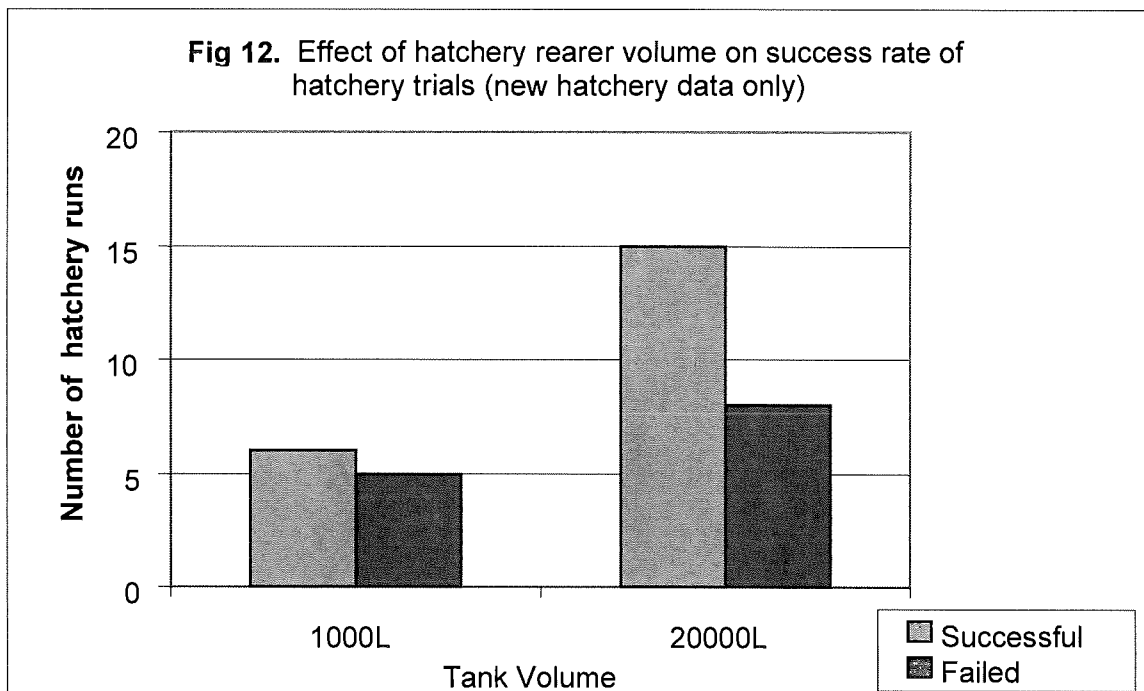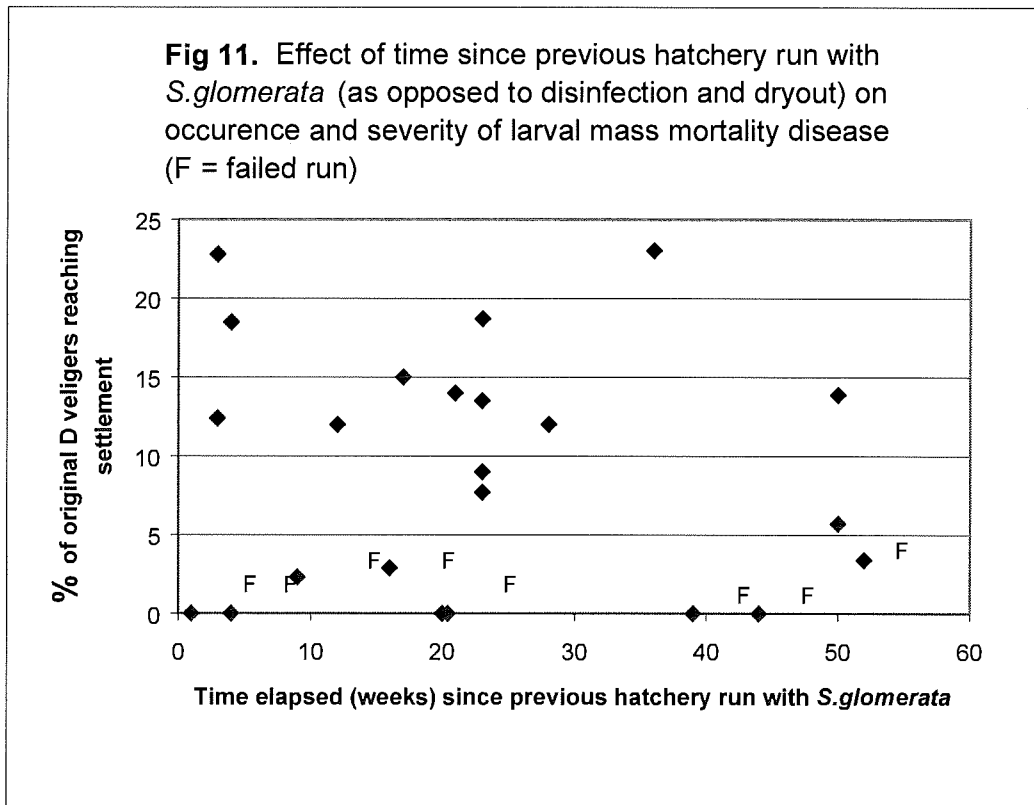
    -   During the second half of year (July to December) chances of success, based on records of 34 attempted hatchery runs, are much improved ranging from 40 to 100%.

    -   Dry-out and disinfection of the new bivalve hatchery had a marked positive influence on the relative incidence of the larval disease of *S. glomerata*. Following a minimum hatchery disinfection and dry-out period of 4 weeks, the probability of a successful hatchery operations decreased over successive runs (Fig 10) from about 60% on the first run to 40% on the second and 25% on the third. By contrast the time elapsed from the previous hatchery run did not effect the incidence or severity of the disease (Fig 11). These findings highlight the critical importance of regular programmed dry-out and disinfection of hatcheries intending to produce *S. glomerata* and the desirability of a modular design for any future commercial bivalve hatchery in NSW.

2. As indicated in Fig 12, use of 20 000L tanks rather than 1 000L tanks within the new hatchery has increased the probability of successful hatchery runs. Of successful hatchery operations conducted in the new hatchery for which comprehensive survival data are available, 4 were conducted in small (1000L) experimental tanks and 10 in commercial scale (20 000L) rearing tanks. Within this data set, survival rates from first feeding D veliger stage to ready-to-set pediveliger stage was higher in 20 000L rearing tanks (mean ± s.d., 15.7± 4.8%; range 9 – 23%) than in the 1000L tanks (11.7± 3.5%; range 8 to 14%). However the reverse trend was true for post-settlement survival that was higher for 1 000L tank reared larvae (46.3± 38.6%;

range 5 to 86%) than for the 20 000L tank reared larvae (29± 7%; range 20 to 37%). Thus larger 20 000 L tanks have substantially improved the proportion of successful runs but within successful runs they have not provided a higher net yield of spat from D veligers (average yield 4.6%) than the smaller 1000L tanks (average yield 5.4%).

Fig 9. Seasonal variation in relative incidence of successful hatchery runs from 1985/86 -1999

number of runs

Jan Feb Mar Apr May Jun Jul Aug Septr Octr Nov Dec

◻ number of successes
▨ number of failures

Fig 10. Probability of success for sequential larval runs following minimum dryout periods of 4 weeks for the years 1985 to 1999

% Success

1 2 3

Sequential run since previous dry-out and disinfection of hatchery

Fig 11. Effect of time since previous hatchery run with
S.glomerata (as opposed to disinfection and dryout) on
occurence and severity of larval mass mortality disease
(F = failed run)

Fig 12. Effect of hatchery rearer volume on success rate of
hatchery trials (new hatchery data only)

5.3 Pathology (Histopathological and micro-biological investigations)

Samples of live apparently healthy (asymptomatic) feeding larvae and moribund anorexic larvae of *S glomerata* were sent to Associate Prof. Peter Hanna (Deakin University, Victoria) in February 1994. These samples were subjected to FITC immunofluorescence tests with a panel of monoclonal antibodies that identify *Vibrio* bacteria to species level (Hanna et al., 1991). In these tests most of the seawater was removed from the larvae and then centrifuged to concentrate the bacteria for testing. Prior to testing, the larvae were sonicated for 5 seconds to open any closed shells, thereby allowing an exchange of solutions. The results of the normal *S glomerata* larvae showed that *V. alginolyticus* bacteria were present on the larval shells (see Appendix 3), but that bacteria were not detected in the water.

By contrast, the samples of diseased *S glomerata* larvae had *V. alginolyticus* present both on the shells and in the tissues and in numbers about three times greater than in asymptomatic larvae. In addition the tissues of diseased larvae often appeared to be necrosing. Test of water surrounding diseased larvae also contained considerable numbers of *V. alginolyticus*, not seen in the asymptomatic larvae. Prof. Hanna concluded that *V. alginolyticus* appears to be associated with diseased larvae. Of concern was that asymptomatic larvae also had *V. alginolyticus* present on their shells.

In May 1995, the anorexia disease occurred in *S glomerata* larvae. This outbreak was followed by succession of six more disease episodes involving a batch of clam larvae (*Tapes dorsatus*) and a batch of scallop (*Pecten fumatus*) larvae as well as further batches of *S. glomerata* larvae. Bacteriological staining (Sudan black) of live mounts and independent histopathological examination of diseased *S. glomerata* larvae by Dick Callinan (NSW Fisheries) showed patchy degeneration and necrosis of the alimentary tract epithelial cells in 4 to 5 day old larvae.

Diseased *S. glomerata* larvae from both genetically selected and control non selected breeding stock exhibiting classic symptoms of anorexia and stalled growth were sent to Prof. Hanna in May 1996. These were again subjected to FITC – immunofluorescence tests as described above and found to carry at least 3 main types of bacteria including *Vibrio alginolyticus* but low numbers precluded a definite diagnosis.

Diseased larvae were sent to Dr Judith Handlinger, and subjected to conventional histopathology and electron microscopy examination. These failed to detect the presence of a herpes virus disease of a type recently found to have caused total mortality of 5 to 7 day old clam larvae in a Tasmanian hatchery (Handlinger, pers. comm). Dr Handlinger also reported that gut epithelium cells of these larvae, were characterised by irregular long brush borders with some sloughing and apparent vacuolation. (Vacuolation of the gut epithelium, illustrated in Appendix 4, was also reported by Dick Callinan and by Dr Alex Hyatt).

Additional consignments of diseased larvae were sent to Dr Mike Hine (NIWA, NZ) and to Dr Alex Hyatt at AAHL for EM examination for possible viral infection. Again results failed to implicate viruses as a cause of the disease. Likewise Dr Tristan Renault at the IFREMER research institute La Tremblade, France and Dr Mike Hine (NIWA, New Zealand) were unable to confirm the presence of a herpes virus known to cause a similar disease symptoms in Pacific oysters.

5.4 Experimental Investigations

5.4.1 Challenge tests with Vibrio isolates

In June 1996, bacteriological plating of rearing water and of diseased larvae on marine agar and Harris media was used to isolate three *Vibrio* species. All three were propagated, purified (two passages), identified by Dr Lachlan Harris (Oonoonba Veterinary Research Station (Qld. DPI), successfully cryopreserved and used in challenge tests. The three species identified as *V. spendidus, V. mediterranei and* Vibrio sp. phenon 10/85 did not include *Vibrio alginolyticus* which had been persistently associated with previous disease outbreaks of scallop larvae and spat at the PSFC. Indeed none of the bacterial species isolated were generally recognised as causing disease symptoms of the type encountered and none were clearly capable of generating disease when used in subsequent challenge tests at 10^6/ml.

5.4.2 Experiment to further elucidate the suspected involvement of an infective agent(s)

This experiment was conducted in the PSFC hatchery in December 1997 in parallel with a commercial scale *S. glomerata* hatchery production operation. Four experimental rearing treatments were run in a total of twelve (3 replicates per treatment) 80L cylindro-conical polyethylene rearing vessels. Four of the 80L containers were accommodated in each of three, 1,000L water baths operating at 24°C with one replicate per treatment located (randomised for position) in each water bath.

To minimise the probability of inadvertent transmission of a possible disease agent(s) between the 4 treatments:
- each rearing vessel was fitted with drip, spray and aerosol proof lid.
- harvesting equipment was thoroughly cleaned, chemically disinfected with 100 ppm active chlorine solution and rinsed.
- all vessels including airlines were thoroughly cleaned between uses, chemically disinfected with chlorine solution, rinsed with bore water and air-dried between successive uses.

The four experimental treatments comprised seawater subjected to a range of preparation steps as follows:

Treatment 1 (control) EDTA (1 mg/L) treated seawater that had been trucked as usual from local beaches at either Anna Bay or Shoal Bay and stored for 7 to 14 days in outdoor 50 000 L tanks before being pumped to the hatchery via successive polyester felt filter socks with a nominal pore sizes of 5 and 1 microns respectively.
Treatment 2 As for Treatment 1 but passed through 2 x 1μm (nominal) wound cartridge depth filters mounted in series .
Treatment 3 As in Treatment 2 with additional passage through activated charcoal to remove possible bio-toxins.
Treatment 4 As for Treatment 2 except that fresh seawater was sourced from the abalone R&D facility at Tomaree Headland and conveyed in a sterilised 350L fish transporter.

Fertilised eggs from the same batch used in the above hatchery experiment were also reared in quarantine at the abalone research facility at Tomaree Head. Seawater used to rear these larvae in a 1000L vessel was treated in the same manner as in Treatment 4 of the bivalve hatchery trial.

All other hatchery rearing protocols including rearing temperature of 24°C and feeding regimes were the same as usually applied to *S. glomerata* larvae at the PSFC.

5.4.3 Results

Larvae in all four experimental treatments (in common with the commercial batch of larvae reared in a 20, 000 L vessel) succumbed to the wasting diseases (ubiquitous cessation of feeding and growth) by day 5. By contrast, larvae reared in a 1,000L vessel in quarantine at the Tomaree Head facility remained asymptomatic, feeding, growing and surviving normally to and beyond metamorphosis. (Frankish et al., 1991).

Diseased larvae subjected to SEM, TEM by Dr Alex Hyatt and conventional light microscopy histopathological examination by Dr Handlinger failed to detect significant numbers of bacterial, protozoan or viral agents in or on affected larvae, nor indeed any other obvious histopathology.

5.4.4 Conclusions

These results in conjunction with other evidence already presented indicate that the anorexia disease of *S. glomerata* larvae:

– Is not vertically transmitted
– Is not caused by seawater quality, dietary or stock quality factors and cannot be ascribed to invasive disease agents.

It is therefore probable that the cause of this wasting disease is cytotoxic and appetite suppressing compounds (exotoxins), probably *in situ* bacterial in origin and probably associated with bacteria persisting on wet surfaces within the bivalve hatchery or associated seawater storage and plumbing system at the PSFC between consecutive production cycles.

5.5 Disease prevention through optimised temperature regimes for gamete storage, incubation and larval rearing of S. glomerata – Summary of results

Experiment 1 – Evaluation of the effects of storage temperature on sperm motility.
To ensure retention of a high degree of motility, sperm should be stored at or below 15^0C and preferably used within an hour of collection.

Experiment 2 - Effect of temperature and storage time on fertilisation.
Storage periods for eggs over which high rates of fertilisation (>90%) are assured decrease from 2h at 15^0C to less than 30 mins at 30^0C.

Experiment 3 – Effect of temperature (Fig 13) on growth and survival of larvae.
Optimum larval rearing temperature was found to be $29^0 C$ which is 5^0C higher than the routinely practised rearing temperature of 24^0C

Fig 13. Effect of temperature on growth and survival of *S.glomerata* larvae

$y = 0.1061x^4 - 3.697x^3 + 30.333x^2 - 62.792x + 52$

$R^2 = 0.9909$

✱ Mean Growth(um) after 13 days   ◆ Mean % Survival
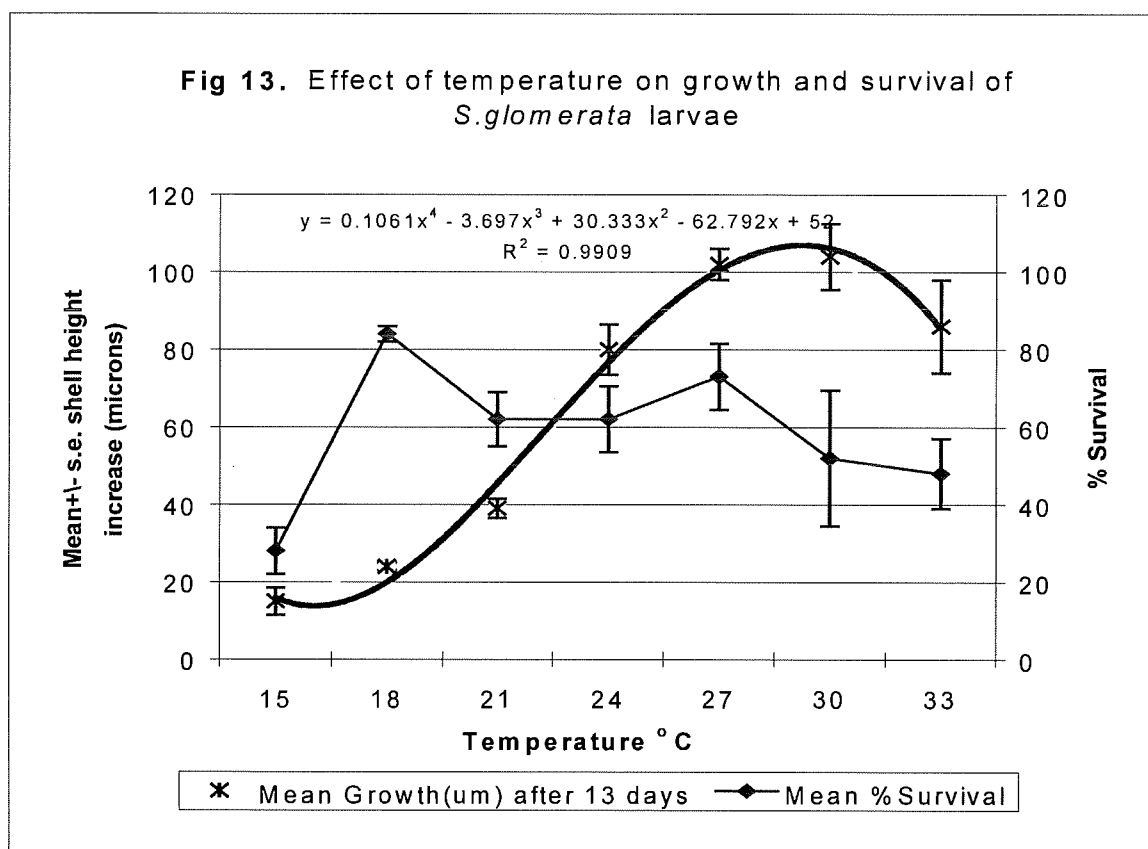
5.6 Use of Probiotics

From 1994 to 1996 intellectual property constraints prevented importation and evaluation of an array of probiotic bacteria shown by colleagues in the USA (Dr Philippe Douillet, University of Texas at Austin) and Japan (Nogami and Maeda) to be effective in combating vibriosis in marine larvae including those of another rock oyster, *Crassostera virginica* (Douillet, 1994). An opportunity to evaluate probiotic bacteria for combating the suspected cause (exotoxin forming bacteria) of mass mortality diseases of larval and juvenile *S. glomerata*, arose in 1998 from the work of Dr Lewis Gibson and post graduate students at the Dept of Cell Biology at the University of Technology (UTS), Sydney. Dr. Gibson's team had identified several strains of *Aeromonas* bacteria with demonstrable inhibitory effects on marine *Vibrio* bacteria of known pathogenicity to finfish and invertebrate larvae including those of the Pacific oyster (Gibson et al., 1998).
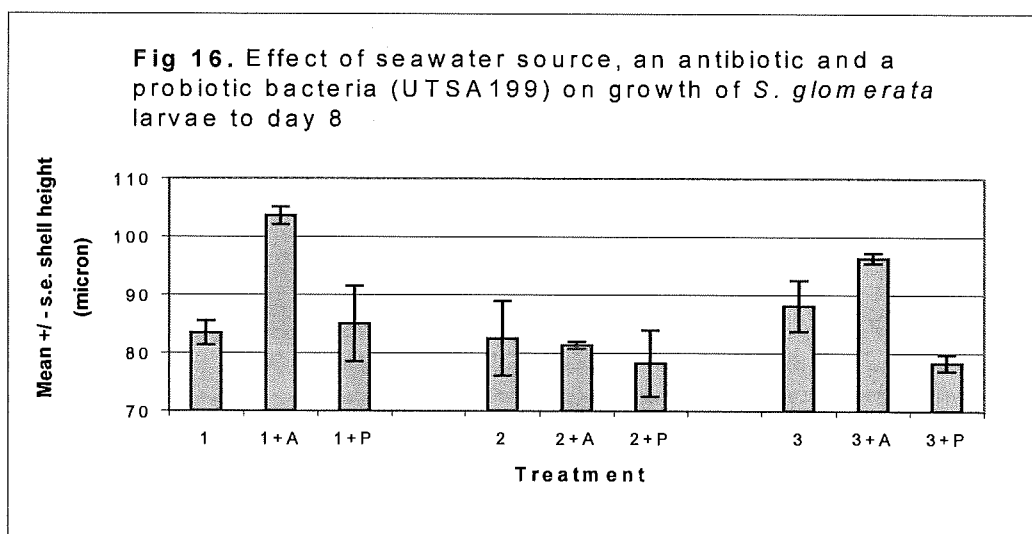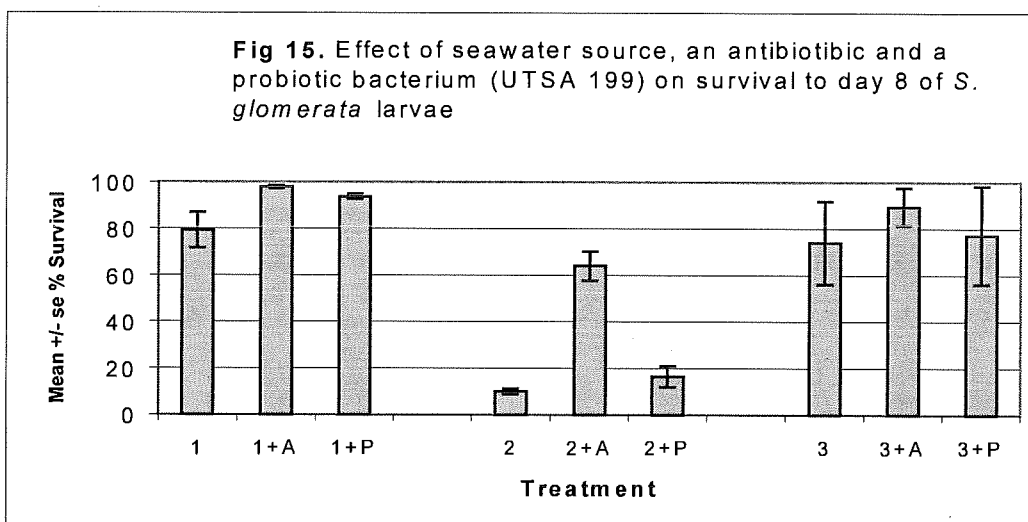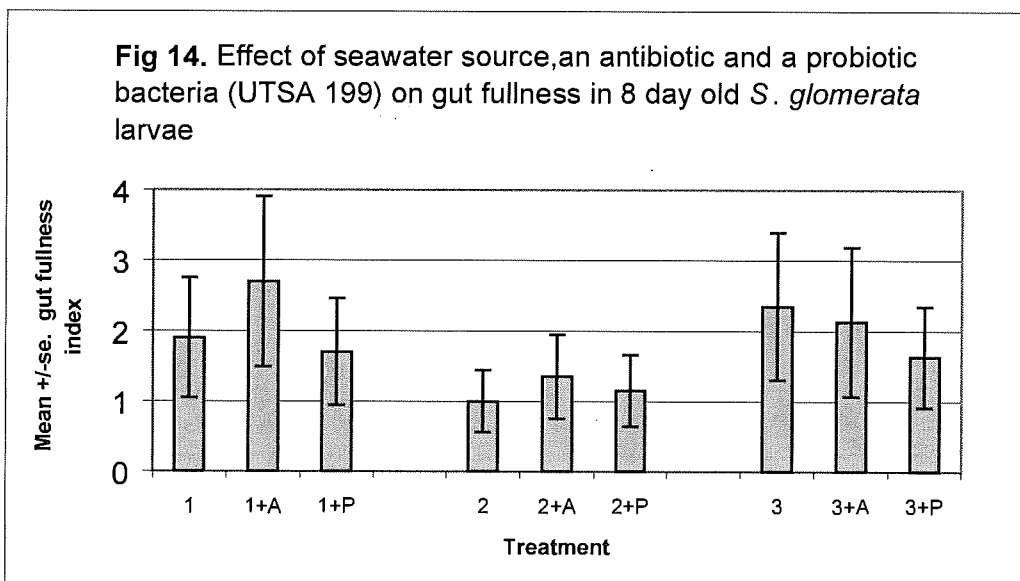
Preliminary research to evaluate probiotics in combating larval disease was conducted in collaboration with Dr Lewis Gibson and BSc Hons. student Mr Edward McGregor. Eighteen species/strains of bacteria isolated from seawater and from surfaces of vessels containing diseased larvae were identified as either *Vibrio* or *Aeromonas* species. All but one strain were found sensitive to tetracycline and thirteen were significantly inhibited by a probiotic strain of Aeromonas (UTS-1999) bacteria (McGregor, 1999). A 3x3 factorial design experiment involving the use of seawater from three alternative sources, namely:
1. normally sourced, stored and 1μm filtered seawater
2. contaminated seawater used to rear diseased larvae
3. stored seawater sourced from the marine fish hatchery and prefiltered to 1 μm.

These seawater sources were used alone or in combination with either a probiotic bacteria (UTSA 1999) at 10^3 cells/mL, or an antibiotic (chloramphenicol at 5 mg/L) of broad spectrum efficacy in inhibiting vibriosis in bivalve larvae (Lodeiros et al., 1987; Jeanthon et al., 1988). Five replicates for each of the nine treatments comprised 250 ml conical flasks containing 150 ml of seawater stocked with 1 day old *S. glomerata* larvae at 5/ml and held in a shaker incubator at 25±1°C for 8 days.

Results presented in Figs 14, 15 and 16) showed that:
*   Contaminated water reduced food ingestion and increased mortality compared with other treatments.
*   The antibiotic generally improved food consumption reduced rate of mortality and marginally improved growth rate.
*   Use of UTSA 1999 did not generally improve food ingestion or growth rate but may have marginally reduced mortality although not significantly.

Fig 14. Effect of seawater source,an antibiotic and a probiotic bacteria (UTSA 199) on gut fullness in 8 day old *S. glomerata* larvae

Fig 15. Effect of seawater source, an antibiotibic and a probiotic bacterium (UTSA 199) on survival to day 8 of *S. glomerata* larvae

Fig 16. Effect of seawater source, an antibiotic and a probiotic bacteria (UTSA199) on growth of *S. glomerata* larvae to day 8

Seawater source: 1 = mollusc hatchery store; 2 = contaminated; 3 = marine fish hatchery
Additive: A = antibiotic; P = probiotic

These results together with those of the earlier research of Gibson et al., (1998) with *C. gigas* were sufficiently encouraging to prompt a successful application for an ARC/SPIRT grant to develop probiotic techniques to combat mass mortality diseases of larval and juvenile *S. glomerata*. Dr Gibson serves as chief investigator; with Dr Mike Heasman and Dr Tony Maurice as co-investigators and NSW Fisheries as a commercial partner. BSc Hons. graduate Mr Cheok Tan was selected as the successful PhD candidate for the project in December 1999. The first experiment, to evaluate the use of several bacteria as probiotics to prevent and combat the larval disease was conducted in March 2000.

In the inaugural 2x8 design experiment of the project, seven bacterial flora manipulation treatments plus a control were applied in combination with two methods of pre-treating seawater used to rear *S glomerata* larvae for 8 days post hatch. The bacterial flora treatments comprised the pre-inoculation of rearing water with single, paired or all combinations of three different bacteria. The first of these was a UTSA 1999, previously shown by Gibson et al. (1998) to counteract pathogenic strains of *Vibrio tubiashi* when applied as a challenge to Pacific oyster (*Crassostera gigas)* larvae. The second was, a bacterium previously shown to be non pathogenic and of substantial nutritional value (a food bacteria) to *S glomerata* larvae by Nell et al. (1993). The third bacteria was an archived strain of *Vibrio alginolyticus* held by UTS that was added as a challenge rather than as an alternative probiotic.

Results (Fig 17) showed that sterilization (autoclaving) of sea water followed by re-inoculation with one or a combination of the 3 different selected species of bacteria raised survival of 8 day old *S, glomerata* larvae from a base level of only 9% (typical of disease episodes) in control larvae reared in conventionally treated sea water (stored for ≥ 7 days and $1\mu m$ filtered), to a range of 45 to 68%. The latter survival rates are among the highest achieved with *S. glomerata* larvae. Benefits conferred by sterilization and re-inoculation of seawater with non pathogenic bacteria were equally dramatic in relation to enhanced larval growth (Fig 18).

Fig 17. Effect of seawater sterilization and added bacteria on mean+\- s.e. % survival of day 8 *S. glomerata* larvae [note: prefix N=stored, 1um filtered seawater; prefix S=sterilized (autoclaved) seawater]
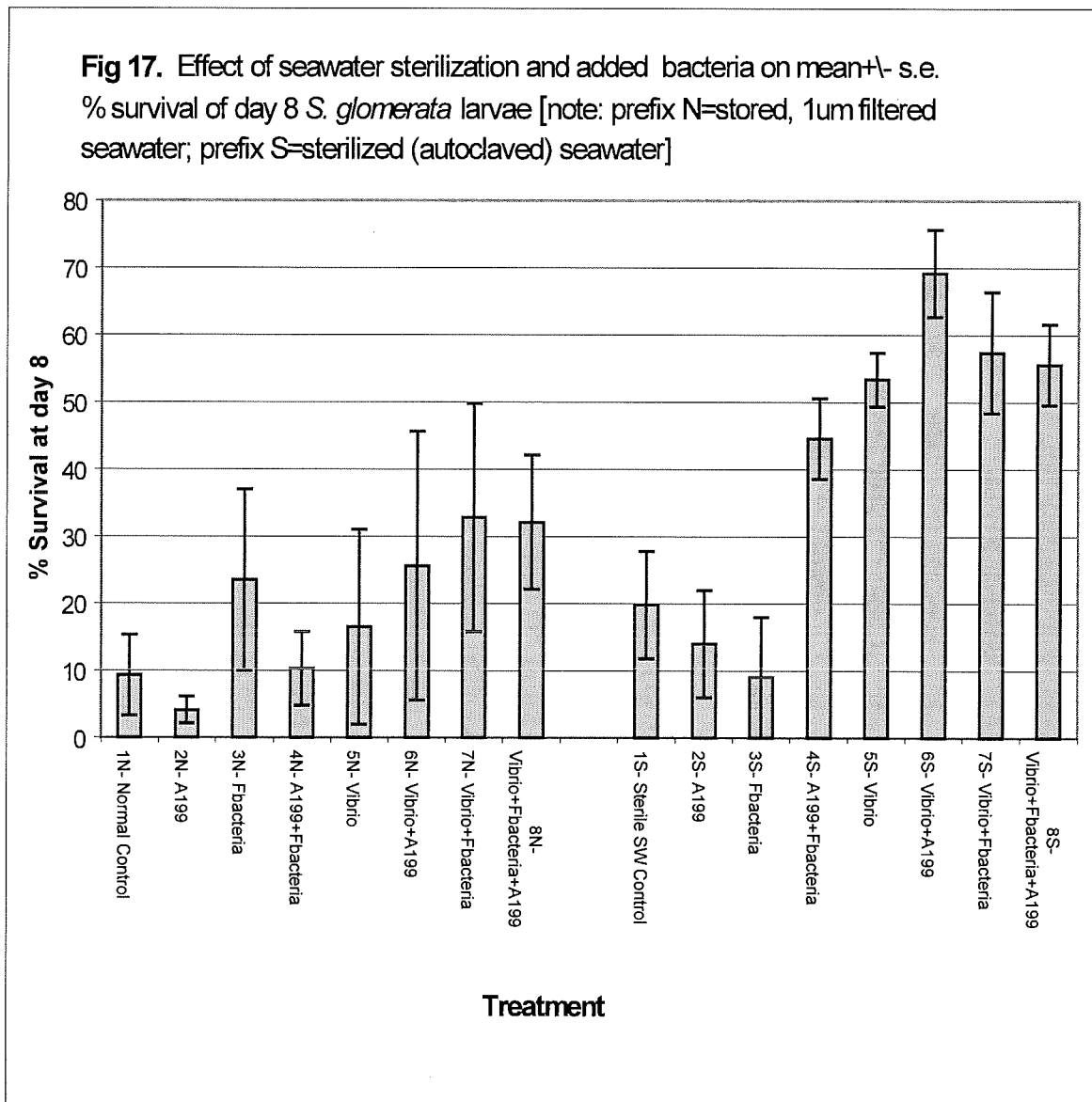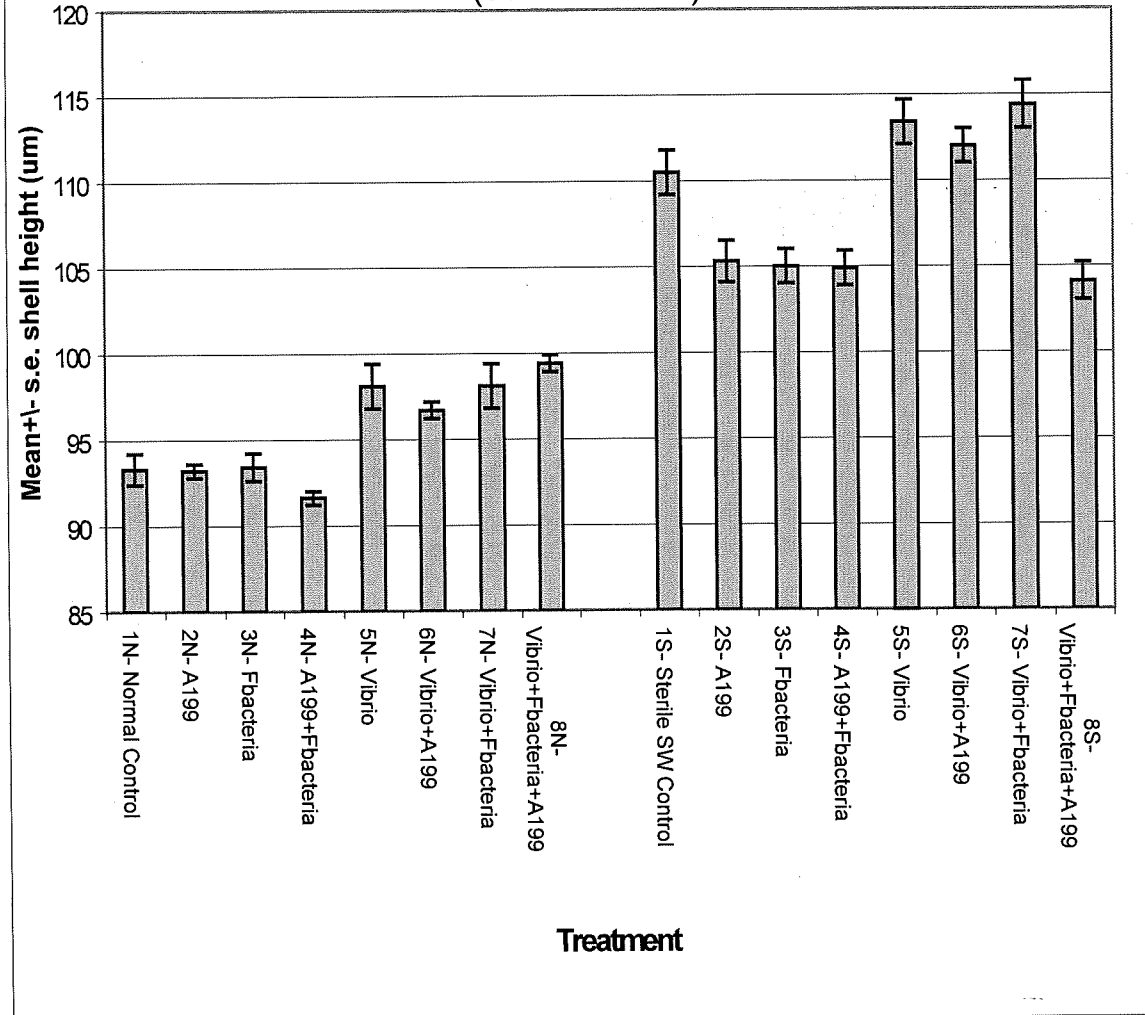
Fig 18. Effect of seawater sterilisation and added bacteria on growth of 8 day *S. glomerata* larvae shell height (error bars = s.e.)

6.0 INVESTIGATION OF MASS MORTALITY SYNDROME OF JUVENILE *S. GLOMERATA*

6.1 Epidemiological Investigations

6.1.1 Background

As with the larval mortality disease, all available hatchery data (Appendix 2) were extracted from diary records dating from September 1985. Reports compiled by and by Goard (1990), Nell et al. (1991) and by Frankish et al. (1991) were also reviewed in the light of more recent experience and information gathered. A search of published literature was also conducted in an attempt to uncover disease case- histories and research findings bearing close apparent resemblance to those afflicting spat of *S. glomerata*.

In 1987, the still current down-weller set system was installed at the PSFC. This incorporated the use of ground and graded (250 – 500 μm) scallop shell culch within 450 mm diameter 210 μm mesh screens each holding 100 000 to 200 000 single seed spat. Using this system to maintain single seed and to prevent doubles or triples etc., spat are separated from larvae using a 350 μm mesh screen. After approximately two weeks, spat are large enough to be retained on a 350 μm mesh and are removed from this hatchery system and placed in outdoor upwellers. From 1987, passive upweller systems located either in the field and fed raw estuarine water or on site at the PSFC and fed re-circulated brackish pond water, have been used.

While the introduction of ground scallop shell single seed settlement culch in conjunction with a Bayes (1981) downweller nursery system enabled consistent large scale (>1 million) production of single seed *S. glomerata* , these large batches often suffered intermittent mass mortalities. Over the period 1987 to 1990, these mass mortalities first manifest 8 to 30 days after the larvae were introduced to the settlement system. Apparently healthy spat moved from the hatchery at the PSFC to upwellers in different estuaries also experienced sudden and severe mortalities within 5 to 7 days. These outbreaks always coincided with mortality of spat remaining in the hatchery. On one occasion however spat, some of which were moved out of the hatchery 3-4 days after settlement (size >350 μm), subsequently survived in the upwellers while those retained in the hatchery died.

The investigation of Nell et al. (1991) evaluated intensified hatchery hygiene protocols, especially increased rinsing of spat and disinfection of settling tanks and equipment. The latter did not significantly reduce total bacteria, presumptive *Vibrio* bacteria, or the subsequent survival (14%) of spat relative to controls. It did however reveal very large concentrations (14 to 18 $\times 10^4$ g^{-1}) of presumptive *Vibrio* bacteria on conventional ground scallop shell culch used to induce settlement of *S. glomerata* pediveligers as single spat.

In an associated report to that of Nell et al. (1991), Goard (1990) made the following key observations:
- Mortalities occur suddenly and severely, (up to 95% in 36 hours) in spat that show no obvious signs of stress, are actively feeding and producing good quantities of faeces, have dark coloured guts and are exhibiting good shell growth.
- Survivors on-grow normally during and after rapid pulses of mortality.
- Spat set on the sides of downweller screens and not removed appear to be least effected.

In reference to an experimental investigation of the disease, Goard (1990) also stated that all spat were on 670 μm and 500 μm screens exhibited mortality one day after their first multiple grading and counting with cumulative mortality increasing to approximately 90% over the next 6 days. By contrast spat that had set on flat fibrous cement slats that had been laid flat on the screens on top of beds of scallop shell, or on the smooth plastic walls of the nursery screens, suffered negligible mortality. While there was no absolute proof that bacteria cause or contribute to spat mortalities, a bacterial linkage to the disease was supported by the far superior survival of spat that had set on the slats and walls.

6.1.2 Results

Evaluation and re-evaluation of hatchery records 1988-2000

Examination of general hatchery records useful epidemiological information extracted from records for operations conducted in the new hatchery 1988 to 1999 were as follows:

1. Seasonal Factors

In contrast to the larval disease, no seasonal trends in the occurrence or absence of mass mortality of juvenile *S. glomerata* were apparent from these data (Fig 19).

Fig 19. Seasonal occurrence or absence of mass spat mortality episodes 1988 to 1999 (n=19)
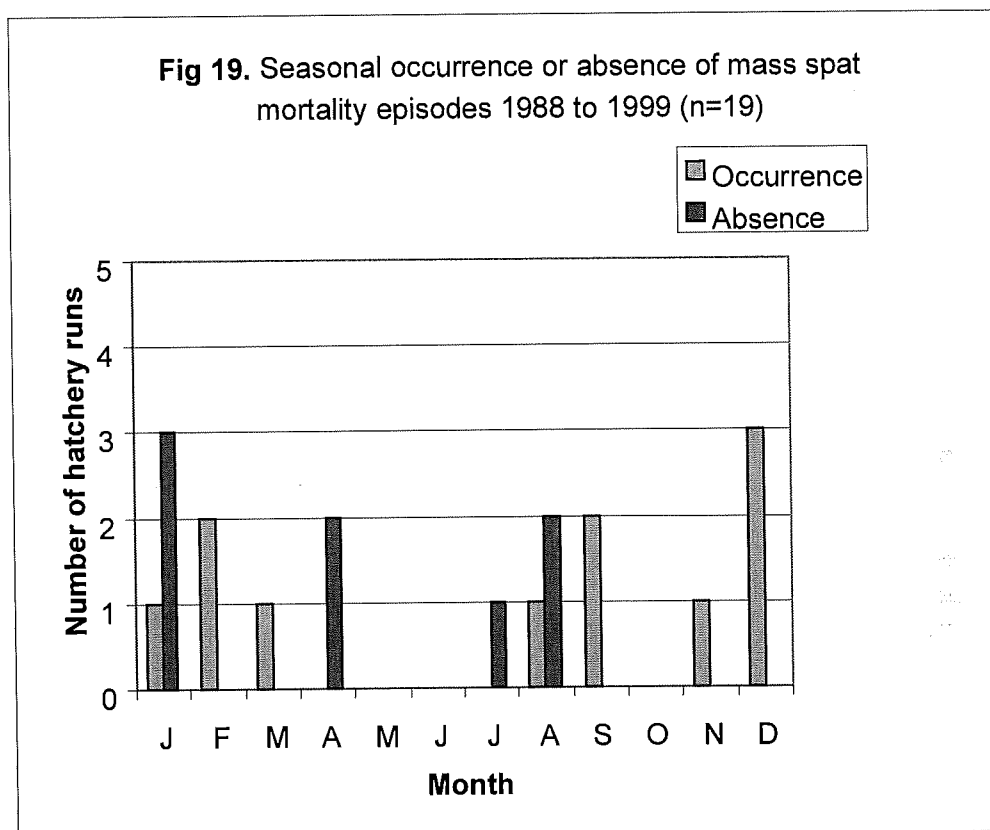
2. Effect of larval rearing tank size

Also in contrast with the larval disease, juvenile *S. glomerata* reared as larvae in commercial scale 20 000 litre tanks experienced much higher relative incidence of mass mortality (9 out of 11 documented cases) than their counter parts reared in much smaller 1000 litre tanks (only 1 out of 6 cases).

Fig 20. Occurrence or absence of mass spat mortality episodes in relation to size of larval rearing tanks 1988/1999 (n = 19)

Fig. 21. Effect of batch size (millions of pediveligers put to set) in relation to incidence of mass spat mortality (n=19)
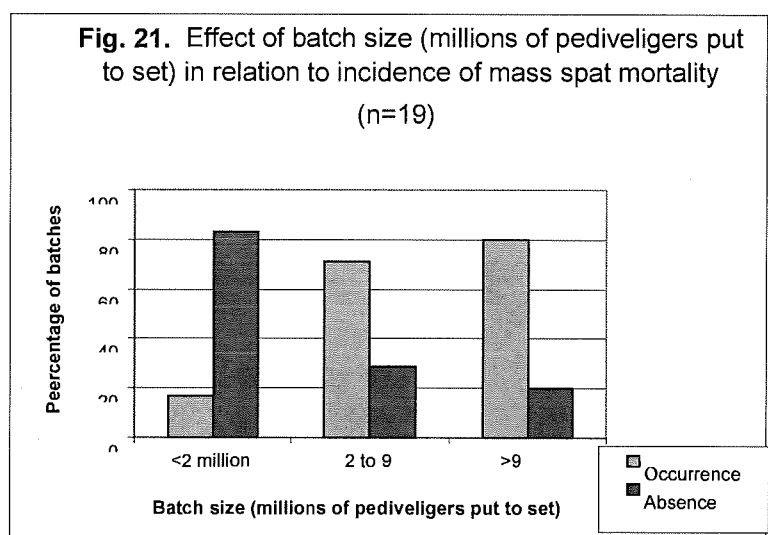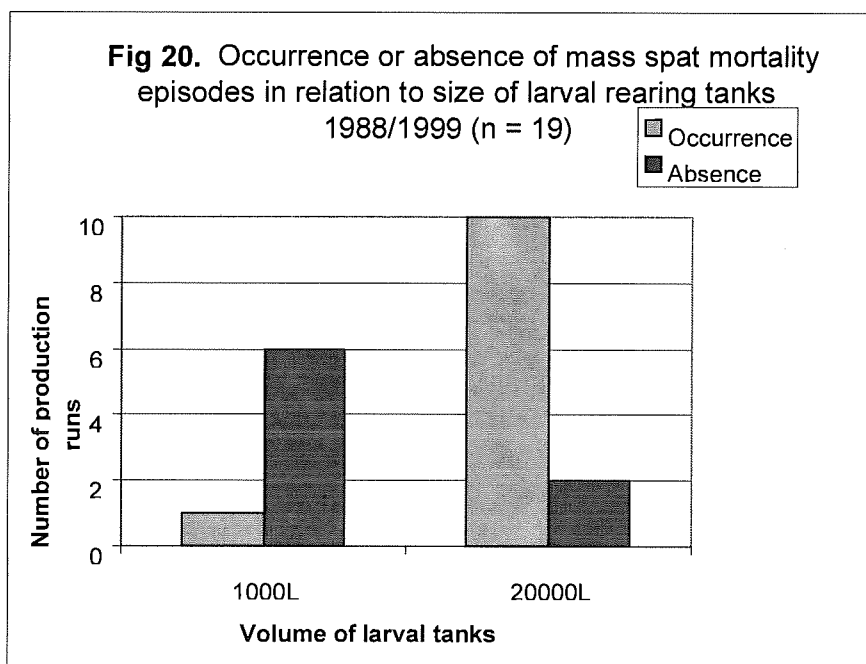
A possible explanation for this unexpected observation was density factors associated with larger batch sizes of juveniles reared in 20 000 litre tanks. This explanation was supported by data collected from the same 17 batches of spat referred to in Fig 20, that were pooled according to batch size and regardless of the volume of the larval tank used. These data (Fig 21) strongly suggest that the probability of mass spat mortality episodes did indeed increase with the absolute size of batches and probably was not directly influenced by larval tank volume.

Intensive monitoring of spat during epiozooitics

Case History 1- Production of triploid of *S. glomerata*- February 1994
The first recurrence of the Mass Mortality Syndrome in more than three years was experienced during the February 1994 hatchery production run. No deaths or any other signs of disease were observed prior to the detection of mass mortality rates of 40% and 53% respectively in preserved diploid and triploid of *S. glomerata* spat sampled on April 11 (day 42 in Fig 22). By this time all

dead spat were free of tissue remnants suggesting that all deaths had occurred as a short sharp pulse within four days of initial multiple grading and counting (day 38 in Fig 22).

The mortality data presented in Fig 22 are suggestive of a possible link between the mass mortality syndrome and stress and physical damage sustained by small spat during protracted sieve grading and counting using volumetric methods. Growth rates of surviving spat, as in previous epizootics discussed above (Goard, 1990 and Nell et al., 1991), appeared to continue normally both during and beyond periods of sudden and severe mortality.

Fig 22. Mortality data *S.glomerata* mass mortality episode Feb.94
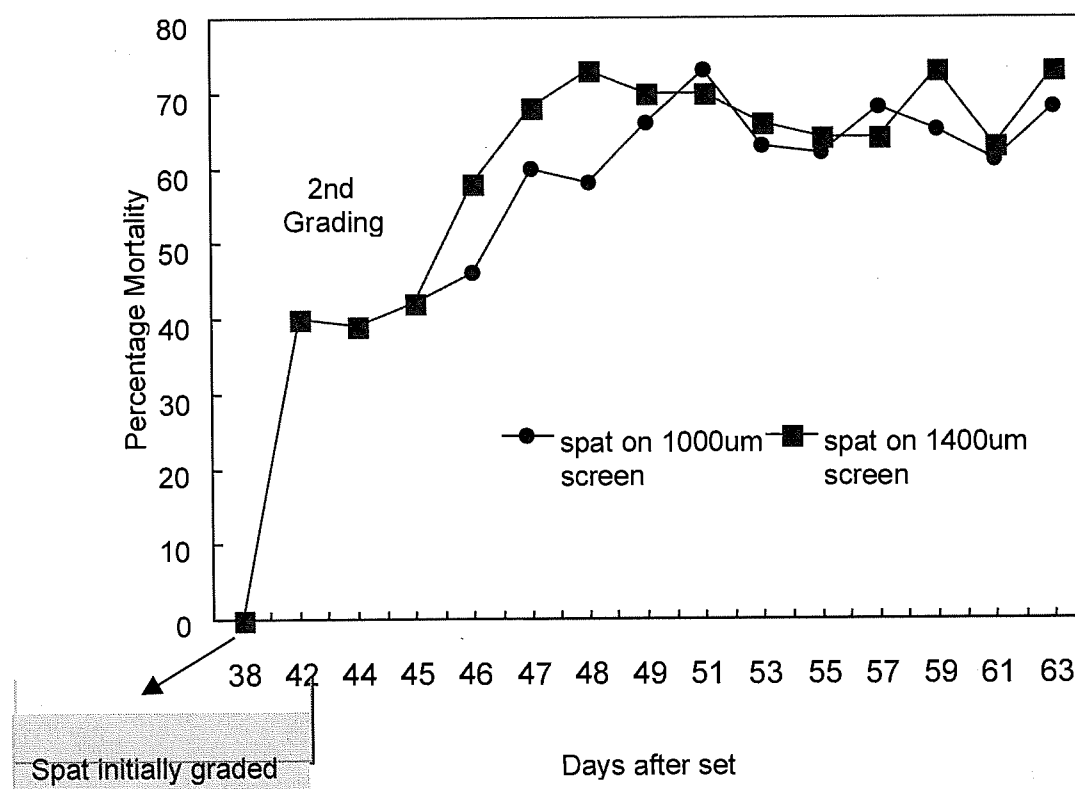
Case History 2- Monitoring of early juvenile stock produced during the course of production of 3rd generation mass selection lines of *S. glomerata*-in January and February 1995

In response to the recurrence of the mass mortality episode in February 1994 and possible implication of disturbance and trauma sustained in grading and counting, the progressive growth and mortality of broods of spat from 6 separate breeding lines of *S. glomerata* were closely and frequently monitored prior and subsequent to successive grading and volumetric counting operations in January and February 1995. These grading and counting operations were conducted 12 days apart and were applied as juvenile stock attained mean shell heights in the order of 600 to 800 microns and again at 900 to 1100 microns in accordance with what was then standard practice.

As illustrated in Figs 23 to 24, up until days 9 to 11 post-set (18th and 19th January) when all 6 genetic lines and grades thereof in the size range 500 to 800 micron were re-graded, cumulative mortality remained below 8%. However over the 1st to 3rd days after grading it rose markedly (15 to 38%) across all breeding lines in the smaller grade spat and across three of the six breeding lines
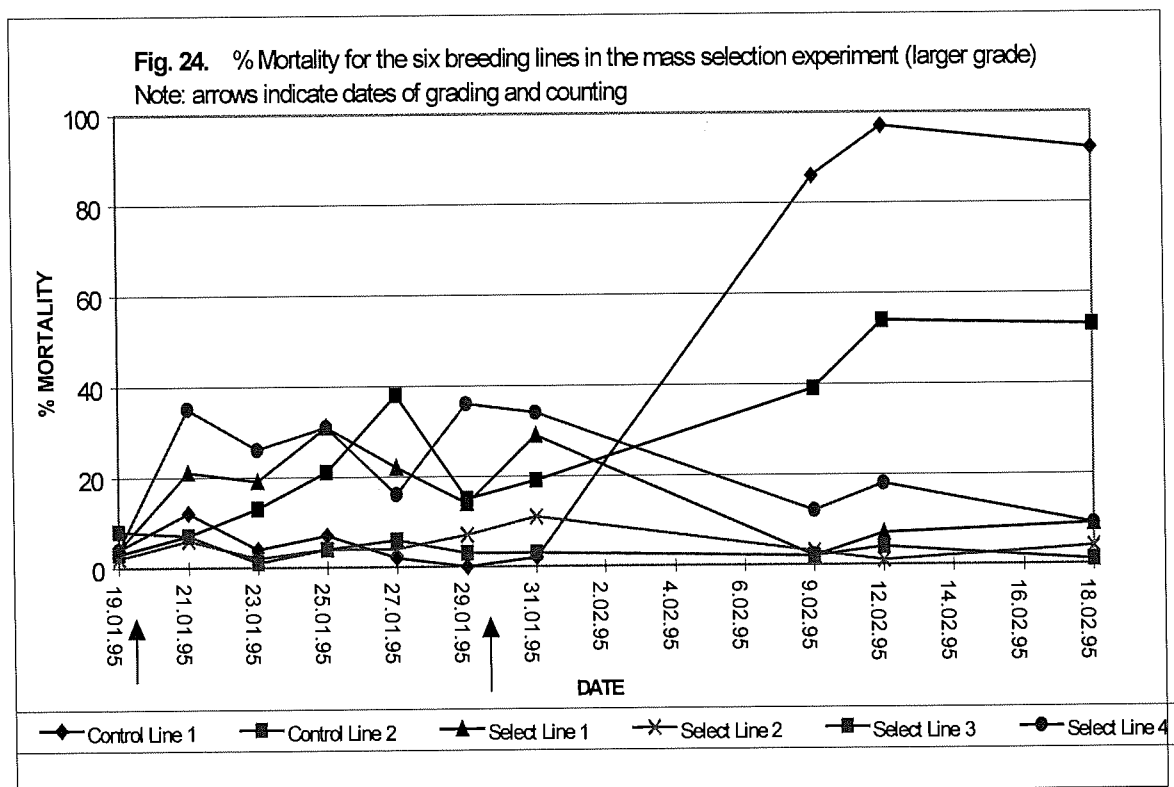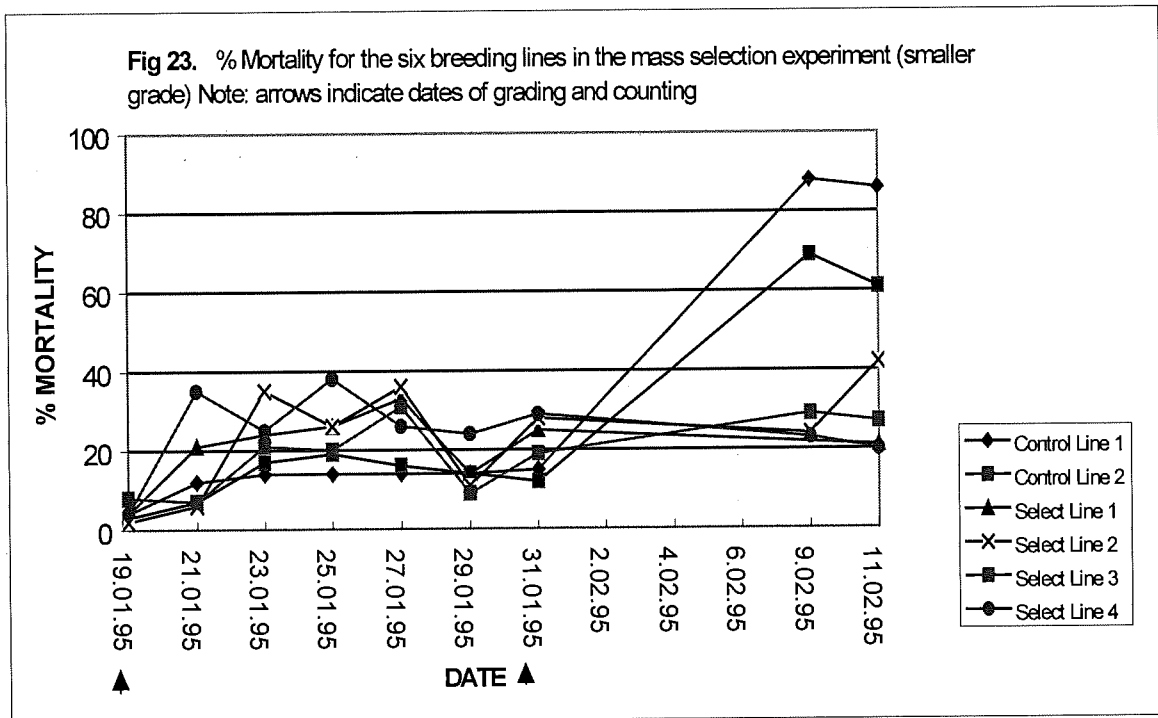
in the case of larger grade spat that had already been transferred from the hatchery to the field upweller nursery at Wanda Head, Port Stephens.

During the 9 days between successive gradings on the 18th and 19th and on the 29th and 30th January, a major disparity developed between cumulative mortality in the four genetically selected lines of spat and the two control lines of spat those produced from non genetically selected parent stock. This disparity was consistent across the larger and smaller size grades but was most pronounced in relation to the larger grade. By the 11th February, cumulative mortality for larger spat across all four genetically selected lines remained at or below 10% while the two control non selected lines of spat exhibited mortality rates of 61 and 87%.

These results in conjunction with histopathological evidence presented below, supported the initial hypothesis, that mass mortality episodes occur as pulse events that are caused by bacterial infections triggered by such things as trauma and physiological stress sustained by small spat during mechanical sieving. As observed in previous episodes, growth rate of surviving spat did not appear to be checked prior to, during or following pulse mortality events i.e. the disease appears to manifest as an all (rapid death of afflicted spat) or nothing (no sub-lethal effects on surviving spat) phenomenon.

An additional pertinent observation of growth rate data presented in Figs 25 and 26 is that good growth rates of 45 to 50 micron/day were exhibited by all breeding lines of both size grades from the time of settlement to the time of transfer to the field nursery 9 days later. Once in the field nursery growth rate slowed to a universally low rate of 20 to 25 microns/day and may have reflected dietary or density related suppression of growth that otherwise would be expected to be in the order of 50 to 100 microns /day under the high (23 to 25°C) ambient summer temperatures prevailing at the time.

A possible explanation for the markedly lower rates of mortality sustained by genetically selected lines of spat was that four generations of selection for fast growth and survival has inadvertently conferred some genetic resistance to the mass mortality disease. On the other hand, slight inadvertent differences in handling or density factors may have been responsible. The only way to confirm genetic resistance was to gather further evidence during future selective breeding operations.

Fig 23. % Mortality for the six breeding lines in the mass selection experiment (smaller grade) Note: arrows indicate dates of grading and counting

Fig. 24. % Mortality for the six breeding lines in the mass selection experiment (larger grade) Note: arrows indicate dates of grading and counting

Case History 3- Monitoring of juvenile stock produced during the course of a hatchery operation to produce a batch of triploid spat for sale to farmers in late 1998 /early 1999.

A batch of triploid *S glomerata* spat were put to set on the 10 to 14th October 1998. These were retained in the hatchery then transferred to the Wanda Head field upweller nursery. The health of these stock was monitored every few days. Significant numbers of dead or dying (gaping) spat were first detected following a very protracted period of low temperature suppressed growth. Confirmation that the batch had suffered a mass mortality episode was confirmed over the following week as daily estimates of cumulative mortality rapidly rose to about 70%.

To check whether or not mass mortality had occurred as short duration pulse and that those spat that did not die continued to grow normally, the status (live or dead) and size frequency distribution of 300 spat randomly sampled from each of three separate upweller screens, was determined on the 18th of December and again 17 days later on the 4th January 1999.

The initial mean± s.e. percent of dead spat on 18th of December (Fig 27) was 79.8±4.3%. This remained unchanged when re-assessed as 73.8± 0.8% on the 4th January (see Fig 28), indicating that no additional mortality had occurred over the interim. Likewise, mean± se. shell height of dead spat on the 4th January (869± 8 µm) was almost identical as on 18th December (849±7 µm). By contrast, mean ± se. shell height of live spat had increased from 1390± 29 µm to 2490± 46 µm over the 17 days. This constituted a very respectable mean growth rate of about 80 µm/day and probably reflected reduced densities of spat on the upweller screens following grading and the massive reduction of densities created by the mass mortality.

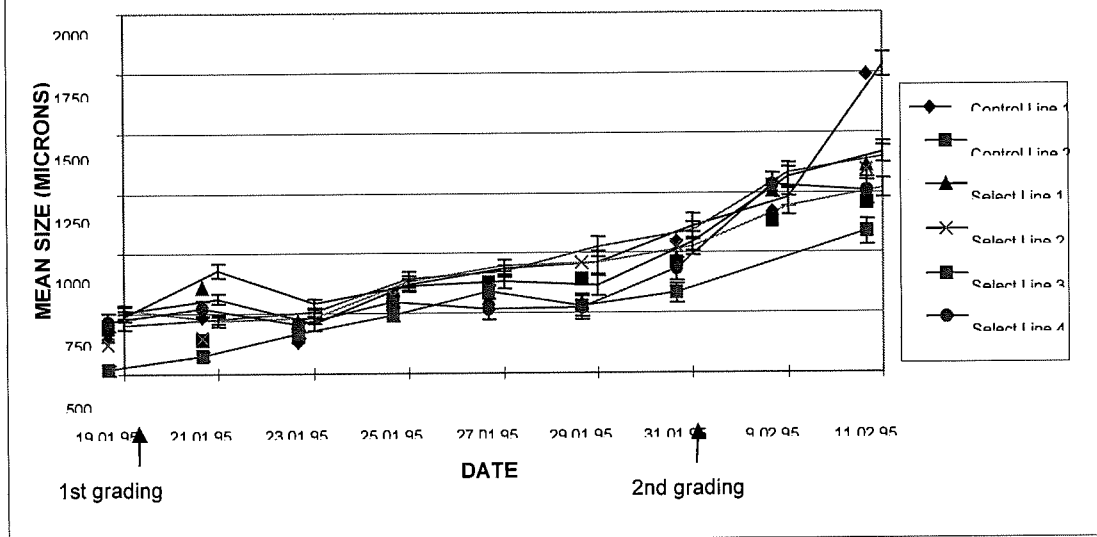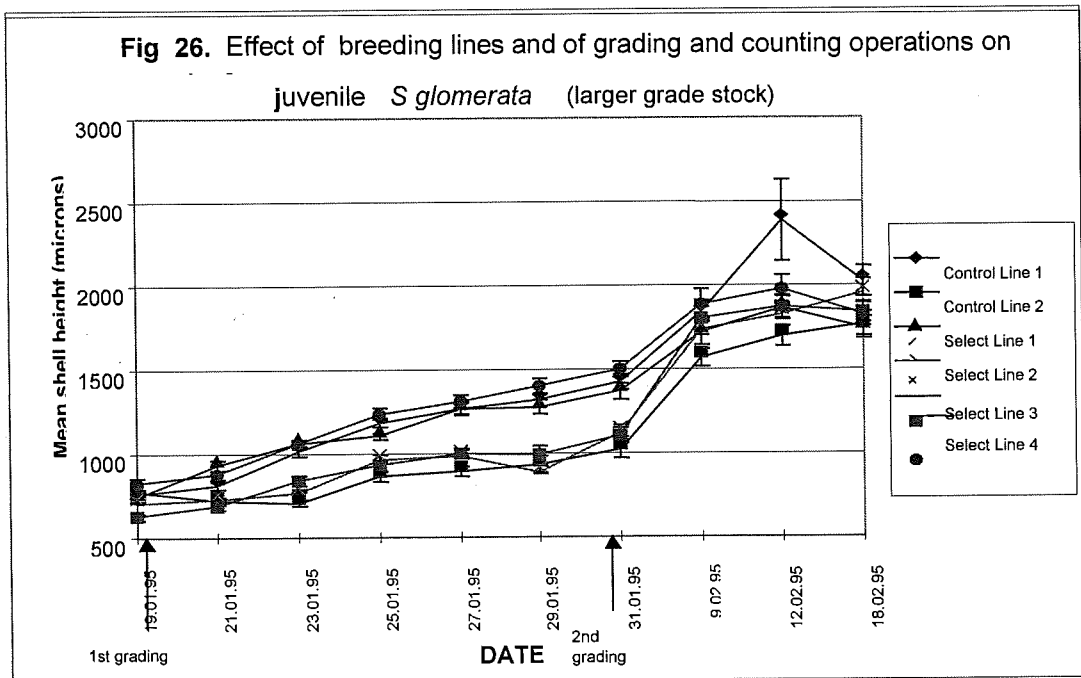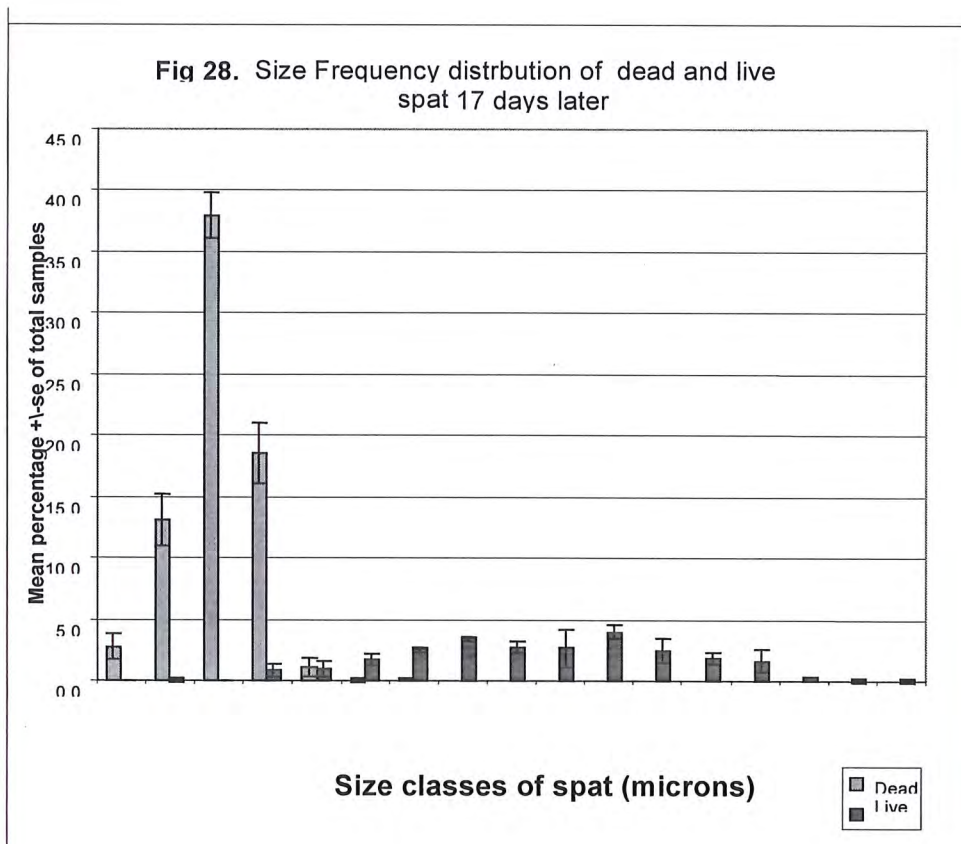Fig 25. Effect of breeding lines and handling on growth of *S. glomerata* (smaller grade)
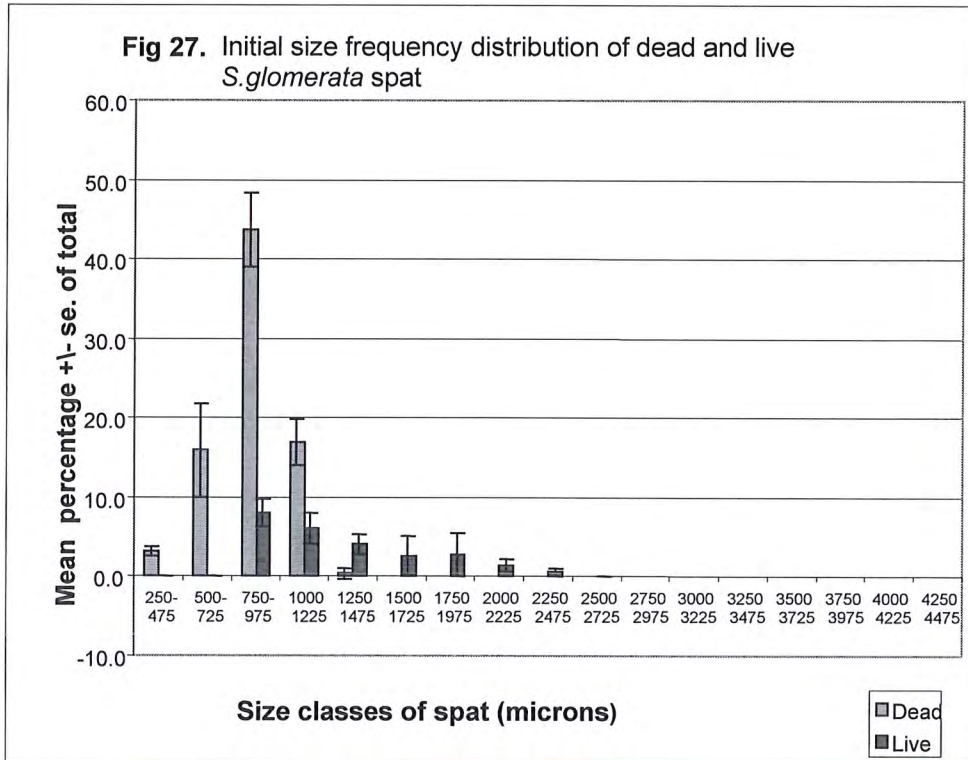

Fig 26. Effect of breeding lines and of grading and counting operations on juvenile *S glomerata* (larger grade stock)

Fig 27. Initial size frequency distribution of dead and live
S.glomerata spat

Fig 28. Size Frequency distrbution of dead and live
spat 17 days later

6.2 Pathology

6.2.1 Background

In February 1994 (triploid S *glomerata* project) and again in December 1994 (mass genetic selection project) serial sampling was made of all size grades of spat that sustained mass mortality during routine hatchery production cycles. In both cases standard larval rearing, settlement on scallop shell and downweller/upweller nursery techniques described by Frankish et al., (1991) were employed. Sampled spat were fixed and stored in either 5% formal seawater or 3% each of glutaldehyde and paraformaldehyde in seawater for Scanning Electron Microscopy (SEM). Progressive growth and mortality data were derived from these samples and sub-samples submitted for specialist histopathological examination to Dick Callinan (NSW Fisheries Fish Pathology Unit, Wollongbar), for SEM to Dr Alex Hyatt (AAHL/CSIRO) and for specific bacteriological analysis using monoclonal antibodies that identify *Vibrio* species commonly isolated from shellfish (Chen et al., 1992) to Assoc. Prof. Peter Hanna (Deakin University, Geelong Victoria).

6.2.2 Histopathology reported by Dick Callinan

Histopathological examination of haematoxylin and eosin stained thin sections by Dick Callinan in 1994 revealed a range of conditions from asymptomatic to severely diseased individuals among mass mortality spat. These observations were consistent with pathology described by Dick Callinan for earlier epizootics and reported by Nell et al., 1991. Lesions seen in all obviously diseased individuals comprised mild to severe focal to diffuse inflammation in connective tissue (see Appendix 4). The parietal mantle surface was often severely effected with exudation of proteinaceous fluid and cell debris. Many spat showed partial dissolution of the hinge ligament associated with moderate to many bacteria of various morphological forms including filamentous. Many spat from the mass mortality batch also showed focal to locally extensive necrosis in connective tissue associated with rod shaped bacteria. No other potential causative agents were seen associated with the above lesions. Similar but less common and severe lesions to the above were seen among the batch of apparently healthy spat. Dick Callinan concluded, "It is possible that the non inflammatory changes seen in all spat mortality episodes so far examined and in apparently healthy groups, were due to bacterial toxins originating from culch and/or spat shell surfaces".

6.2.3 Histopathology reported by Dr. Alex Hyatt (AAHL/CSIRO)

Spat from a mass mortality episode in April 1994s were examined by negative contrast electron microscopy (NCEM), by scanning electron microscopy (SEM) and by examination of ultra thin sections. All three techniques failed to reveal any viruses. Examination of the surface of spat (see Appendix 5) revealed significant erosion and the presence of large numbers of rod shaped bacteria plus prokaryotic fungal filaments and fruiting bodies.

6.2.4 Histopathology reported by Assoc. Prof. Peter Hanna (Deakin University)

S. *glomerata* spat sampled from an earlier apparently healthy batch reared at the PSFC in January 1993 and diseased spat sampled during a period of rapidly increasing cumulative mortality (15/4/94) was sent to Prof. Hanna with a view to identify the surface bacteria (originally identified by Dr Hyatt as cited above) using *Vibrio* species specific FITC immunofluorescence tests. Apparently healthy larvae samples showed *Vibrio alginolyticus* on their external surfaces, but not

in supernatants (seawater). The diseased samples by contrast contained at least three times the level of immunofluorescence associated with *V. alginolyticus* on the external surfaces (see Appendix 3). High numbers of *Vibrio* bacteria were found in association with extensive soft tissue lesions in diseased spat.

6.2.5 Conclusions

These findings thus collectively supported the initial favoured hypotheses of Dick Callinan reported in Nell et al. (1991) and Goard (1990) that mass mortalities of *S. glomerata* spat are associated with heavy bacterial biofouling of spat and residual scallop shell cultch in early stage spat. Toxins produced by fouling bacteria may have caused the apparently non specific connective tissue inflammation and mantle necrosis in both apparently healthy and diseased groups and subsequent systemic invasion by bacterial opportunists resulting in tissue necrosis of the diseased group.

On the basis of epidemiological evidence reported above it is also postulated that the recurrence of the mass mortality syndrome after a three year absence in 1994 was partly a consequence of at least two predisposing factors, namely:

- A failure to subject the hatchery to an annual dry-out cleaning and disinfection on the scheduled date of October 1993 and the use of stocking rates about three times recognised upper biomass limits (Goard - pers. comm.,) or downweller systems of this type. The lapse in implementing annual hatchery dry-out, cleaning and disinfection protocols was brought about by intense operational demands imposed by concurrent scallop, clam and oyster breeding research at the PSFC bivalve hatchery.
- Physiological stress and physical abrasion sustained by small (<2 mm) *S. glomerata* spat during multiple grading and volumetric counting operations in the hatchery, was also viewed as the likely trigger to such outbreaks. As already discussed above, a similar coincidence of sudden catastrophic mass mortality within one to several days of first grading and counting of spat was revealed in a re-examination of the Goard (1990) report.

6.3 Experimental Investigations

6.3.1 Spat mortality experiment 1

Investigation of effects of settlement technique mechanical grading and prophylactic use of an antibiotic on the incidence and severity of the mass mortality disease of early *S. glomerata* spat

Background and aims
This experiment was prompted by the above described results of epidemiological and histopathological evidence that:
- Manifestation of the mass mortality disease of juvenile *S glomerata* is caused by exotoxins of *Vibrio* bacteria followed by fatal secondary invasive bacterial infections.
- Disease manifestation triggered by additional stress factors especially high accumulation of organic matter and bacteria associated with the use of ground scallop shell in settlement screens and the practice of mechanical sieve grading and volumetric counting of small delicate spat.

Experimental Design

The nested factorial experimental design comprising 8 treatments represented schematically in Fig. 29 involved three factors:

1. Mode of settlement used i.e. culchless epinephrine induced settlement and metamorphosis (treatment code E) or scallop shell induced settlement and metamorphosis (treatment code S).
2. Grading (treatment code G =graded).
3. Use of antibiotic(treatment code A = antibiotic (chloramphenicol at 5 mg/l)).

This experimental design was devised to test the hypotheses that:

- The primary cause of mass mortality of small (0.3 to 2 mm) *S. glomerata* spat is the development of large concentrations of virulent strain(s) of *Vibrio* bacteria associated with the use of ground scallop shell to produce single spat in conventional downweller and upweller nursery systems.
- Manifestation of mass mortalities (as opposed to sub-clinical disease in apparently healthy stock) depends on a triggering factor especially trauma or other stress factors such as those associated with multiple grading and volumetric counting of small *S. glomerata* spat.
- Mass mortality if caused by *Vibrio* bacteria should be prevented or ameliorated with the prophylactic use of an appropriate antibiotic namely chloramphenicol at 5 mg/l.

Fig 29. Design of experiment to test effect of settlement method, grading and prophylactic use of an antibiotic on the occurrence and severity of spat mortality

Treatment Factor

Method of induced
Settlement

Scallop shell chips (S)   Epinephrine induced
culchless settlement (E)

Grading of spat   not graded   Graded (G)   Not graded   Graded (G)

Antibiotic   Not used   Used   Not used   Used   Not used   Used   not used   Used

Treatment no.   1 (S)   2 (SA)   3 (SG)   4 (SGA)   5 (E)   6 (EA)   7 (EG)   8 (EGA)
and(code)
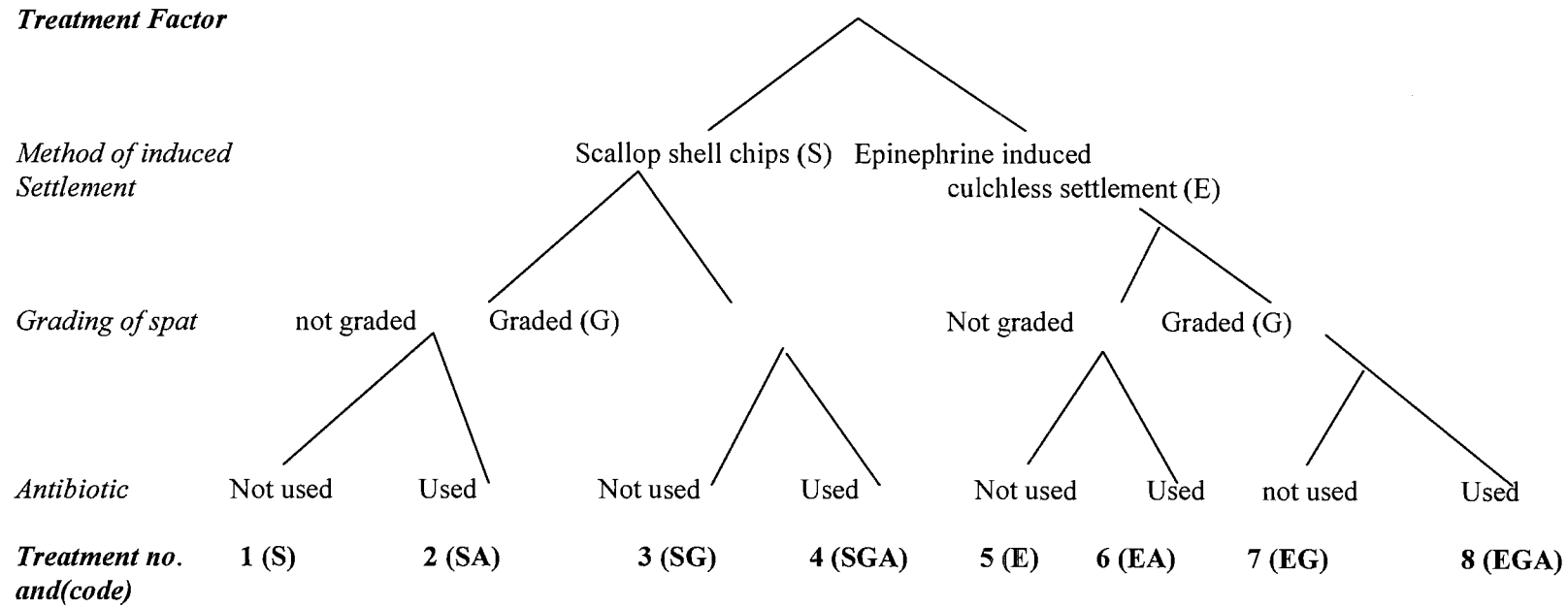
General Husbandry

Ripe broodstock oysters were supplied by a commercial *S. glomerata* farmer from Pambula on the mid south coast of NSW and strip spawned on 23rd of June 1995. Competent pediveligers were put to set on 13th and 14th of July in 6 downweller units (4 screens per unit and 50 000 larvae /screen).

In treatments 5 to 8 involving culchless (epinephrine induced) metamorphosis of spat, competent larvae were exposed to epinephrine (bitartrate salt) at 10^{-4}M in seawater for 45 minutes, on the 13th and 14th of July and again one week later on the 19th July.

For treatments 1 to 4 in which competent larvae were set onto scallop shell culch, spat were first separated from residual shell over a 350 μm mesh size screen on the 17th and 18th July and a for a second time on the 20th and 21st July. On the latter occasion, treatments 1, 2, 5 and 6 subjected to grading were additionally screened over 500 μm mesh.

Graded treatments 1, 2, 5 & 6 were graded over 350, 500 and 670 μm screens and separated size classes counted volumetrically and redeployed onto individual screens on the 24th and 25th of July. Grading for these treatments was repeated the 3rd of August using 500, 670, 1000, 1250, and 1400 μm screens.

At the conclusion of the experiment on 17th August all screens of spat from all eight experimental treatments were graded over 500, 670, 1000, 1250, 1400, 1800, 2240 and 3000 μm screens into separate size classes then counted.

Treatments 1, 3, 5 & 7 involving prophylactic use of antibiotics were continuously exposed to 5mg /L of chloramphenicol. This protocol has been found extremely reliable and is a commonly used method of combating larval Vibriosis in several bacteria prone species especially the European scallop *Pecten maximus* (Corre et al., 1993; Jeanthon et al., 1988).and the flat oyster *Ostrea edulis* (Tubiash et al., 1965; Lodeiros et al., 1987).

Results

The absence of mass mortality from all of the eight experimental treatments, which may in part have been a consequence of the reintroduction of an annual disinfection and dry out of the bivalve hatchery in November 1995, precluded any additional definitive findings on the cause(s), control and prevention of the disease. Nevertheless, the following useful information was gathered on the following issues:

- The relative benefits and costs of epinephrine (culchless) induced settlement as opposed to scallop shell culch production of single spat.
- The need or otherwise to grade and count spat before reaching a size (>2 mm) at which they are apparently no longer susceptible to the mass mortality syndrome.
- General effects of prophylactic use of an antibiotic on growth and survival of spat.

At the time of the first multiple grading and counting of spat (treatments 3, 4, 7 & 8) on 24th and 25th of July, mean ± s.d. survival (Fig 30) ranged from 60.3 ±2.6 to 72.3 ± 3.0%. Survival was not influenced by method of induced settlement but was apparently enhanced by prophylactic use of chloramphenicol although differences were not statistically significant at the P 0.05 level of confidence. Mean sizes of single spat settled on scallop shell (Fig 31) were similar to those of epinephrine induced culchless settlement spat.

Comparing size frequency data for shell and epinephrine settled spat (Fig 32), it is apparent that grading of spat does not enhance average growth rate nor reduce growth variability of spat. The antibiotic did appear to marginally improve growth rate of spat settled on scallop shell but the opposite appeared true in relation to epinephrine settled spat.

Another important inadvertent finding of this experiment was that adoption of culchless settlement induced with epinephrine and omission of periodic grading of small *S. glomerata* spat drastically reduces hatchery labour requirements. Although hard evidence was not forthcoming, it is nevertheless likely that culchless settlement will reduce risks of mass mortalities by avoiding traumatic handling and elevated levels of pathogenic *Vibrio* bacteria associated with large accumulations of amounts of organic matter created by use of scallop shell culch.

Fig 30. Effect of rearing method on mean survival (Bag = s.e.)

Fig 31. Effect of rearing method on growth
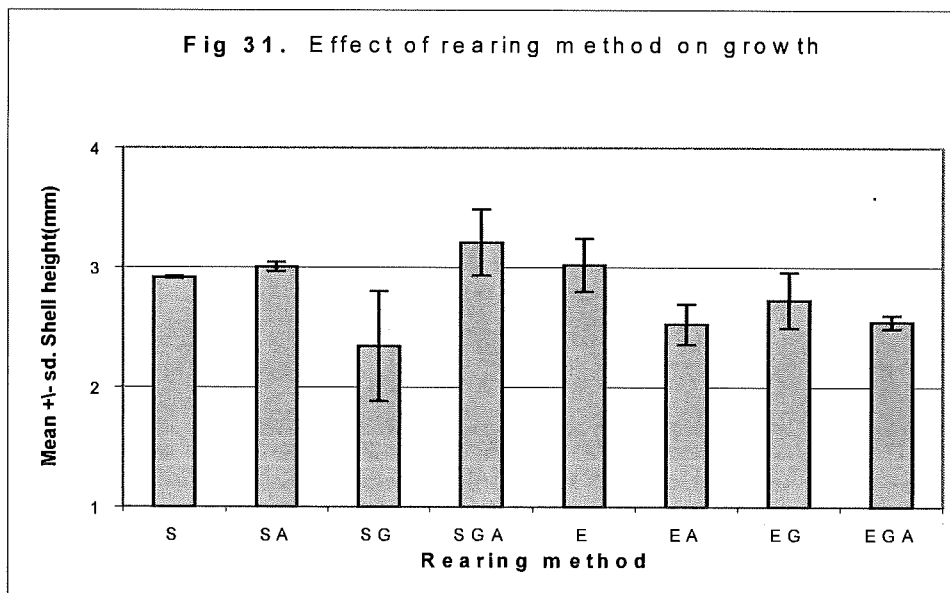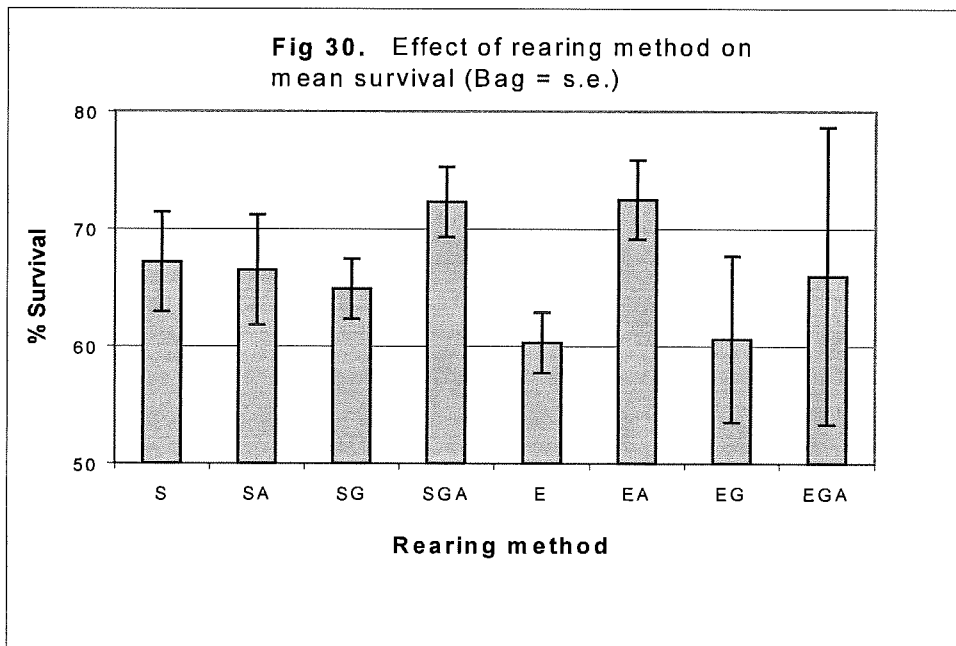
Fig 32. Effect of settlement technique, use of an antibiotic, and grading on the size frequency distribution of *S glomerata* juveniles

1 (S)
scallop shell, not graded , no antibiotic

2 (SA)
scallop shell, not graded, plus antibiotic

3 (SG)
Scallop shell, graded, no antibiotic

4 (SGA)
scallop shell, graded, plus antibiotic

5 (E)
epinephrine, not ungraded, no antibiotic

6 (EA)
epinephrine, not graded, plus antibiotic

7 (EG)
epinephrine, graded, no antibiotic

8 (EGA)
epinephrine, graded, plus antibiotic
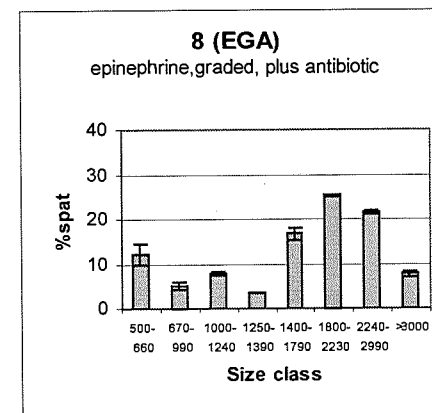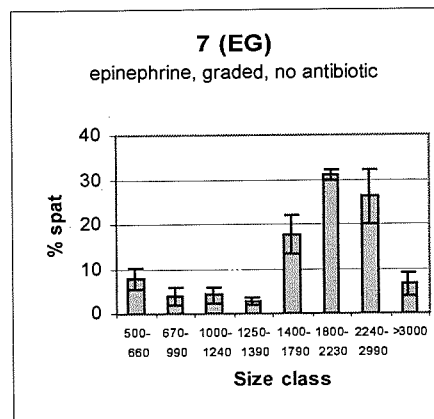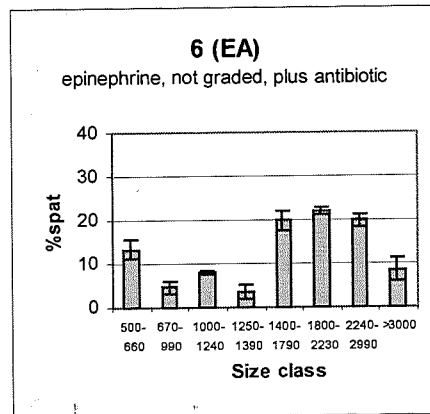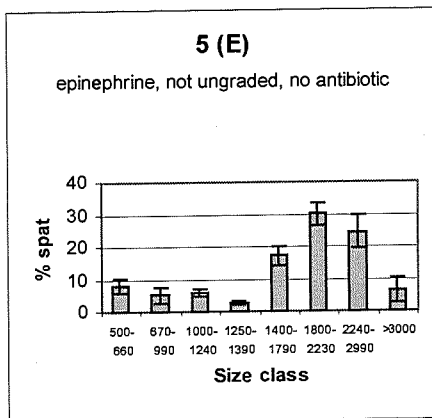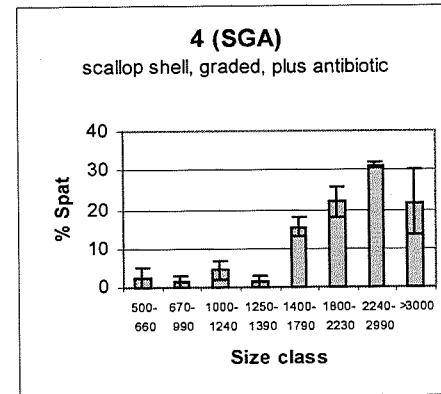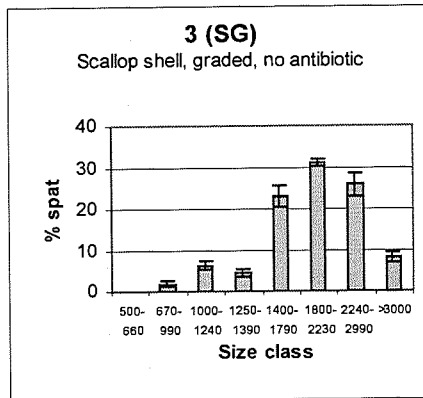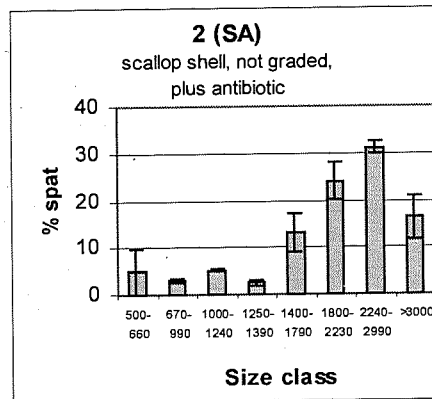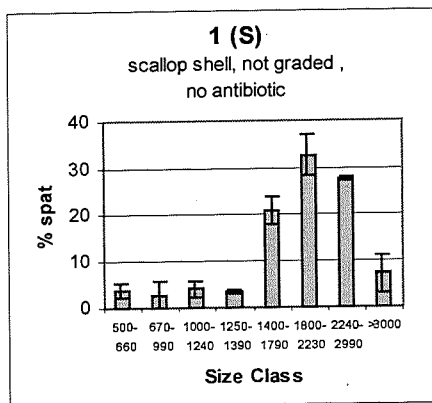
6.3.2 Spat mortality experiment 2

Investigation of effects of settlement technique, genetic selection and induced triploidy on the incidence and severity of the mass mortality disease of early *S. glomerata* spat

Background and aims
This experiment was run in conjunction with a large scale experiment (FRDC Project 93/15) to evaluate whether advantages conferred by selection for faster growth in *S. glomerata* (Nell et al., 1998) could be combined with and added to those conferred by cytochalasin C induced triploidy (Hand and Nell, 1999).

Methods
To meet the needs of the primary experiment aimed at assessing the single and combined effects of genetic selection and triploidy on the growth and survival and seasonal market condition of *S. glomerata*, the following four lines of larvae were separately reared in duplicate experimental scale (1 000L) vessels.
1. Non genetically selected diploid stock (code CTL-2N)
2. Non genetically selected triploid stock (code CTL-3N)
3. Genetically selected diploid stock (code L2-2N)
4. Genetically selected triploid stock (code L2-3N)

In addition to these, a much larger commercial batch of line 4 larvae i.e. genetically selected triploid stock (L2-3N) that initially yielded an estimated 12 million spat was separately reared in a 20 000L tank. Of the latter mass reared L2-3N larvae, three sub batches of competent larvae were stocked on separate screens and subjected to culchless settlement as single seed using epinephrine. Three sub batches of L2-3N larvae were also stocked onto each of three screens loaded with scallop shell culch to produce single seed. In all cases yields of single seed on each screen were similar.

Subsequently all six groups of spat were transferred to downweller nursery screens in the hatchery and thence to upweller screens in the field nursery system at Wanda Head, Port Stephens. All other rearing methods employed were identical to those described above for spat mortality experiment.

Results
Unlike experiment 1 most lines of spat sustained high rates of mortality. However manifestation of the disease, as indicated by cumulative mortality data was atypical. Unlike earlier episodes in which mortality occurred as one or a series of intense pulses over a few days, cumulative mortality data presented in Fig 33 and Fig 34 showed that mortality of spat on this occasion was progressive and protracted.

This atypical pattern of mortality occurred regardless of whether spat were settled on scallop shell or through culchless induction using epinephrine (Fig 30) and regardless of whether the stock were the progeny of 4th generation genetically selected parent stock or not (Fig 34).

These atypical results were however readily explained by pathology reports based on examination of samples of diseased spat sent to Dick Callinan (NSW Fisheries), Dr Judith Handlinger (Tas. DPIF), Dr Mike Hine (NIWA) and Dr Alex Hyatt (AAHL/CSIRO). All four pathologists concurred that the disease was clearly caused by invasive infections of a ciliate (*Uronema* sp.) (Appendices 6 & 7). Ciliates of the type had previously been identified by Dr Handlinger as responsible serious losses of pacific oyster (*Crassostrea gigas*) spat commonly experienced by commercial hatcheries in Tasmania over the past decade. Re-examination of archived samples of spat from previous mass mortality episodes as early as 1990, by Dick Callinan, Dr Handlinger and

Dr Hyatt however proved entirely negative for the involvement of *Uronema* sp. or any other type of ciliate. Unfortunately this involvement of *Uronema* sp. undermined any meaningful conclusions from this experiment on the likely benefits or otherwise of genetic selection, triploidy or method of settlement on bacterial mediated mass mortality disease of small juvenile *S. glomerata*.

Fig 33. Effect of settlment method on mortality of genetically selected triploid *S. glomerata* spat (L2-3N)

Legend: Scallop Shell (GRIT 1), Epinephrine (EPI 1)

Fig 34. Effect of genetic selection and triploidy on mortality of *S. glomerata* spat (error bars when included =s.d.)

Legend: Control Diploid(CTL-2N), Control Triploid(CTL-3N), Selected Diploid(L2-2N), Selected Triploid(L2-3N)
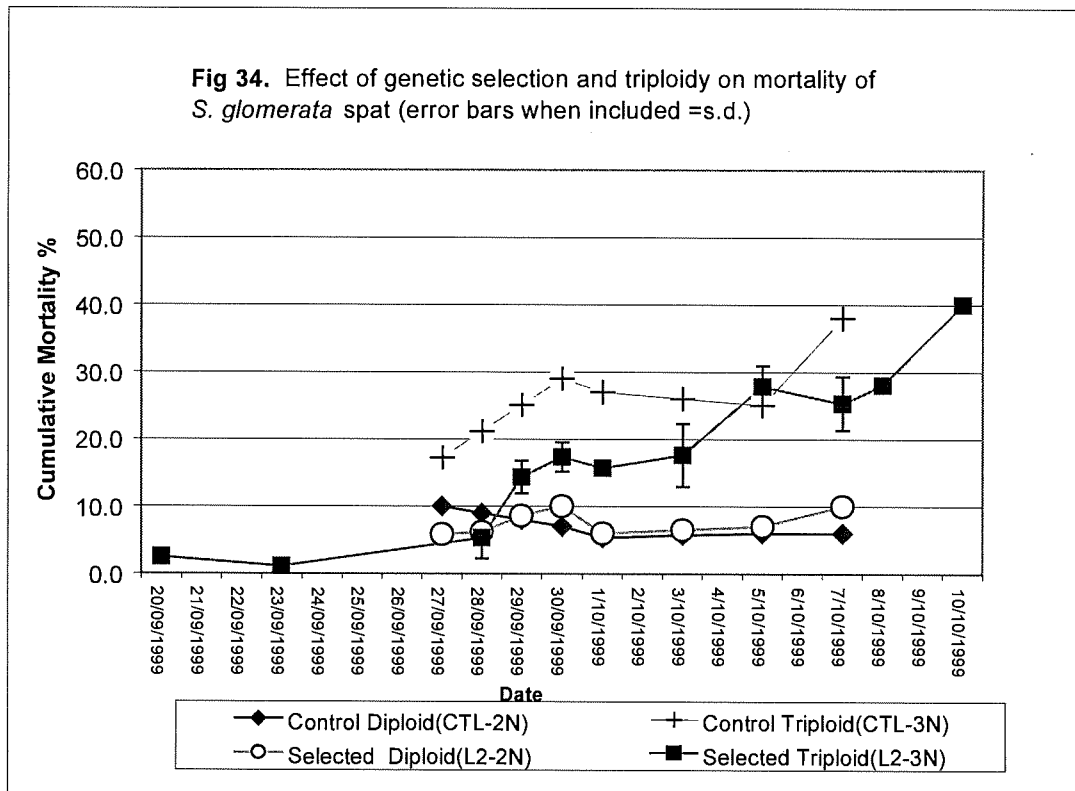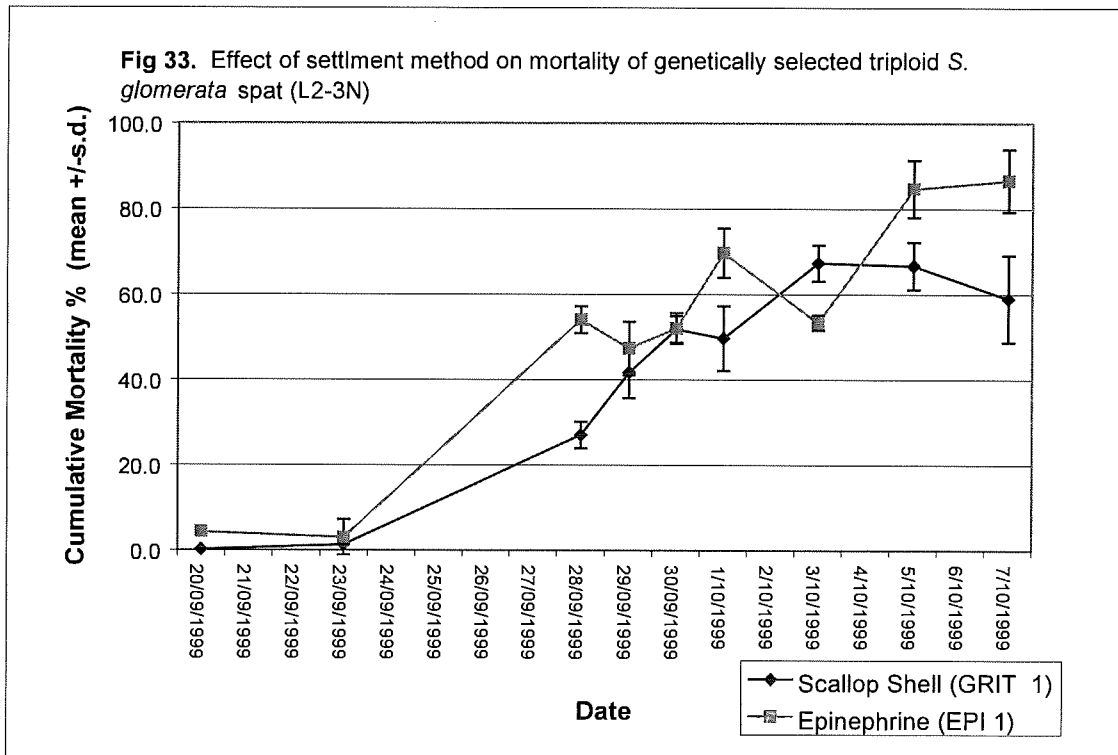
7.0 OVERALL CONCLUSIONS AND RECOMMENDATIONS

7.1 Mass Mortality Syndrome of Larval *S. glomerata*

- Results of epidemiological, histopathological and experimental investigations collectively point to the conclusion that this disease is caused by bacterial (*Vibrio* spp.) exotoxins that impact on the gut epithelium of young larvae and hence their ability to feed, grow and survive.
- The disease is highly seasonal and exacerbated through successive hatchery cycles. It is therefore recommended that future bivalve hatcheries comprise two or more fully independent modules that can be used and disinfected/dried out in alternation. It is also recommended that future hatchery production of *S. glomerata* in existing hatcheries be confined as much as is practicable to the lower risk season of July to December, that numbers of hatchery runs between consecutive cleaning, disinfection and 4 to 6 week dry-out operations be limited to one or two.
- Very encouraging experimental results have been attained when *S. glomerata* larvae were reared in seawater that had been sterilized then re-inoculated with selected non-pathogenic bacteria. Results showed that survival and growth of larvae over the first critical 8 days could be elevated from very low levels in control treatments typical of disease episodes to some of the highest rates ever experienced with *S glomerata* larvae over the past 15 years. It is therefore strongly recommended that this research being conducted in collaboration with the Dept of Cell Biology of the University of Technology, Sydney, be continued.
- It is recommended that future commercial scale hatchery protocols for *S. glomerata* be amended based on results of small-scale experimental trials on effects of temperature on fertilisation, incubation and hatchery rearing protocols. Of particular importance is a need to evaluate higher larval rearing temperatures than currently practised.

7.2 Mass Mortality Disease of Small (<2 mm) Juvenile *S. glomerata*

- Results of epidemiological, histopathological and experimental investigations collectively point to the conclusion that this disease is caused by high organic matter and *Vibrio* spp. bacterial loads associated with dense stocking rates in nursery screens and the use of ground scallop shell culch to produce single spat seed.
- Recommended strategies of overcoming this disease are to combine:
  - cultchless production of single seed using epinephrine induction of metamorphosis or
  - develop commercially practical ways of setting oysters on flat surfaces that also enable creation of single seed once juveniles exceed 2 mm shell height
  - an avoidance of grading, counting and all other unnecessary handling of spat below 2 mm
  - development of low trauma high growth rate promoting rearing systems incorporating spat bubbler columns, controlled and optimised rearing temperatures and if necessary supplementary feeding with micro algae concentrate diets recently developed at PSFC (Heasman et al., 2000)
  - adapt and evaluate probiotic technology being developed to combat larval mortality diseases.

8.0 FURTHER DEVELOPMENTS

Mass Mortality Syndrome of larval S. glomerata
Continued research being conducted b NSW Fisheries in collaboration with the Dept of Cell Biology of the University of Technology, Sydney, to develop probiotic methods of preventing this disease is scheduled for completion in September 2002.

Mass mortality disease of small (<2 mm) juvenile S. glomerata
Development of low trauma and high growth rate promoting rearing systems incorporating spat bubbler columns and optimised rearing temperatures will be pursued over the same time frame as the larval disease R&D and will also incorporate probiotic technology as a means of combating the disease.

In addition, practical methods of propagating spat on two dimensional surfaces that can be fragmented in such a way as to generate single seed will be appraised.

9.0 REFERENCES

Chen, D., Hanna, P.J., Altmann, K., Smith, A., Moon, P., Hammond, L.S., 1992. Development of monoclonal antibodies that identify Vibrio species commonly isolated from infections of humans, fish, and shellfish. Appl. Environ. Microbiol., 58(11): 3694-3700

Corre, S., Ansquer, D., Nicholas, J.L., 1993. Mortality of scallop larvae and preventive use of antibiotic. In: Diseases of Fish and Shellfish. 6th International Conference. European Association of Fish Pathologists, Brest, France Sept. 5-10 (Abstract only).

Frankish, K. R., Goard, L. J., O'Connor, W. A., 1991. The development of hatchery rearing techniques of the Sydney rock oyster *(Saccostrea commercialis)*. Internal Report, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, NSW. 28 pp.

Gibson, L.F., 1997. Probiotic bacteria can prevent death of pacific oyster larvae infected with pathogenic vibrios. Austasia Aquaculture, 11(5): 65-66.

Gibson, L.F., Woodworth, J., George, A.M., 1998. Probiotic activity of Aeromonas media on the pacific oyster *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. Aquaculture, 169: 111-120.

Goard, L., 1990. Sydney Rock oyster spat mortality experiment. Unpublished Report. NSW Fisheries. 11pp.

Hand, R.E., Nell, J.A., 1999. Studies on triploid oysters in Australia XII. Gonad discolouration and meat condition of diploid and triploid Sydney rock oysters *(Saccostrea commercialis)* in five estuaries in New South Wales, Australia. Aquaculture, 171(3-4): 181-194.

Hand, R.E., Nell, J.A., Smith, I.R., Maguire, G.B., 1998. Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters *(Saccostrea commercialis* (Iredale and Roughley)) through outbreaks of winter mortality caused by *Mikrocytos roughleyi* infestation. J. Shellfish-Res., 17(4).

Hanna, P.J., Altmann, K., Chen, D., Smith, A., Cosic, S., Moon, P., Hammond, L.S., 1991. Development of monoclonal antibodies for the rapid identification of epizootic *Vibrio* species. J. Fish. Dis., 15(1): 63-69.

Heasman, M., Diemar, J., O'Connor, W.A., Sushames, T., Foulkes, L., 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs - a summary. Aquaculture Research, 31: 1-23.

Jeanthon, C., Prieur, D., Cochard, J.C., 1988. Bacteriological survey of antiobiotic treated sea waters in a *Pecten maximus* hatchery. Aquaculture, 71: 1-8.

Nell, J., Heasman, M.P., Goard, L.J., 1999. A quest for super oysters. Fisheries NSW, 2: 10-11.

Lodeiros, C., Bolinches, J., Dopazo, Toranzo, A.E., 1987. Bacillary necrosis in hatcheries of *Ostrea edulis* in Spain. Aquaculture, 65:15-29.

Maeda, M., Nogami, K., Ishibashi, N., 1992. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus* Bull. Natl. Res. Inst.Aqacult. Japan Yoshokukenho 21: 31-38.

Nell, J.A., Maguire, G.B., 1994. Evaluation of Triploid Sydney Rock Oysters *(Saccostrea commercialis)* and Pacific Oysters *(Crassostrea gigas)* on Commercial Leases in New South Wales and Tasmania. Final Report to the Fisheries Research and Development Corporation (FRDC). 282 pp.

Nell, J.A., O'Connor, W.A. Goard, L J., Callinan, R.B., 1991. An evaluation of hatchery hygiene protocols and bacterial concentrations for hatchery reared Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) spat. Unpublished report of NSW Fisheries.10pp.

Nell, J.A.; Smith, I.R.; Sheridan, A.K., 1999. Third generation evaluation of Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) breeding lines Aquaculture, 170(3-4): 195-203.

Nogami, K., Maeda, M., 1992. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus* . Can. J. Fish. Aquat. Sci., 49(11): 2373-2376.

Paterson,K.J., Nell, J.A., 1997. Effect of different growing techniques and substrate types on the growth and survival of the clams *Tapes dorsatus* (Lamarck) and *Katelysia rhytiphora* (Lamy). Aquaculture. Res., 28(9): 707-715.

Renault, T., Le-Deuff, R.M., Cochennec, N., Chollet, B., Maffart, P., 1995. Herpes-like viruses associated with high mortality levels in larvae and spat of Pacific oysters, *Crassostrea gigas*: A comparative study, the thermal effects on virus detection in hatchery-reared larvae, reproduction of the disease in axenic larvae. Vet. Res., 26(5-6): 539-543.

Tubiash H.S., Chanley, P. E. Leifson, E., 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. J. Bacteriology, 90(4): 1036-1044.

10.0 STAFF

NSW Fisheries Staff engaged on this project

Permanent staff
Dr Mike Heasman - Senior Research Scientist, Principal Investigator
Dr Richard Callinan - Departmental Veterinary Pathologist
John Diemar - Fisheries Technician Grade 2/3
Lindsay Goard, - Fisheries Technician Grade 2/3

Temporary staff
Celina Gartrell (1995) - Fisheries Technician Grade 1
Tanya Sushames (1996-1998) - Fisheries Technician Grade 1
Melissa Walker (1999-2000) - Fisheries Technician Grade 2

Collaborating UTS Staff

Dr Lewis Gibson -Principal Researcher –SPIRT/NSW Fisheries, Probiotic Project
Edward McGregor. BSc. (Hons) student (1999)
Cheok Tan, PhD student (2000)

11.0 APPENDICES

APPENDIX 1 Summary of hatchery data July 1988 to Dec 1999															
MS=mass selection; 3n=triploid; 2n=diploid; W=wild/control stock;? =unknown															
Date	Operation	Larval Success (S/F)	Nursery / Spat Success (S/F)	Broodstock conditioned (Y/N)	Tank Size (L)	Eggs (x10^6)	D-veligers x10^6 (% yield)	Number to set system x10^6	Spat x10^6 (%yield)	Net spat from D veliger	Spat >2mm x10^6	Day larvae ceasedf feeding)	time since past SRO larval run / weeks	Days post set to spat mortality	Comments
24-Jul-88	?	s	s		20000		61		13.98		11.3	5	new	nil	Excellent larval run. 11.3 million spat sold
5-Sep-88	?	s	f	?	20000		51.34		16.92		1.6		5	?	Large numbers moved to Vales Pt Nursery 14th October 88, 90% spat mortality
20-Mar-89	?	s	?	n	20000	?	34.4	?				5	18	?	Satisfactory larval run. Numbers to settlement unknown
24-Sep-89	?	s	s	y	20000/1000 (D's)	?		2.88	0.96 (33%)				22	?	5% Spat mortality at 500um. No record after this observation. Successful run
6-Feb-90	MS	s	f		20000	?	55.6	8.36 (15%)			0	6	17	7 & 14	Larvae pale day 6. Severe mortality in upwellers 5/3/90-severe mortality in downwellers 12/3/90
31-Jan-91	MS	f		n	20000		96.8	0			0	5	44		Batch discarded. Larvae ceased feeding day 5 & did not resume. Strip spawned
14-Jan-92	MS	s	s	n	1000 x 12	96	60.8 (63%)	8.43 (13.9%)	6 (71%)	9.9%			50	nil?	Minor mortality 19 days post set. Good run

5-Jan-93	MS, Georges R. Wallis L. Hawkesbur y R. Port S.	s	s	n	1000 x 12		70.59	4 (5.7%)	3.42 (86%)	4.9%				50	Nil ?	Overall set rate 86%. Poor larval growth and survival - satisfactory run.
11-Jan-94	MS	f		n	1000 x 16	48	40.9 (85%)	1.39 (3.4%)			0	10	52	?	Regarded as a failure. Guts light and dropout day 10
8-Feb-94	3N	s	f		20000		36.18	4.5 (12.4%)				11	3	?	60-70% spat mortality
	3N	s	f	n	20000		26.33	6 (22.8%)				4	3	10	Few pale larvae - but otherwise successful larval run. Mass mortality of spat. 10 days post set
22-Apr-94	MS	f	s	n	1000 x 6	96	60 (62.5)	1.37 (2.3)	0.065 (5%)	1.2%		6	9	nil	Regarded as a failure. Day 6, light guts. No spat mortality. Very few larvae put to set.
19-Dec-94	MS	s	f	n	1000	8		1.5			0		36	35	1.5 million pedivelligers put to set for each line. Good larval run. Severe mortality 5 weeks post set (90 - 20 %)
23-Jun-95	Mass mortality	s	f		20000	130	13.21 (10%)	1.24 (9%)			0.22 (17%)		23	35	Strip spawned. Good larval result. Spat mortality 5 weeks post set, spat < 1800um almost all dead
12-Apr-96	Mass mortality	f		n	20000	100	36.4 (36.4 %)	0			0	5	39		Run aborted. Larvae failed to feed.
12-Apr-96	Mass mortality	f		n	20000	100	30.2 (30.2 %)	0			0	5	39		Run aborted. Larvae failed to feed.
25-Apr-96	Mass mortality	f		n	20000	60	57 (95%)	0			0	6	1		Run aborted. Larvae failed to feed. Poor water quality
25-Apr-96	Mass mortality	f		n	20000	60	54 (90%)	0			0	6	1		Run aborted. Larvae failed to feed. Poor water quality

31-May-96	Mass mortality Wild 2N	f	f	?	20000			0			0	2	4		No gut colour at all day 2. Run aborted
12-Nov-96	Antifoul exp.	s	?	?	10000	30	28 (93%)	4 (14%)				21	?		Good larval survival and food consumption
12-Dec-96	3N	s	?	?	20000	100	50.4 (50.4%)	9.3 (18.5%)	2.4 (26%)		?	7	4	?	Small larvae not feeding well. Poor set on scallop shell. Algal blooms in tank.
17-Apr-97	3N	s	s	n	20000		64.13	1.86 (2.9%)	1.04 (56%)			6	16	?	Pale slow growing larvae early in run. Improvement observed after day 10
26-Nov-97	Wild 3N	s	f	n	20000		118.4	14.7 (12%)	5.5 (37%)		0.4 (7%)	2	28	11	Larvae appear light AM day 2. Improve afterwards. Good larval run. Massive Mortality of spat Jan 27th 1998
18-Sep-98	Wild 3N Manning R.	s	f	y	20000	95	72.24 (76%)	16.7 (23%)			0.745 (4.5%)	2	36	?	No spat grading or counting to try & avoid spat mortality. Spat suffered severe mortality over a 6 week period. Good numbers through larval cycle
13-Jan-99	MS 2N	f			20000	80	73.6 (91%)	0.1 (.001%)	0		0	2	20		Day 11, clear guts. Large size range. Large dropout of larvae day 12
13-Jan-99	MS 3N	f			1000	10	4.6 (46%)	0	0		0	6	20		Larval dropout and cessation of feeding day 6. Run aborted day 12
25-Feb-99	W 2N	f			1000	10	2.3 (23%)	0	0		0	5	new		Light guts. Larvae failed to feed. Tomaree site used. Run aborted.
26-Feb-99	MS 2N	f			250 FT	100	91.3 (91.3%)	0	0		0	4	new		Light larvae. Failed run. Velum deformities - Tomaree. Run aborted
18-Aug-99	W 2N	s	f	y	1000	8	6.9 (86.2%)	0.53 (7.7%)			0.054 (10%)	3	23	?	Slight paling of guts day 3. Successful larval run.

|															
20-Aug-99	MS 3N	s	f	y	20000	160	108.1 (67.6%)	20.3 (18.7%)	?		?		4	23	14	Spat mortality 28/9/99 - 2 weeks post settlement. Continued until December 99 caused by Uronema ciliates
20-Aug-99	MS 2N	s	f	y	1000	10	4.3 (43%)	0.58 (13.5%)	0.146 (25%)	3.40%		4	23	14	Larval run successful. Guts pale day 4. Mass mortality of spat 2 weeks post settlement.
17-Nov-99	Ciliate Mortality Trial MS 2N	s	f	n	20000	60	45.7 (76%)	5.61 (12%)	?		?	2	12		Good larval run. Settled spat numbers unknown.
Dec-99	MS FRI	s			4000										Mass Mortality of spat in Upwellers at Sans Souci

APPENDIX 2. SPAT PRODUCTION DATA					
Year	**Month**	**F**	**S**	**Days**	
1988	*July*		S		20000
1988	*Sept*	F		?	20000
1989	*Mar*	F		?	20000
1989	*Sept*		S?		1000?
1990	*Feb*	F		7 & 14	
1992	*Jan*		S		1000
1993	*Jan*		S		1000
1994	*Jan*				1000
1994	*Feb*	F		10	20000
9194	*April*		S		1000
1994	*Dec*	F		35	1000
1995	*Dec*	F		35	20000
1996	*Dec*	F			
1997	*April*		S		20000
1997	*Nov*	F		43	20 000
1998	*Sept*	F		?	20000
1999	*Aug*	F		18	20000
2000	*Jan*	F			
1999	*Nov*				

Appendix 3. Photomicrograph of a diseased juvenile *S. glomerata* showing *Vibrio alginolyticus* labelled using FITC immunofluorescence. Courtesy of Peter Hanna.
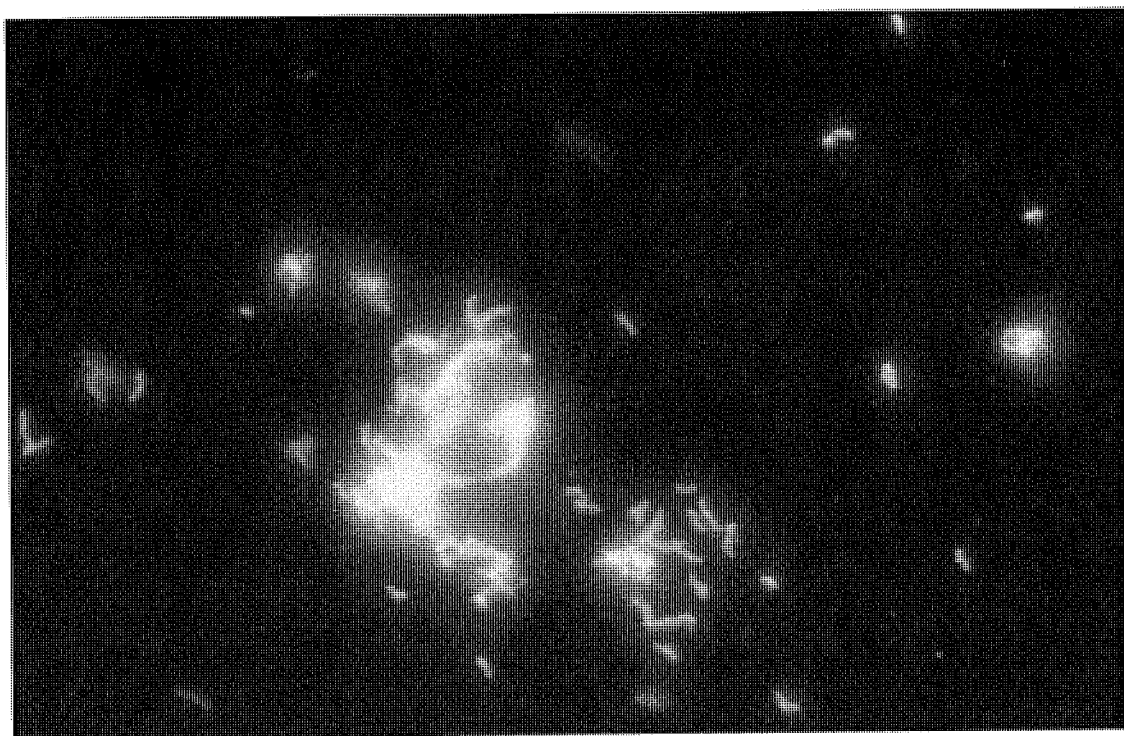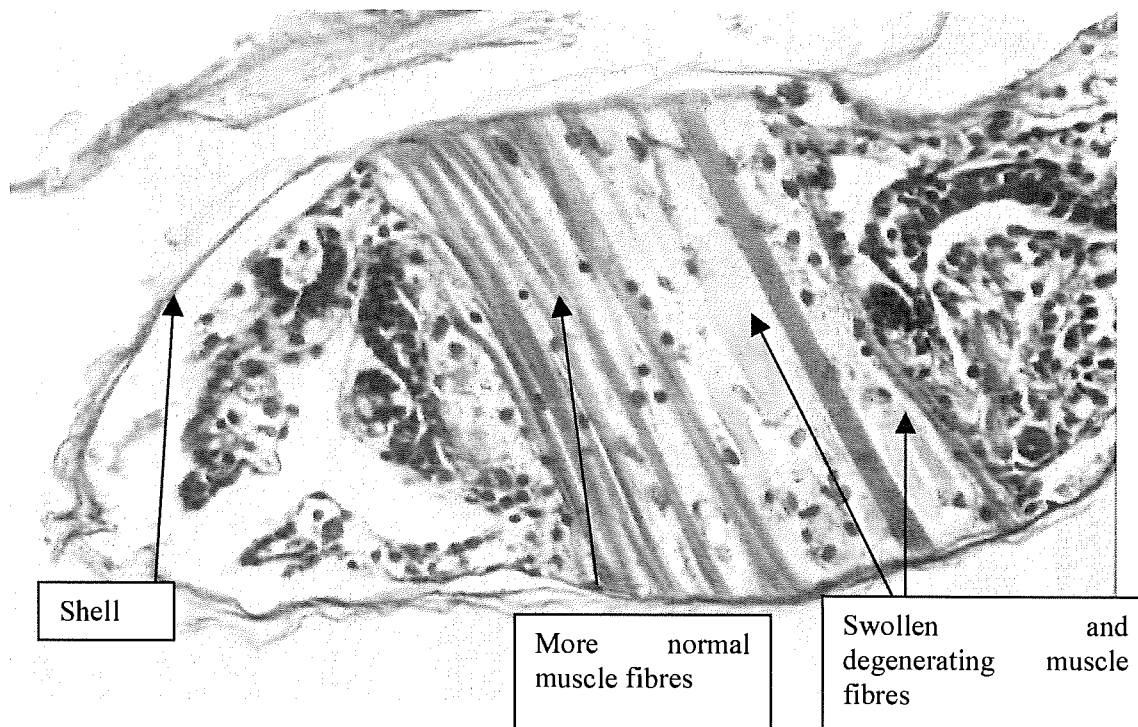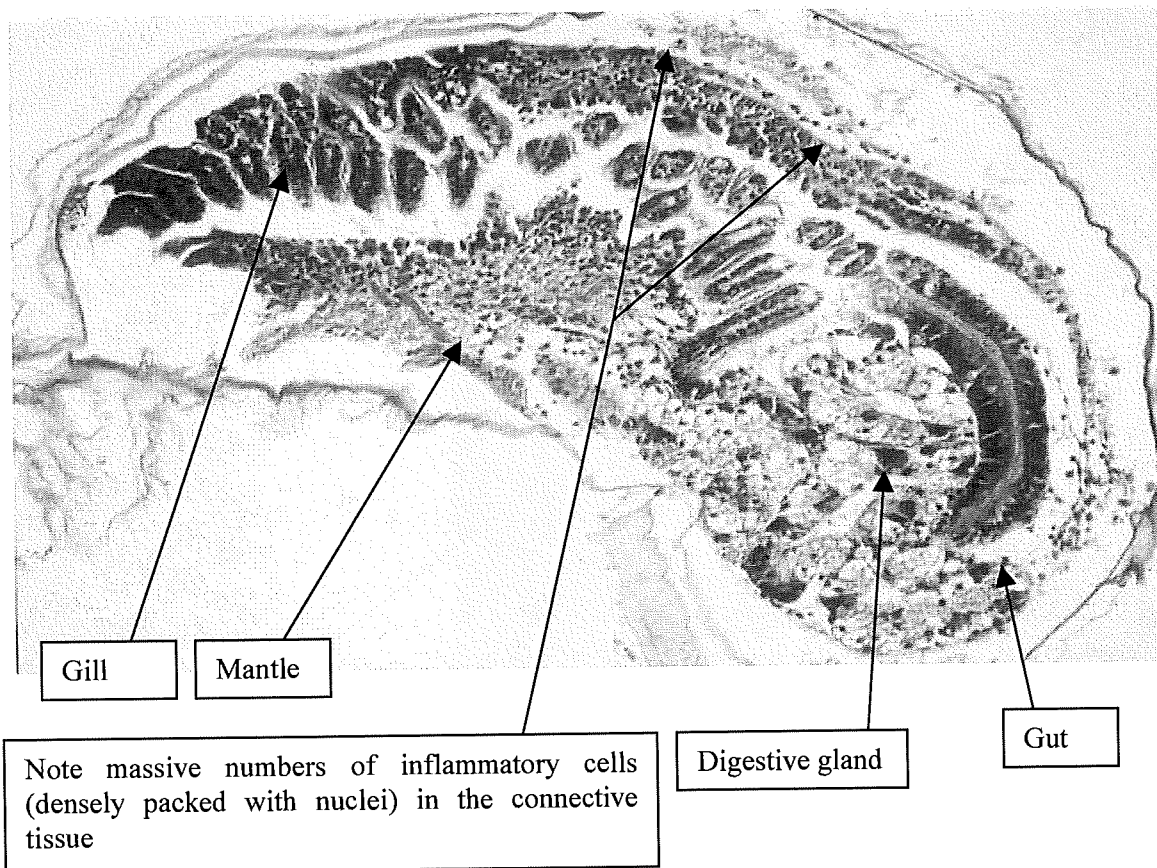
Appendix 4. Stained H & E section of healthy and diseased juvenile *S. glomerata*.
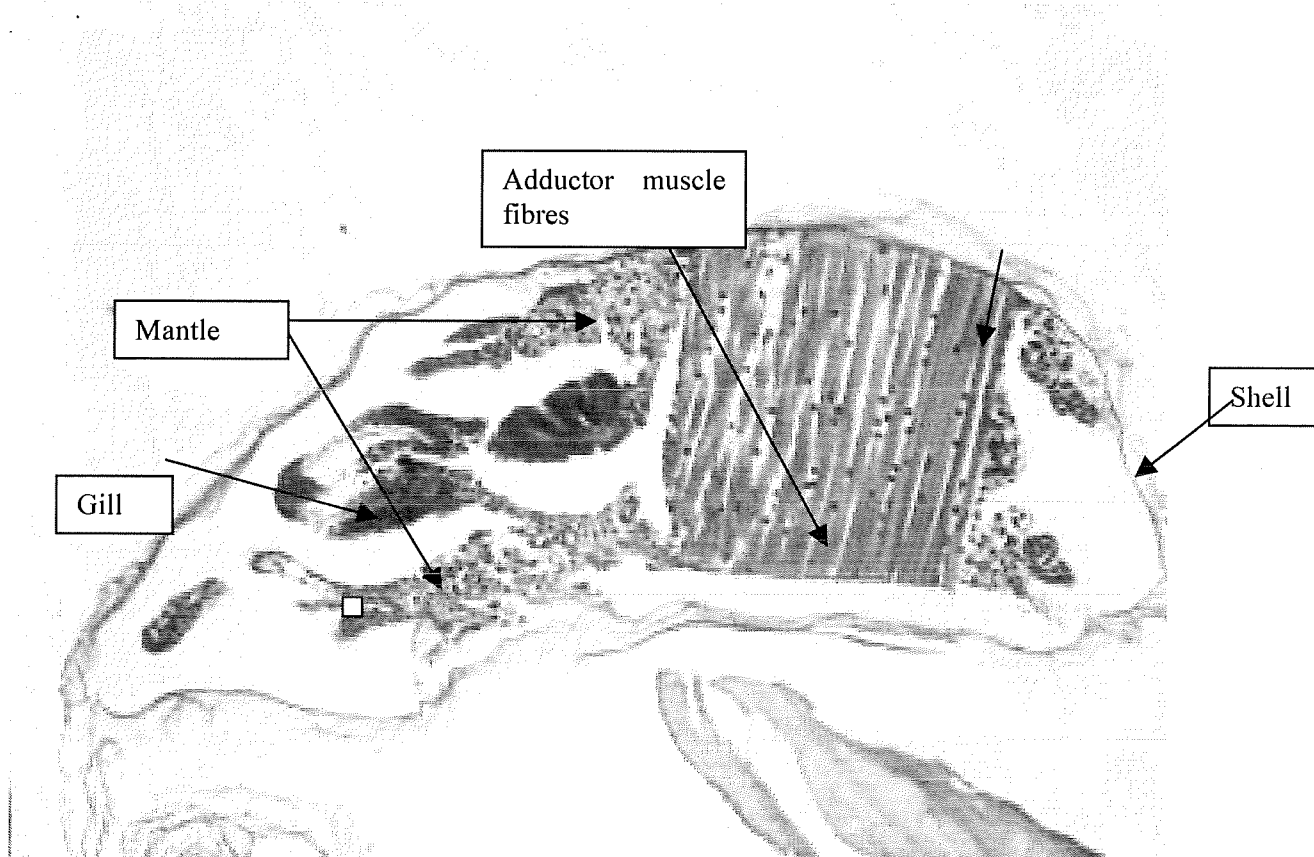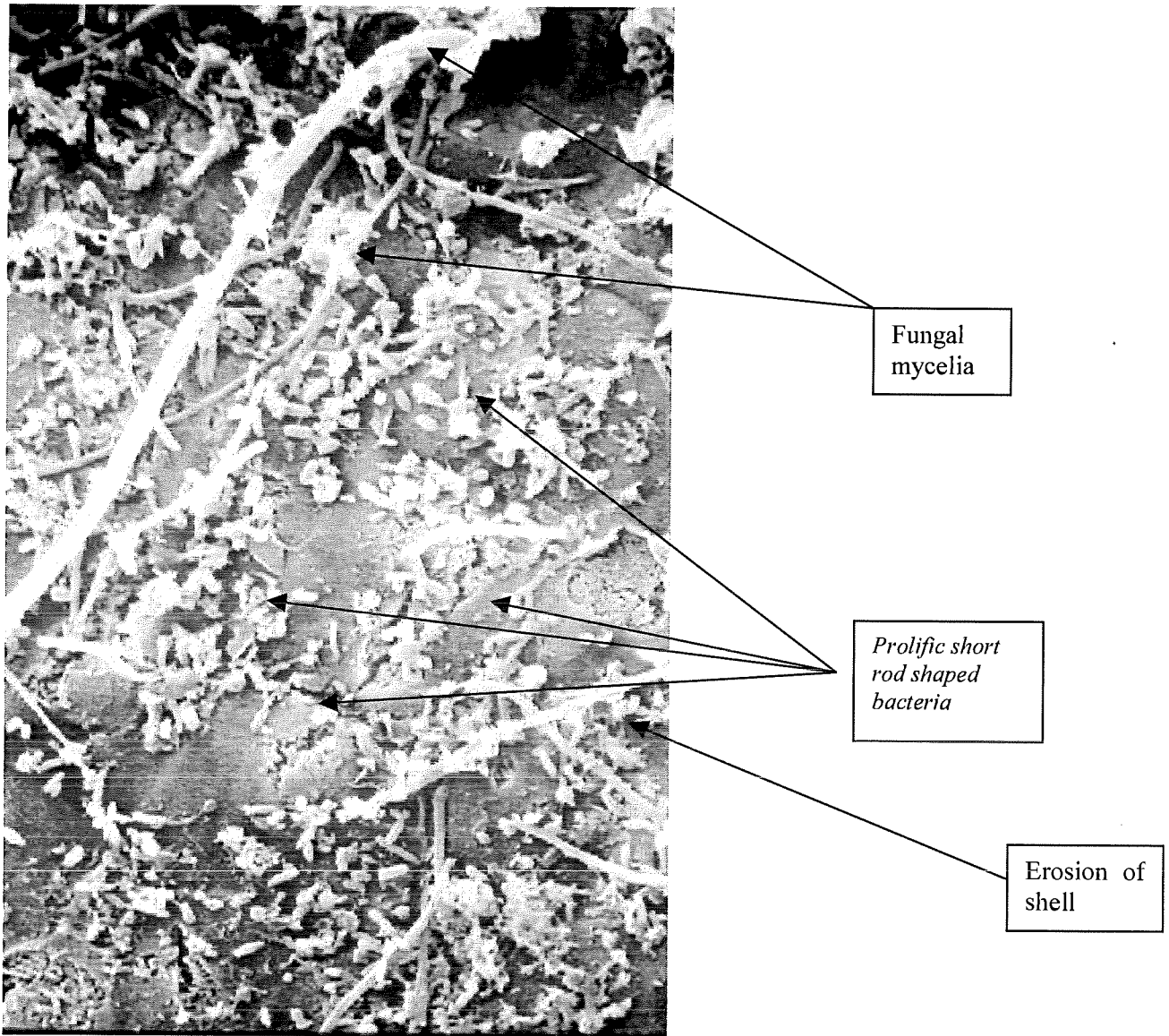

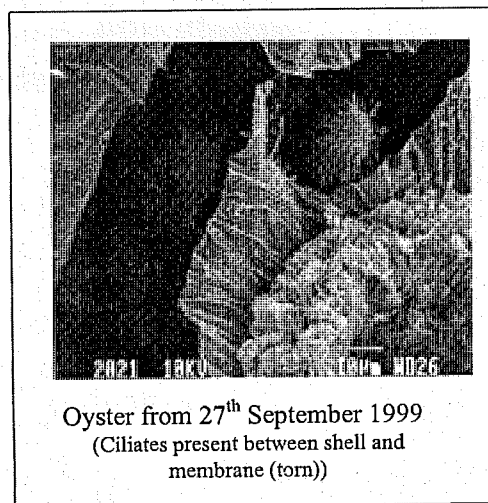Above: Relatively normal *S. glomerata* juvenile in cross section (Courtesy of Dick Callinan).

Below: Diseased gaping spat exhibiting degeneration of the adductor muscle fibres.
(Courtesy of Dick Callinan).

Appendix 4. Continued- Diseased juvenile *S. glomerata.*

Gill

Mantle

Note massive numbers of inflammatory cells (densely packed with nuclei) in the connective tissue

Digestive gland

Gut

Appendix 5. Electron photomicrograph of diseased juvenile *S. glomerata* showing massive numbers of rod-shaped bacteria and fungal strands. Note erosion of shell. Courtesy of Alex Hyatt. (AAHL/CSIRO).

| Ciliate within the cavity of an oyster harvested on the 27th September 1999 | Oyster from 27th September 1999 (Ciliates present between shell and membrane (torn)) |
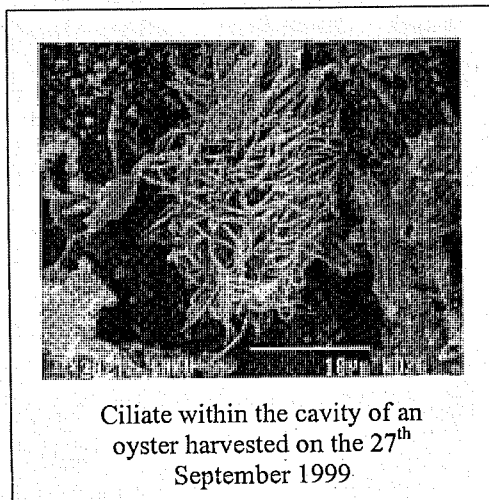
Appendices 6 and 7 – Electron photomicrographs of invasive ciliates (*Uronema* sp.) in juvenile *S. glomerata*. Courtesy of Alex Hyatt.(AAHL/CSIRO).

Appendix 3. **Call for Expressions of Interest to conduct three separate hatchery and nursery production runs with Sydney rock oysters** *Saccostrea glomerata.*

Call for Expressions of Interest
to conduct three separate hatchery and nursery production runs
with Sydney rock oysters *Saccostrea glomerata*

Preamble

Recurrent mortality of the larvae and juveniles of Sydney rock oysters (SRO) has been experienced by the oyster hatchery at the Port Stephens Fisheries Centre over the past 15 years. Unfortunately reasons for the mortalities have defied definitive diagnosis let alone prevention or cure. This is in spite of the fact that no significant problems have been encountered in the successful production of many other bivalves in the same facility over the same period. As a consequence, commercialisation of a 10 year SRO breeding program by NSW Fisheries, that has developed fast growing disease resistant strains for the benefit of the NSW Oyster Farming Industry, is in jeopardy.

Two major strategies are being pursued to address these hatchery problems with SRO.

The first is to draw on the best available expertise to assess current information on the pathology and epidemology of these particular mortality events and to review factors that cause similar losses in production of other bivalves throughout the world. This review will take the form of a workshop to be held in on the 8th and 9th August, 2002 at the Port Stephens Fisheries Centre (PSFC). The workshop will involve leading national and international experts in bivalve hatchery and nursery health and production and will help guide the formulation of a new 3 year R&D plan to overcome these problems. This plan will serve as the framework for a research grant application to be submitted to FRDC by December 2002.

The second strategy – identified during the course of a recent NSW Oyster Research and Advisory Committee (ORAC) meeting – is to engage a reputable commercial bivalve hatchery to produce 3 consecutive commercial scale batches of SRO spat (exceeding 2 mm, beyond the maximum size at which the mass mortality has occurred in the past). This strategy is designed to test the possibility that the problems are site specific, possibly caused by the diverse operational tasks and constraints imposed at the PSFC bivalve hatchery. If a hatchery can produce 3 successive batches without experiencing mass larval or spat mortality, this will be taken as supporting evidence that problems are site specific. Conversely, if mass mortality of larvae or spat occurs, this will be taken as evidence that the problem(s) are not just specific to PSFC.

Specification

NSW Fisheries hereby invites your company to submit a tender to address this second strategy in accordance with the following specifications.

1   NSW Fisheries will supply the successful tenderer with a minimum of 100 fifth generation *Saccostrea glomerata* broodstock Sydney rock oysters selected for rapid growth. Transport costs will be met by NSW Fisheries.

2   The successful tenderer is to conduct all hatchery operations under full quarantine (bio-secure) conditions to minimise the risk of possible translocation of infectious disease.

3   The successful tenderer is to reproductively condition supplied broodstock for 8-10 weeks at a temperature of 22°C prior to attempted induction of spawning.

4   Methods used to rear the larvae and spat will be at the discretion of the successful tenderer but will need to be discussed in detail with NSW Fisheries staff.

5   A comprehensive daily diary recording hatchery and nursery operations and observations is to be kept through to the completion of each batch.

6   A genuine attempt will be made by the successful tenderer to produce a minimum of 3 successive broods of larvae and juveniles each with a target of 5 million spat >2mm.

7   Daily samples of eggs, larvae and spat are to be collected and appropriately preserved for pathological examination using methods prescribed by NSW Fisheries.

8   Hatchery and nursery rearing operations are to commence no later than 15th November 2002 and completed no later than 31st March 2003.

9   Subject to full quarantine (bio-secure) procedures having been applied and any resultant spat ≥2mm having been given health certification, all spat is to be returned to NSW Fisheries with transport costs to be met by NSW Fisheries. The tenderer will be paid 1 cent for each spat.

10   The tenderer must notify NSW Fisheries as soon as mortality of >80% for larvae or >60% for spat occurs. This will be the criteria used to characterise the run as having suffered mass mortality.

11   The tenderer may be notified in writing not to attempt another batch should mass mortality occur – in this situation the engagement would be considered to have concluded on the notified date and no further claims would be accepted by NSW Fisheries in regard to batches proposed but not produced.

12   The successful tenderer will be required to indemnify NSW Fisheries against all claims and actions arising from or due to their involvement in this project other than the prices accepted in the quotation related to batch production.

Information to be included in tender submission

  i.   Tenderers should include quotes for the production of 3 successive batches. For each batch, quotes should be prepared for:

        a)   rearing larvae that die after 3 days but before 18 days after hatch
        b)   rearing larvae and then spat that die after settlement but before spat reach 2mm (ie after 3-6 weeks of rearing)
        c)   successfully rearing larvae and spat and producing high numbers of spat greater than 2mm (NOTE: all spat greater than 2 mm will be purchased by NSW Fisheries for 1 cent each).

  ii.   A short description of the hatchery and quarantine facilities at which production is to take place. This should demonstrate that the applicant has the capability to successfully and safely produce the spat required. Details of relevant mollusc/bi-valve production history and outcomes should be supplied where appropriate.

iii. Details of the qualifications and experience of key staff involved.

iv. A brief outline of the production methodology proposed including biosecurity arrangements.

v. An endorsement by the CEO confirming that the tendering company has the ability and resources to meet the timeframes and other conditions specified.

Submissions received will be evaluated and ranked based upon the above information.

Submissions must be in a sealed envelope and mailed or placed in the Tender Box marked:

<div align="center">

TENDER BOX
Sydney Rock Oyster Spat Production
Port Stephens Fisheries Centre
Private Bag 1
Nelson Bay NSW 2315

By 11am 30 August 2002

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Submissions must be received in the Tender Box by 11am Friday 30 August 2002 or they will be ineligible for consideration. No faxed, emailed or unsealed submissions will be accepted.

If you have any questions or require further information please contact Jo Pickles on 02 4916 3901; Fax: 02 4982 1107 or Email Jo.Pickles@fisheries.nsw.gov.au.

Yours sincerely

Nick Rayns
Director Aquaculture

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No. 7   Heasman, M., 2003. Proceedings of the Sydney Rock Oyster Hatchery Workshop held on 8 and 9 August 2002 at Port Stephens, NSW.