Sydney Rock Oyster Hatchery Health Workshop
8-9 August 2002, Port Stephens, NSW

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FRDC Project No. 2002/206
February 2004

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ACKNOWLEDGEMENTS

We wish to acknowledge the contributions of delegates to this workshop, all of whom made significant contributions from a broad range of perspectives. We are particularly indebted to Dr Ralph Elston who drew on 25 years of practical experience of bivalve hatchery siting, design and operational problems.

Major insights into nutritional issues were provided by Drs Malcolm Brown and by Peter Thompson who also provided some very useful comments on current algal production practices at the Port Stephens hatchery.

Dr Elston's expertise on factors contributing to the health of hatchery reared molluscs were complimented by that of other leading researchers in the field, namely Drs Robert Adlard, Dick Callinan, Judith Handlinger and Brian Jones.

Expert commentary on disease manifestation, prevention and control provided by various health experts was greatly enriched by that of past and present commercial hatchery managers; Jonathon Bilton, Rod Grove-Jones, Martin John, John Mercer and Richard Pugh all of who contributed their own time in attending the workshop.

We were delighted by the excellent representation and contributions to debate by representatives of the NSW Oyster Farming Industry. This included the entire membership of the NSW Oyster Research Advisory Committee (ORAC), namely; Geoff Diemar, Laurie Lardner, Tony Troup and Ray Tynan together with Chairperson Professor Ian White. Also amongst this group were representatives of both NSW Oyster Farmers associations namely; Glenn Browne, Roger Clarke, Andrew Phillips, Robert Diemar, Mark Shepphard and Dominic Boyton. As with the hatchery managers, all contributed their own time to attending the workshop.

We thank FRDC sponsorship of this workshop and Dr Patrick Hone's valuable contribution to discussion sessions, his innovative suggestion that hatchery operators run a parallel session to formulate changes they would impose to overcome mass mortality of Sydney rock oysters experienced at the Port Stephens Fisheries Centre and for his suggested guidelines for developing a long term strategy to overcome these problems.

Finally, we applaud the enormous task 'behind the scenes' organisation of the workshop provided by Jo Pickles and for Jo's subsequent invaluable assistance in compiling the workshop proceedings. I would also like to thank Helena Heasman for her assistance in the preparation of this final report.

NON-TECHNICAL SUMMARY

2002/206	Sydney Rock Oyster Hatchery Health Workshop

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OBJECTIVES:

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.

NON TECHNICAL SUMMARY:

Outcomes Achieved

Unfortunately, chronic mass mortality episodes of stock that manifest either as anorexia of 2 to 8 day old larvae or as sudden shell gaping and death of small spat below 2 mm, 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 same problems have also forced the abandonment of SRO production operations by several commercial hatcheries elsewhere in NSW. Although a considerable body of valuable information on the epidemiology has been compiled in relation to larval and spat mass mortalities, both have to date defied positive diagnosis or prevention (Heasman, 2000; 2003).

Over the past 18 months, three complementary strategies have been developed and major resources marshaled to address this issue. In the first strategy, we have begun to implement relatively simple but potentially significant modifications to existing bivalve hatchery facilities, rearing equipment and operating protocols at PSFC. These changes are being made in accordance with recommendations of the Review of Hatchery Production Technology and Breeding program for Sydney Rock Oysters, FRDC Project 2001/213 (Benzie *et al*., 2001 – Appendix 8.2), with advice received at the Sydney Rock Oyster Hatchery and with conclusions and recommendations of the Sydney Rock Oyster Nursery Health Workshop, FRDC Project, 2003/206 (Heasman, 2003), and on the basis of a recently commissioned independent hatchery audit prepared by Mr Martin John of Shellfish Hatchery Consultancy (Appendix 8.3) and a HACCP plan developed in conjunction with of AusVet Animal Health Services (Appendix 8.4).

The second strategy is assessing whether or not 'in-house factors' at PSFC, namely site and facility design and operational constraints (especially inherent plumbing design faults and hygiene constraints imposed by year-round competing demands for limited hatchery resources), have been responsible for variable and generally poor hatchery production of SRO spat. To do this, New

Tech Aquaculture at Hervey Bay in central Queensland, was commissioned as successful respondent to an Australasian- wide call for expressions of interest, to conduct a series of external SRO seed production trials, using NSW sourced brood-stock. Overall results of these trials, conducted between February and May 2003, have been encouraging with valuable insights being gained into improving current hatchery facilities, equipment and practices.

Results of particular importance were as follows. The first of four hatchery runs encountered an extreme episode of the larval anorexia and mass mortality on Day 4 thereby confirming that the larval syndrome is not confined to NSW. The subsequent 3 runs were deemed moderately to highly successful with a three-fold increase in yield of ready-to-set larvae having been demonstrated. Results of the 1st and 4th hatchery runs strongly suggested that the success of particular batches of SRO larvae, is not only profoundly influenced by the seasonal cycle of gametogenesis and nutritional history of brood-stock, but also by physiological stress, particularly extremes of temperature, leading up to spawning. Results and observations made over the course of the 4 runs collectively suggest that substantial improvement in production efficiency appears possible with implementation of relatively minor refinements to conventional batch production equipment and protocols used at PSFC and by most oyster hatcheries globally. Results also suggest that if larval rearing conditions, especially quality of source water, nutrition and hygiene, are generally conducive to good health, then satisfactory growth and high rates of survival can be achieved independent of temperature over the wide range 20 to 26ºC.

The third strategy, a 3-year program of systematic experimental investigation, developed by myself and Dr Wayne O'Connor, has been designed to optimise a wide spectrum of husbandry factors, particularly those identified as of high probable significance during the course by an international health workshop that I convened in August, 2002. This strategy mimics a successful recipe that I used previously to develop reliable and efficient hatchery technology for another problematic hatchery species, the commercial scallop *Pecten fumatus*. This experimental program also attempts to identify specific causes and predisposing factors of larval and spat mass mortalities and hence a formula for their prevention.

This experimental program, that has recently attracted a $700K grant (FRDC project 2003/209), will build on progressive results and successes such as those already achieved by New-Tech Aquaculture in Hervey Bay under Strategy 2, by refocusing research on those issues revealed as most likely to improve the reliability and/or efficiency of spat production. The program is also geared to 'fast-track' commercialisation of SRO breeding program with the first large sale production run to be attempted in Sept/Oct, 2003. A priority objective is rapid assessment of alternative settlement and spat rearing technologies, including use of spat bubblers and estuary-based field nursery systems, to promote fastest possible growth of spat to a size of 2 mm beyond which they appear to overcome susceptibility to the mass mortality syndrome. A critical outcome of the program will be to ensure the portability of improved commercial production technology developed.

KEYWORDS:
Sydney rock oyster, *Saccostrea glomerata*, hatchery, nursery, health.

1. BACKGROUND AND NEED

This workshop brought together a small number of the most experience 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.

The 120 year old Sydney rock oyster industry directly employs about 1200 people, more than any other form of aquaculture in Australia. However, it has suffered a 40% decline from peak production 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.

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. S*elected 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.*

Significant resources have been allocated to attempt to solve these problems but clearly they have been insufficient. A recent review of the hatchery breeding program and hatchery problems at the PSFC (FRDC 2001/213) identified that the potential benefit cost of solving hatchery problems was very positive and that a concerted, new research effort was warranted. A specific recommendation was to convene an expert workshop to systematically analyse past data and practices and, after carefully considering international developments in oyster larval and spat diseases, nutrition, epidemiology and hatchery technology, to design a research project to solve the problems.

2. OBJECTIVES

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.

3. METHODS

The objectives were addressed by bringing together in a highly focussed workshop the best available international and national experts with relevant experience in oyster hatcheries, specifically with mortality problems in those hatcheries. The foremost expert in the world in this field is Dr Ralph Elston. The workshop was organised around his availability and after consultation with him on the best way to achieve objectives. Several other experts on oyster health or epidemiology included Drs Judith Handlinger, Brian Jones, Dick Callinan and Rob Adlard. Larval nutritionists, Drs Malcom Brown and Peter Thompson and commercial mollusc hatchery managers, Martin John, Richard Pugh, Jonathon Bilton, Rod Grove-Jones, John Mercer, Ken Frankish and Steve O'Connor attended. All members of the NSW Oyster Research Advisory Committee attended to keep industry fully involved with the problem and to ensure their voice is heard on future directions. Other NSW Fisheries scientists and technicians who participated in the workshop included Drs Mike Heasman, Nick Rayns, Geoff Allan, John Nell, Wayne O'Connor and Lindsay Goard, John Diemar and Lynne Foulkes.

4. RESULTS AND DISCUSSION

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.

5. 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.

6. BENEFITS AND ADOPTION

The Sydney Rock Oyster industry directly employs about 1200 people, more than any other form of aquaculture in Australia. However, it has suffered a 40% decline from peak production levels 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. This workshop was the first step towards solving hatchery problems so NSW oyster farmers can purchase faster growing, disease resistant spat. The internal rate of return was estimated to improve from 3% to 14% for oyster farmers who shift from current practices to growing genetically improved hatchery-produced oysters that reach market-size one year earlier.

7. PLANNED 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 SROs (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.

8. REFERENCES

Benzie, J.A.H., Grove-Jones, R.P. & Adlard, R.D. (2003) Review of hatchery production technology and breeding program for Sydney rock oysters. Final Report to Fisheries Research & Development Corporation, Project No. 2001/213. Oyster Research Advisory Council.

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

Heasman, M.P. (2003) Proceedings of the Sydney Rock Oyster Hatchery Health Workshop held on 8 and 9 August 2002 at Port Stephens, NSW. NSW Fisheries Research Report Series No. 7. NSW Fisheries.

9. APPENDICES

9.1 Intellectual Property

Not applicable.

9.2 Review of Hatchery Production Technology and Breeding Program for Sydney Rock Oysters by John Benzie *et al.*

9.3 Hatchery Audit Report by Martin John

9.4 Hazard Control Plan by Animal Health Services

Appendix 9.2

REVIEW
OF
HATCHERY PRODUCTION TECHNOLOGY
AND
BREEDING PROGRAM
FOR
SYDNEY ROCK OYSTERS

for

Oyster Research Advisory Council

by

John A. H. Benzie, Rodney P. Grove-Jones and Robert D. Adlard

FRDC PROJECT REPORT 2001/213

Contents

1. Terms of reference

The objectives of the review are:

1. To critically review objectives methodology and results to date of the Sydney rock oyster breeding program.

2. To critically review practices and procedures for Sydney rock oyster hatchery technology at the Port Stephens Fisheries Centre and problems associated with larval and post-settlement mortality.

3. Prepare a cost benefit review of the Sydney rock oyster hatchery program.

4. Provide recommendations for either continuation or discontinuation of the Sydney rock oyster hatchery R&D and breeding program.

2. Background

The Sydney rock oyster industry is the oldest aquaculture industry in Australia and employs several hundred people in regional New South Wales**.** In the last twenty years the industry has declined in size from a peak production of 130, 000 bags in the 1970's by about 40%, representing a loss of annual revenue of $20 million**.** A major contributing factor to the decline has been escalating costs of production of Sydney rock oysters relative to Pacific oysters**.** Significant influences on the cost of production are the lower growth rate and higher susceptibility to diseases of Sydney rock oysters.

Research work on genetic improvement of Sydney rock oysters was started with a view to addressing these key issues by producing a faster growing, disease resistant oyster**.** This has been attempted using three separate approaches: by selective breeding for faster growth, by manipulating the chromosome number to produce triploid animals that grow faster than the normal diploids, and by selectively breeding animals that have survived disease exposure**.** An essential part of all these approaches is the hatchery production of young oysters and research has also been undertaken on developing appropriate hatchery technology**.** The total investment in selective breeding and triploid work from 1989 to 2000 is estimated to be $3.5 million.

Hatchery technology had been developed that has allowed sufficient numbers of animals to be produced to maintain experimental scale mass selection lines**.** Several batches of triploid spat were also provided to the industry**.** However, when commercial scale production of genetically improved stock was attempted to supply industry, production was inadequate to meet demand**.** In fact, production was highly variable, unpredictable and largely failed**.** This led to considerable disappointment in the industry, which had understood that sound technology was available, and led to questions as to the adequacy of the hatchery technology, breeding programs and impediments to their commercialization.

These concerns led to the request for a review of these programs**.** The review panel was to include experts in mollusc genetics, hatchery technology and disease.

The panel selected to undertake the review consisted of:

The Chair, Prof. John Benzie from the University of New South Wales who has extensive expertise in aquaculture genetics, including oversight of Pacific oyster genetic improvement programs in the former Aquaculture CRC,

Mr Rodney Grove-Jones who has extensive experience in mollusc hatcheries with many years experience in the South Australian Oyster Industry and,

Dr Robert Adlard from the Queensland Museum who is expert in mollusc diseases and who has direct experience of diseases of Sydney rock oysters.

3. Scope of investigation

The review panel collected information principally through reading a number of reports that were available summarizing the research programs and industry plans, through meeting with the research group and managers at NSW Fisheries and through meetings with the two oyster farming associations. Discussions were also held by telephone with other industry members and with other experts brought in to advise the research group from time to time.

The principal meetings are recorded below:

31 October – 2 November in Port Stephens

- Meeting with NSW Fisheries managers and the research team.
- Detailed inspection of hatchery facilities and genetically improved stock.
- Presentation on probiotics by University of Technology, Sydney.

[Staff interviewed: D. Ogburn (Principal Manager - Aquaculture), G. Allen (Principal Scientist - Aquaculture), M. Heasman (Scientist - Head of Mollusc Hatchery), J. Nell (Principal Research Scientist, Oysters), J. Diemar (Fisheries technician algae), L. Goard (Fisheries technician mollusc hatchery), L. Foulkes (Fisheries technician algae)]

15 November in Port Stephens

- Panel available for meeting with Industry.
- Telephone conference with industry members.
  (L. Lardner, T. Troup, A. Phillips)
- Meeting with D. Liska of Pisces Marine (operators of Broome's Head hatchery).

16 November in Sydney

- Meeting with NSW Farmers Association - Oyster Section.
  [Farmers in attendance: A. Sciacca (President), M. Sheppard, J. Croucher, T. Dent, G. Browne, S. Feletti, G. Diemar, F. Knudson, J. Manson, D. Flemming, M. Dejoi, J. Collinson, A. Collinson]
- Meeting with Oyster Farmers Association of NSW.
  [In attendance: R. Clark (President), R. Roberts, R. Tynan, R. Drake, G. Campbell]

Electronic/telephone discussions by individual members of the panel were also held with:

- Oyster farmers, N. Ellis, S. Verdich, R. Moxon, I. Crisp, P. Clift, and G. Barclay.
- Disease experts Dr M. Hine, Dr R. Callinan.
- Genetics expert B. Sheridan (geneticist on Sydney rock oyster project).
- Economic expert C. Catt (developed economic models for the Sydney rock oyster industry).
- Hatchery expert Mr J. Bilton (Albany Hatchery Manager).

Key reports accessed were:

ACIL Economics. (1997) Oysters at the crossroads: a strategic plan for the New South Wales Oyster Industry. Unpublished report. 84 pp. plus appendices.

Anon., (2000). NSW Coastal and Estuarine Sustainable Aquaculture Strategy. Natural Waters Based Aquaculture. Preliminary Draft. Unpublished 244 pp.

Anon., (2001). Oyster breeding review information package. NSW Fisheries.

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Johnston, B. (2001). OysterProfit version 1.0. NSW Fisheries.

Nell, J. and Maguire G.B. (1998). Commercialisation of triploid Sydney rock oysters. Part 1: Sydney rock oysters. FRDC Project No. 93/151. NSW Fisheries final report series. Report no. 10. 122 pp.

ORAC (2001). Adding value to the NSW Oyster Industry through research and development. The 2001-2006 R&D Strategic Plan. 45 pp.

White, I. (2001). Safeguarding environmental conditions for oyster cultivation in New South Wales. Draft unpublished final report for the NSW Healthy Rivers Commission. 83 pp.

Additional material supplied by the Fisheries Department research group is provided in the appendices.

4. Review of the Sydney rock oyster breeding program

The panel was asked to assess the objectives, methods and results to date of the Sydney rock oyster breeding program. These are dealt with in separate sections below.

4.1. Objectives of the breeding program

4.1.1. Description of the objectives of the breeding program

The NSW Fisheries oyster research program is based on the development of genetic lines of oyster stock that are:

mass-selected for increased growth rate.
mass-selected for resistance to marteiliosis (QX disease).
mass-selected for resistance to mikrocytosis (Winter Mortality).
induced to be triploid.

The objectives for the selected lines are concordant with those identified as being of high priority among the research topics listed in the Industry R&D Strategic Plan for 2001-2006 (ORAC, 2001) as follows:

Under Program 2: Diseases, pollution and pests

- to develop QX disease resistant oyster through genetic selection
- to develop winter mortality resistant oyster through genetic selection

Under Program 4: Hatchery and farm technology

- to develop through selective breeding an oyster which reaches market size (40-60g) by at least one season earlier

Triploidy is not mentioned in the R&D Strategic Plan, as much of this research was completed prior to 2000. Triploid oysters have been successfully grown on farms, and have demonstrated improved growth, and maintained their condition for longer, enabling sales, and better prices at some times of year.

4.1.2. Conclusions

The panel concluded that the general objectives of each of these program elements are clearly and appropriately targeted at issues of key economic and strategic importance for the Sydney rock oyster industry.

4.2. Methodology of the breeding program

4.2.1. Description of selection program for improved growth

The current lines mass selected for increased growth rates were first set up in February 1990 from oysters collected from Wallis Lake, Port Stephens, the Hawkesbury River and Georges River. This approach was designed to include as much of the genetic variety as possible that might have existed in a range of populations utilized by the industry in NSW.

A total of eight separate mass spawnings of females, utilizing in total some 222 females and 52 males, was used to generate larvae that were reared in one tank. Again, the several separate mass spawnings, using different sets of males and females, were an attempt to reduce potential inbreeding. The total spat from all the spawnings were then split at random into eight populations. These constituted the base populations of four replicate lines maintained at Port Stephens and four replicate lines maintained at Georges River. Each replicate line consisted of three trays of oysters in each of three locations within the estuary (i.e. nine trays in all for each replicate line in each estuary).

The largest progeny (those with heaviest shell weight) from several trays within a line were then selected for breeding when mature, and four separate mass spawnings/fertilizations were used within each replicate line to provide the progeny for the next generation. In order to assess whether any genetic gain was being made the selected lines were compared with "control" lines that consisted of hatchery-reared progeny produced from parents taken at random from wild stock. For logistic reasons, and to spread the workload, the Georges River ones in even years (1992, 1994, 1996 etc) and the Port Stephens lines were generally spawned in odd years (1993, 1995, 1997 etc).

A summary the breeding history of the lines including spawning times, number of adults used in mating is given in Appendix I. Details of the tray stocking densities and of measurements are given in relevant research papers summarized in Nell *et al.* (2000).

4.2.2. Description of selection programs for disease tolerance

In 1995, the Georges River lines were reorganized following severe QX disease mortalities (98%) in two of the sites at Georges River, (Oyster Bay and Lime Kiln Bar). The survivors from these two sites were pooled and used to breed animals presumably tolerant to QX disease as they had survived the disease event. Animals from the Woolooware Bay site, where winter mortality was known to occur were treated as survivors of Winter mortality and bred to improve tolerance to that disease. Matings within and between these populations were attempted (see Appendix I, 2nd page).

4.2.3. Description of triploid program

The program uses chemical treatment to prevent the extrusion of the second polar body during reduction division during cell replication in the fertilized egg, giving an animal that has three sets of chromosomes (triploid) instead of the normal two (diploid). The improvement in production results from the fact that triploids are usually sterile. As a result of this lack of reproduction triploids do not divert energy into egg development but into growth. The oysters therefore grow faster, and do not suddenly lose condition because of spawning. These animals can maintain their condition into and beyond the breeding season and so extend the time/price for which these oysters can be provided to markets. Full descriptions of the techniques used are given in Nell and Maguire (1998).

4.2.4. Breeding philosophy

The approach used to set up the breeding program for improving growth was based on using mass selection – in this case the best growing animals from the population are used as parents for the next generation. The advantage of this approach is that it is relatively cheap compared with family-based or pedigree approaches. However, there is a risk of inbreeding as a result of mating close relatives, and it is not possible to directly select for characteristics that can only be measured in dead animals (disease response, meat weight at harvest etc.). The program has used procedures that are likely to reduce inbreeding such as basing the initial population on collections from a variety of sources and using multiple mass spawnings to produce each generation.

Inbreeding can be more closely managed, and direct selection for characters valuable to the industry can be achieved using pedigreed breeding programs. These programs are more expensive to run but could give more targeted, more reliable results, and achieve specific breeding goals more quickly, than mass selection programs.

The philosophy of why a mass selection approach was chosen is not discussed in the published papers on the program. However, the decision appears to have resulted in part from the difficulty of reliably producing spat from single pair matings because of the difficulties in hatchery production. The approach to selection for disease tolerance is largely a reaction to the existence of survivors in areas affected by disease.

The selection practiced for disease resistance is poorly controlled in that the precise level of exposure of animals to the disease is not known, and may be affected by a variety of variables in Georges River. In addition, animals are still being selected on size, so selection is not based solely on disease tolerance and the degree to which these variables interact is not known and not measured. Similarly, the choice of shell weight as the character to be selected to achieve growth improvement simplifies data collection but has yet to be confirmed as meeting the ultimate breeding goals.

The choice of mass selection as the approach used for improving growth probably reflects original and continuing limitations in available resources and hatchery technology. The methods aimed at developing disease tolerant lines are a practical response to disease events limited by what can be done with available resources rather than optimally designed to achieve specified results. While mass selection designs can be effective in achieving genetic gains, they are unlikely to be optimal in the circumstances described for the Sydney rock oyster, where there are significant practical constraints in the hatchery technology and lack of precision in characters being selected.

In contrast to selection programs, ploidy manipulation does not depend on maintaining stocks continuously**.** Broodstock can be collected from the wild and treated on spawning. Ploidy manipulation can also be used in addition to selective breeding potentially combining the advantages accruing from both techniques.

4.2.5. Conclusions

The panel concluded that the methods used could well provide genetic gain, but that the present approach has a number of deficiencies:

- **The mass selection designs reflect what can be done on limited resources rather than the outcome of optimal designs for specific goals.**
- **The approach used specifically excludes possibilities of direct selection for key characters of commercial value such as meat weight or disease resistance.**

- **The characters being selected have not yet been tested as to their adequacy as proxies for the characteristics of principal commercial interest.**
- **The level of inbreeding is unknown and the adequacy of measures used to reduce inbreeding are unknown.**
- **The selection regime for disease resistance is effectively uncontrolled and the "hit or miss" nature of this approach may not yield the desired outcomes.**

4.3. Results to date of the breeding program

Despite the potential limitations of the designs, and the considerable practical constraints confronting the research team there is evidence the improvements have been achieved.

4.3.1. Selection for improved growth

Detailed descriptions and analyses of the results of selection for growth after different numbers of generations are given in (Anon., 2001). Summary information of key results is given in Appendix I, Tables 3 and 4. These suggest that 0-8.5% improvement in total weight was achieved at the end of generation one (Table 3) and that 14.2-22.7% improvement over control lines was observed by the second generation (Table 4). A survey of allozyme genetic variants suggested that no substantial loss of genetic variation had occurred in the selected lines. There was also an indication that the selected oysters grew with greater metabolic efficiency than the controls.

However, the results have to be qualified because it is difficult to assess the extent of genetic gain as the controls differed from year to year, the actual level of inbreeding is unknown and it must also be pointed out that the character being selected is total weight, and there has yet been no check made that this character is working as a valid proxy for the real character of interest - meat weight (and quality).

4.3.2. Selection for disease resistance

Detailed descriptions and analyses of the results of selection for growth and disease tolerance after different numbers of generations are given in (Anon, 2001). Summary information of key results is given in Appendix I, Tables 1 and 2. Little improvement was observed after one generation (Table 1) but mortality in the second generation appeared to be significantly reduced relative to the controls by about 35%. Growth appeared to be greater in the QX and Winter Mortality selected lines, but not in the line assumed to be selected for both (Table 2).

4.3.3. Triploid production

Details of the research work on triploids, their performance in trials and aspects of their commercialization are given in Nell and Maguire (1998). Methods have been developed that reasonably reliably achieve some 80% triploids in the batch of eggs treated. Chemical methods never provide 100% triploid spat, and there has been research attempted to produce tetraploid Sydney rock oysters. Tetraploids mated with diploids give 100% triploids, but to date the attempts to develop tetraploids have failed.

A large-scale experiment considered to be on a commercial scale was carried out in 1994-1996 where 25,000 triploids and 25,000 diploids were provided to each of thirteen farms for growout under normal production environments. The triploids have been demonstrated to grow faster by some 30-40% better then diploids on average, and maintain better condition than diploids at two to

two and a half years age. There was also some evidence that the triploids suffered less mortality then diploids in the face of winter mortality disease.

The results, for both growth and mortality, did differ from site to site however, and the triploids have a greater incidence of brown discolouration of the gonad, that again varied from site to site and season to season. Economic analysis of the benefit concluded triploids were likely to be of benefit but that greater growth margins were required for the benefit to be more than marginal, but it was noted that key economic information on the industry that was available had been collected almost ten years previously, and that industry practices may have changed since that time (see Catt pp 100-119 in Nell and Maguire 1998).

In subsequent work it has been demonstrated that the gains obtained by selective breeding are additive with those achieved by ploidy manipulation (Appendix II, Table 5). Selected line diploids were some 21% heavier than control diploids, triploids developed from the control diploids were 25% heavier than the control diploids, and the triploids developed from the selected diploids were 58% heavier than the control diploids.

Despite these promising results, no further commercial scale triploid spat have been provided to industry despite some industry interest because of difficulties in rearing commercial quantities of spat.

4.3.4. Challenges in the production of breeding lines

In interviews with project staff they noted that, while batches of young could be produced sufficient to maintain an experimental sized breeding program, hatchery survival was highly unpredictable and often low. There were larval mortality and spat mortality problems, which prevented successful large-scale production. There were difficulties in achieving measurements at all sites at all times. Although planned when setting up the breeding program, there has not yet been a check as to whether the selection for shell weight is highly correlated with meat weight (the ultimate target for selection). There was also a strong reduction in early growth of hatchery spat that would also influence results of selection. Farmers that had reared hatchery spat particularly noted this issue. Details of the hatchery production issues will be dealt with in a later section, but the high mortalities in the hatchery phase may well be imposing strong selection of unknown direction on the mass selection lines. They were certainly a complete impediment to the commercial scale production of spat that will be required to deliver the benefits of genetic improvement to the industry.

Further work on overcoming these hatchery issues was constrained by a priority to produce sufficient progeny to keep the genetic improvement program going at the expense of detailed study of hatchery problems.

The genetic improvement programs appear to have made significant advances in the growth of selected lines and in the growth and mortality of animals in areas exposed to disease. However, because of shortcomings in experimental design it is not clear to what extent progress has been made for total weight, let alone the ultimate character it is wished to improve (meat weight), and the extent to which apparent disease tolerance may be a site-specific response. It is unlikely that present approaches are optimal. Alternative approaches that may better target ultimate breeding objectives and provide faster selection responses need to be considered. Deficiencies in larval hatchery production severely restrict the range of genetic improvement strategies that could be applied to Sydney rock oysters and may also adversely affect levels of inbreeding and the nature of selection in the current mass selection program. These deficiencies are a barrier to cost-efficiencies in the genetics program and are an absolute impediment to successful commercialization of genetic improvements.

4.3.5. Further consideration of the characters to be selected

The key characters that industry has targeted for genetic improvement are reflected in the high priority topics listed in the Industry R&D Strategic Plan for 2001-2006 (ORAC, 2001):

Under Program 2: Diseases, pollution and pests

- to develop QX disease resistant oyster through genetic selection
- to develop winter mortality resistant oyster through genetic selection

Under Program 4: Hatchery and farm technology

- to develop through selective breeding an oyster which reaches market size (40-60g) by at least one season earlier

It is worth considering some general issues with respect to these characters that need to be taken into account in determining the appropriate means to dealing with them, either whether they should be the subject of genetic improvement and, if so, by what strategy.

Improved growth is of obvious commercial value. However, because oyster leases are of finite size, a faster growing oyster that utilizes the same amount of food per unit growth does not provide for any increase in production if the lease is near its carrying capacity. It is unlikely that many leases are in this position given the recent declines in production. Nevertheless, it is important that selection is not just for increased size, but for increase efficiency of food utilization, and this be targeted to meat growth and condition. There has been some evidence that the faster growing selected oysters are more efficient metabolically but there is a need to discuss the most effective ways of achieving goals of efficient growth – goals that involve matching several characters related to high production performance.

QX disease resistance is also considered of importance because this disease has the potential to wipe out Sydney rock oyster operations. Although the life-cycle of this disease is incompletely known, there are compelling data from Georges River and southern Queensland estuaries that infection occurs from an, as yet, unidentified alternate host during a short-lived (2-4 weeks) pulse in mid to late summer. Infected oysters then shed developed spores of the pathogen into the environment 6-8 weeks postinfection, spores are short-lived (maximum 35 days at optimal temperature and salinity – experimental data) presumably then are taken up (ingested?) by the alternate host and undergo development prior to the next pulse of infection in mid to late summer of the following year.

It is thus likely that disease dynamics are responsive to changes in the abundance and distribution of either the definitive (oyster) or alternate host with concomitant changes in the selection pressure at a particular site. The prevalence of marteiliosis in ‘upriver’ leases of Limekiln Bar, Double Bay, Oyster Bay in 1995 reached an average of 90.1%, indicative of high infection rates and a high selective pressure on oysters exerted by the pathogen during that period (data from the Georges River epizootics). However, additive resistance in successive generations of stock through mass selection (under pressure from the pathogen) may be unpredictable through fluctuating intensity of the pathogen. Furthermore, other biological and/or physical parameters exert selective pressure in an uncontrolled experimental location (i.e. in an estuary rather than a laboratory).

Currently, the pathogen cannot be cultured in the laboratory, because its life cycle involves multiple hosts, one of which is unknown. For this reason controlled disease challenge is not an option at present for selecting oyster genetic lines. The relative merits of investing in processes to

culture this species to allow such a targeted approach in time, or into other control measures, is required prior to deciding on genetic improvement strategies.

Level of QX disease resistance: Mass selection results for QX disease resistance have indicated a mortality of 55% in second generation selected stock compared with a mortality of 73% for unselected controls. For a commercial product with equivalent survivorship to what is currently accepted by growers (5-10% mortality accepted from 'natural' sources), selection must deliver at least a further doubling (36%) of reduction in mortality for a genetically value-added product to be commercially viable. If selected resistance increases additively at the same rate as already indicated, such a result would be expected in approximately 8 years time.

Winter Mortality disease resistant stocks would also be of benefit to the industry but less is known of the disease dynamics of mikrocytosis, than of QX. Onset of the disease appears to be correlated with high salinity (more oceanic) areas of estuaries and it requires low water temperatures (10-12ºC) for clinical signs (focal lesions) to occur. Transmission is direct as evidenced from successful transmission by association and inoculation of uninfected controls with naturally infected stock or homogenate.

In the Georges River in 1995, cumulative mortality in downriver sites (Quibray Bay) peaked at 50%, with the Winter Mortality disease agent, *Mikrocytos roughleyi* thought to be largely responsible. However, this organism is difficult to diagnose through traditional methods (molecular diagnostic method now available, see Adlard and Lester 1995) and its presence is more often implied than unambiguously established. As such, it can be difficult to determine whether selective pressure is derived from a single selective origin. Furthermore, anecdotal observations on patterns of mortality at both oyster lease and oyster tray scale, suggest a patchy distribution of the disease resulting in variable, rather than equivalent, selective pressures on individual oysters.

Since transmission of mikrocytosis can be induced between oysters through association and inoculation – selective pressure on genetic lines can be controlled in the laboratory by disease challenge from an infected source population. The infected source population can be maintained or developed through repeated inoculation (hyperinfection) from naturally infected oysters.

Market uptake of WM resistant stock: Winter Mortality impacts on oyster production from the southern NSW border to Port Stephens on the central coast of NSW. The mortality is temporally unpredictable and growers respond where possible by local translocation of oysters during high risk periods (late March to September) into areas known to be free of clinical disease. This practice is labour intensive and requires the ability to rotate stock on leases (i.e. effectively doubles the required growing area). Provision of a winter mortality disease resistant rock oyster would significantly benefit growers, particularly in southern NSW estuaries.

4.3.6. Conclusions

The panel concluded that:

- **Significant gains in growth rate, possibly disease resistance, and certainly improved growth and condition in triploids, had been achieved to date.**
- **The strength of the gains in the mass selection programs was difficult to establish given the lack of control on selection levels, the variation in "control" groups and the use of proxy characteristics as the focus of selection.**
- **Given these difficulties, the design of the program, and the speed of selection are unlikely to be optimal.**

- **The lack of effective hatchery technology is a key impediment to:**
    1) **the effective direction of genetic selection in the current program,**
    2) **the cost-effectiveness of the current program,**
    3) **precludes the use of alternative approaches to genetic improvement and,**
    4) **prevents effective transfer of benefits to industry.**

4.4. Recommendations

While the present programs have appeared to make some gains, there is doubt as to the true extent of these because of the difficulty of estimating the true amount of genetic gain. The lack of a reliable hatchery technology prevents:

- soundness and cost-effectiveness in the current program.
- precludes the use of alternative approaches to genetic improvement and,
- prevents effective transfer of benefits to industry.

Alternative approaches to genetic improvement have not been discussed in detail with industry. Some genetic improvement approaches that might be far more targeted in meeting the industry needs will depend on overcoming the present hatchery problems. However, given the high level of investment needed to achieve a sound genetic improvement program, the long time scale that is required for return on investment, and the need to integrate a genetic program into an overall industry strategy, it is vital industry is intimately involved in deciding the nature of the program put in place. It is vital the industry be suitably informed so it is enabled to make the decision as to whether to develop a genetic improvement program, and how this might be structured.

The panel therefore recommends:

That no further work, other than necessary basic maintenance, be carried out on genetic improvement until:

1. **The problems in hatchery technology are overcome.**

2. **A workshop for industry and researchers has been held to assist the development of a sound strategy for genetic improvement of Sydney rock oysters.**

3. **The workshop should utilise external genetic experts, and members from other industries where genetic programs are in place (e.g. pacific oysters, abalone), to inform the Sydney rock oyster industry of the range of approaches available and the practical situations experienced by other programs.**

5. Review of Sydney rock oyster hatchery technology

5.1. Introduction

Attempts to rear Sydney Rock Oysters (SRO) began at the Port Stephens Fisheries Research Centre (PSFRC), then known as the Brackish Water Fish Culture Research Centre (BWFCRC) in the early 1970's with the aim of supplying an alternative source of spat and selectively breeding faster growing disease resistant oysters (Frankish *et al.*, 1991). A pilot scale hatchery was built in 1981 and a larger "commercial scale" hatchery commissioned in 1988. Difficulties in rearing SRO larvae and spat were encountered from the very beginning (Frankish *et al.*, 1991). These difficulties persist despite numerous attempts to solve the issue (Appendices IV and V, Heasman *et al.*, 2000).

This section of the report reviews the practices and procedures for SRO hatchery technology at the PSFRC and compares them with contemporary and progressive commercial techniques. The problems associated with larval and post settlement mortality are discussed and directions for future research are suggested. The review is divided up into several aspects for separate discussion, viz., Algae, Larval Rearing and Settlement Systems.

5.2. Algae System

5.2.1. Stock and working cultures

Stock and working cultures are maintained in an insulated room with controlled conditions of light and temperature. The 100 ml stock cultures are originally sourced from suppliers of axenic cultures and replaced annually. They are grown in reduced light conditions and without aeration. Working cultures are grown in larger aerated flasks and carboys. All containers and media used for the stock and working cultures are autoclaved before use and inoculated in a laminar flow cabinet to reduce the likelihood of contamination by extraneous bacteria.

The technique for flask culture as employed at the PSFRC and many other Australian laboratories may be regarded as one that has been a standard and apparently unchanging practice over many years. While there may be good procedural reasons for this in a research laboratory, the trend in commercial hatcheries has been to reduce the amount of labour and capital expenditure on equipment required to maintain the stock and working cultures. For example, many fewer working cultures are needed when the production cultures generated from the working cultures last 2-3 months rather than 1-2 weeks. Even fewer working cultures are needed when systems are in place that allow for the sanitary inoculation of production cultures from other production cultures rather than starting from fresh working cultures each time. The labour requirement to maintain cultures can be greatly reduced using such methods, freeing trained staff from routine work. Some commercial hatcheries have reduced the total labour requirement to produce algae (including labour to maintain stock, working and production cultures harvesting 5-10,000 litres per day, mix nutrients, maintain and administer the system) to less than 15 hours per week. In addition, a much smaller autoclave is then needed and the constant temperature room can be reduced to a small controlled environment cabinet.

Another trend in commercial hatcheries has been to replace axenic cultures with cultures known to contain a balanced suite of non pathogenic bacteria. This is based on the premise that bacteria free cultures are unstable in a microbiological sense and that it is not practical to maintain axenic

conditions in the production phase. Cultures derived from axenic stocks are therefore likely to be colonised by unknown and possibly deleterious bacteria during the production phase. Cultures derived from non axenic stocks already have a commensal bacterial flora and are less likely to be colonised by opportunistic bacteria during the production phase.

5.2.2. Production Cultures

Seawater for use in production cultures is trucked in from off site and stored in covered tanks for at least four days, then pumped and filtered through nominal one micron cartridges. The 1000 litre tanks or 500 litre clear polythene bags are filled and aerated. Sodium Hypochlorite solution is added to chemically disinfect the seawater neutralised with Sodium Thio Sulphate the next morning. The tank or bag is inoculated from a flask or carboy and (modified F/2) nutrient added. The air supply is CO_2 enriched to achieve a pH of 7.8-8.4 in the culture medium. The 1000 litre tanks are illuminated with 400 W metal halide lamps and the 500 litre bags with fluorescent tubes, both on a 16:8 light:dark cycle. Cultures are harvested by gravity through clear vinyl tubing to larvae tanks located one floor below.

In contrast, modern algal systems;

- reliably and effectively kill pathogens in the incoming water supply by pasteurisation;
- reliably sterilise glass pipework without chemical residue using steam;
- reduce handling and therefore the opportunity for error and contamination at each step;
- simplify procedures;
- automate harvest and distribution;
- use all natural light;
- extend the useful life of cultures from 1-3 to 8-12 weeks;
- harvest cultures of the same age and cell density by continuous overflow.

None of these improvements or any other significant improvements in algal production technology developed in recent years appear to have been adopted at the PSFRC. In addition, it is clear that such commercially orientated goals as reducing labour, capital and operating costs have not been pursued.

Not withstanding this, the algae unit at PLFRC has continued to produce enough algae to meet the needs of the projects it supports, including SRO production. However, it does so at a cost. The system is adequate to supply existing needs while production costs are not at issue and algal consumption remains near its current level. However, the panel does not consider it is a suitable model on which to base a commercial hatchery.

5.2.3. Conclusions

The panel concluded that, while the hatchery has successfully produced algae for a variety of purposes in the last decade that the hatchery had not kept up to date with changes in hatchery technology and practice. In particular, it was thought that the following changes should be adopted:

- **Non-axenic cultures should be used in preference to axenic cultures in the production system.**

- **The algal team needs to develop a plan to modernise its production system and release labour for more productive work.**

- **The PSFRC should also consider the path to commercialisation (beyond semi commercial trials) for its SRO genetic research. This will ultimately involve the development of a commercial algal facility at an oyster hatchery in NSW. The panel does not consider the PSFRC algal production facility in its present form to be a suitable model on which to advise a commercial hatchery.**

5.3. Larval Rearing and Settlement System

The production technique is described in Frankish *et al.* (1991) and in Appendix III**.** The system employed can be generally described as the low density batch culture of larvae followed by settlement on ground scallop shell and the culture of "cultchless" spat in a recirculating closed system.

The larval and spat production systems in use at the PSFRC are similar to designs popular during the 1970's and described by Wilson *et al.* (1984), Dupuy *et al.* (1977), O'Sullivan and Wilson (1976) and Curtin (1979). More cost effective and efficient systems began to emerge during the 1980's, e.g. Holliday (1985), Coon *et al.* (1986) and continued to evolve in commercial hatcheries during the 1990's, becoming increasingly efficient and reliable. Few of those developments have been incorporated into the larval rearing and settlement systems at the PSFRC such that the hatchery is technically out of date from a commercial perspective.

5.3.1. Spawning and Incubation

The PSFRC system uses coastal seawater trucked in and settled in covered tanks for four or more days rather than water direct from the adjacent estuary. Gravid broodstock is induced to spawn by thermal stimulation and salinity reduction. Fertilised eggs are incubated at 3 per ml in 1 micron filtered seawater with the inclusion of 1ppm di-Sodium EDTA. The hatchery does not have a dedicated broodstock conditioning system.

In commercial hatcheries there has been a tendency to reduce the use of chemical additives such as EDTA unless a positive benefit can be clearly demonstrated. Most hatcheries would have a dedicated broodstock conditioning system to insure a continuous supply of gravid adults independent of variations in the natural environment. Those hatcheries involved in genetic selection tend to use a spawning system that contains the gametes of each adult separately and allows for individual pair matings. In addition, it has been shown that for other bivalve species, eggs can be incubated at much higher densities (>250 per ml) than SRO are incubated at (3 per ml) providing steps are taken to keep eggs in suspension, maintain oxygen levels and mitigate bacterial numbers. Successful high density incubation has not been demonstrated for SRO.

It is often overlooked that incubation is a period when fertilised eggs develop in warmed and aerated seawater that has had much of its natural microbiological flora removed by water treatment. It is also a period when any opportunistic bacteria present in the system and particularly those associated with the broodstock are mixed with the organic material from spawning. These are ideal conditions for opportunistic bacteria to multiply. **The importance of stabilising the microflora at this early stage and during the first days of larval growth cannot be overstated.**

Trials that aim to stabilise the microbiological community during incubation, create unfavourable conditions for opportunistic bacteria or favourable conditions for beneficial bacteria are likely to be worthwhile avenues for research. This would include probiotic research, targeted trials with antibiotics to better understand the role of bacteria in the larval mortality syndrome, and investigations into the effects water treatment (i.e. filtration, dark storage and settlement) has on the composition and stability of bacterial communities in the incubation tank water.

5.3.2. Larval Rearing.

Larvae are cultured in 20,000 litre tanks, initially at 2-3 per ml but reducing to 1 per ml when ready to set at day 16-18. The larvae are sieved on progressively larger screens every second day and stocked into new tanks with clean water. Larvae are fed algae twice per day, with the ration determined from a "feed curve". The batch system of larval culture as described has been in use around the world for 20-30 years at least. It is still in use in many hatcheries but there are much more efficient methods now available.

The alternative system can be described as a high density continuous larval culture system. Culture vessels are typically much smaller than for batch cultures but larval densities are much higher. Typical tank volumes are less than 500 litres and stocking densities (for Pacific oysters) from 250 to a minimum of 100 larvae per ml. Thus a 200 litre high density tank contains the same number of eyed larvae at 100 per ml as a 20,000 litre batch tank at 1 per ml (both with 20 million larvae). The high density tank is however, much easier to handle, being quick to drain, clean and refill. It is also cheaper to buy and takes up much less room so requires less floor area and smaller drains.

New water is added to the high density tank continuously (24 hrs per day), and exits via a screened outlet to prevent loss of larvae. Food is added continuously and automatically in a measured quantity and the ration maintained within a defined range throughout the larval cycle. Such systems are much cheaper to build and operate than batch systems. In addition they require much less tedious labour to operate than batch systems, so freeing up skilled staff to network with other hatcheries, conduct more trials or investigate new techniques and modernise systems.

Regardless of the larval rearing system used, there should be a much more rigorously defined rearing protocol than the one described in Appendix III and by Frankish *et al.* (1991). The protocol should define the process exactly and the process should be done exactly the same way each time, without exception. The protocol should form part of an Operations Manual prepared by the hatchery team and there should be in place a formal process for updating the Operations Manual.

It should be noted that while the adoption of more efficient rearing systems would free up staff to investigate the larval mortality syndrome, it is unlikely to solve the problem alone. The panel believes that solving the larval mortality syndrome and the need to update hatchery technology to be separate issues. The existing system, though cumbersome, should be capable of producing, at will, the few tens of millions of larvae required to support the SRO genetics program. The question of whether or not to invest in modernisation will be determined by the importance industry and senior management places on containing or reducing running costs and the degree of professional importance they attach to being technically current.

It was considered more likely that the primary problem lies in the day to day procedures whereby the production system removes an essential requirement unique to SRO larvae, or adds a debilitating step that weakens and predisposes larvae to bacterial attack, or increases the opportunity for bacterial proliferation. Thus, it was considered that a thorough and detailed review of each step of the procedures should be undertaken in a workshop environment with other scientists and hatchery specialists. This should question each step of the production process with particular regard to reducing anthropogenic stress factors such as the adequacy or otherwise of nutrition, opportunities for bacterial proliferation and effects of handling and water processing. The product of the workshop would be a range of alternative protocols to be assessed in the research scale larval rearing unit at the PSFRC.

Research into probiotics with an emphasis on establishing beneficial bacteria and stabilising bacterial communities in the larval rearing tank was considered a useful approach and the SPIRT grant on this issue should be pursued.

In addition to the semi commercial larvae production system, there is a research scale batch culture system consisting of multiple small tanks (approx 10 litre) contained in a temperature controlled water bath. This system appears to be suitable for achieving experimental aims.

5.3.3. Settlement

Settlement is effected by holding larvae that are competent to metamorphose in downwellers (Appendix III) containing crushed and sieved scallop shell as a substratum. The downwellers are contained in tanks of recirculating seawater which are drained and cleaned every second day and to which algae is added as necessary. Settled spat are screened off on days three, five and seven and kept in a separate downweller. Spat that are retained on a 500 micron screen leave the hatchery on day seven for the field nursery. Holliday (1991) also describes a system in which SRO larvae are set on plastic slats.

The alternative method of settlement of using the hydrochloride or bi-tartrate salts of epinephrine to induce larval metamorphosis without settlement on a hard surface (Coon *et al.*, 1986) is well established and routine in many hatcheries. This method has been used at the PSFRC but is not routine. The advantages of this method are that there is no need for downwellers and associated tanks, settlement is spread over a shorter period (typically 1-2 days instead of 7 days) and that the spat is truly "cultchless", i.e. not attached to a shell particle. The scallop shell "cultch" greatly increases the surface area available for colonisation by sedentary bacteria during the first critical days following metamorphosis.

Spat newly settled using epinephrine are transferred directly into free fall spat culture units ("spat bubblers") or fluidised bed "bottle" tanks. Both differ from downwellers fundamentally in that they are flow-through and self cleaning systems whereas the downwellers are closed systems that trap faeces and encourage bacteria to multiply. Spat are removed from the fluidised bed tanks at a size where they are retained on an 1800-2000 micron screen rather than 500 micron screen for the downwellers. Large spat produced in bottles should have a better survival rate than smaller spat produced by other methods when transferred to the field nursery.

While it cannot be ruled out that SRO may have specific requirements that predispose them to early spat mortality, the symptoms are similar to those that frequently arise in hatcheries for other species, particularly in spat smaller than 2 mm shell height. Bearing this in mind, **it is suggested that profitable lines of investigation would be those designed to maximise growth rate while also reducing the organic load and bacterial numbers** in the culture system. An investment in technical improvements (open flow spat bubblers and/or fluidised bottles) will reduce the organic load in the system and is therefore likely to contribute to improved spat survival. This must, however, be combined with appropriate handling protocols and culture temperatures, water flows and (continuous) feeding rates that maximise the growth potential of the species.

An alternative line of inquiry would be to revisit the use of flat plates for settlement and early post larval rearing. Most hatchery operators have observed that spat settled to a flat surface grow faster during the first few weeks post metamorphosis than cultchless spat. This has been confirmed for SRO spat (Holliday, 1991 p99). Moreover, PSFRC staff indicated to the review panel that spat mortality did not occur or was much reduced in spat set on a flat surface relative to spat set on crushed scallop shell or using epinephrine. Given this, settlement on a flat surface would appear to the panel to be a worthwhile line of inquiry to produce the numbers of spat required, particularly if combined with improved hygiene procedures.

The panel thought that the early spat mortality is most likely the result of culturing the organisms in a growing system that:

- depresses growth rates,
- elevates the retention of organic matter,
- compromises water quality,
- and promotes the proliferation of bacteria,

during the critical early post metamorphic stage. Suggested alternatives are to invest in more advanced flow through technologies and handling systems or to revisit the use of flat surfaces for settlement. The spat culturing system should be the subject of a workshop procedural review as previously described for larval rearing.

It was also thought that the PSFRC field nursery (Appendix III) is effectively replaced by the in-hatchery fluidised bed system. Modern field nurseries for commercial hatcheries are larger versions of the PSFRC nursery. While no two systems are alike, the on-shore systems tend to grow spat to a size where they are retained on a 5-8000 micron screen and the in-sea raft systems take them up to 15000 screen. There is a tendency towards mechanisation and the use of heavy lifting gear as the individual upwellers in a raft system may be over 1.2 metres in diameter and each contain hundreds of kilograms of spat.

5.3.4. Conclusions

The panel concluded that:

- **Unstable microbiological communities in the incubation and larval rearing tanks may be a significant factor contributing to the later onset of larval disease syndrome.**

- **The existing larval rearing and settlement systems are out of date from a commercial perspective and should not be used in their present state as a model for developing commercial systems.**

- **The larval mortality syndrome was considered to be a separate issue to that of system modernization.**

- **The existing larval rearing system should be capable of producing the few tens of millions of spat required to support the SRO genetics project providing that appropriate steps are taken to critically review the hatchery procedures in a workshop environment and there is action on the findings.**

- **The research scale larval production system was considered suitable for achieving experimental aims.**

- **The spat culturing system and procedures in use at PSFRC provides sub-optimal growing conditions for SRO spat.**

- **The existing spat system and procedures should be the subject of a workshop to review these.**

- **Subject to the findings of a procedural review, the spat system may need replacing, either with open flow fluidised bed / spat freefall culture units or a system utilising settlement to a flat surface.**

5.4. Larval and juvenile oyster disease

Prior to, and since the construction of the PSFRC hatchery in 1988, mortality of larval oysters on days 3-5 has been a recurrent problem**.** Spat mortality appears to be able to be overcome by the use of slats for setting.

5.4.1. Disease agent investigations

Investigations by specialist pathologists have failed to unambiguously identify the aetiological agent of disease. However, the evidence suggests that bacillary necrosis caused by *Vibrio* spp. is the most likely candidate, while it must be recognised that this may not have been the primary cause of mortality for all events (e.g. Mike Hine demonstrated the presence of herpesvirus from moribund larvae, contamination with *Uronema* sp. ciliates was confirmed in one mortality event). Bacillary necrosis has been recorded from mollusc hatcheries around the world for the last 2 decades with vibriosis being identified as the primary disease agents in some reports while being implied as the disease agent in others. Most promote the use of UV sterilisation of water and/or the application of antibiotics as prophylactic measures.

While unambiguous diagnosis of the cause of primary pathogenicity may be complicated by synergistic effects of a number of agents, it is classically approached in a systematic manner. It is recommended that the disease syndrome should be reproduced by challenge with the range of potential pathogens against otherwise healthy individuals in the absence of other potential pathogens (i.e. Koch's postulates tested). However, this classical approach should be tempered by cost/benefit analysis – do we need to identify the pathogen? NSW Fisheries staff have attempted this approach - exclusion of the pathogenic agent through sterilisation of hatchery water followed by inoculation with probiotic bacteria. Results of preliminary experimental work using probiotics and antibiotics as 2 treatments indicate enhanced survival for 3 water treatment regimes. This line of inquiry should be pursued by employing broodstock conditioning techniques to allow multiple small-scale trials throughout the year.

5.4.2. Susceptibility factors

Mortality in the hatchery, whether caused by vibriosis or not, is likely to be linked to parameters within the rearing protocol. Overwhelming evidence from veterinary and medical studies confirm the link between nutrition and health status.

A review of the research into optimal rearing conditions for rock oysters undertaken by NSW Fisheries reveals that most was done in the first 4 years of hatchery operation, between 1988 and 1992. Nell and Holliday (1988) investigating optimal salinity for growth and survival, Nell and O'Connor (1991) evaluating 10 algal species as food for larval rock oysters, and a year later, O'Connor, Nell and Diemar (1992) evaluating 12 algal species as food for juvenile rock oysters. In 1991, Nell *et al*. evaluated improved protocols for hatchery hygiene but found no significant difference in mortality.

Further attempts at optimisation and disease investigations have been made more recently by Heasman *et al*. (2000) in their Aquaculture CRC project, while some findings are promising (e.g. probiotic studies), definitive outcomes were precluded by absence of disease in experiments designed to test particular treatments.

It is surprising to note that given the underpinning role of transfer of hatchery technology to the commercial sector, that relatively few test runs (even at research levels) have been undertaken with rock oysters (Appendices IV and V). Conditioning of broodstock for experimental rearing studies should be undertaken year round with water source, water treatment, nutritional parameters, source of broodstock (i.e. genotype) all considered.

5.4.3. Conclusions

The panel concluded that while diseases have played a role in the problems experience by the hatchery, that:

- **diseases were not necessarily the primary problem.**
- **attempts to document diseases and establish their roles were limited and were not the subject of a concerted effort.**

5.5. Project management and related issues

5.5.1. Project activities

It is apparent that a combination of, competing research agendas for available resources, and unclear lines of responsibility between research staff, technical staff and management of the Port Stephens facility have had a negative impact on the optimization of hatchery protocols for rock oyster production. This has led to a lack of focus on overcoming hatchery mortality, while research staff (with pressure to continue scientific publication and maintain funding opportunities) have produced promising results from their genetics programs they are unable to complete extension of this technology.

At the same time, technical staff in the hatchery have been required to service a range of research needs (e.g. fish culture, flat oyster production, Pacific oyster production, pearl oyster production) in addition to the production of sufficient rock oyster juveniles to service the genetics program, and have neither been resourced sufficiently, nor directed clearly, to address the mortality problems of rock oysters.

Appendix V summarizes the investment of time in Sydney rock oyster work for 1999, and the panel was informed by the research group that this was reasonably representative of the annual effort in the last five or six years. It can be seen that around 60-80% of the time of the technical staff involved in the hatchery is taken up by projects other than Sydney rock oysters. Of the time allocated to Sydney rock oysters, hatchery and disease issues were addressed using only 5-10% of technicians time. The rest of their time was spent getting through adequate numbers of spat for the genetic lines.

While focus should doubtless be placed on hatchery production of rock oysters, some NSW Fisheries research staff argued that genetic lines should be maintained simultaneously to avoid loss of continuity. However, it appears that this strategy has already had a negative impact on the hatchery program under the current resource and project management regime, and any benefits developed are meaningless unless they can be delivered to industry.

The difficulty of having to tackle the same recurrent hatchery problems with limited time and resources has led to low morale in the research group, and some difficulty in determining novel

approaches to problems in isolation. In discussion with the group it was apparent that although many issues had been considered they had not been dealt with in sufficient depth to resolve the problems. Members of the research group expressed the desire for discussion of a number of topics with other scientists and other experts in order to work through new ideas. The group reacted positively to suggestions of the involvement of additional expertise, of brainstorming sessions, and the opportunity to take a fresh look at problems they confront.

5.5.2. Industry interactions

Industry responses were variable concerning the level and quality of interaction with the research group. Some industry members that have been involved closely with the research consider the PSFRC group to have put in considerable work on some of the issues. Others complained about a lack of follow-up and assistance with oysters they had been provided to grow as part of experimental trials.

The commercial hatchery growers that had made an attempt to produce commercial quantities of oysters for the industry considered they had received considerable attention and help from Fisheries in setting up their operation, and in having technical questions and operational difficulties addressed over time.

Overall, though, there was disappointment that the promise of delivery of commercial scale quantities of improved spat could not be achieved, and that the extent of the hatchery problems had not been highlighted to industry earlier. There was little understanding by industry of the genetics work although some members were keen to get improved (mainly triploid) oysters, while others rejected genetic improvement given the disappointment they had experienced with lack of supply. It is clear that communication with industry could be improved, and the panel is aware that this is a two-way street. However, communication difficulties between the industry and Fisheries researchers has already been identified in previous reports (ACIL Economics, 1997).

5.5.3. Conclusions

The panel concluded that:

- **There has been a lack of focus on overcoming hatchery mortality as a result of conflicting requirements for hatchery and research staff.**

- **This has been exacerbated by a lack of clear lines of responsibility, and a developing low morale in the face of continuing lack of success in trial runs.**

- **Communication between industry and the research group has been flawed.**

5.6. Recommendations

While the PSFRC has successfully reared several bivalve species over the years it remains unable to reliably produce SRO at will. In addition, the panel considered that the various algae, larvae and spat production systems were technically out of date but that this alone was not the primary cause of the rearing problems. Consequently, it considered that the production problems would not be solved simply by modernising the hatchery systems.

The panel does believe the larval and early spat mortality issues can be solved. It does not claim to know the specific remedy, however, it does suggest research directions that will advance the solution.

It was considered that the underlying problem lay in the day to day procedures whereby a debilitating step or steps weaken larvae or provides opportunities for invasive bacteria to multiply. In order to identify the offending steps it will be necessary to systematically review each step of the hatchery Operations Manual. This should be done in a non threatening workshop environment that includes outside specialists. The output of the workshop should provide the appropriate direction for experimental trials that assess the impact of routine procedures on rearing success. These findings should be further assessed at a production scale and then incorporated in an update the Operations Manual.

Specifically, the panel recommends that:

1. **A workshop be convened to analyse each step of the procedures in the hatchery Operations Manual. Initial focus should be on procedures during the first seven days following incubation and the first seven days following metamorphosis. The output of the workshop should be:**

    - **a statement of the goal for each step,**
    - **an evaluation of the success of the step in achieving that goal,**
    - **a list of unplanned secondary effects of the step,**
    - **an evaluation of secondary effects on the health of the oysters,**
    - **a list of alternative, less intrusive procedures to achieve the same goal,**
    - **the design of trials to experimentally assess the reliability of the step to achieve its goal (using the PSFRC research scale larval rearing unit).**

2. **Research be supported that increases the understanding of the dynamics of microflora communities associated with the water treatment, incubation, larval rearing and early spat rearing procedures. This would include research into;**

    - **Probiotics,**
    - **identifying taxa of beneficial microflora,**
    - **identifying taxa of deleterious microflora,**
    - **identifying practices that inhibit the growth of deleterious microflora,**
    - **identifying practices that promote the growth of beneficial bacteria,**
    - **identifying practices that stabilise microflora communities in the rearing system.**

The output of such research would be an increased range of alternative procedures to be evaluated for inclusion in the Operations Manual

3. **The outputs from recommendations one and two relating to incubation and larval rearing be evaluated in a series of trials using the research scale larval rearing unit and later using the existing commercial scale tanks. This will involve continuous access to sufficient resources including the provision of broodstock and support staff and facilities for a period of up to six months following completion of the workshop.**

4. **The Operations Manual be updated to include the findings above.**

5. **A plan be developed to:**

    - **modernise hatchery equipment,**
    - **enable regular exchange of staff and networking with other progressive hatcheries interstate and overseas,**
    - **regularly update the Operations Manual.**

6. **Clear lines of project management, responsibility and reporting be established to focus on overcoming the problems in hatchery production of rock oysters.**

6. Cost benefit review of the Sydney rock oyster hatchery program

The panel was not asked to conduct a detailed cost-benefit analysis, given the time limitations of the review, and the difficulty of gathering the detailed information required for such an approach. Given the failure of the Sydney rock oyster hatchery program to deliver improved stock in commercial scale to the industry, the present program is clearly not cost effective.

However, it was considered important that some assessment be made as to whether a hatchery program or a genetic improvement program is likely to be of benefit to the industry, in order to provide a context for more specific advice the panel may have in relation to the current R&D projects in hatchery technology and genetic improvement.

It was also necessary to determine the likely scenarios for the future of the industry, particularly in light of the considerable changes that have occurred in the industry over the last few years, and are being demanded of the industry in the immediate future. Aspects of industry structure, such as scale of development, the introduction of new technologies, the nature of farming, the possibility of farming alternative species such as the Pacific oyster are all likely to influence the need for a hatchery, the goals for a genetic improvement program and the likely benefit from such a program.

6.1. The industry future

The last decade has seen a decline in the number of leases in the industry, a decline in production from peaks in the 1970's, a decline in water quality in some estuaries as a result of exposure of acid sulphate soils and because of sewage input to estuaries, a decline in access to sites for aquaculture, pessimism with respect to further investment in the industry and an ageing population of industry members (White 2001). Given these strong negative trends the first question was to establish whether there was a reasonable future for the industry.

While it was considered that there were significant hurdles to overcome, discussions with farmers demonstrated that many members, and both industry associations, and NSW Fisheries saw a future for the Sydney rock oyster industry, and believed they could deal with the challenges in front of them (White 2001). The planning being undertaken by government for integrated coastal zone planning that will include zoning areas for oyster growing activity is a positive step (Anon., 2000). Its implementation will aid planning for estuarine aquaculture in the same way that its sister documents has for land-based aquaculture (Stone *et al.* 2000). Making some simplifying assumptions about the area available to Sydney rock oyster growers, and that plans to improve water quality can be met, a conservative projection considered that some 100,000 bags of oysters per year could reasonably be produced in 5-10 years time, and this would represent a value of around $40 million per year. The panel accepted these positive views, and used this estimate of industry size/value in making estimates of the potential value of genetic improvement programs.

6.2. Industry views on hatchery produced spat, and on genetically improved spat

Industry views on the advantages of hatchery produced spat, and whether hatchery produced spat were likely to be taken up by industry were mixed. This depends on a variety of factors including the degree of liquidity available to a given farm, and whether farmers used sticks or single spat methods to stock their farms. The former use wild spatfall at very small unit cost (<<0.01 cent per spat), and could see no value in accessing hatchery spat (circa 2 cents per unit). It is worth pointing out that some rejected hatchery spat simply because hatcheries, by default, provide single spat and stick farms would need to change their production approach to accommodate such spat.

All industry members expressed considerable disappointment that the trials attempted in the late 1990's failed. Many had been keen to take up improved stock, and had been surprised when it transpired that the hatchery technology available was inadequate to achieve this goal. Many were deeply frustrated by the lack of communication they perceived had occurred in this process. Their views as to whether they would take hatchery spat were affected strongly and negatively by this experience. When asked whether they would buy hatchery spat (assuming the technical difficulties were overcome) the resounding response was that unless they offered a demonstrated advantage to wild caught spat (= genetic improvement) they would not purchase hatchery spat, since the price difference relative to wild spat was too high. If there were some added value from genetically improved spat, then they would be keen to acquire them. The industry hatchery supplier and Fisheries noted that there was demand for triploid and improved spat of several million per year, however this demand could not be met as a result of the inability to reliably supply commercial quantities of spat.

Industry views were divided on the value of genetic improvement programs. The return on investment would take time to achieve, and those that might expect to leave the industry soon considered that they would not receive the benefit of such a program. Those whose work was based primarily on sticks could not see how this would benefit them, as hatchery produced spat are dealt with by another growout method they could not use. On the other hand, there was enthusiastic support from others who viewed genetic improvement technologies to be the way of the future and, without which, the industry could not succeed in the long term. However, the level of understanding of genetic improvement methods, their strengths and weaknesses, costs and benefits, and value relative to other technology or management regimes was poor.

It is clear that, if there is to be an investment in hatchery technology and in a genetic improvement program, that industry makes that decision, and needs to be empowered to do so. This will require greater transparency and communication between the research group and industry, and greater understanding by industry of genetic technologies. Some industry members felt strongly that commercial spat supply should be provided by a private sector operator to ensure a commercial focus and appropriate industry support.

6.3. Economic value of genetic improvement for growth

The panel utilized the simple economic model by Johnston (2001) recently made available to the industry to make a crude assessment of the benefit of genetic improvement to a Sydney rock oyster farm. The final target of selection was to achieve a harvest of the same size of oyster one year ahead of unimproved animals (i.e. at 2 years instead of 3 years).

An example of a farm with a total lease area of 28 hectares, with a nursery lease of 5 ha, first stage growout of 8 ha, final growout of 15 ha, and annual gross production of 143,624 dozens giving an annual gross revenue $570,666 for a production cost of $520,052, was used. The unimproved stock in this system were assumed to take three years to grow and gave an annual return of $50, 614, an internal rate of return of 11.03% and benefit to cost ratio of 1.10.

When improved stock assumed to take only two years to grow to harvest, the annual return was $384, 852, the internal rate of return of 34.17% and benefit to cost ratio was 1.65, for an annual production cost of $593,802 giving a gross return of $978,654. The production cost per dozen was $2.41 as opposed to $3.62 in the unimproved stock.

The additional returns are immense. However, the model is crude. Basically it assumes the same annual labour costs in the two situations, and that harvest one year ahead saves a full year of labour costs. This is unlikely to be the case as a faster growing crop will require more sorting within the two years before harvest (although not necessarily as much as over three years). Taking this factor into account will reduce the benefit. However, the model does not take account of other positives such as the fact that mortalities as a result of disease are more damaging financially during the final year of growout since costs (infrastructure and labour) are temporally additive, with most mortalities occurring in the last year at a time when improved stock will have been harvested. There are also improved economic benefits for a small business in gaining more rapid turnover of cash that would follow from the ability to harvest one year earlier. If one halves the estimated benefit calculated above ($384,852 - $50,614 = $334,238), the gain is still substantial for that farm ($141,812), at almost three times the profit from the unimproved stock. Even assuming that a premium would need to be paid to the hatchery for improved stock the farm would be better off.

The cost of maintaining a genetic improvement program depends on a variety of parameters including the scale of the program, the nature of the characters selected. Assuming a modest approach but one with sufficient investment to achieve the desired outcomes in a timely fashion, the panel assumed a cost of around $250,000 per year. If it is assumed that the industry-wide benefit of genetic improvement is 10% of the industry value ($40 million as noted above), the benefit is $4 million – some 16 times the cost required to achieve it. Even if the cost of genetic improvement were $500,000 per year the return at $4 million is still eight times the investment and, therefore, of a value likely to interest investors.

These figures are rough, and the panel notes that Catt (in Nell and Maguire 1998) was particularly cautious about the extent to which projected savings in labour might achieve in reality when he considered the benefits of faster growing triploids. It will be necessary for a far more detailed economic assessment to be carried out before embarking on a genetic improvement program, and that this be integrated with other approaches to industry management on a variety of fronts.

6.4. Scenario example - QX spread

It is pertinent to examine some potential scenarios that illustrate this point that genetic improvement programs need to be integrated with other aspects of industry strategy. The desire of the industry to have some strategy in place to deal with QX disease, and that disease resistant lines might provide security against an outbreak, is used as an example. The panel recommends that proposed workshops include scenario building exercises as exemplified below to provide a perspective for Industry response to disease issues.

Background: Currently, leases in QX endemic estuaries (e.g. Georges River) are being surrendered and it is unlikely that they will ever again support commercial oyster culture due to competition from other resource users (e.g. recreational boating/real estate). In northern NSW and southern Queensland, growers in estuaries in which QX disease has been recorded could benefit from a consistent supply of disease resistant oyster seed. Currently these estuaries produce a relatively small proportion of the total production of rock oysters but have opportunities for expansion if a QX disease resistant oyster was available.

Scenario 1 – new outbreak with resistant oysters available: In the event of a new QX disease outbreak in a major production area (e.g. Hawkesbury River, Wallis Lake) in which all oyster age classes are affected and stock previously sourced from wild spat capture: Immediate availability of

commercial numbers of hatchery-reared disease resistant oyster seed would allow the Industry in that estuary to restock with juvenile oysters.
Requirement: Ability of effected industry members to absorb a minimum of 3 years without income during growth of new stock to market size. Adjusted cultivation methods to address differences in growout for hatchery-reared spat versus wild-caught spat (e.g. nursery areas).
Constraint: Commercial hatcheries are driven by demand and unlikely to maintain disease resistant stocks against a potential need. As such NSW Fisheries would be required to maintain genetically resistant lines against future need, then transfer technology immediately to commercial sector for large scale production.

Scenario 2 – new outbreak without resistant oysters available: In the event of a new QX disease outbreak in a major production area (e.g. Hawkesbury River, Wallis Lake) in which all oyster age classes are affected: Industry abandons cultivation of rock oysters and replaces with alternate species e.g. Pacific Oysters, Flat Oysters.
Requirement: Alternate species refractory to QX disease. Source of alternate species spat. Uptake of appropriate growout infrastructure. Absorb unspecified (?2-3 years) time without income while alternate species grows to marketable size.
Constraint: Legislative barriers for cultivation of Pacific Oysters.

Scenario 3 – precautionary use of hatchery reared disease resistant oysters: Stocking of estuaries with hatchery-reared disease resistant oysters regardless of disease outbreak. Allows commercial operations without QX disease risk.
Requirement: Acceptance of cost of hatchery-reared spat (industry estimates of 2c/spat from hatchery versus .01c/spat for wild caught) and comparison with 'normal' cost of stocking. Estuary specific considerations must be addressed e.g. does this estuary have sufficient and consistent natural spatfall? Individual risk assessment undertaken for intended commercial location to determine need (cost/benefit) for disease resistant stock (e.g. level of importation of stock for growout, physical/biological parameters may be correlated with susceptibility to disease).

Each of these (and other) scenarios must be analysed pragmatically and accepted or rejected as likely outcomes. Accepted scenarios can then be applied to prioritise decisions on the timing and need for such programs as genetic improvement.

6.5. Recommendations

While the industry has declined in recent years and still faces a number of problems, it does have a future in which growth can be achieved, and general developments in coastal and environmental planning should allow for farming in natural waters. A crude individual farm-scale cost/benefit analysis also supported the value of genetic improvement of oysters for increased oyster growth. A crude estimate of possible benefit demonstrated the likely costs of a genetic improvement program to be much less than the benefits. The industry has developed a strategic plan and an R&D strategy. However, these plans have yet to integrate several aspects of priority work, and it is clear that genetic improvement programs must be planned with an integrated approach to industry and technology development.

The panel therefore recommends that:

1. **Scenario building exercises be included in the Industry/NSW Fisheries/independent researchers workshops recommended in section 4.4 of this report to help develop such an integrated approach and an understanding of the outcomes required from a genetic improvement program. These scenarios should include consideration of the response to disease threats, production efficiency and commercialisation paths for improved stock.**
2. **More accurate economic models of the benefits of genetic improvements be undertaken once the general approaches have been decided.**

7. Summary of findings

1. To critically review objectives methodology and results to date of the Sydney rock oyster breeding program.

The general objectives for each of the elements in the genetic improvement program (growth, disease resistance and triploids) are appropriately targeted at issues of key economic and strategic importance for the Sydney rock oyster industry.

The methods used, and results achieved to date, are probably not optimal. Although the results have been promising, it is hard to judge the actual genetic gain achieved and to predict likely future achievements. This is because the characters of real commercial interest are not selected directly, the validity of the proxy characters being used has not been established, the controls used are themselves variable and the degree of selection (e.g. for disease) is unknown and uncontrolled. In addition, the high mortalities in the hatchery may impose a strong selection not related to the main characters of interest, and may impose high and uncontrolled levels of inbreeding.

Most importantly though, the difficulties in hatchery rearing are such that they pose an absolute impediment to the practical commercialisation of any gains either from improvements through selective breeding or ploidy manipulation.

2. To critically review practices and procedures for Sydney rock oyster hatchery technology at the Port Stephens Fisheries Centre and problems associated with larval and post-settlement mortality.

The present hatchery technologies that have been developed are inadequate to provide spat on commercial scales, and are not appropriate models upon which to base commercial operations. The panel considered that the various algae, larvae and spat production systems were technically out of date but that this alone was not the primary cause of the rearing problems.

Despite the time period over which research has been conducted, attempts to solve the problems of larval rearing and spat mortality have been fitted in between other priorities, and have not been approached either systematically or as exhaustively as would be required to overcome the problems.

Much of the activity on Sydney rock hatchery work has been placed on producing sufficient spat for the genetic improvement programs almost to the exclusion of work on the hatchery problems themselves. The problems are seen to be resolvable with effort and the encouragement of lateral thinking on the problem.

In order to identify the offending steps it will be necessary to systematically review each step of the hatchery Operations Manual. This should be done in a workshop environment that includes outside specialists. The output of the workshop should provide the appropriate direction for experimental trials that assess the impact of routine procedures on rearing success.

Clear lines of project management, responsibility and reporting need to be established to focus on hatchery production of rock oysters, and the incorporation of additional expertise to revitalise work in this area.

3. Prepare a cost benefit review of the Sydney rock oyster hatchery program

The current program is not cost effective in that commercial quantities of spat cannot be produced.

However, crude economic analysis suggests genetic improvements can have a significant impact of farm profitability, and an overall industry benefit that outweighs any extra cost required to produce improved stock.

The utility of research on genetic improvement or hatchery technology will depend not only on the extent to which these meet critical industry needs, but how these needs are prioritized relative to other industry requirements. It is critical that industry understands these technologies and makes the decision itself as to whether to proceed with them and to interact effectively with research agencies.

4. Provide recommendations for either continuation or discontinuation of the Sydney rock oyster hatchery R&D and breeding program.

These are provided in the next section.

8. Recommendations for the hatchery R&D and breeding program

The panel concluded that NSW Fisheries has made significant contributions to research on rock oysters in a climate where research agendas were competitive, individual performance indicators structured to preclude outcomes, institutional management dynamic, and relationships with industry variable.

It also concluded, though, that there were serious shortcomings in the approach to genetic improvement, major impediments in the hatchery technology that prevent transfer of research benefits to industry, and a loss of impetus and clear focus in the research leading to low staff morale, and difficulties for the research group in identifying new solutions.

The panel did not consider that these problems were insurmountable, but that their solution will require greater communication and involvement with industry to determine research strategies, and the involvement of a greater range of expertise than currently available at Port Stephens Fisheries Research Centre in the research team. A re-examination of research priorities and management of the hatchery and genetics research at PSFRC will be required in order to provide the financial, logistic and intellectual resources necessary to re-invigorate the research and the focus required to achieve successful outcomes for the industry.

The panel also considered the industry had a solid future and could benefit from a genetic improvement program.

The panel therefore recommends that research in hatchery technology and genetic improvement of Sydney rock oysters is continued subject to the implementation of the three sets of recommendations outlined below:

INDUSTRY EDUCATION AND OWNERSHIP

- **A workshop (or series of workshops) on the strengths and weaknesses of genetic improvement programs, and the costs of these, be undertaken in the next twelve months.**

- **The workshops include presentations from industry groups where programs are in place (e.g. abalone, pacific oysters) and briefings from researchers from a number of institutions to provide a wider range of scenarios than those to which the industry has been exposed to date.**

EVALUATION OF GENETIC IMPROVEMENT METHODS

- **Work on genetic improvement be halted until effective hatchery procedures are developed that will allow cost effective production of single pair matings, and commercial scale production of spat.**

- **While the hatchery research is being undertaken, a full evaluation of a range genetic improvement plans is made to assess the benefits and their speed of delivery to industry (see recommendation under industry education and ownership).**

FOCUS ON HATCHERY TECHNOLOGY

- **Research focuses on the hatchery rearing problems, and that a concentrated program of work on those issues be developed.**

- **A workshop series involving scientists/experts external to Fisheries should be implemented as a matter of urgency to generate fresh approaches, to define and prioritise objectives and to determine operational environments prior to the start to any further hatchery research.**

- **That any new project on hatchery work be led by an expert steering committee with the project PI being either external to Fisheries, or a senior Fisheries Officer, assuming an administrative role.**

- **A time limit of one year for realizing initial outcomes with a maximum of three years for more strategic outcomes should be set.**

9. Acknowledgements

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11. APPENDICES

Appendix I

Oyster breeding program summary

John Nell

November 01

Base populations

Locally oyster collected from Wallis Lake, Port Stephens, Hawkesbury River and Georges River. Base population spawning February 1990 – 8 separate mass spawnings and fertilisations with a total of 222 females and 51 males spawned. All larvae reared in one tank. Spat split into 4 breeding lines for Port Stephens and another 4 for Georges River. Lines were bred approximately in alternate years as follows:

The Mass Selection Breeding Program

Breeding Season	**Program Outline**		
	Georges River Lines (4 lines, 3 sites)		Port Stephens Lines (4 lines, 3 sites)
1989-1990	G1	*1st Generation*	P1
1991-1992	G2	*2nd Generation*	
1992-1993		*2nd Generation*	P2
1993-1994	G3	*3rd Generation*	
1994-1995	Line reorganized due to QX outbreak	*3rd Generation*	P3
1995-1996	G4 2 QX lines 2 winter mortality lines 2 combined disease lines	*4th Generation*	
1996-1997		*4th Generation*	P4
1998-1999	G5	*5th Generation*	

Georges River breeding lines

- First generation spawning – January 1992.
- 216 heaviest survivors selected on a within tray basis from 9 trays for each line.
- Four separate mass spawnings/fertilisations per lines.
- Two lines with at lease 1000 oysters stocked loose on trays (Loose lines) and another two lines with exactly 280 oysters glued on slats within trays (Slat lines).
- Three trays/line on each of three sites namely:
  - Woolooware Bay – mild winter mortality site, east of the bridges.
  - Oyster Bay – low winter mortality site, west of the bridges.
  - Lime Kiln Bar – no winter mortality site, west of the bridges.

	Oysters spawned	
Line	Females	Males
Loose 1	106	12
Loose 2	104	13
Slat 1	13	7
Slat 2	13	9

Second generation spawning – January 1994

	Oysters spawned	
Line	Females	Males
Loose 1	69	23
Loose 2	96	37
Slat 1	33	17
Slat 2	54	35

QX first detected in Georges River in 1994 with a 98% kill of oyster at Oyster Bay and Lime Kiln Bar and no kill at Woolooware Bay.

Breeding lines reorganised in 1997. Survivors from Oyster Bay and Lime Kiln Bar were pooled as 'QX survivors' and those from Woolooware Bay were pooled as 'winter mortality survivors'. 216 heaviest survivors were selected for each of the following spawnings/mass fertilisations QX x QX, WM x WM and WM x XQ (WQ).

Spawning Georges River – February 1997

	Oysters spawned	
Line	Females	Males
QX	41	31
WM	64	30

In case of the QX and WM crosses the matings shown below were carried out:

Females		Males
34 QX	x	21 WM
44 WM	x	32 QX

The spat produced for each group were split into two to produce the following disease resistant breeding lines:

- QX 1 at Lime Kiln Bar – QX disease site
- QX 2 at Lime Kiln Bar – QX disease site
- WQ 1 at Woolooware Bay – combined winter mortality and QX disease site
- WQ 2 at Woolooware Bay – combined winter mortality and QX disease site
- WM 1 at Quibray Bay – winter mortality site
- WM 2 at Quibray Bay – winter mortality site

All oysters were placed loose on trays and the 'slat' system was discarded.

Evaluation of progeny of first generation 'disease' resistance breeding lines, spat produced in February 1997, is shown in Table 1. After only one generation of selection for disease resistance, mortality of QX breeding line oysters at Lime Kiln Bar was reduced by only 3%.

Spawning first generation 'disease' resistance lines – January 1999

	Oysters spawned	
Line	Females	Males
QX 1	37	55
QX 2	52	56
W 1	55	47
W 2	91	48
WQ 1	32	50
WQ 2	59	51

The larvae rearing was a disaster because of *Vibrio* contamination because of water from fishpond being used to fill larvae rearing tanks. Some spat were produced for all lines and these were kept as broodstock, but as numbers were rather low for some lines, another larvae/spat rearing was done over the following summer.

Repeat spawning first generation 'disease' resistance lines – January 2000

	Oysters spawned	
Line	Females	Males
QX 1	26	18
QX 2	38	20
W 1	11	6
W 2	14	6
WQ 1	45	11
WQ 2	37	13

The number of WM broodstock was greatly reduces because of mortality.

Evaluation of progeny of second 'disease' resistance breeding lines, spat produced in January 2001, is shown in Table 2. After only two generations of selection for disease resistance, mortality of QX breeding line oysters at Lime Kiln Bar was reduced by 35%. This experiment will be terminated around February/March 2001.

Spawning for second generation 'disease' resistance line will commence on Monday 7 January 2001 at Georges River.

Port Stephens breeding lines

- First generation spawning – February 1993.
- 216 heaviest survivors selected on a within tray basis from 9 trays for each line.
- Four separate mass spawnings/fertilisations per lines.
- Two lines with at lease 1000 oysters stocked loose on trays (Loose lines) and another two lines with exactly 280 oysters glued on slats within trays (Slat lines).
- Three trays/line on each of three sites within Port Stephens.

	Oysters spawned	
Line	Females	Males
Loose 1	64	40
Loose 2	79	25
Slat 1	59	31
Slat 2	72	21

Evaluation of progeny of first generation Port Stephens breeding lines, spat produced in February 1993, is shown in Table 3.

Second generation spawning – December 1994

	Oysters spawned	
Line	Females	Males
Loose 1	67	44
Loose 2	77	36
Slat 1	56	39
Slat 2	86	42

Evaluation of progeny of second generation Port Stephens breeding lines, spat produced in December 1994, is shown in Table 4.

Third generation spawning – March 1998

	Oysters spawned	
Line	Females	Males
1	45	19
2	43	21
3	66	39
4	119	46

The slat 'system' was discarded and the lines renamed as follows: Loose 1 = Line 1, Loose 2 = Line 2, Slat 1 = Line 3 and Slat 2 = Line 4.

Evaluation of diploid and triploid progeny of a third Port Stephens breeding line (Line 2 above), is shown in Table 5.

Fourth generation spawning – January 2001

	Oysters spawned	
Line	Females	Males
1	162	36
2	165	24
3	167	27
4	127	23

An experiment to compare growth and survival of all four breeding lines with a control was established in Port Stephens in July 2001.

Appendix II

Oyster breeding program results

Table 1. Evaluation of progeny of first generation Georges River 'disease' resistance breeding lines July 2000 – September 2001. Data are means ± se.

	Mortality (%)		Whole weight (g)	
Breeding lines	Woolooware Bay	Lime Kiln Bar	Woolooware Bay	Lime Kiln Bar
QX	71±6.8	96±0.8	43±13.6	39±10.4
WQ	76±5.0	98±0.9	42±14.2	38±9.5
WM	80±3.9	99±0.3	38±14.2	32±10.3
Control*	73±8.3	99±0.9	36±12.2	29±8.2

*Wild caught spat from Wooli Wooli River matched for both shell height and whole weight.

Table 2. **Preliminary data only, experiment will be terminated in February/March 2001.** Evaluation of progeny of second generation Georges River 'disease' resistance breeding lines from July 1997 – April 1999. Data are means ± se.

	Mortality (%)			Whole weight (g)		
Lines	LKB	WWB	QBB	LKB	WWB	QBB
QX	56±5.3	51±4.7	60±8.9	29±3.1	30±1.0	20±2.3
WQ	72±2.4	56±8.0	64±6.9	26±2.6	25±2.6	20±1.5
WM	81±15.0	61±2.8	61±19.7	20±3.3	22±2.3	17±2.9
Control*	91±2.2	70±7.4	69±4.2	20±1.1	20±1.5	15±1.5

*Hatchery produced controls.

Table 3. Evaluation of progeny of first generation Port Stephens breeding lines from August 1993 – January 1995. Data are means ± se. **Paper published in Aquaculture 144 (1996) 295-302.**

Lines	Whole weight (g)	Difference (%)
Loose 1	35.3±0.23	2.9
Loose 2	37.2±0.24	8.5
Slat 1	34.3±0.21	0.0
Slat 2	36.0±0.25	5.0
Control 1	34.0±0.22	
Control 2	34.6±0.22	

Table 4. Evaluation of progeny of second generation Port Stephens breeding lines from November 1995 – May 1997. Data are means ± se. **Paper published in Aquaculture 170 (1999) 195-203.**

Lines	Whole weight (g)	Difference (%)	Mortality (%)
Loose 1	43.2±0.40	18.0	11±1.0
Loose 2	44.9±0.39	22.7	10±1.3
Slat 1	41.8±0.32	14.2	8±1.1
Slat 2	42.6±0.35	16.4	11±1.9
Control 1	37.0±0.33		16±2.2
Control 2	36.1±0.34		13±1.0

Table 5. **Preliminary data only, experiment will be terminated when the slowest group of oysters reaches an average weight of 50 g.** Evaluation of diploid and triploid progeny of a third Port Stephens breeding line from March 2000 – October 2001.

Oysters	Dec. 00 Mean wt (g)	Dec 01 Gain (%)	March 01 Mean wt (g)	March 01 Gain (%)	June 01 Mean wt (g)	June 01 Gain (%)	Sept. 01 Mean wt (g)	Sept. 01 Gain (%)
Controls diploids	6.5	-	13.3	-	18	-	24	-
Control triploids	7.0 (+7%)	8	18.1 (+36%)	36	25 (+39%)	31	30	25
Selection line diploids	7.8	20	17.0	28	23	29	29	21
Selection line triploids	8.4 (+8%)	29	22.6 (+33%)	70	31 (+35%)	71	38	58

The weight gain of the selection line triploids over the controls was maintained over winter and it will be interesting to see if this difference gets larger over summer. It is still expected that the selection line diploids and triploids will reach market size (40 – 60 g) 6 and 12 months earlier respectively than the usual 3½ years. This means that as expected the growth advantages of triploidy and selective breeding are additive, because they are achieved through different means.

Appendix III

HATCHERY PROCEDURES-SRO-PSRC

LARVAL

Day:

-2 -hatchery has been disinfected and dried (4weeks) and water storage tanks filled and settled.

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

0 -spawning with cleaned 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 and 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.

1 - larval sample under microscope used 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.

2 – batch water change using wet screen of 45µm. Washed larvae are flushed into 20L bucket and sampled to establish the total numbers, hatch rate (%) and size. The known number of larvae stocked into the new tank that has been fed with 50% of their daily ration. The balance of the feed ration is added late afternoon. Larval counts are done on ¼ of the tank at a time.

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 an am. and pm. feed.

4 – 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. and pm. feed. New larval tank filled (as above) and heated.

5 – batch water change using wet screen of 63µ m and 53µ m backup. Washed larvae flushed into 20Lbucket 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.

6 – 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. and pm. feed.

7 – As for day 6. New larval tank filled (as above) and heated.

8 – batch water change using wet screen of 85µ m and 63µ m 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.

9 - the above pattern is continued throughout the larval cycle until day 16-18. 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.

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. Retained 212µm larvae transferred to set screens. If significant number of larvae retained on 180µm screen they are on-grown in new larval tank following above protocol for further 1-2 days, screened on 212µm mesh and transferred to set system.

SET

-2 - screens, tanks and equipment sterilised.

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

0 - retained 212µm larvae to set screens @ 200-250,000/ screen – thin layer of shell (pass 350µm, ret.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. and pm. Screens removed from system pm. and rinsed with ambient temp. seawater.

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

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

3 – screens rinsed am. and pm. with saltwater, 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 350 µm screen returned to set system.

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

5 – shell set screens washed am with salt water, spat screens am. and pm. freshwater. Total sea water exchange to spat downweller systems. Systems fed as per previous days. All shell systems graded over 350µm screen. Ret. 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.

6 – screens removed from system and spat washed with fresh water am. and pm., fed as per previous days. Water change of 100% or 50% depending on previous sequence.

7 - spat screens removed from system and washed am. with freshwater, fed as per previous days. All spat graded over 500 screen, ret. spat counted and transferred to field nursery upwellers.

FIELD NURSERY

- 500 micron spat transferred to upwellers (350µm screen). Approximately 400,000 spat per screen, flow rate approximately 6L/min (500µm spat) to12L/min.(3mm spat). 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.)
- 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.
- 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.

Appendix IV

CHRONOLOGY OF ACTIVITIES USED TO ADDRESS SRO LARVAL AND SPAT MASS MORTALITY DISEASES 1990 2001

prepared by Mike Heasman (Nov 2001)

DIAGNOSTICS

General Histopathology

Since 1990 samples of diseased larvae and spat have been sent to one or more of the following pathologists: Drs Dick Callinan(NSWF), Judith Handlinger (TasDPIF), Alex Hyatt (AAHL/CSIRO) or John Norton (QDPIF) on the majority of episodes of both the larval and spat disease.

Larval disease

Typical example June 1996

- Dick Callinan report patchy degeneration of the alimentary tract cells with no evidence of causative (suggestive of virus or bacterial exo toxins).
- Judith Handlinger irregular brush border and some sloughing of gut epithelial cells but not obvious agents present also suggestive of virus or bacterial exotoxins?
- John Norton – 3 species Vibrio isolated *V. spendiferous, V. mediterranei* and phenon 10/85.

Spat disease examples

Original report 1991 (Callinan as reported in Nell *et al.*, 1991) diffuse moderate to severe degeneration and of adductor muscles already present in some settlement stage larvae (indicating spat probably predisposed to disease prior to metamorphosis).

April 1994 Dick Callinan. Many diseased spat showed focal to locally extensive necrosis in connective tissue and or partial dissolution of the hinge associated with small rod shaped bacteria.

Nov 1999 *Uronema* infection of dying spat detected by Judith Handlinger and Alex Hyatt but whole disease episode was atypical of mass mortality disease and *Uronema* not subsequently detected in archived samples held by Callinan, Handlinger or Hyatt.

TEM and SEM examination for Viruses

April 1994 Earliest investigation (Hyatt/AAHL) Negative contrast EM and TEM of ultra thin sections of spat no viruses detected but numerous bacteria on external surfaces of shells

November 1999 TEM and SEM no viruses detected but invasive infection *Uronema* ciliate diagnosed.

Feb 1999 and Oct 1999 Mike Hine /Ben Diggles - no virus detected.

March 1999 diseased larvae exhibited "rough cells" and virus like particles. Lysing and degenerating detached cells showed presence of herpes viruses (toroidal DNA capsid hexagonal in cross section, tegument and envelope).

Most recent Jan 2000 AAHL Alex Hyatt examination of anorexic larvae. No viruses detected.

Other Viral Investigations of larval disease

October 1999 Tristan Renault /IFREMER. Samples sent at same times as to Mike Hine but only samples arrived in La Tremblade and these arrived a full week later and having thawed- were compromised wrt testing for herpes virus using mono-clonal antibodies. The negative result to date must therefore be viewed as equivocal.

Specialised bacterial testing

9h Feb1994 Prof. Peter Hanna, Deakin Uni. TCBS plating and in situ mono-clonal antibodies (FITC immuno fluorescence) *Vibrio alginolyticus* at 10^{4-5} discovered in both samples of diseased diploid and triploid larvae and in rotifers being cultured in the algae area and drained via the hatchery floor. No Vibrio detected in 7 species of bulk micro algae (also oxolinic acid treated larvae free of *V alginolyticus*)

18 –23rd Feb1994 Diseased larvae with *V alginolyticus* in tissues but healthy larvae on the outside of shell only. Same story with diseased scallop larvae in Nov 1994

April/May 1996 *V alginolyticus* and two other species of bacteria isolated from diseased 7 to 11 day old larvae

Evaluation of bacterial probiotics

1. Three attempted collaborative projects with O/S researchers unsuccessful due to IP constraints:
   - August 1993 Dr K Nogami. Japanese Sea Farming Assoc. Tomano, Kayama and Dr M Maeda, Nat Res Institute Aquaculture, Nansie, Mie, Japan.
   - September 1993 Dr K Inoue. Yeast related business developments, Miyahara Takasaki, Gunma , Japan.
   - Jan 1994 Philippe Douillet – Oregon State Uni. USA.
2. May –June 1996 Our own work to isolate and passage bacteria and to conduct challenge experiments initiated in collaboration with Dr Lachlan Harris (JCU/Seafarms P/L).
3. Feb 1998 UTS B.Sc. Hons Student Edward McGregor under supervision of Dr Louis Gibson initiated isolation passaging and challenge tests using diseased larvae from failed 20000L tank batch.
4. June 1999 Formal collaborative SPIRT project initiated in PhD thesis project involving Keong Tam (supervisors Dr Louis Gibson).
5. Nov 2000 Collaboration on Vibrio R&D established with Dr Jeremy Carsons (FRDC project to establish National Vibrio Reference Centre in Launceston by Tas DPIWE).

Uronema Ciliates

Nov 1999 Diagnosis and experimental disinfection investigations of *Uronema* infection of spat. Tas Hatchery operators (Camerons P/L) offered to assistance at a fee of $10000) and claimed to have foolproof preventative treatment. Several disinfection protocols evaluated but none were effective.

Appendix V

OTHER INVESTIGATIONS AND PROCEDURAL CHANGES

Nutritional Factors

May 1991 Joint venture with Dr Paul Southgate JCU to determine energy reserves and consumption rates of eggs and early (pre and post feeding) and thence developing larvae and spat. Sampling protocol not implemented as multiple sample size of 30 000 larvae viewed as threat to genetic selection trials and due competition from other externally funded projects (FRDC Scallop project) prevented dedicated hatchery run.

Nov 1996 Proposed Biochemical assays on eggs/larvae reared in the "larvitron fed alternative diets to show critical points in development wrt protein, lipid and carbohydrates(poly and mono saccharides) and lipids reserves. Detailed CRC joint project with Drs Kevin Williams and Frances D'Sousa proposal prepared but not pursued due to series of larval disease incidents and priority decisions to preserve surviving stock in genetic selection trials.

Increased hatchery hygiene

- Late 1989 use only of outer bay and ocean beach seawater 7day pre settlement 1 micron filtration and EDTA.
- Dec 1990 Experiment with spat High hygiene comprised:
    1. 4 hourly rinsing of screens
    2. daily cleaning and disinfection of tanks and total water change

    Results No improvement with high hygiene with or without feeding
- 1990 on Carboy food for first 3 days and optimised 3 species larval micro algae diet introduced.
- 1993 First formal complete dry-outs and disinfections imposed.
- 1994 filtration of larval rearing water to 1 micron nominal introduced.
- Feb 1998 Quarantine wall between larval rearing and nursery areas of hatchery installed and footbaths introduced.
- May 98 rotifers moved out of bivalve hatchery.

Improved handling /general husbandry

- August 1999 Flow through small vessel vs static large vessel batch system rearing for larvae and spat bubblers vs downweller screens was to be evaluated in as part of the CRC funded initiatives etc. John Diemar and Lindsay Goard sent to SAABDEV P/L for specialist training and documentation of systems. Initial implementation trials with SRO's unsuccessful. Follow up trials pending subject to adequate resourcing.
- Feb 1997 Dedicated experiment to evaluate culchless (epinephrine) induced settlement and avoidance of early grading of spat below 2mm completed. Demonstration of possible advantages of culchless settlement and omission of grading in reducing the incidence or severity of the disease was precluded by lack of disease manifestation in any treatment. However some useful findings on the comparative efficiency and costs of using culch and culchless settlement were achieved.
- Nov 1997 Optimisation of sperm storage temperature and time and optimised larval rearing temperature experiments completed.

- 1998 Development of non-traumatising fluidised grader in collaboration with University of Newcastle Encouraging results but additional refinement of equipment and its operation required.
- October 1999 Experimental settlement on slats experiment proved unsuccessful when pediveligers failed to set on slats.

Appendix VI

NSWF CR Annual Expenditure on Bivalve Hatchery and Related Operations especially SRO Genetics and Health R&D

	SRO Oyster Breeding R&D	SRO Disease R&D	Other bivalve hatchery activities
Permanent Staff Costs			
Mike Heasman	$7,090 (10%)	$3,545 (5%)	$46,085 (65%)
John Diemar	$10,146 (20%)	$5,073 (10%)	$38,046 (75%)
Lindsay Goard	$18,786 (40%)	$2,348 (5%)	$25,831 (55%)
Lynne Foulkes	$7,908 (20%)		$31,631 (80%)
John Nell	$76,500 (90%)		$8,500 (10%)
Ben Perkins	$38,000 (100%)		
Temp Staff Costs			
Ian Diemar	$4,880 (16.7%)		$9,760 (33.3%)
Overtime ($10000)	$3,333 (33.3%)		$6,667 (66.7%)
Subtotal	**$129,443**	**$10,966**	**$166,520**
Oncosts = + 94% loading on salary costs)	$121,676	$10,308	$156,529
RandM $40, 000	$10,000 (25%)	$2,000 (5%)	$7,000 (70%)
Hatchery Operating ($31500)	($7,875) (25%)	$3,150 (10%)	$22,050 (70%)
Rent on $2million capital facilities and equipment @10%pa	$50,000 (25%)	$10,000 (5%)	$140,000 (70%)
NSWF Contribution to SPIRT Probiotics R&D		$5,500	
Total	**$318,994**	**$41,924**	**$492,100**

NB External funding for breeding R&D by FRDC and for health R&D from the Aquaculture CRC of $4004-$12000 per annum from 1994 to 1998 not included)

Appendix 9.3

Hatchery Audit Report

NSW Fisheries - Port Stephens Fisheries Centre
Sydney Rock Oyster (*Saccostrea glomerata*)

Report prepared for

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February 2003

TABLE OF CONTENTS

EXECUTIVE SUMMARY

The scope of this document is to carefully inspect all aspects of the Port Stephens Fisheries Centre hatchery used or associated with the production of Sydney Rock Oysters from a commercial operators view point. This includes the building and associated facilities, equipment, methods and incorporated systems such as algal and larval units. It will briefly identify what is currently done and an opinion on what needs to be changed to accommodate specific needs for Sydney Rock Oysters.

It will not describe in detail the procedures for each aspect of production, as this has been done to a degree in a 1991 document, titled, 'The Development of Hatchery Rearing Techniques of the Sydney Rock Oyster' at the Brackish Water Fish Culture Research Station, Salamander Bay, NSW by K.R.Frankish, L.J.Goard and W.A.O'Connor, 1991. A later document, titled, 'Improved Early Survival of Molluscs: Sydney Rock Oyster *(Saccostrea glomerata)* by M.P Heasman, L.Goard, J.Diemar and R.B.Callinan in 2000, describes hatchery problems, investigations and conclusions.

There are a number of other documents that have been written to describe various methods, procedures and techniques that may be utilised within the hatchery, however there is a definite need to compile a comprehensive procedures manual for the production of Sydney Rock Oysters.

It is also not a document that will withstand scientific scrutiny, however it is based on the authors experience as a successful commercial operator using current best practice to produce various species of bivalve shellfish.

ACKNOWLEDGEMENTS
I am most appreciative of time and information provided by NSW Fisheries staff John Diemer, Lynne Foulkes, Lindsey Goard, Dr Michael Heasman and Dr Wayne O'Connor.

1. Overview

There is an occasional problem with the larval rearing of *S. glomerata*, particularly in the first half of the calendar year from January to June, with successful larvae batches achieved less than 40 percent of the time. The second half of the year from July to December often results in improved overall larval survival.

The larval problem or disease is known as anorexia, is characterised by most larvae in a batch not eating, with up to 95 percent dying within an average of 14 days of going off the diet. According to published data, the first non specific clinical signs of the disease, anorexia, occurs most commonly on days 3 to 5 but also occurs as early as day 2 and as late as day 8. The surviving larvae usually go on through to settlement and nursery rearing without suffering catastrophic spat mortality.

A post settlement spat problem or disease is known as mass mortality of juvenile *S. glomerata*, and is characterised by catastrophic mortality in settled spat less than 2mm in shell length from 7 to 43 days after settlement. Although there are no seasonal trends apparent in the relative occurrence of mass mortality, it is interesting to note that spat that have survived larval anorexia usually do not suffer spat mass mortality.

The Port Stephens Fisheries Centre hatchery was constructed between 1986 and 1987 at an estuarine site near Salamander Bay. The majority of equipment and systems appear to be original. There is evidence of routine facility and equipment maintenance, however there does not appear to have been a consistent capital equipment replacement program or regular hatchery system evolution.

There has not been a hatchery on the East Coast of Australia that has successfully produced Sydney Rock Oysters on a regular basis. A hatchery in Western Australia has successfully produced a local oyster thought to be the SRO.

2. Source water

The location of the hatchery does not allow good quality oceanic seawater to be constantly delivered to the facility. This is a fundamental flaw in the original proposal to locate the hatchery at this site, and I suspect this was known by some NSW personal even prior to construction. Hatchery personnel need to contend with this, which at times will be a difficult task. The only way to overcome this primary issue is to relocate the hatchery to a more suitable site.

It was decided some years ago that the seawater pumped from an adjacent estuary was of inconsistent quality, in that salinity varied and it was often quite turbid. Since that time almost all seawater is trucked to the hatchery site and stored for varying periods of time.

Sites

The water is collected from a number of places including Anna Bay, Little Beach and Shoal Bay. All seawater is pumped from shallow sites, which may be a rocky or sandy bottom, and on occasion appears to be of poor quality based on colour and turbidity. Because seawater supply is limited, it is often necessary to use this water, even though it is considered to be inferior on occasion. Trucking water in to the PSFC site is a necessary evil. *(Verbal: Lindsey Goard)*

PSRC has limited access to ocean and outer bay beaches and the seawater can be of variable quality based on seasonal factors, weather and sea conditions, rainfall and runoff, three marinas and large fishing fleet and general run-off into Port Stephens. The salinity of trucked water is 33 to 36.5 parts per thousand. (Written: Mike Heasman)

Methods of collection

The water is pumped from a depth of approximately one metre using a conventional centrifugal pump in to a 7000-litre stainless steel tank mounted on a truck. It is not unusual for several loads to be delivered each week.

Storage

Trucked seawater is delivered using the same pump on the truck to 4 x 40,000 litre and 2 x 45,000 litre fibreglass tanks. It is apparent that seawater is often stored for less than a week, often for only a couple of days during peak demand. The tanks are more than ten years old and appear to be in quite good condition both internally and externally. They are regularly cleaned by hosing, brushing or scrubbing.

Fibreglass seawater storage tanks

Filtration

Seawater is not filtered prior to storage. It is filtered through 5-micron cartridge style filters immediately after entering the hatchery building.

Hatchery seawater filters

Seawater delivery to hatchery

Water is delivered to various parts of the hatchery via four centrifugal pumps. Three of the pumps are more than 15 years old, and although they are still running due to repairing when necessary, they are rusty and in poor overall condition. The pump house is approximately 30 metres from the hatchery seawater inlet point. On the suction side of each pump, the water is drawn from one of the storage tanks through an outlet located approximately 300mm above the base of the tank. Some of the plumbing is shown in the photograph below.

Water storage tank plumbing

When a Sydney Rock Oyster batch is to be run in the hatchery, all pipe work from the storage tanks to the hatchery are filled with chlorine solution, and left for a day or so before being thoroughly flushed out with clean water. The pipes, valves and fittings have never been thoroughly cleaned or pulled apart in 15 years and will be almost certainly dirty.

Three pumps draw water through common manifold

Recommendations:

Priority

- Replace all existing pipes and fittings with new material, and thoroughly clean valves if using again. Replace in such a way that the whole system can easily be dismantled for cleaning. i.e. Above ground with valves that can be taken apart and cleaned.
- Develop strict protocols for maintaining seawater delivery pipes in good and clean condition.
- Collect seawater from one site only. This will remove one variable.

Proposed

- Increase storage tank capacity and monitor for known SRO disease and pathogens.
- Relocate hatchery or outsource hatchery work where applicable.
- Constant water flow in to storage/header tank is ideal if possible.
- Do not use sand filters. (Not used at present)
- Filter all seawater to 20-micron utilising drum, disc or screen before entering the hatchery.

3. Broodstock

Sources

Sourced from mass selection SRO program. (Oysters have been reared in the Port Stephens hatchery before being grown on a lease the in Port Stephens area.)

Wild broodstock may have diseases and pathogens associated with them. Suggest use farmed stock from known clean area.

Handling

All broodstock are cleaned of excess fouling prior to introduction to the holding/conditioning system. Conversely, if broodstock is collected from the farm the day before a spawning is to be attempted, the shells are cleaned before overnight desiccation.

Conditioning

PSRC normally undertake only one or two spawnings per year when oysters are in peak condition. This means that it is not necessary to condition oysters in the hatchery. The broodstock is often collected from the farm the day before use. These oysters are sometimes spawned in conjunction with oysters that have been held in the hatchery for several weeks in ambient seawater and fed an algal diet.

Quarantine Operations

There is no quarantine system in the facility.

Spawning Induction

The procedure for spawning Sydney Rock Oysters is described in the 1991 hatchery rearing techniques document, and is a quite acceptable method of inducing ripe oysters to spawn.

Water Temperature

Ambient seawater temperature on 18 February 2003 was 24ºC.

Recommendations:

Priority

- Install a conditioning system to be able to produce quality gametes all year round.
- Only use SRO broodstock with known pedigree for tracking purposes.
- Install quarantine/depuration system to keep broodstock collected from farms for a period prior to spawning.
- Consider program to investigate SRO egg quality.
- Determine any genetic difference between NSW oysters and WA rock oysters.

Proposed

- Install boiler for instant seawater heating.
- Install chiller for seawater cooling.
- Naturally spawn only. Keep numbers constant. Keep good eggs only. (This is done)

4. Algae

Since the hatchery was completed in 1988, microalgae has been grown using much the same methods and principals right up to 2003. F2 media is utilised for all cultures of all sizes.

The PSRC algal facility produces algae for a wide variety of purposes throughout the year and has been dried out only once in 15 years. *(Verbal: Lynne Foulkes)*

Species

Port Stephens Fisheries Centre cultures a range of microalgae used to feed a variety of species. The primary species utilised for the rearing of Sydney Rock Oyster larvae are:

Pavlova lutheri
Isochrysis species (T.Iso)
Chaetoceros calcitrans

Several other species of microalgae are utilised to feed broodstock, settling larvae and spat. These are:

Skeletonema costatum (CSIRO CS-252)
Chaetoceros muelleri
Tetraselmis chui

Age of Cultures

Stock cultures are maintained in 250ml erlemeyer flasks and are transferred approximately every two to three weeks. The older culture is retained until the algae technician is satisfied the new culture is growing well and is healthy.

Working lines are cultured in two litre Schott bottles. These cultures are used to start new Schott bottle cultures and 10-litre plastic carboys. The average age of the Schott bottle cultures used for transfers are four to six days. The cultures look good visually.

The average age of the carboy cultures utilised for feeding and for innoculums of 500 litre bags and 1000 litre tanks is also four to six days.

All working lines are aerated through 0.45 micron GFC or hydrophobic filters.

According to algae manager, it is the intention of the PSFC to purchase replacement cultures from CSIRO Marine Laboratories in Hobart annually. New cultures have recently been purchased for the first time in two years.

2 litre Schott bottle cultures. *Pavlova lutherii* at 0, 2 and 4 days post innoculum from a single Schott bottle culture.

Feeding rates and protocols

There has been substantial work done on developing suitable feeding rates for Sydney Rock Oysters over the years. A graph of feeding rates based on algae cells per larvae is strictly adhered to during the entire larval cycle. I believe this is based on residual algae counts remaining in the larvae tanks at the end of a one day feeding period. This may well be acceptable, however the method of feeding once or even twice per day should be compared with trickle feeding a larvae tank over a standard feeding period.

There remains work to be done on developing the ideal diet and feeding regime for SRO's:

Culture conditions

The stock and working cultures are maintained in a controlled temperature room set at 21ºC. This temperature is a compromise temperature to accommodate the range of algae species. The bag and tank room is maintained at approximately 23ºC.

Seawater treatment

There are two distinct methods of treatment of seawater.

1. Stock cultures and working lines
   Raw seawater is filtered to a nominal size of one micron through cartridge filters. Schott bottles and carboys are filled, covered and autoclaved. The seawater is allowed to cool for a day or so before having nutrients, phosphates and vitamins added, and inoculated with 20 percent of total volume innoculum in a laminar flow cabinet.

2. Bag and tank culture
   Raw seawater pumped from storage tanks is filtered through two cartridge filters rated nominally at one micron. Filtered seawater is then directed to a 500-litre bag or 1000 litre tank for filling. When full, the water is chlorinated and dechlorinated using a method developed at PSFC.

Algae bag and tank filtration through
two 1-micron cartridge filters

Bacterial monitoring and quality assurance

There does not appear to be any regular bacterial monitoring regime in the hatchery. Some bacterial monitoring does occur when a new experiment or program is being established.

Routine bacterial monitoring was undertaken in the algal section some years ago, however this ceased as Vibrio species of bacteria were found very infrequently.

Alternative production techniques and systems

The main alternative to a batch culture system for algae is a semi-continuous system. The primary difference is when a culture vessel such as a large plastic bag is set up, the culture can be routinely harvested for several weeks rather than be used only once.

The reason that so many commercial aquaculture ventures have moved over to a semi-continuous system is one of cost and efficiency. This is often a trade off with the quality of the algae, as a batch culture system generally yields higher quality algae. This is usually not a major issue, however, it depends entirely on the target species being fed and the species production system.

One of the industry's major exponents of a semi-continuous algae production system is Mr. John Bayes who operates a commercial shellfish company in the United Kingdom. He has developed an entire shellfish hatchery system based on particular algae strains cultured in several different configurations of semi-continuous algae systems designed to feed high density/low volume batches of oysters and clams. This technology was developed in an attempt to improve efficiency in a commercial hatchery and because of limited space within the hatchery.

It is fair to say that the system works for his company as well as many others he has sold this technology to. It is also fair to say that the batch culture of algae and the low density/high volume larvae culture works well in many major commercial ventures.

I suggest that any new shellfish hatchery facility would seriously consider the introduction of certain components of this more intensive system, particularly the semi-continuous algae system and the post settlement spat bottle system. Both are proven performers.

There is a great deal more to this than described so far, however I do not believe the current method of culturing algae is contributing to the intermittent larval problems or the occasional catastrophic spat mortality.

Probiotics e.g. Bayes x flagellate species

Unable to comment on this subject.

Semi-continuous algae bag system filled with pasteurised Seawater at the same rate as algae is harvested.

Recommendations:

Priority

- Develop comprehensive Procedures Manual for algae culture and production. Make a start now. Any changes in the future can be added.
- Introduce routine sampling of algae for Vibrio species of bacteria. This would involve the batches of algae targeted as the food source for SRO larvae.
- Optimise feeding regime and protocols.
- Separate algae production unit seawater supply from rest of hatchery to allow regular dry out.

Proposed

- Upgrade algae system to a continuous flow system.
- Investigate several commercial hatchery algae production methods with the view of improving efficiency.
- Develop methods to reduce manual handling.

5. Larval Culture

Equipment

1. Larval Tanks – Four 20,000 litre fibreglass tanks approximately 15 years old. The interior of all these tanks appear to be in reasonable condition with no apparent signs of delamination, however they are showing signs of age through discolouration and staining of the fibreglass.

One of the four 20,000 litre larvae rearing tanks

2. Larval Tanks – Several 1,000 litre plastic tanks. Some are quite old.

3. Larval Screens – A complete range of larval screens exist. All mostly late 1980's originals.

4. Larval tank Valves – 50mm outlets flanged to larvae tanks. The valves are glued, and are not able to be taken apart for cleaning and inspection. It is likely that internal parts of the valves have a layer of scum on them, which has been known to cause larval problems in other hatcheries. The valve handles are quite difficult to turn. Require replacement with good quality valves.

Drain valve apparatus on 20,000 litre larval rearing tank

5. Larvae 'pull-down' apparatus – Although this apparatus allows for the removal of larvae from a tank through screens, it is slow, cumbersome and inefficient. A similar improved apparatus could be used for the first few days of the larval cycle, however when the larvae can be caught on a 60-micron screen, a rapid pull-down screen should be used.

Existing apparatus used to catch larvae on screens

Rapid pull-down screen

6. Air Blowers - Two air blowers located in the main pump house building supply air supply for the hatchery facility. The filters for the blowers are also located inside the pump house. The air is delivered in to the hatchery in PVC pipe located underground. At certain times of the year, particularly warm and humid days, there is a substantial build up of condensation water in the pipes. A couple of bleed off valves are present on the second floor of the hatchery in the algae facility, however there does not appear to be a bleed off valve at the lowest point of the plumbing.

Fertilisation and handling of eggs

As described in existing documents. This methodology is acceptable.

Incubation (protocols)

The following parameters were observed following successful spawning on day 0 of the larval cycle.

- Larvae tank filled one day prior to use.
- EDTA was added at the rate of 1gm per 1000 litres of seawater.
- 25ºC constant temperature maintained with electric over-the-side-heaters.
- Very slight aeration for first 24 hours of cycle.
- 3 - 5 fertilised eggs per ml in a 20,000-litre tank.

In general, the development percentage of fertilised eggs to D-veliger larvae is quite high, so I must assume that the conditions within the tank are acceptable. However, as SRO larvae take less than 24 hours to develop from eggs, it is good practice to drain the D-larvae from the tank on day 1 rather than day 2. This is a standard practice in commercial hatcheries for virtually all bivalve species.

Water treatment

As far as I can determine, the only water treatment undertaken for larvae rearing is the variable aging of collected seawater from several sites, and filtration through 5 micron cartridge type filters.

The storage and collection of water has been mentioned previously. If these two variables were removed from the equation, and water was filtered to 5-microns only, then this would be

acceptable. In fact, oceanic seawater filtered through a basic filter bag with a nominal rating of 10 – 20 microns should be quite OK.

Heaters, temperature control/logging

All heating of seawater in larvae tanks is achieved with electric heating elements located directly in the larvae tanks. This is often a slow process, particularly in the colder months, and so requires a larvae tank to be filled, up to a day before use.

The rearing temperature for SRO has been determined by staff at PSFC to be suitable, and although I am sure there is some fine tuning to be done in this area, the temperatures for both incubation and larval rearing appear to be in the correct tolerance level. I believe the incubation temperature should be 2ºC less than the larval rearing temperature. Say 25ºC and 27ºC respectively.

It is up to the particular hatchery as to whether the temperature is logged and what degree of control of temperature is necessary. It is fair to say that when large volume larvae tanks are utilised, the temperature will not vary greatly over two days, and will probably not adversely affect larvae growth.

The use of a boiler/heat exchange system to heat seawater is strongly recommended. It allows rapid filling of tanks in the event of the necessity to do so, and removes heating elements from tanks. I believe a boiler to be more efficient and definitely more versatile than element heaters. The same can be said about a chilling unit.

Sanitation

The cleaning of tanks and equipment within the hatchery appears to be satisfactory. The liberal use of chlorine based chemicals to soak filters, tanks and pipes as well as use in footbaths will certainly kill any surface bacterial contaminants.

I have found the best way to keep all these things clean is to manually clean by scrubbing or etching followed by a chlorine rinse or soak for about ten minutes before hosing with fresh water and allowing to dry.

All staff should be wearing gumboots around the hatchery, rather than ordinary shoes. The gumboots are left at the facility.

Examples:

- Larvae tanks – Manually clean with detergent/chlorine agent with a soft broom and rinsing with fresh water.
- Pipes – Etch with a suitable chemical for several hours, before pigging of simply rinsing.
- Screens – Manually scrub with a soft scourer, rinse with chlorine solution, leave for ten minutes and rinse with fresh water.
- Similar procedures can be used for all pieces of equipment. I am not sure if footbaths have any impact, rather than cause staff an inconvenience.

Water quality/chemistry – monitoring DO, pH, NH_3, bacterial

Measuring of these parameters should be done periodically, however dissolved oxygen levels should always be high because of aeration, pH of raw seawater will not vary much and NH_3 levels will be very low in a large tank because of the minimal biomass present.

Use of antibiotics? (flow through)

I am unable to comment on the use of antibiotics in any bivalve shellfish system.

Stocking Densities

The larval density graph I have seen appears to be OK. There is probably more work to be done in this area, however the figures closely resemble good commercial practice.
If in doubt, stock less.

Quality assurance (monitoring and sampling and archiving of larvae and eggs)

Of course this should be done and probably is done, but is only useful if a program is in place to examine larvae and eggs against benchmarks.

The following is the existing hatchery procedures at PSFC:

HATCHERY PROCEDURES FOR SYDNEY ROCK OYSTERS AT PSRC
(in conjunction with hatchery manual, Frankish et al., 1991)

Dr Mike Heasman
NSW Fisheries, Port Stephens Fisheries Centre
Taylors Beach, NSW, 2316, Australia

LARVAL

Day:

-2 Hatchery has been disinfected and dried (4weeks) and water storage tanks filled and settled. *I believe this is the intention, however it is practically difficult with so many other programs running simultaneously.*

-1 Incubation 1 larval tank filled with 1um filtered seawater from settlement tanks and heated to
25ºC.EDTA added @ lg/1000L, low aeration.

0 Spawning with cleaned mature broodstock (wild or conditioned) using thermal stimulation to 28ºC 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 50um screen and pooled into a 20L bucket, fertilised with selected-pooled sperm (5-10 sperm/egg), homogenised and counted (replicated I ml samples). Incubation tanks stocked at 3/ml.

1 Larval sample under microscope used 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. *The D-larvae should be removed from this tank at this stage.*

2 Batch water change using wet screen of 45um. Washed larvae are flushed into 20L bucket and sampled to establish the total numbers, hatch rate (%) and size. The known number of larvae stocked into the new tank that has been fed with 50% of their daily ration. The balance of the feed ration is added late afternoon. Larval counts are done on ¼ of the tank at a time.

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 an am. & pm. feed.

4 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.

5 Batch water change using wet screen of 63um and 53um 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 is 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. *Consider using a rapid pull-down larval screen to remove larvae from rearing tank from this stage to the end of the larval cycle.*

6 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.

7 As for day 6. New larval tank filled (as above) and heated.

8 Batch water change using wet screen of 85um and 63um 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.

9 The above pattern is continued throughout the larval cycle until day 16-19. 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 - 100,118,150 180,212 micron.

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

Discussion with Mr. Lindsay Goard - was the senior larvae technician in the hatchery facility for many years. The following points are opinions and facts based on extensive experience working with SRO as interpreted by Martin John for the purpose of this report.

- The plumbing, including pipes, fittings and valves from the seawater storage tanks to the hatchery is not accessible, and has never been thoroughly cleaned or replaced or I suspect, inspected since installation. Any replacement plumbing should be above ground and be able to be dismantled for cleaning.

- Lindsey was adamant that when a batch of Sydney Rock Oysters are successfully produced in the facilities 20,000 litre larval tanks to settlement, then there is often a catastrophic mass mortality of spat between one and four weeks post settlement. There have only been a couple of batches, including the first ever batch in 1988 that have been reared successfully to larger spat.

- Conversely, if the problem known as Factor X is suspected, it does not affect all larvae. Subsequently when the majority of larvae die, there are often up to 25 percent survivors that appear to be initially suffering from lesser degree of anorexia but recover after a few days and resume eating. They settle well, and almost always do not suffer a catastrophic spat mortality.

Note: The larval mortality problem is known as Factor X. is attributed to cause anorexia in the larvae around Day 4 of the larval cycle. Factor X is suspected when the gut of the majority of larvae lightens, and the larvae do not feed and die within a couple of days.

Recommendations:

Priority

- Drain D-larvae from tank on day 1. i.e. 24 hours incubation period.
- Do not feed until D-larvae are in new tank of water. i.e. Day 1
- Use seawater from one collection site only.
- Commence equipment capital replacement program. This will include the replacement of larval rearing tanks.
- Replace tank outlet valve apparatus with a system that can be dismantled and cleaned.

- Handle larvae as little as possible. This means the use of a rapid pull-down larvae screen after day 5.

Proposed

- Consider constant trickle feeding at a pre-determined cell density over 24-hour period. This will stop larvae gorging on food and the potentially starving when food is cleared.
- Diet to 50% diatoms: 50% flagellates after day 5.
- Maintain constant temperature in larvae tanks using one small immersion heater if necessary.
- Consider a boiler for heating seawater prior to delivery to tank rather than heating water with over-the-side immersion heaters.
- Adopt a strict protocol for larvae production. i.e. Procedures Manual.
- Compare wild larvae with cultured larvae for similar histological symptoms. Do wild larvae suffer from anorexia?

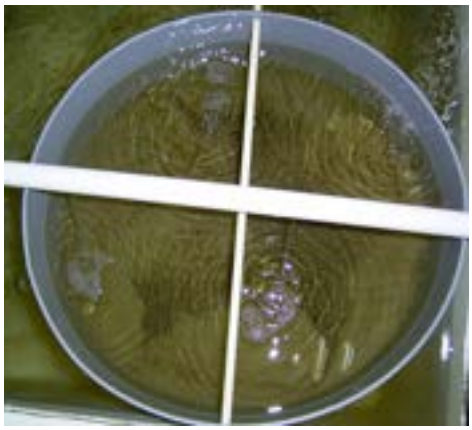
6. Settlement

Equipment

1. Settlement Screens – Round PVC plastic bodies with 200 micron mesh bases attached. (Approximately 350mm diameter). The screens are in good condition, and although quite small for settlement screens, they are adequate. Each screen can hold approximately 250,000 pediveliger larvae.

2. Settlement Tanks – Fibreglass rectangular tanks that are quite old, but are adequate for the job. This setting tank holds ten PVC screens, or around 2.5 million pediveliger larvae.

Setting tank holding 10 settlement screens.



Settlement screen downwelling

3. Settlement substrate – aged scallop shell is crushed and sieved to a size of between 200 micron and 350 micron screen size.

Competence criteria

The normal criteria in a commercial hatchery for the removal of eyed and pediveliger larvae for settlement is as follows:

a) Make sure larvae counts have been done in the previous two days, as it is virtually impossible to accurately count ready to set larvae because they are very 'sticky'.
b) Take a larvae sample from the valve at the base of the tank for inspection. There should be approximately 30% pediveliger larvae present in the sample before introducing to the set system. The larvae should have a good reserve of lipid droplets stored in the umbone region, and be actively crawling.
c) If you are not sure if the larvae are ready to set, leave them in the tank for a few more hours, and inspect again. It is better to lose a small percentage in the tank than introduce them to early to the set system.
d) When harvesting eyed and pediveliger larvae from the tank, it must be done very quickly, so a rapid pull-down screen is required.
e) To enhance a rapid settlement, it is often a good idea to place the larvae to be settled in to a 150-micron mesh bag wrapped in a damp cloth for a few hours in the refrigerator. They will need to be acclimatised before introduction to set.

Culch and culchless settlement

I think a book could be written on this subject, however it is clear that the use of large culch, flat or curved surfaces, shell grit culch and chemical or culchless methods all work for a number of bivalve species, including most edible oysters.

The restaurant market in Australia demands a half shell oyster, which must be of satisfactory appearance to the consumer. The most effective way to achieve this is by producing an oyster that will be farmed in such a way that the shell shape and meat to shell ratio parameters are optimised.

In my opinion, the best way to produce a superior seed oyster is in a hatchery, which is able to control the entire production process by the introduction of improved broodstock, larvae selection, settlement techniques and nursery rearing.

Achieving rapid settlement of competent larvae of pacific oysters is best done in a number of commercial hatcheries by the use of epinephrine. I am not sure if the same technique can be applied to SRO, but I would be surprised if it could not. Rapid settlement further selects the strongest larvae, which usually produces the strongest spat. Competent larvae should settle within two to three days. A lot has been written about this chemical and why it works, so it will not be discussed in this document.

Following is a summary of a standard commercial practice for the use of epinephrine. No shell grit is used with this method.

The use of Epinephrine in a hatchery to enhance settlement of Pacific oysters

Chemical: Epinephrine Bitartrate Salt

Sigma E-4375
$C_9H_{13}NO_3$-$C_4H_6O_6$ FW=333.3

General use of epinephrine: 1.2 grams of epinephrine per 50grams of larvae in 20 litres of seawater in a conical tank for 60 minutes treatment.

Method: Culture Pacific oyster larvae under normal condition until good percentage of pediveligers visible. (Say > 20%)
Note: 212-micron screen size – 1M = 18grams

1. Determine number of oyster larvae to be treated by weight and bag.
2. Weigh out quantity of epinephrine based on 1.2g per 50g larvae and dissolve in small volume of fresh water.
3. Fill conical tank to required volume at about 20 litres per 50g larvae with seawater at settlement temperature.
4. Add larvae and epinephrine solution
5. Drain tank after 60-minute treatment on to suitable screen.
6. Add treated larvae to settlement screen in set system.

It is often desirable to treat the larvae again on a daily basis depending on settlement percentage achieved.

1. Day 2 set. Determine percentage spat. (Say 25%)
2. Drain settlement system and rinse screens with fresh water to remove faeces and uneaten food.
3. Refill system so water is just covering larvae in screens.
4. Weigh out quantity of epinephrine based on 75% of original concentration of 1.2g per 50g larvae. In this case it equates to 0.9g per 50g larvae.
5. Add epinephrine solution to each screen and treat for 60 minutes.

6. Turn on settlement system flow. There is no need to drain and rinse system again.

What method is best for Sydney Rock Oysters? The conventional use of aged shell grit sieved to a suitable size and placed on downwelling screens is currently used by PSFC. This method has been used for a number of years and appears to do the job, however I am unsure of the average percentage settlement of larvae to spat over the years.

Alternative flow through techniques including tubular upwellers and spat bubblers.

Although a few hatcheries around the world have tried a number of variations of tubular upwellers and spat bubblers post settlement, I am not aware of any commercial hatcheries that are still pursuing these alternative systems.

Stocking rates in conventional upwelling flexible downwelling set systems.

PSFC current stocking rate of 250,000 competent ready to set larvae is well within the parameters of commercial hatchery criteria. In fact, the set screens utilised by the PSRC hatchery could handle up to 500,000 larvae.

Water treatment

Water filtered to 10 microns is adequate. No other treatment is necessary.

Aeration

A settlement system does not require aeration, as long as water is continually introduced to the system by a spay bay above the downwellers.

Heaters, temperature control logging

Constant temperature in the settlement system is essential. This temperature should be the same as the larvae culture temperature. The best way to achieve this is by the before mentioned boiler/heat exchanger system, and the use of a header tank supplying the system with new water.

Sanitation

On a daily basis it is recommended that the following tasks be undertaken:

a) The complete draining of the settlement tank followed by a freshwater rinse. (After screens)
b) While leaving the set screens in place, rinse with fresh water.
c) Refill tank, settle swimming larvae by flicking the surface with seawater.
d) Turn on overhead spray bars.
e) Commence adding food by trickle feeding.

Water exchange and sanitation in conventional set systems

The introduction of new clean seawater at the rate of 2 litres per minute per million larvae is recommended.

Use of antibiotics? – flow through systems

Unable to comment on the use of antibiotics during settlement.

Quality assurance

Examine settling larvae and spat from each upweller on a daily basis. This can be as simple as taking a small sample from each set screen in a small petri dish, and having a brief look through the microscope to determine general health, percentage set, and any obvious problems. This is not currently done on a daily basis.

Feeding – species array and amounts and methods of administration especially batch vs trickle feeding.

Following the introduction of larvae to the settlement system, it is not necessary to feed them immediately, particularly if they are healthy competent larvae. Commence feeding after a couple of hours. This is best done by attaching a feed line to the existing spay bar, with a small valve directing food to each set screen and trickle feeding at the desired rate.

The diet should consist of 70% diatoms and 30% flagellates that could be transferred from the tanks or bags to a holding tank, diluted to say 1 – 2 million cells/ml and pumped to the set system with a small submersible pump. One million larvae will require approximately 250 litres of food per day.

The current method of batch feeding a set tank appears to work to a degree, however I believe trickle feeding to be a better alternative for the larvae.

Recommendations:

In summary, settlement percentage and survival will be enhanced with the use of shell grit and most likely with epinephrine using the following techniques.

- Maintain temperature at larval rearing temperature.
- Use Epinephrine only. May be able to avoid using scallop shell altogether.
- Flow through new seawater at the rate of 2 litres per minute per million larvae.
- Trickle feed at desired rate with 70% diatoms and 30% flagellates.
- Remove settled spat from downwelling system after four days.

7. Post Settlement and Nursery

The current method of retaining newly settled spat in a downwelling system may be causing some problems down the track. It is possible that some fouling on both the oyster shells and scallop shell is occurring and the spat should be removed as soon as practicable, usually after four days.

Following the removal of newly settled spat from the settlement system by screening out any dead shell, larvae and shell grit, they can be introduced directly to a spat bottle system.

Standard spat bottle system.

This method of rearing spat up to 2mm has been a great break through for both growth and survival of young spat during the most vulnerable stage. The system requires large quantities of seawater and algae and may be difficult to operate at PSFC. It may be feasible to utilise estuarine water in this system.

The only alternative is to remove the newly settled spat from the settlement system and use a conventional indoor upweller system utilising existing tanks. This requires less flow through water and algae, but commercial practice has clearly shown this method results in a higher incidence of mass spat mortality.

Recommendations:

- Instal a spat bottle system.

Overall Recommendations

Source water

Priority

- Replace all existing pipes and fittings with new material, and thoroughly clean valves if using again. Replace in such a way that the whole system can easily be dismantled for cleaning. I.e. Above ground with valves that can be taken apart and cleaned.
- Develop strict protocols for maintaining seawater delivery pipes in good and clean condition.
- Collect seawater from one site only. This will remove one variable.

Proposed

- Increase storage tank capacity and monitor for known SRO disease and pathogens.
- Relocate hatchery or outsource hatchery work where applicable.
- Constant water flow in to storage/header tank is ideal if possible.
- Do not use sand filters. (Not used at present)
- Filter all seawater to 20-micron utilising drum, disc or screen before entering the hatchery.

Broodstock

Priority

- Install a conditioning system to be able to produce quality gametes all year round.
- Only use SRO broodstock with known pedigree for tracking purposes.
- Install quarantine/depuration system to keep broodstock collected from farms for a period prior to spawning.
- Consider program to investigate SRO egg quality.
- Determine any genetic difference between NSW oysters and WA rock oysters.

Proposed

- Install boiler for instant seawater heating
- Install chiller for seawater cooling.
- Naturally spawn only. Keep numbers constant. Keep good eggs only. (This is done)

Algae

Priority

- Develop comprehensive Procedures Manual for algae culture and production. Make a start now. Any changes in the future can be added.
- Introduce routine sampling of algae for Vibrio species of bacteria. This would involve the batches of algae targeted as the food source for SRO larvae.
- Optimise feeding regime and protocols.
- Separate algae production unit seawater supply from rest of hatchery to allow regular dry out.

Proposed

- Upgrade algae system to a continuous flow system
- Investigate several commercial hatchery algae production methods with the view of improving efficiency.
- Develop methods to reduce manual handling.

Larval Culture

Priority

- Drain D-larvae from tank on day 1. I.e. 24 hours incubation period.
- Do not feed until D-larvae are in new tank of water. i.e. Day 1
- Use seawater from one collection site only.
- Commence equipment capital replacement program. This will include the replacement of larval rearing tanks.
- Replace tank outlet valve apparatus with a system that can be dismantled and cleaned.
- Handle larvae as little as possible. This means the use of a rapid pull-down larvae screen after day 5.

Proposed

- Consider constant trickle feeding at a pre-determined cell density over 24-hour period. This will stop larvae gorging on food and the potentially starving when food is cleared.
- Diet to 50% diatoms: 50% flagellates after day 5.
- Maintain constant temperature in larvae tanks using one small immersion heater if necessary.
- Consider a boiler for heating seawater prior to delivery to tank rather than heating water with over-the-side immersion heaters.
- Adopt a strict protocol for larvae production. i.e. Procedures Manual.
- Compare wild larvae with cultured larvae for similar histological symptoms. Do wild larvae suffer from anorexia?

Settlement

Priority

- Maintain temperature at larval rearing temperature.
- Use Epinephrine only. May be able to avoid using scallop shell altogether.
- Flow through new seawater at the rate of 2 litres per minute per million larvae.
- Trickle feed at desired rate with 70% diatoms and 30% flagellates.
- Remove settled spat from downwelling system after four days.

Post Settlement and Nursery

- Instal a spat bottle system.

Other Recommendations

- Replace air delivery plumbing from air blowers to hatchery entry point. Locate air bleed off valves at strategic points in the delivery system, with main valve at lowest point in system at entry point to hatchery. This should be slightly open at all times.
- Relocate air blowers to dedicated building, or alternatively draw air from outside of existing pump house.
- Procedures Manual on all aspects of Sydney Rock Oyster production, and associated issues.

Appendix 9.4

NSW Fisheries

Port Stephens Fisheries Centre

Sydney Rock Oyster Hatchery

Hazard Control Plan

FINAL DRAFT

Version 1

28 March 2003

Prepared

with

NSW Fisheries

by

AusVet Animal Health Services

with the financial support of the

NSW Oyster Research Advisory Committee

Contents

PLEASE NOTE

This Hazard Control Plan was developed following standard HACCP methods but the resulting Plan differs from a standard HACCP plan in that the significance of many of the hazards and the need for controls is uncertain. Many of the factors listed in the Hazard Control Table as hazards and the controls in place are based on experience with previous runs of SRO at PSFC and with other cultured marine molluscs at PSFC and elsewhere and on knowledge from experimental work conducted at PSFC and the published literature.

Glossary

The terms used in this Plan are to be interpreted as described.

C. muelleri	*Chaetocerus muelleri*
Calcitrans	*Chaetocerus calcitrans*
D-veliger	First shelled larval stage
Larvae	Juvenile SRO from fertilisation to metamorphosis as set spat
Pav	*Pavlova lutheri*
PSFC	Port Stephens Fisheries Centre
Set	Process of metamorphosis
Spat	Juvenile SRO from metamorphosis to 5 mm
SRO	Sydney Rock Oyster *(Saccostrea glomerata)*
T. iso	*Isochrysis* species

1. Hazard Control Team

NSW Fisheries appointed the following staff members at PSFC as the Hazard Control Team:

Hatchery Biologist	Michael Heasman
Biologist	Wayne O'Connor
Hatchery Manager	John Diemar
Algae Technician	Lynne Foulkes
Oyster Technician	Ian Diemar

The Team has a sound knowledge of the SRO Hatchery process and developed the Plan in March 2003.

With David Kennedy of AusVet Animal Health Services, the Team conducted a Hazard Analysis on 25 March 2003 and developed the following plan over the following three days.

The Hatchery Manager will be responsible for the future verification and updating of the analysis and plan as procedures in the Hatchery change.

The Plan will be reviewed annually in March at the end of the production season and in time to make changes to the production system and the Plan where required.

2. Scope of the Hazard Control Plan

This Plan addresses the hazards identified in the process of rearing SRO spat in batch culture at the PSFC.

The process starts with the selection and collection of broodstock from leases in Port Stephens and concludes with spat attaining a minimum size of 2mm. Viable spat of a smaller size (minimum 0.5mm) may be distributed to intermediate commercial oyster nurseries in coastal NSW but NSW Fisheries maintains an interest in their scientific and commercial success.

Note: At the time of the hazard analysis spat were not commercially marketed. One of the purposes of this Plan is to help develop a commercially viable process.

The animals included in this process are the broodstock and their progeny as they develop through larval stages to spat. The process uses 20,000L larval incubation tanks.

It also includes the production of algae as food for the larvae and spat.

This Plan has been developed as one of a series of projects supported by the NSW Oyster Research Advisory Committee (ORAC) to identify and resolve long-standing problems in the production of juvenile SRO. This process has suffered numerous failures over several years in two separate areas: anorexia and death of larvae in larval tanks during the first week and mass deaths of spat late in the process. The causes of these are unknown but they are probably multifactorial.

This Hazard Control Plan was developed following standard HACCP methods but it differs from a normal HACCP plan in that the significance of many of the hazards and the need for control at

many points is uncertain. Many of the factors listed in the Hazard Control Table are based on experience with previous runs of SRO at PSFC and with other cultured marine molluscs at PSFC and elsewhere and on knowledge from experimental work conducted at PSFC and the published literature. As a result it has not been possible to determine critical limits and corrective actions for many of the factors considered to be hazards or potential hazards.

The Hazard Control Table does include a large number of recommended actions to resolve the status of some hazards either by research or by improvements that address deficiencies in the premises. These vary in cost from minor to substantial investments in research and capital investment.

Other components of NSW Fisheries' current approach to solving mortality problems in the hatchery have included a SRO Hatchery Health Workshop held in August 2002 to explore the problem with other scientists and commercial hatchery operators. In February 2003, practices in the hatchery and potential hazards were reviewed and rated for significance during an audit by Mr Martin John (Shellfish Hatchery Consultancy, Wallington, Victoria). The sources of the knowledge or assessment of the importance of hazards and controls are cited in the Hazard Control Table. A research program has also been submitted to Fish Research and Development Corporation.

The Hazard Control Team considers that the following factors present significant hazards to the successful production of SRO spat but their control is considered to be outside the scope of this Plan:

1. Competition for hatchery facilities

All in – All out stocking systems are an important strategy to reduce the impacts of disease in intensive animal production systems. Because of the heavy demand on facilities in the hatchery by various PSFC projects two hazards arise:

- The concurrent holding of other species on the premises increases the potential for cross-infection between species; and
- The inability to effectively disinfect and dry out the hatchery between runs or between seasons increases the potential for cross-infection between batches.

2. Staffing and training

The levels of staffing of the hatchery and of training of staff in new technologies are considered inadequate for successful SRO production. Additionally, improvements can be made in managing the existing staff including improved planning, communication, allocation of responsibilities and reporting.

3. The Product

This Plan addresses hazards in the production of

> A commercial batch of (nominally) 5 million genetically selected Sydney Rock Oyster spat of a minimum shell size of 2 millimetres.

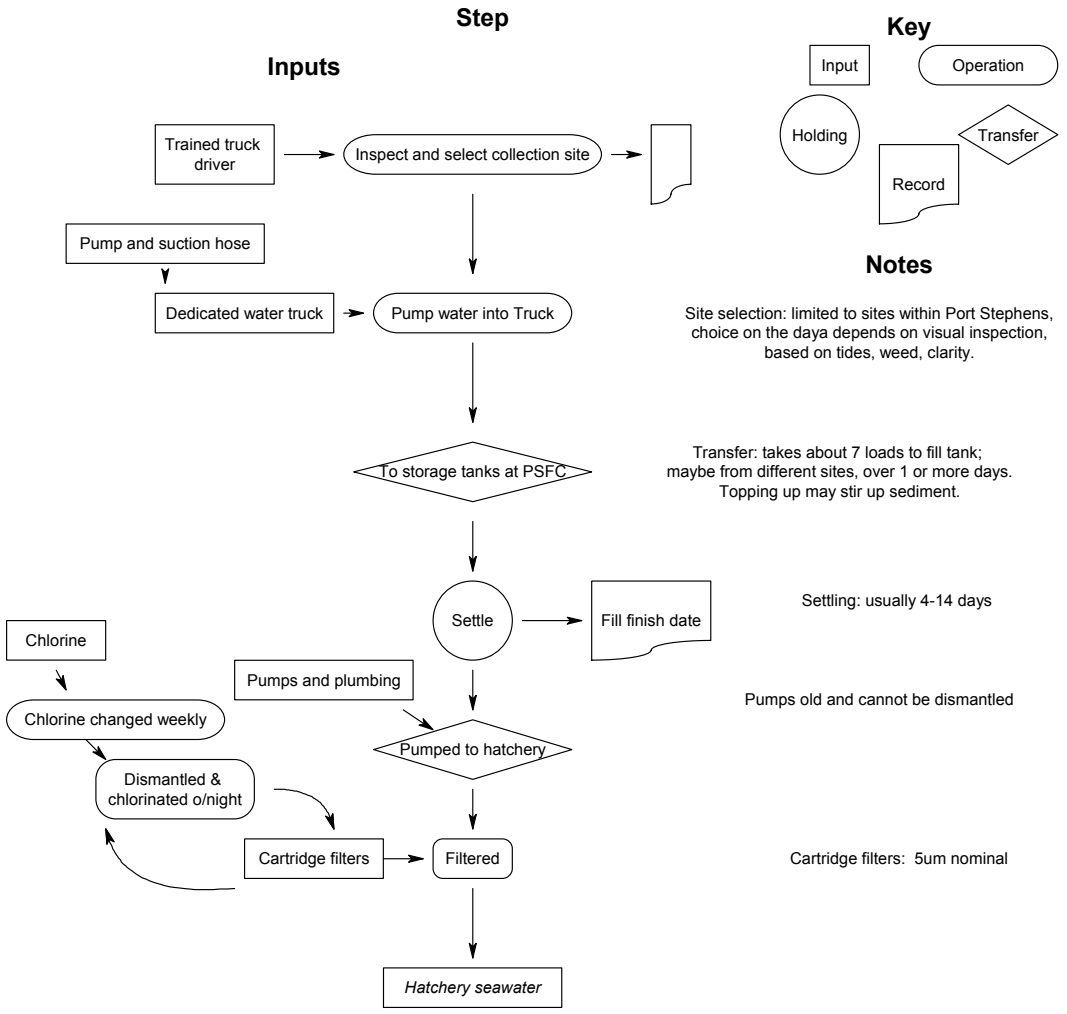
They are supplied to commercial field nurseries in coastal NSW.

During transport the spat are maintained at 8 to 16 degrees Celsius and damp in robust insulated boxes.

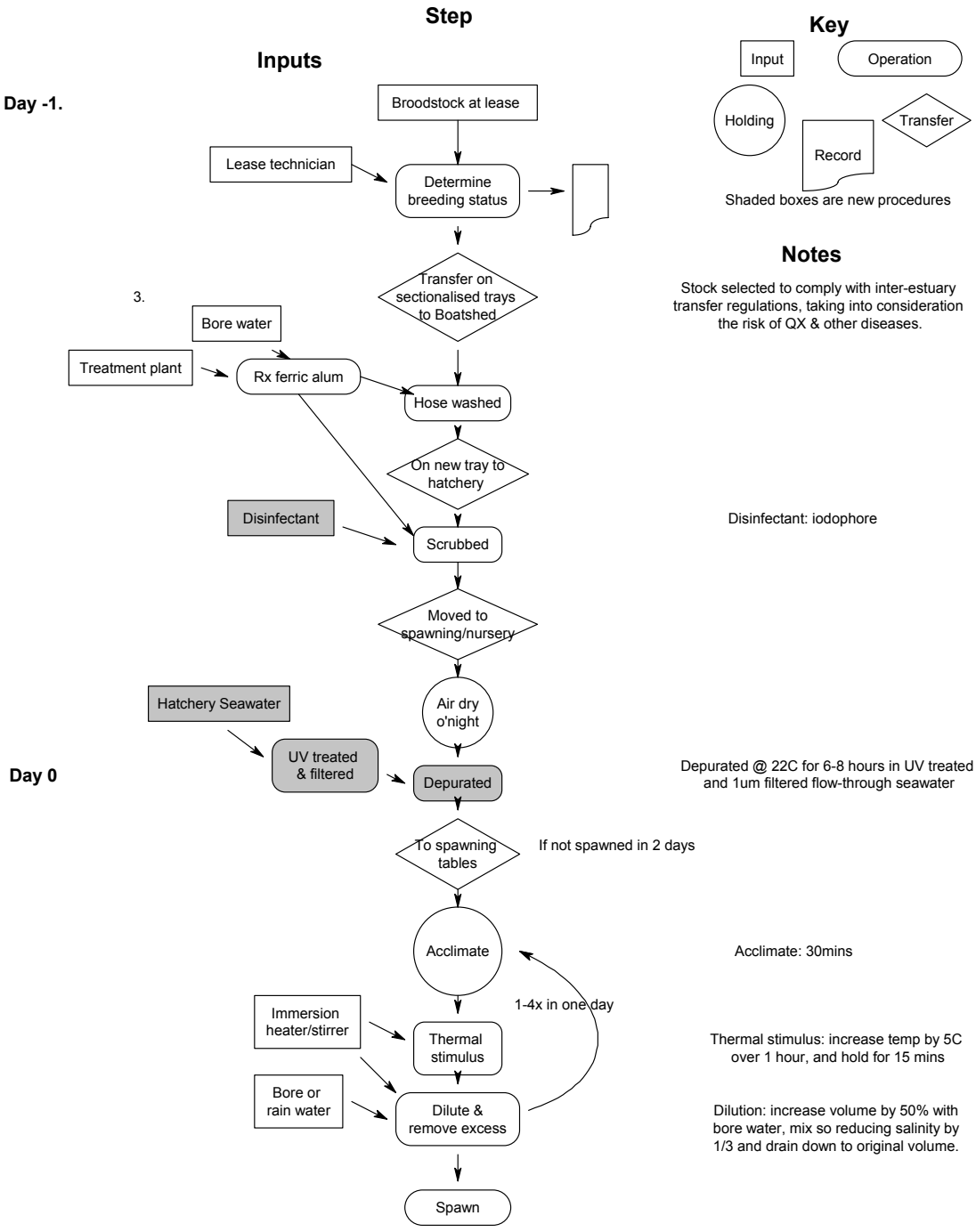
4. Hatchery Flow Diagrams

Flow diagrams for various stages in the process are outlined on the following pages.

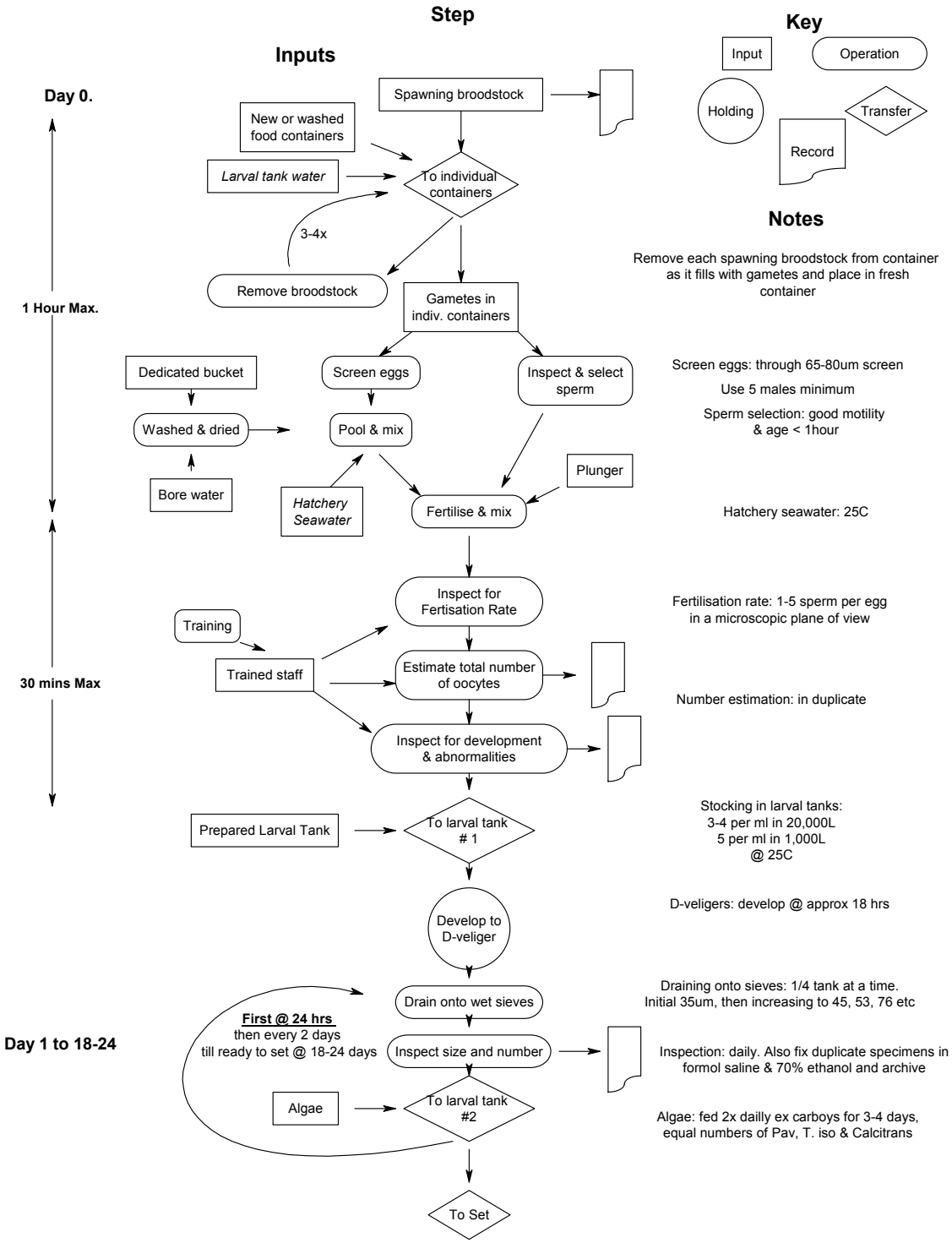
Seawater Preparation



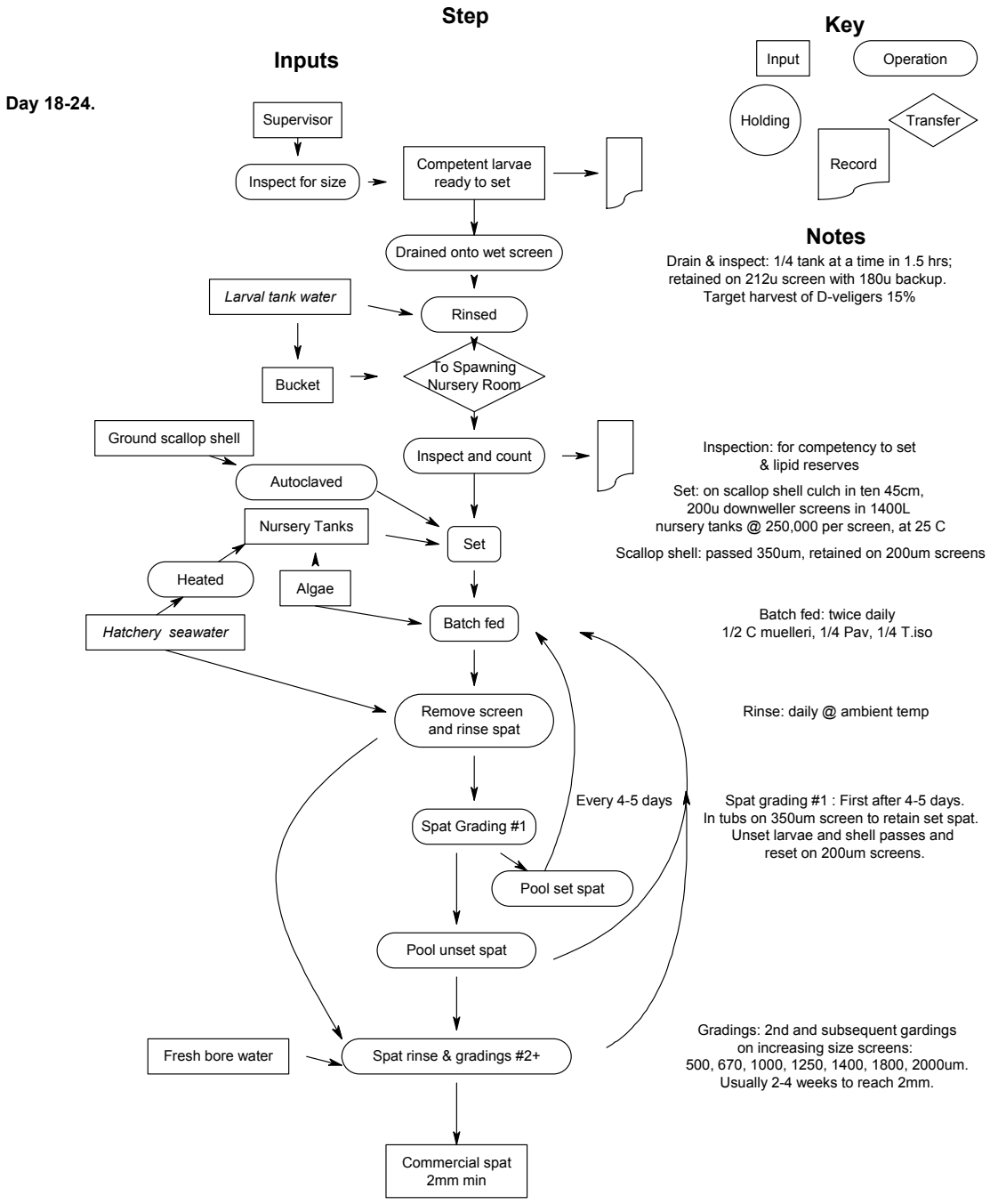
Broodstock


Step
Inputs
Key
Input
Operation
Holding
Transfer
Record
Shaded boxes are new procedures
Day -1.
Broodstock at lease
Lease technician
Determine breeding status
Transfer on sectionalised trays to Boatshed
Notes
Stock selected to comply with inter-estuary transfer regulations, taking into consideration the risk of QX & other diseases.
3.
Bore water
Treatment plant
Rx ferric alum
Hose washed
On new tray to hatchery
Disinfectant
Disinfectant: iodophore
Scrubbed
Moved to spawning/nursery
Hatchery Seawater
Air dry o'night
UV treated & filtered
Day 0
Depurated
Depurated @ 22C for 6-8 hours in UV treated and 1um filtered flow-through seawater
To spawning tables
If not spawned in 2 days
Acclimate
Acclimate: 30mins
1-4x in one day
Immersion heater/stirrer
Thermal stimulus
Thermal stimulus: increase temp by 5C over 1 hour, and hold for 15 mins
Bore or rain water
Dilute & remove excess
Dilution: increase volume by 50% with bore water, mix so reducing salinity by 1/3 and drain down to original volume.
Spawn

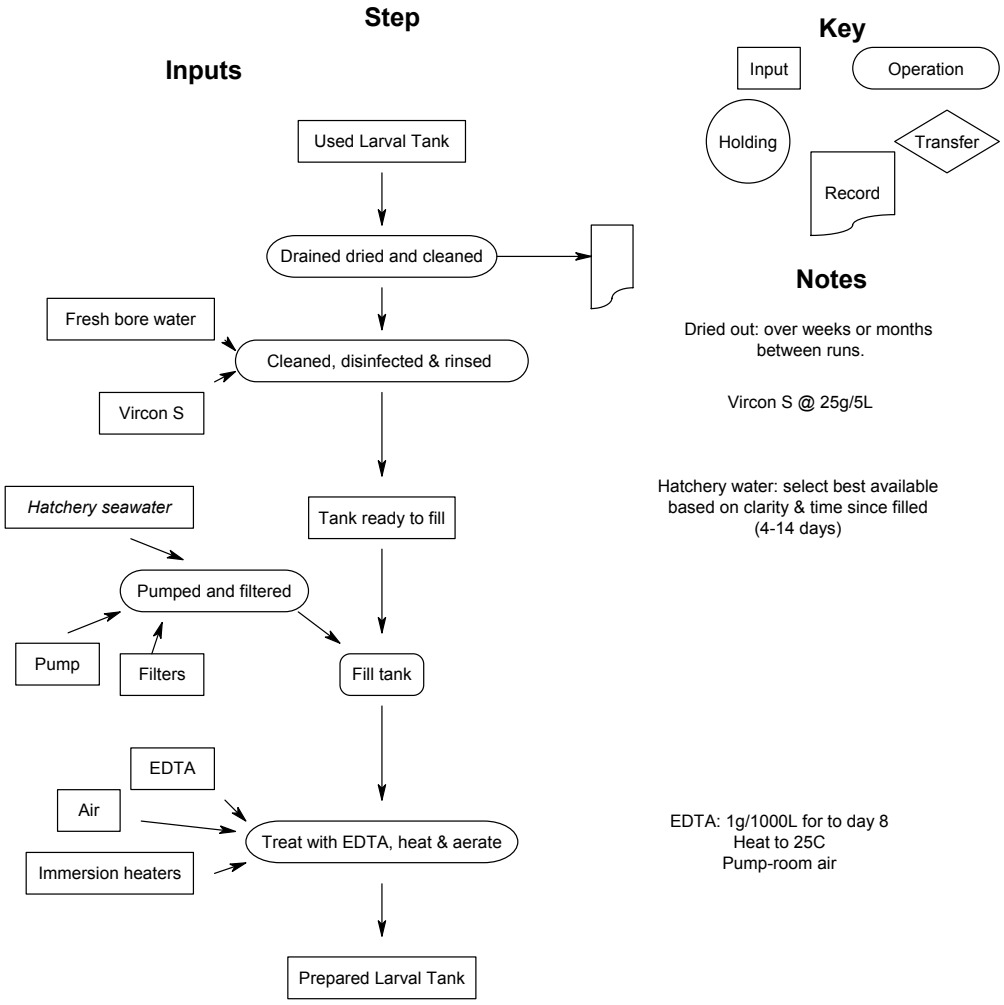
Spawning - Fertilisation - Incubation



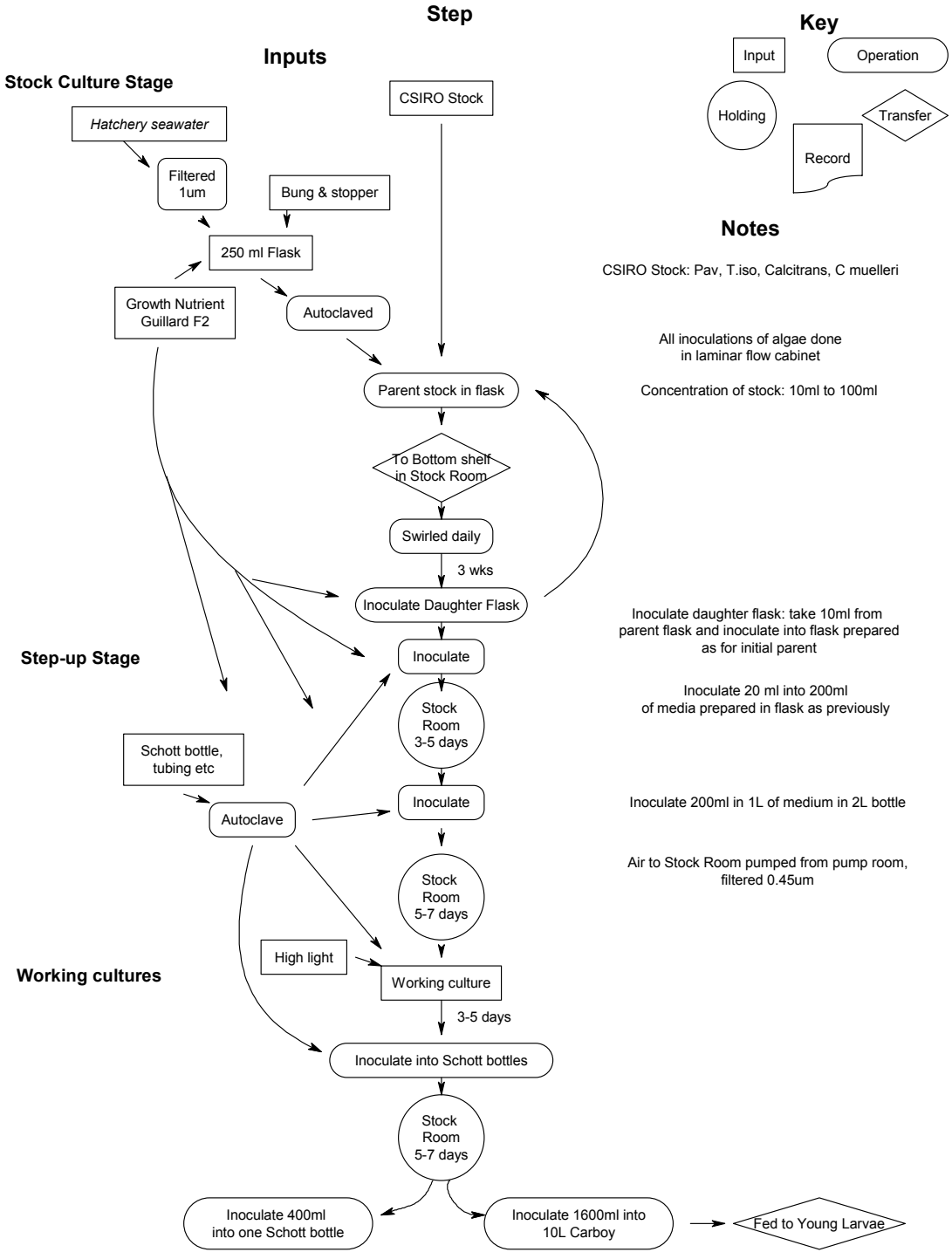
Settlement


Step
Inputs
Key
Input
Operation
Holding
Transfer
Record
Day 18-24.
Supervisor
Inspect for size
Competent larvae ready to set
Drained onto wet screen
Larval tank water
Rinsed
Bucket
To Spawning Nursery Room
Ground scallop shell
Autoclaved
Inspect and count
Nursery Tanks
Set
Heated
Algae
Hatchery seawater
Batch fed
Remove screen and rinse spat
Spat Grading #1
Pool set spat
Pool unset spat
Every 4-5 days
Fresh bore water
Spat rinse & gradings #2+
Commercial spat 2mm min
Notes
Drain & inspect: 1/4 tank at a time in 1.5 hrs; retained on 212u screen with 180u backup. Target harvest of D-veligers 15%
Inspection: for competency to set & lipid reserves
Set: on scallop shell culch in ten 45cm, 200u downweller screens in 1400L nursery tanks @ 250,000 per screen, at 25 C
Scallop shell: passed 350um, retained on 200um screens
Batch fed: twice daily 1/2 C muelleri, 1/4 Pav, 1/4 T.iso
Rinse: daily @ ambient temp
Spat grading #1 : First after 4-5 days. In tubs on 350um screen to retain set spat. Unset larvae and shell passes and reset on 200um screens.
Gradings: 2nd and subsequent gardings on increasing size screens: 500, 670, 1000, 1250, 1400, 1800, 2000um. Usually 2-4 weeks to reach 2mm.

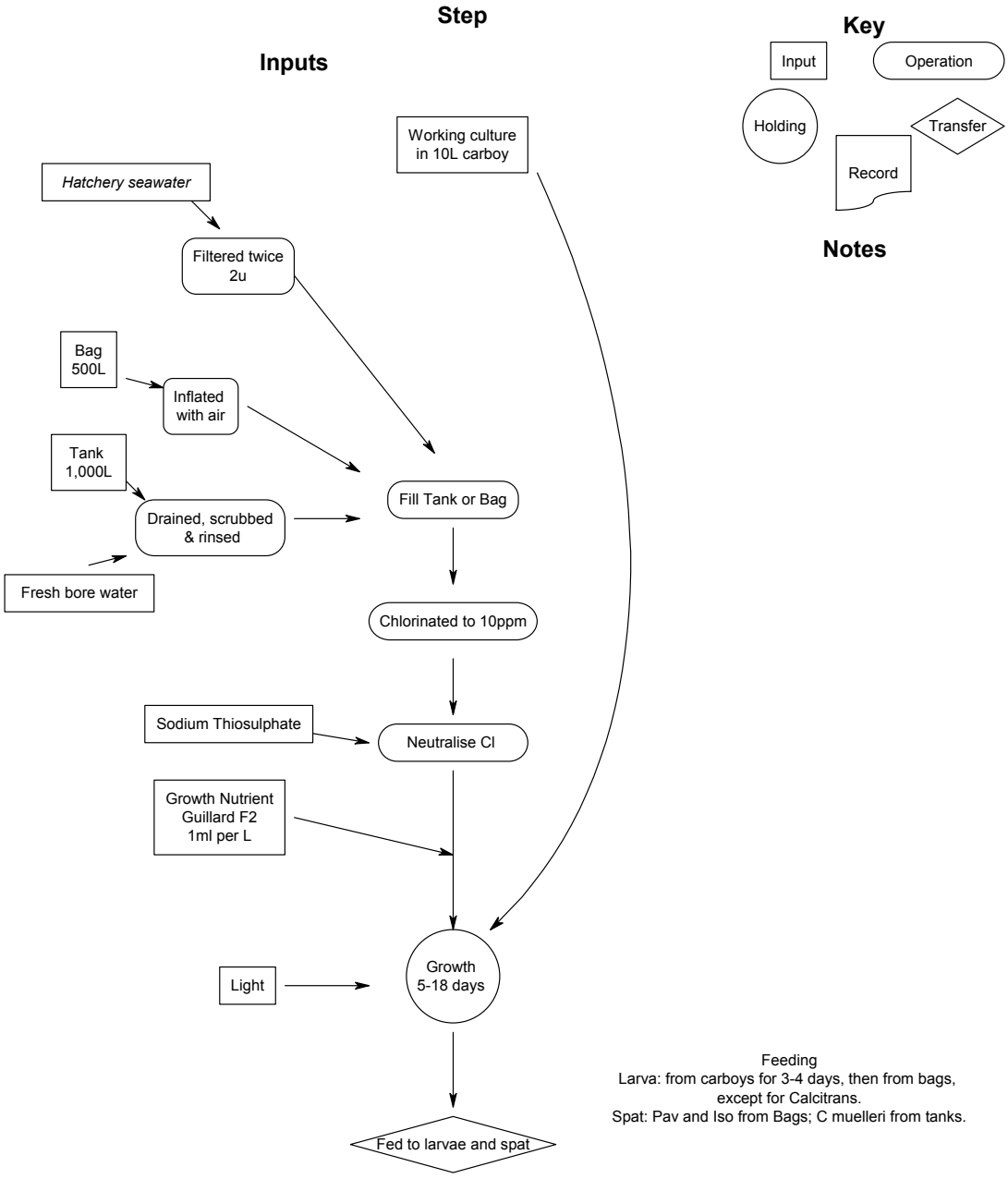
Larval Tank Preparation



Algae Preparation



Algae Production Room



5. Hazard Control Table

1. Seawater Supply

Step	Hazard	Control	Ref	Critical? Critical Limit	Monitor	Further Action/Research *I = Capital Improvement, R = Research*	Records
Collection	Excess metals and hydrocarbons	Select most suitable site. Treat water with EDTA			Visual inspection by truck driver	*Filter water into truck (I)*	Description of site/water
	Contamination in pump and hoses	Drain tank of seawater, flush with fresh, chlorinate, rinse with fresh.					
Stored water	High organic content & associated bacteria & bio-toxins.	Settle water in tanks. Select clearest settled water.		Yes, 4 days min.	Visual inspection when water needed. Vibrio count 2 days before use.	*Source from deepwater site(I)*	Record tank and fill date.
	Valves, manifolds, pumps and pipes contaminated by microbes and metabolytes					*Redesign to purge residual water and pig system(I).* *Re-plumbing and replace pumps every 3 years (I)* *swab and culture water and hazard points in lines.*	
	Suspended biotoxins in supernatant water	Use water up to 14 days old.				*Add dispersal plate at water intake to reduce turbulence (I)* *Investigate impact on larvae and embryos (R).*	
	Contaminated filters	Daily dismantle, clean and chlorinate. Change chlorine weekly Replace before new run		Yes	Monitor residual chlorine	*Investigate use of hydrogen peroxide or Vircon S as disinfectant (R).*	

2. Broodstock - Incubation

Step	Hazard	Control	Ref	Critical? Critical Limit	Monitor	Further Action/Research *I = Capital Improvement, R = Research*	Records
Preparation	Inter-batch infection			Yes		*Dryout and disinfection(I)*	Time dry
	Introduction of pathogens to hatchery	Staff wash hands and wear dedicated protective footwear in SRO hatchery					
	Cross-infection between spat and larvae	Keep broodstock, spat and larvae in separate air spaces.					
Selection	Broodstock not breeding	Train staff in identifying breeders		Yes		*Investigate methods of conditioning (R).*	Record source of broodstock and their condition.
	Failure to meet statutory health requirements	If out of season, condition broodstock		Yes, 2 weeks			
		Select from estuaries free of QX disease and Pacific oysters					
Handling	Poor egg quality	Stimulate to spawn naturally		Yes			
	External pathogens on broodstock	Wash, scrub with disinfectant (eg iodophore)		Yes			
	Internal pathogens	Flow through depurate		Yes			
Spawning	Temperature shock on eggs from temperature cycling.					*Investigate benefits of rain water and bore water for rinsing and effect of cooling(R).*	No. of cycles needed to spawn
	Gametes too old	Proceed from spawning to fertilisation quickly		Yes, 1 hour			Record time.
Fertilisation	Anoxia of fertilised eggs	Conduct inspections quickly		Yes, 30 minutes		If longer aerate gently, with sterilised airline.	Record time and if aerated.

	Contaminated equipment infecting oocytes	Dedicated buckets etc washed in fresh water and dried for weeks/months before use before run. During run, hold in chlorine bath @ 100ppm, rinse with fresh and Hatchery Seawater		*Investigate relative toxic effects of residual chlorine and iodophores (R)*
Incubation	Contaminated larval water	Clean and chlorine wash plumbing and tanks.	Yes	*Replace plumbing (I)*
	Residual toxic chlorine.	Rinse thoroughly.		
	Larvae affected by passing direct immersed heater.	Inspect larvae daily		*Pre-heat water rapidly to 25°C before entering tank (I)* *Investigate means of maintaining temperature in tanks.*
	Larva shocked by turbulence passing out through valves.	Inspect larvae daily		*Investigate effect of draining by syphoning on larvae (R)*
	Sub-optimal diet for larvae from day 5.	Inspect larvae daily. Feed 50:50 diatoms: flagellates		*Investigate optimal diet eg 50:50 diatoms:flagellates(I)*

3. Settlement

Step	Hazard	Control	Ref	Critical? Critical Limit	Monitor	Further Action/Research *I = Capital Improvement, R = Research*	Records
Nursery Tanks	Thermal stress on young spat of ambient water wash and intermittent heating system	Maintain temperature at larval rearing temperature. Heat bore water		25°C	Temperature logging.	*Monitor 1400L tank temperature (R)*	Temperature log. Bore water temp.
	Bacteria and metabolytes build up on scallop shell					*Investigate epinephrine stimulation, ie without shell (R)*	
	Bacteria and metabolytes build up in pseudofaeces					*Trickle feeding system (I)* *Change to upwelling spat bottles (I)*	
	Bacteria and metabolytes build up on walls of old tanks	Drain, hose with fresh water, wipe, air dry for 1.5 days before reusing.				*Investigate effectiveness of upwelling banjo screen system(R).*	
	Bacteria and metabolytes build up in submersible pumps and hoses.	Dismantle and clean at end of and before each run.				*Duplicate system so can clean by pigging hoses and dry out and alternate equipment every 2 days during runs (I)*	
Grading	Stress and abrasions allowing bacterial infection	Handle gently and quickly		Yes, Less than 1.5 hours		*Review need for grading in downweller screen set (R).*	Record time taken at each grading.

4. Algal production

Step	Hazard	Control	Ref	Critical? Critical Limit	Monitor	Further Action/Research	Records
Stockroom	Microbial contamination of hardware.	Autoclave		Yes, 121°C for 15-20 mins	Self regulating		Automatic
	Environmental contamination of flasks and carboys	Train operators. Open vessels only in laminar flow cabinet.		Yes	Visual inspection		
	Cross-contamination of algal cultures.	Train operators in correct use of laminar flow cabinets.			Visual inspection		
	Contaminated air					*Drainage bleed-off point so that condensation can be drained continually(I)* *Replumb inlet pipe so that it can be pigged for cleaning (I)* *Source air from outside, not from pump house(I)*	
	Culture too old to be quality food for larvae.	Roll over cultures @ 4-7 days Have a spare bottle of culture. Use best available cultures					
Production room	Residual chlorine in filled tanks or bags.	Neutralise with sodium thiosulphate.		Yes, NIL	DPD tablet test for chlorine	*Investigate effectiveness of pasteurisation.*	Chlorine concentrations.
	Environmental contamination in filled bags or tanks			Yes, No clumping or ciliates present	Visually inspect algae for viability and count cells prior to feeding from each bag		Inspection and count results.
				Negative TCBS	Plate test for *Vibrio* spp on TCBS media 2 days before feed out.		TCBS results.
	Contaminated feedlines to bags	Clean with pig sponge and air dry before attaching to bag.					

6. Verification Schedule

Area/ Activity	Method Check and enter data in spreadsheet or database from:	Frequency	Responsible	Records
Seawater preparation	Water collection/delivery running sheet Storage Tank Management and Test sheet	Weekly Weekly	Hatchery Mgr Hatchery Mgr	Truck/Office Hatchery Office
Broodstock	Broodstock Record Sheet	Each batch	Hatchery Mgr	Hatchery Office
Fertilisation to competent larvae	Batch Record sheet	Each batch	Hatchery Mgr	Hatchery Office
Algae production				
Despatch of spat	Movement sheet	Each batch	Hatchery Mgr	Hatchery Office
Batch Review - product & scope - hazards and hazard analysis -critical limits -monitoring and corrective action	Hazard Control Team meeting	Within one week of finishing each batch	Hatchery Mgr	Hatchery Office
Verify or Update flow diagram Audit Hazard Control Plan		Annually in March	Manager PSFC	Hatchery Office

7. Supporting Knowledge & Documents

The following sources are cited in the *Ref* column of the Hazard Control Table.

Ref	Source/Document	Location
1		
2		
3		
4		
5		
6		
7		
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11		
12		
13		
14		
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ISSN 1440-3544

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