REVIEW OF

HATCHERY PRODUCTION TECHNOLOGY

AND BREEDING PROGRAM FOR

SYDNEY ROCK OYSTERS

for the

Oyster Research Advisory Council

by

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

THE UNIVERSITY OF
NEW SOUTH WALES

Project No. 2001/213

Fisheries Research and Development Corporation

Review of hatchery production technology and breeding program for Sydney rock oysters.

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NON-TECHNICAL SUMMARY

2001/213	**Review of hatchery production technology and breeding program for Sydney rock oysters**

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OBJECTIVES (terms of reference): 2001/213:

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.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

Wide consultation within the Sydney Rock Oyster Industry and SRO researchers allowed a critical review of the methodology and results of the Sydney rock oyster breeding program, the practices and procedures for Sydney rock oyster hatchery technology at the Port Stephens Fisheries Centre and problems associated with larval and post-settlement mortality, and the preparation of a cost benefit review of the Sydney rock oyster hatchery program. The review panel found a number of shortcomings in the research and commercialization process, but considered there was sufficient benefit to the industry to continue the Sydney rock oyster hatchery R&D and breeding program subject to a set of processes being implemented. If these are achieved, significant benefit should accrue from improved communication and involvement of industry in the research and better focus of the research work on overcoming key technical problems for the industry.

Genetic improvement of Sydney rock oysters was started with a view to producing a faster growing, disease resistant oyster and included the development of hatchery technology to produce sufficient numbers of animals for the maintenance of breeding stock. However, when commercial scale production of genetically improved stock was attempted, production was highly variable, unpredictable and largely failed to meet demand. There was considerable disappointment in the industry, which had understood that sound technology was available. This outcome led to questions concerning the adequacy of the hatchery technology and breeding programs, the impediments to their commercialization, and led to the request for a review of these programs.

The review panel was to include experts in mollusc genetics, hatchery technology and disease and was intended: 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.

After widespread consultation with industry and in-depth discussion with researchers the review panel concluded that:

1) 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. However, the methods used, were probably not optimal, and it is hard to judge the actual genetic gain achieved and to predict likely future achievements. Most importantly, the difficulties in hatchery rearing pose an absolute impediment to the practical commercialisation of any gains from genetic improvements.

2) The present hatchery technologies were technically out of date, inadequate to provide spat on commercial scales, and are not appropriate models upon which to base commercial operations. 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. Revised procedures and clear lines of project management, responsibility and reporting need to be established to focus on hatchery production of rock oysters, and additional expertise incorporated to revitalise work in this area.

3) 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 panel concluded that NSW Fisheries, the Fisheries R&D Corporation, the CRC for Aquaculture, other funding agencies and industry have made significant contributions to research on rock oysters but 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.

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 (PSFRC). 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 considered there was enough evidence to consider it was worth continuing with work on hatchery production technology and breeding programs for the Sydney Rock Oyster, but that, without improved hatchery management and control these technologies will not be effective and the industry will not be able to grow. At present industry has limited capacity to take up any new hatchery technology because of the limited hatchery capacity in New South Wales, but the industry can utilize the spat that has been supplied by hatcheries to date. The lack of adequate hatchery production has been the major impediment to commercialization, but better planning for technology introduction through involvement of the industry at early stages would assist technology transfer.

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

1) Industry education and ownership: a) 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. b) NSW Fisheries improve communications and collaboration with the SRO industry regarding the development of any genetic improvement program for the SRO. c) 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.

2) Evaluation of genetic improvement methods: a) 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. b) 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).

3) Focus on hatchery technology: a) Research focuses on the hatchery rearing problems, and that a concentrated program of work on those issues be developed. b) 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. c) That any new project on hatchery work be led by an industry and expert steering committee whose method of operation will be specified by ORAC, with the project PI being either external to fisheries or a senior fisheries officer. d) A time limit of one year for realizing initial outcomes with a maximum of three years for more strategic outcomes should be set.

KEYWORDS: **Sydney Rock Oyster, Aquaculture, Hatchery technology, Genetics.**

FINAL REPORT

2001/213	**Review of hatchery production technology and breeding program for Sydney rock oysters**

1. Terms of reference

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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 Stevens

- 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 Stevens

- 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. Prliminary Draft. Unpublished 244 pp.

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

Heasman, M.P., Goard, L., Diemar, J and Callinan, R.B. (2000). Improved Early Survival of Molluscs: Sydney Rock Oyster (*Saccostrea glomerata*). NSW Fisheries Final Report Series No 29, 62 pp.

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:

1. mass-selected for increased growth rate.
2. mass-selected for resistance to marteiliosis (QX disease).
3. mass-selected for resistance to mikrocytosis (Winter Mortality).
4. 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. 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 of 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 (human and financial) 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, or workshops, 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 vital 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 managed effectively, 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 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 workshop(s) 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 workshop(s) 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, the Fisheries R&D Corporation, the CRC for Aquaculture, other funding agencies and industry have 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 considered there was enough evidence to consider it was worth continuing with work on hatchery production technology and breeding programs for the Sydney Rock Oyster, but that, without improved hatchery management and control these technologies will not be effective and the industry will not be able to grow. At present industry has limited capacity to take up any new hatchery technology because of the limited hatchery capacity in New South Wales, but the industry can utilize the spat that has been supplied by hatcheries to date. The lack of adequate hatchery production has been the major impediment to commercialization, but better planning for technology introduction through involvement of the industry at early stages would assist technology transfer.

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.**

- **NSW Fisheries improve communications and collaboration with the SRO industry regarding the development of any genetic improvement program for the SRO.**

- **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 industry and expert steering committee whose method of operation will be specified by ORAC with the project PI being either external to fisheries or a senior fisheries officer.**

- **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

The review panel would like to express their thanks to those individuals who made their time available to the panel, and for the assistance provided by many individuals in Fisheries and in the Sydney rock oyster Industry to enable the panel to undertake the review in a relatively short time frame. The openness of all those who provided information greatly facilitated the work of the panel.

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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	Georges River Lines (4 lines, 3 sites)	Program Outline	Port Stephens Lines (4 lines, 3 sites)
1989-1990	G1	*1st Generation*	P1
1991-1992	G2	*2nd Generation*	
1992-1993		*2rd 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 1?m 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 50?m 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 45?m. 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 63? m and 53? 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 85? m and 63? 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 – 100,118,150 180,212?m.

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

SET

-2 - screens, tanks and equipment sterilised.

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

0 - retained 212?m larvae to set screens @ 200-250,000/ screen – thin layer of shell (pass 350?m, ret.200?m) 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 350?m 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 ?m 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 350?m screen. Ret. 350?m 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 (350? m screen). Approximately 400,000 spat per screen, flow rate approximately 6L/min (500? m 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 3000? m. 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 detatched 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

9th 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

Nutitional Factors

May 1991 Joint venture with Dr Paul Southgate JCUto 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	$7090 (10%)	$3545 (5%)	$46085 (65%)
John Diemar	$10146 (20%)	$5073 (10%)	$38046 (75%)
Lindsay Goard	$18786 (40%)	$2348 (5%)	$25831 (55%)
Lynne Foulkes	$7908 (20%)		$31631 (80%)
John Nell	$76500 (90%)		$8500 (10%)
Ben Perkins	$38000 (100%)		
Temp Staff Costs			
Ian Diemar	$4880 (16.7%)		$9760 (33.3%)
Overtime ($10000)	$3333 (33.3%)		$6667 (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%)	$2000 (5%)	$7000 (70%)
Hatchery Operating ($31500)	($7875) (25%)	$3150 (10%)	$22050 (70%)
Rent on $2million capital facilities and equipment @10%pa	$50 000 (25%)	$10 000 (5%)	$140000 (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)			