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Project 1997/321

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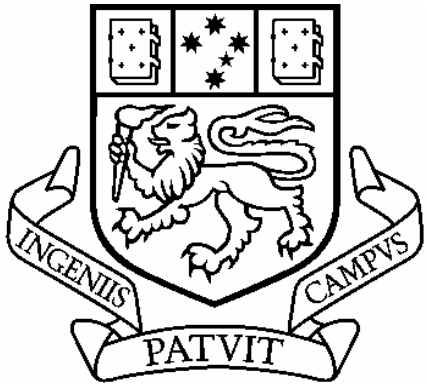
Dr. G. B. Maguire



UNIVERSITY OF TASMANIA

Australian Government

Fisheries Research and Development Corporation

Project No. 1997/321

Selective Breeding of Pacific Oysters

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Non-technical Summary

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Objectives (in combination with Aquaculture CRC Project D1)

1. To evaluate groups of Pacific oysters mass selected for growth rate.
2. To establish family groups that allow combinations of desirable characteristics to be selected.
3. To establish how these selected groups perform in different areas and production systems.
4. To ensure that improvements are sustainable by undertaking molecular genetic analysis of the progeny.
5. To ensure that the benefits of the molecular genetics "revolution" e.g. DNA markers for performance, can be adopted within an industry breeding plan based on separate breeding lines.
6. Overall, to improve profitability and production in the Pacific oyster farming industries on a genetically sustainable basis.

<u>Outputs relative to Objectives</u>

Objective #1: The project produced 2 generations of mass selected oysters with (up to) four mass selection lines per generation that grew faster than commercial controls. At market size in their second generation they were 12.5% greater in whole weight than the commercial controls.

Objective #2: Traits such as growth rate, meat weight, shape, sex ratio, colouration and frill all showed significant segregation between family lines. The second and third generation of family lines used a variety of techniques to "fix" or combine desirable traits including inbreeding, backcrossing and outcrossing. Preliminary indications are that these approaches were highly successful.

Objective #3. In general all control, family and mass selection lines performed better on intertidal sites relative to the one site selected for subtidal grow-out. In general, all control, family and mass selection lines grew faster on the South Australian sites than in Tasmania. In general, high performing lines were consistently high performing regardless of site or production system. For example ranking by mean weight at market size (~ 2 years) produced a similar order for all families from all 4 intertidal sites. A few family lines performed considerably better in subtidal grow-out than they did in intertidal grow-out.

Objective #5. Oysters from all lines were available to the CRC Aquaculture project D1 for the purpose of developing genetic markers. Progeny were genetically tested to confirm parentage. Potential broodstock for the second generation of mass selection were genotyped in an effort to select those with the greatest genetic diversity. Genetic markers were used to assess potential for improvement in growth.

Objective #6. On a trial basis in 2000 the project supplied broodstock to a commercial hatchery. The hatchery produced about 5 million seed oysters in two batches. One batch was from inbred family lines considered free from the undesirable shell characteristic (called by industry "curl back"), and the second line derived from the best performing first generation of family lines. These oysters are now on commercial farms and form the first commercial scale trial of the selected oysters. In 2001 expressions of interest were received from Shellfish Culture Ltd of Bicheno (Tasmania), Geordy River Aquaculture of St. Helens (Tasmania), Camerons of Tasmania, and the South Australian Oyster Hatchery. All 4 hatcheries were sent some broodstock in 2001 but only one hatchery produced commercial quantities of juveniles. These were eventually sold, their performance was followed and reported on in Ward et al. (2005). The formation of

a joint venture company (Australian Seafood Industries) by the two oyster growing bodies (Tasmanian Oyster Research Council and South Australian Oyster Growers Association), The University of Tasmania, CSIRO and FRDC) dedicated to the commercialisation of this science should provide for continued adoption of the improved selected lines by industry. Further details of the joint venture operation and commercial trials can be found in Ward et al. (2005).

Acknowledgments

The principal investigators would like to acknowledge support from FRDC, the Aquaculture CRC and the University of Tasmania. Very significant additional support was provided by the Tasmanian and South Australian oyster industries (through the Tasmanian Oyster Research Council [TORC] and the South Australian Oyster Growers Association [SAOGA]) and a number of individual growers (Poke, Zippel, Sumner, Whillas, Murdoch and Rainer). The Aquaculture CRC and CSIRO Marine Research provided additional support through project D1 (Oyster Genetics). The main individuals from these organizations that contributed to the success of this project are recognized below.

- Greg Kent and Mat Willis (University of Tasmania, School of Aquaculture) were senior technicians at the University of Tasmania (UTas) who assisted with all hatchery and farm operations.
- Dan McGoldrick while a CRC-Aqua supported post-doc at CSIRO-UTas was responsible for much of the molecular genetic work including construction of marker and QTL maps, and estimates of heritability.
- Bronwyn Innes (CSIRO Marine Research) assisted with the production of the new lines of oysters, rearing of the juvenile oysters and carried out much of the microsatellite and AFLP laboratory work in CRC Aqua-D1.
- Bob Ward (CSIRO Marine Research) was the Principal Investigator on CRC-Aqua Project D1 and co-supervised Dan McGoldrick.
- John Benzie (AIMS) as Genetics Program Manager at CRC-Aqua attended many meetings of the project and helped guide its progress.
- Cam McPhee (Queensland Department of Primary Industry) and Jordan Howarth (CSIRO Mathematics and Information Science) provided quantitative genetics advice.

Of course, the project would not have progressed without the continuing support of industry:

- We thank Michael Whillas as project co-investigator and in his capacity of Chair of SAOGA for his continuing support.
- Colin Sumner acted as Industry Liaison for the project and provided highly valued input into the project over its duration.
- Barry Ryan, as Chair of TORC, continued to support the on-going progress of the work.
- Jon Poke kindly permitted the use of significant areas of his oyster lease for nursery growth and was also one of the five grow-out farms used by the project. We also thank the other four oyster farmers who provided space: Di Murdoch, Sebastian Rainer, Mike Whillas and the Zippel brothers (Bruce, Gary and Ashley).
- Hatchery space and advice was provided by Marine Shellfish Hatchery (Miles Cropp) in the project's early stages, and later by Shellfish Culture (Martin John, Rod Kschammer).

Abbreviations and terminology

ABARE	Australian Bureau of Agricultural and Resource Economics
AIMS	Australian Institute of Marine Science
ASI	Australian Seafood Industries
CRC	Cooperative Research Centre
CRC-Aqua	Cooperative Research Centre for Aquaculture
CSIRO	Commonwealth Scientific and Industrial Research Organization
FRDC	Fisheries Research and Development Corporation

grow-out phase	commercial conditions as oysters grow from ~ 6 mm to market size
half sib	family produced from one ♂male sibling separately mated with two ♀ siblings or two ♂ siblings separately mated with one ♀.
MRL	Marine Research Laboratories (TAFI laboratories located in Taroona, Tasmania).
nursery phase	Location for growing oysters from ~ 1.7 mm to ~ 6 mm*; typically from several months to <1 year old*, sometimes these oysters are referred to as 'spat' or seed.
SAOGA	South Australia Oyster Growers Association
SAORC	South Australian Oyster Research Council
seed	oysters < 6 mm*
sib or full sib	family produced from sibling, or brother and sister mating
spat	oysters post metamorphism, > 400 μm but < 6 mm*
TAFI	Tasmanian Aquaculture and Fisheries Institute
TORC	Tasmanian Oyster Research Council
UTas	University of Tasmania

* dimensions and ages are approximate.

Background

It is estimated that at least thirty percent of the increase in land-based protein production has resulted from genetic improvement of agricultural products (Newkirk 1996). Yet in aquaculture today only about 1% of production is based on genetically improved fish and shellfish (Gjedrem 2000). In a few aquaculture industries significant improvements in production have been achieved with the application of selective breeding. In several large-scale experiments and in breeding programmes, 10–15% genetic change has been obtained per generation (Gjedrem 2000).

The Pacific oyster industry in Australia was initiated by the importation of oysters from Japan starting in 1948 (Thomson 1952). The first oysters were put into the wild at Pittwater, Tasmania. These individuals did not establish a viable naturalized population (Thomson 1959) and it was not until oysters were moved to the north of the State (Port Sorell) that naturalized populations became established. Early naturalized populations, mostly in the Tamar River, formed the basis of a small commercial harvest. Like some other naturalized Pacific oyster populations recruitment from year to year was quite variable making commercial harvest unreliable. Not until the development of hatchery based production of seed oysters did the Australian Pacific oyster industry significantly expand. Seed export to South Australia saw industry production expand once again with Pacific oysters reaching a market value of ~ $25 million per annum (ABARE 2001-2002).

In the case of the Pacific oyster industry in Australia almost the entire commercially marketed product is derived from the hatchery-based production of seed oysters (Brown et al. 1997). The fact that both the life cycle and the production are closed makes the industry well suited to gain the benefits of a selective breeding program (Ward et al. 2000). The relatively small number of hatcheries serving the industry (4 in 2001) and their highly efficient technology for hatchery rearing meant that production is consistent and well controlled. The hatcheries and the industry collaborate on setting research agenda through organizations like the Tasmanian Oyster Research Council (TORC) and

the South Australian Oyster Research Council (SAOGA). The activities supported by these research organisations are underwritten by a voluntary levy upon seed oysters produced by the hatcheries. The organized and highly co-operative nature of the industry combined with a sense of vision from the individual growers that hosted the experimental oysters made it possible for this project to succeed. Other aspects of the industry were also beneficial to the project. The fact that the oysters are normally raised in baskets of ~ 100 individuals provided a convenient replicate unit for a scientific experiment. Low rates of disease meant that survival to market size was a normal expectation (unlike the Sydney rock oyster). Given FRDC and industry support plus the industry traits indicated above it was relatively easy to establish a breeding program where the genetic control exercised in the hatchery could be tracked through to market sized oysters.

This project sought to select for genotypes that had phenotypic characteristics desired by industry. From previous CRC-Aquaculture research it was established that the Pacific oyster in Tasmania had sufficient genetic variability to provide the basis of a selective breeding project (English et al. 2001). Based on formal and informal surveys of the Tasmanian and South Australian oyster growers the traits they felt most likely to improve their profitability and, presumably, improve their market share were determined (full list is in Appendix #3). The three most desired traits were meat yield, total growth rate and shell shape (L-W-D). This project used a range of selective breeding techniques in an effort to improve these characteristics. We used mass selection (= individual selection), family selection (as applied in this project the term family selection is more properly termed ‘combined selection’ since the best individuals from a large familial cohort are used to produce the next generation) to selectively breed for specific characteristics.

While the standard approach to breeding livestock is to raise individuals of known pedigree, oyster biology makes this difficult. During early life history stages oysters are grown in groups and throughout their lives they compete for resources. For this reason the unit of replication is not usually the individual and practical oyster breeding is more similar to plant breeding. In our experiments the unit of replication was the bag of

oysters where each bag had the same number of individuals although the results are reported here as mean weight per oyster per genetic line. Our decision to use various types of selection was based on experience and research from other aquaculture and agriculture species. Additive genetic improvement is possible over the long term provided sufficient genetic diversity is maintained. Mass selection is commonly applied where the phenotypic trait of interest can be quantitatively assessed on living organisms. One advantage of working with oysters is the possibility of applying very high levels of selection due to their relatively high fecundity. It is also relatively easy to simultaneously strip spawn many individuals, to keep eggs or eggs and sperm separate so that equal representation is assured at the stage of fertilized eggs and then to rear a relatively large pool of genetic variability in a single management unit (e.g. tank, vessel, bag or basket). Using the genetic markers developed in the CRC-Aqua project D1 we trialled a novel approach to reducing inbreeding, the single largest problem of mass selection. Prior to the production of the second generation of mass selected oysters the largest and best shaped individuals from the first generation were selected. Their shells were drilled, a small tissue sample extracted and the holes sealed. The tissue samples were analysed and the DNA markers used to assess parentage. Thus it was possible to maintain maximum genetic diversity into the second generation of mass selected oysters. If this approach was fully implemented then mass selection becomes a version of combined selection, that should yield greater selection of desired heritable traits for a given amount of effort.

A number of other successful selective breeding programs in aquaculture have used half or full sibling families as the basis of their program; for example salmon in Norway (Gjedrem 2000) and Pacific oysters in North America (Hedgecock et al. 1997). This sort of family selection is considered superior for traits which cannot be quantitatively assessed on living organisms, where environmental variability is relatively high or heritability is low and when family sizes can be large. In oysters the effects of environmental variability on most traits are high and for traits such as meat weight, which can only be assessed by killing the animal, there is relatively little choice in strategies. It is also much easier to have large family sizes in oysters than many other species of livestock. Using half sib or full sib breeding plans means that family

selection can also be used to estimate trait heritability. Establishing the relative degree of heritability was an important goal of the combined projects FRDC 1997/321 and CRC Aquaculture project D1. Some degree of deliberate within-family breeding (inbreeding) was trialled in an effort to "fix" particular traits prior to outbreeding and thus potentially obtain both hybrid vigour and other superior characteristics (Hedgecock et al. 1995). Family selection has the added advantage over mass selection in that it can be used to combine desirable traits.

Every effort was made to ensure that the scientific outputs from FRDC 1997/321 would be directly relevant to the commercial situation. As far as possible, all experimental oysters were treated as though they were commercial product. The performance of experimental oysters was assessed at five different oyster-growing regions spread across two States. Experimental oysters were grown at four widely spaced sites intertidally across two States (relevant to most of the industry) and at one site subtidally. As a general rule the project used the same type of equipment as used by the oyster growers in that region. At the intertidal sites a range of grow-out methods were used; mesh bags, "pillow bags" and several types of baskets. During on-farm grow-out the project team quantified characteristics such as growth rate, weight, meat weight and shell shape of the various selected lines and controls while documenting variation in other characteristics.

Need

Interest in the genetics of the Pacific oyster in Tasmania arose from concern over the possibility of bottlenecks in population size occurring during the naturalization process and potentially reducing the genetic variability of the available stocks. Considerable work had been conducted on this issue by Bob Ward (CSIRO Marine Research) and Louise English (PhD student at UTas) with the conclusion that genetic variability was similar to that seen in parental Japanese stocks. Given the presence of sufficient genetic variability, the oyster industry was interested in a program to selectively breed a "better" oyster. The first step in the breeding program was to formally survey all Tasmanian oyster growers for the traits they considered "better" in an oyster. The assumption being that the survey would reveal what oyster traits would make the industry more competitive and more profitable. This survey was conducted by the Tasmanian Oyster Research Council and listed meat weight, total growth rate and shell shape (L-W-D) (Appendix 3) as the top three desirable characteristics. Growers in South Australia were never formally surveyed although they provided considerable informal advice through the South Australian Oyster Growers Association and the South Australian Oyster Research Council. The traits identified as "better" were then used as the basic criteria to judge any improvement achieved by selective breeding. Given the range of traits identified as desirable it was recognized that a range of selective breeding strategies might be necessary, with some approaches likely to be more successful than others in terms of improvement of particular traits or combinations of traits. Therefore selective breeding using mass selection, between-family selection and individual selection was initiated. The merits of these approaches are reviewed by Rishell (1997) and it is suggested all these techniques are needed to maximize the ongoing benefits of a selection program to the oyster industry.

The Tasmanian and South Australian oyster industries are both successful and growing industries. Like most successful industries they are searching for methods that will allow them to expand their market share and improve their international competitiveness. Genetic improvement of the breeding stock is a proven method of improving overall farm performance in both terrestrial and aquatic crops. A number of

selective breeding programs for table oysters have recently become established around the world. In terms of the heavy involvement with industry, size, scope and approach perhaps the most comparable to FRDC 1997/321 is the Molluscan Broodstock Program (MBP) found on the west coast of the United States (Hedgecock et al. 1997). Although Australian and American oyster growers compete in only a few international markets (mostly in Asia) there can be no doubt that this sort of technology will be increasingly important for any domesticated product. More recently the French and the New Zealanders have embarked upon selective breeding programs for a variety of shellfish. It seems reasonable to assume that the Australian industry will need a breeding program to ensure long term sustainability of their industry.

It is also clear that the benefits of selective breeding will be most evident in industries where the "seeds" are from domesticated brood stock. In the rapidly developing field of aquaculture many of the broodstock and even the production animals are still collected directly from the sea. The fact that the Tasmanian and South Australian Pacific oyster industry is dependent upon hatchery production makes them more capable of gaining the maximum benefit from a selection program. The reliance of the Australian Pacific oyster industry upon hatchery-reared seed oysters also makes them reliant upon good genetic husbandry by the existing hatcheries. Concern over an apparent increase in shell deformity within the existing industry was an important factor in the industry's desire to bring greater quantification to the ongoing process of broodstock selection by the commercial oyster producers.

The Australian oyster hatcheries produce tens of millions of oysters each year. They typically do this in a relatively small number of very large batches. This production technique is not very amenable to the production of a small number of experimental oysters in a large number of separate groups (an essential feature of a selective breeding program based on family selection). It is also true that some of these experimental groups (families), produced early in a selective breeding program are not likely to be suitable for commercial production. In fact some crosses are, by necessity, inbred to test for recessive traits. To assess oyster performance these experimental oysters need to be grown to ~ 2 years of age but there are not likely to be any hatcheries will produce

or oyster growers willing to hold millions of under performing oysters for 2 years. To be successful the selective breeding project needed a small-scale hatchery production system and a grow-out system capable of keeping the various genetic lines separate. Thus the team involved with FRDC project 1997/321, working in collaboration with existing hatcheries, developed suitable small scale culture facilities for the simultaneous production of ~ 50 separate batches of oysters. Now tested and proven these systems are available to industry for the longer term propagation of the broodstock lines desired for commercialisation.

One of the lasting benefits of FRDC project 1997/321 was the creation of a number of lines of oysters with known pedigrees. Some of these lines were tested for genetic markers (by the CRC-Aquaculture project D1). These lines of oysters are already forming the basis for large scale commercialisation of the results from FRDC 97/321.

Objectives

1. To evaluate groups of Pacific oysters mass selected for growth rate.
2. To establish family groups to allow combinations of desirable characteristics to be selected.
3. To establish how these selected groups perform in different areas and production systems.
4. To ensure that improvements are sustainable by undertaking molecular genetic analysis of the progeny.
5. To ensure that the benefits of the molecular genetics "revolution" e.g. DNA markers for performance, can be adopted within an industry breeding plan based on separate breeding lines.
6. Overall, to improve profitability and production in the Pacific oyster farming industries on a genetically sustainable basis.

Methods

The project used two complementary techniques to select for the traits identified by industry as important. The two techniques are mass selection and combined selection (family + individual selection). The overall approach to producing both mass and family selected lines was to alternate production from (mostly) one type one year to the other in the next. This allowed the newly produced lines ~ 2 years to mature and be assessed prior to selection as broodstock for subsequent generations (Fig. 1).

Early mass selection

The general approach was to use a large number of broodstock, select for rapid growth in the progeny and maintain as much genetic diversity as possible. Mass selection commenced in 1996-97 prior to receiving financial support from FRDC for 1997/321. Initially we used oysters from 2 same-age cohorts produced in a previous FRDC project (1993/151) on triploids to provide diploid parents for the first mass selection lines (M1). About 70 oysters from the upper 20% of the overall size distribution were selected to be the broodstock for the "fast" M1 line. Similarly about 70 oysters from the lower 20% of the size distribution were selected to create the first generation of the "slow" line (M1-slow). These were "audited" for heterozygosity and presence of some known alleles. A commercial "control" was established after consultation with industry. Hatchery work was conducted in a commercial hatchery. The control line (comm. control) was spawned from broodstock oysters that commercial hatchery had selected for their own commercial production.

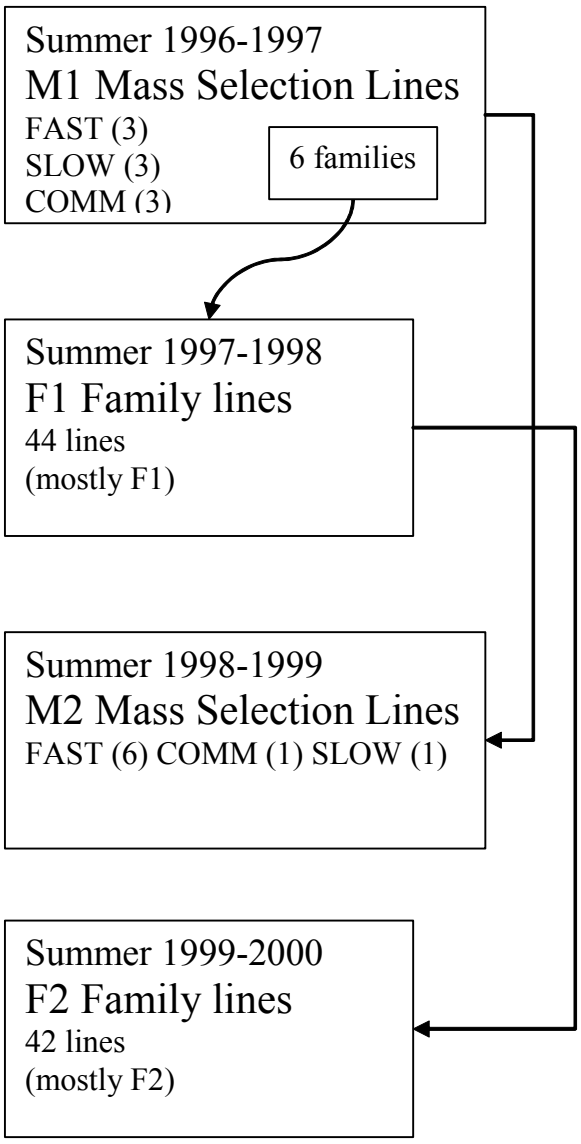

Figure 1. A schematic of the multi-year process of producing various mass and family lines of oysters in FRDC 1997/321. For example, in the summer 1998-1999 the second generation of mass selected oyster lines (M2) was produced. This consisted of 6 fast lines, 1 commercial control line and 1 slow line. See text for additional details.

Initial family lines

Six family lines (i.e. each line with 2 parents) were created in 1996/97 to provide experimental oysters for the related CRC-Aqua project D1 (Oyster genetics-Leader Bob Ward, CSIRO Marine Research). In the same year the first mass selected lines were produced.

It was desirable to start as many lines at the same time as was possible for a number of logistic and scientific reasons. With the young oysters used (1, 2 or 3 years old), the frequency of reproductive females is low. Unplanned or inadvertent matings need to be completely avoided. These considerations meant that strip spawning was much more feasible than induction so all broodstock were strip spawned throughout the project. To produce subsequent generations (i.e. M2 or F2) large numbers (10s to 100s) of oysters were selected as potential broodstock to ensure sufficient numbers of reproductive males and females would be available for breeding purposes.

In 1996-97, for each of the three mass selected lines produced, eggs from all females were mixed and fertilized with sperm from all males. After successful fertilization was observed the fertilized eggs were randomly allocated to three 140-litre tanks (3 lines x 3 replicate tanks = 9). Larvae were fed every day according to the regime of Breeze and Malouf (1975). Tanks were drained and refilled every second day. After each water change all tanks generally received ≈30,000 cells mL^{-1} once per day for larvae 1-6 days old rising to ≈100,000 cells mL^{-1} twice a day as the larvae reached metamorphosis. Some minor adjustments in the amount of phytoplankton added were made if the larval oysters in a particular tank did not clear the water of food prior to the next feeding interval. In general subsamples of larvae were examined under the microscope every second day and their size, density and condition recorded. When larvae reached sufficient size and showed signs of setting (undergoing metamorphosis: i.e. crawling or rafting of pediveligers) they were transferred to downwelling, screened “pots”. Larvae were treated with epinephrine (Coon et al 1985) and set onto finely ground scallop shell to produce single set oysters (typical of the Australian Pacific oyster industry).

The eyed larvae were settled as single seed starting on March 9, 1997and moved to the Tasmania Aquaculture and Fisheries Marine Research Laboratory (TAFI MRL) on March 25, 1997 where they were maintained in upwellers until all individuals were large enough to be retained on an 1800 µm screen. Oysters were moved from TAFI-MRL to a sea-based nursery (Fig 2) at Bolduans Bay Oysters (in Duck Bay, Smithton, Tasmania) on the May 26, 1997 and placed in 1.7 mm seed trays. Oysters were periodically graded to maintain stable densities (per m^2) in appropriate gear (1.7 mm and 3 mm seed trays, 6 and 12 mm soft mesh bags). On March 4, 1998 the various sized oysters within each of the triplicate lines were gathered together from their various trays, pooled and regraded into 6 size groups (10-15; 15-20; 20-25; 25-30; 30-40; +40 mm). From these different size groups of oysters the 25 – 50 percentile and 75 – 100 percentile size groups were created by counting the numbers in each measured size category and recombining the appropriate numbers into these 2 new percentile grades, hereafter termed the 25 and 75 grades. These size- fractioned oysters were bagged and randomly allocated to five different oyster-growing regions across Tasmania and South Australia (Fig. 3). At each of these five grow-out sites we initially placed 3 to 6 replicate bags (300 to 600 oysters) from each of the three initial larval replicates and each line (Fig. 2). Thus the maximum number of bags per line was 3 replicates * 2 size grades * 6 bags * 5 sites = 180 with 100 oysters in each or 18,000 oysters per line. Oysters were grown until they reached market size. Checks were made on the oysters about every 3 months to assess size (used to calculate growth rate), and number surviving (used to calculate mortality). A sacrificial sample of 10 randomly selected oysters per replicate was also undertaken on the final two quarterly samples to ascertain additional parameters including dry condition index, wet and dry meat weight and shell morphology. From October/November 1998, data loggers and water filtering kits were distributed to farms to help measure some of the environmental parameters believed to influence oyster growth.

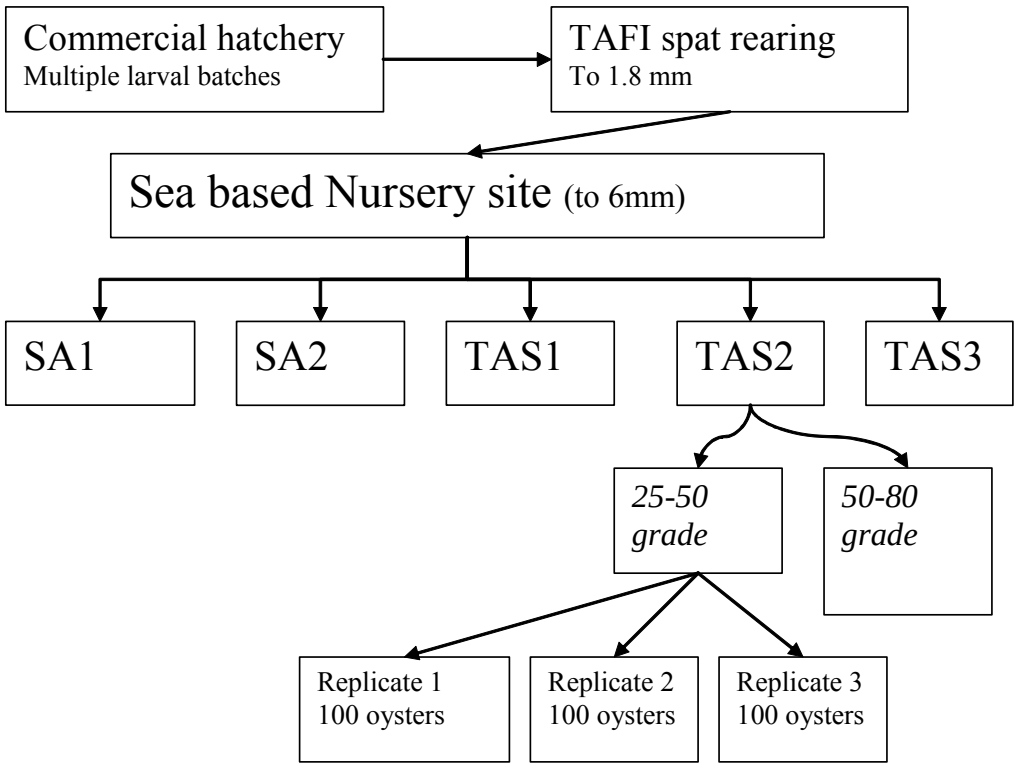

Figure 2. Schematic of the production, movement and allocation of experimental oysters produced by the FRDC Project 1997/321. The process was repeated each year. Most lines had two size grades and up to 6 replicates per line at each site (Tas1, Tas2, Tas3, SA1, SA2).

Family selection in 1997-1998

Family line production was expanded in 1997/98 with the successful production of 44 family lines. Most of these were first generation lines (F1s), but a few were second generation lines based upon the 6 lines produced in 1996/97.

Production of the second generation mass selected oysters

Production techniques similar to those above were used to produce the second generation mass selected and family lines. During November 1998 we selected ~130 of the largest animals from the previous mass selection lines (M1-FAST). These were predrilled and some tissue removed prior to broodstock conditioning. The tissue was used to determine the degree of genetic similarity between the individuals selected. The goal of this work was to maximize the genetic diversity within our second-generation mass selection line (M2). The genetic diversity was assessed by the CSIRO team led by Dr. Bob Ward (from the related CRC-Aqua project D1). After sampling, the animals were placed into a trough with flow-through water supplemented with additional algal food, usually a mixture of the phytoplankton available from the hatchery, including *Isochrysis galbana* (Tahitian strain), various diatoms (*Chaetoceros muelleri*, *Chaetocerous calcitrans, Thalasiosira pseudonana*) and *Pavlova lutheri*; to condition them for breeding.

During the last week of December 1998 and during January-February 1999 we used the hatchery facilities of Marine Culture Pty Ltd at Bicheno on the east coast of Tasmania. We set up ten 140 litre tanks for oyster propagation. The 47 individuals identified as having a 100% probability of being progeny of the M1-FAST parents were opened and their sex confirmed. These yielded 20 females and 27 males. They were randomly assigned to 2 groups (replicates) and eggs + sperm mixed to produce larvae for one new line M2-FAST1. The remaining 80+ animals were opened, sexed and mixed 50:50 with additional sperm and eggs from the first mass spawn and used to create another replicated line with ~ 130 parents (M2-FAST2). Finally the remaining eggs and sperm from these two lines were mixed 1/3, 1/3 and 1/3 with eggs and sperm from 43 (20 male, 23 female) oysters selected from the fastest growing family lines (1 year old

oysters). These were used to produce the third mass selection line (M2-FAST3), also in duplicate.

We also produced a control cross consisting of 20 broodstock oysters that the hatchery had on hand for their own commercial production breeding program. These were mass spawned and then split into three groups (i.e. considered to be replicated 3 times). To complete the mass selection lines we also produced a SLOW line. Although a second generation SLOW line was not required by our FRDC project we had noticed that the previous SLOW lines tended to have better condition than FAST lines (at least at certain times of the year). Given that industry has a strong interest in meat weight as well as fast overall growth it was decided to propagate another generation of this line. In an effort to gain faster growth and improved condition we selected the faster growing individuals from the previous SLOW line and spawned 20 of these (7 males and 13 females) to continue the line (M2-SLOW).

Also in the hatchery during the 1998-1999 season we initiated 20 lines of tetraploids by making multiple crossings of: 4n male x 4n female, 4n male x 2n female and 4n female x 2n male. There were also 10 smaller test crosses (as desired by the CRC-Aqua D1 genetics group: Bob Ward, Dan McGoldrick and Bronwyn Innes) which were grown in either 8 litre plastic bags or 140 litre tanks. These latter crosses reflect the close association of the two research projects (FRDC 97/321 and CRC-Aqua D1) and the need to work jointly to advance the knowledge base useful to this industry.

Site selection

Sites were selected to represent the major Pacific oyster growing areas (in 1996). Four sites were intertidal and one sub tidal representing the two main grow-out strategies. Two of the sites are in South Australia (Coffin Bay and Smoky Bay) while three (Fig. 3) are in Tasmania (Bolduans Bay, Coles Bay [sub tidal site], and Pittwater Lagoon). Once on these farm sites the oysters were managed by the commercial oyster growers in terms of general location on their site and keeping project staff informed about the need to thin and restock the oysters into the various types of gear used. Intertidally grown

oysters were kept in mesh bags when young and various size mesh baskets as they grew. At the time of initial placement and quarterly following data collection for performance assessments the bags (or baskets) of oysters were randomly placed onto the available rack. At any one intertidal site all of the oysters were kept at the same height and in close proximity to each other. At the subtidal site oysters were randomly allocated to two stacks of ~ 10 trays and replicates would have experienced some difference in mean height. At the subtidal site one stack was lost and not recovered leading to a loss of data from some lines. At other sites occasional baskets were also lost for a variety of reasons, mostly due to storms. For most of the grow-out period and at most sites the oyster growers donated the space and equipment needed to grow the experimental oysters and the time necessary to help manage them. Support from growers included the supply of boats and people to assist in the management of the oysters on their site. The use of widespread sites allows the project to measure genetic and environmental (GxE) interactions and ensured the selection program could choose lines that were superior in their performance across the entire industry.

Fig. 3. Map of southeast Australia showing approximate locations (●) of the five grow-out sites used in FRDC 1997/321.

In this report there are data that could be used to infer relative or absolute levels of productivity at the sites used for oyster grow-out. These sites are only identified as SA1, SA2, TAS1, TAS2, and TAS3 due to the possibility that this information could be commercially sensitive.

Commercial scale trials

In 2000 the oyster industry was very keen to trial a commercial scale production of the improved lines. At the time, project 1997/321 had just produced the F2 families and could not yet provide any indication to industry on how the available F1 families would perform when crossed. In spite of this high level of uncertainty the industry undertook a commercial scale trial.

The trial involved producing 2 lines from certain F1 broodstock. The F1 families were selected to offer a certain shell morphology (lack of "curlback") and, potentially, rapid growth (broodstock were sourced from the 4 best performing F1 families). These were spawned in commercial quantities in Feb 2000 under the guidance of project staff (Mr. Greg Kent).

Results

The first oyster lines for FRDC 1997/321 were produced in February 1997 at Marine Shellfish Hatchery (Bicheno, Tasmania). Three replicate cultures of each SLOW, FAST, commercial control (COMM) were successfully created and grown through larval phase (3 treatments * 3 replicates = 9 cultures). The results pertaining to these lines were reported under the Jan 1, 1999 milestone (below). Six family lines were also created during this first spawning season.

The milestone for Jan 1 1999 was: "Complete 2 year growth trial of Generation M1 for mass selection trial".

As discussed above, on February 19, 1997 approximately 200 oysters were stripped spawned to initiate the three lines. The eyed larvae were settled as single seed starting on March 9, 1997 and moved to the Tasmania Aquaculture and Fisheries Marine Research Laboratory (TAFI- MRL) on March 25, /1997. Oysters were moved from TAFI-MRL to a sea-based nursery at Bolduans Bay Oysters (in Duck Bay, Smithton, Tasmania) on the May 26, 1997 and placed in 1.7 mm seed trays. In early March 1998 the seed oysters were graded (as above) into two size categories (25 and 75); from the two treatments (FAST & SLOW) and a commercial control (COMM) yielding 6 categories (FAST75, SLOW75, COMM75 and FAST25, SLOW25 and COMM25). Within grade and treatment these were randomly allocated to batches of 100 and distributed to three Tasmanian and two South Australian farms for on-growing. Data collected in March 1998 just prior to distribution showed that there was no significant difference associated with the allocation process (Table 1). Each farm site received oysters of the same mean size (~ 6 g) with no significant differences across sites (Table 2). Thus there was no bias associated with the process used to select batches of 100 oysters or allocate them to each site.

Table 1. Initial weights (g) for all size grades as of March 12, 1998 just prior to distribution from nursery to grow-out.

Site	Mean weight (g)	Standard error of the mean
Smithton	6.161	0.203
Coles Bay	5.828	0.204
Smokey Bay	6.422	0.229
Pittwater	6.045	0.202
Coffin Bay	6.405	0.229

Table 2. Two way ANOVA for initial weight by site and by line (includes grade).

Source of Variation	DF	SS	MS	F	P
line	5	1394	278.9	202.8	<0.001
site	4	7.434	1.859	1.351	0.254
Interaction	20	16.21	0.811	0.589	0.915
Residual	137	188.4	1.375		
Total	166	1626	9.795		

Bonferroni *t*-tests were used to determine significant differences between lines and grades. In terms of the FRDC 1997/321 Project's objectives probably the most important observation was that COMM75s and FAST75s were not significantly different in initial weight (P = 0.286) with the COMM75s slightly, albeit not significantly, heavier (9.786 versus 9.101 g). Also, the initial COMM25s were not significantly different (P = 1.00) from the FAST25s at 4.189 and 4.222 g, respectively. Their initial similarity in weight indicates that final weight is a suitable measure of performance over the grow-out phase for the first generation of mass selected oysters.

At the end of grow-out phase in terms of wet weight, the FAST lines performed moderately better (P<0.0017) than commercial controls (Fig. 4) and better than the

SLOW lines (P<0.0001). The FAST advantage over controls was 8.3% in the 75 grade and 14% in the 25 grade in terms of wet weight. The estimate of the FAST75 advantage was artificially lowered somewhat by the removal of the largest Fast 75 oysters in December 1998, from one of the Tasmanian sites, for use as broodstock in the production of a second generation mass selection fast line.

Growth rates were estimated over the entire grow-out period from March 1998 to February 1999 in the units of grams per day. Over this period growth rates were greatest for the FAST 75 line and lowest for the SLOW25 (Table 3). Analysis by 2 way (site and line) ANOVA showed significant effects for line (genetics) and site (environment) and a significant interaction between site and line (GxE, Table 4). The site effect was the largest as variation in growth across sites was substantial. Genetic differences included significantly faster growth (Bonferroni *t*-tests) for FAST75 relative to COMM75 (P = 0.014) and FAST25 grew faster than COMM25 (P = 0.007).

Table 3. Growth rates over grow-out period for mass selected oysters and commercial control in two size grades.

Line and Grade	Mean (g/day)	SEM
FAST 75	0.139	0.00292
FAST 25	0.124	0.00279
COMM 75	0.124	0.00391
SLOW 75	0.114	0.00287
SLOW 25	0.0961	0.00277
COMM 25	0.106	0.00462

Table 4. Two way ANOVA for the effects of site (oyster farm) verus line on the growth rate of mass selected oysters over the period from approximately March 1998 to February 1999.

Source of Variation	DF	SS	MS	F	P
Line	5	0.0295	0.00589	25.7	<0.001
Site	4	0.172	0.0431	188.4	<0.001
interaction	20	0.00959	0.000480	2.09	0.008
Residual	121	0.0277	0.000229		
Total	150	0.272	0.00181		

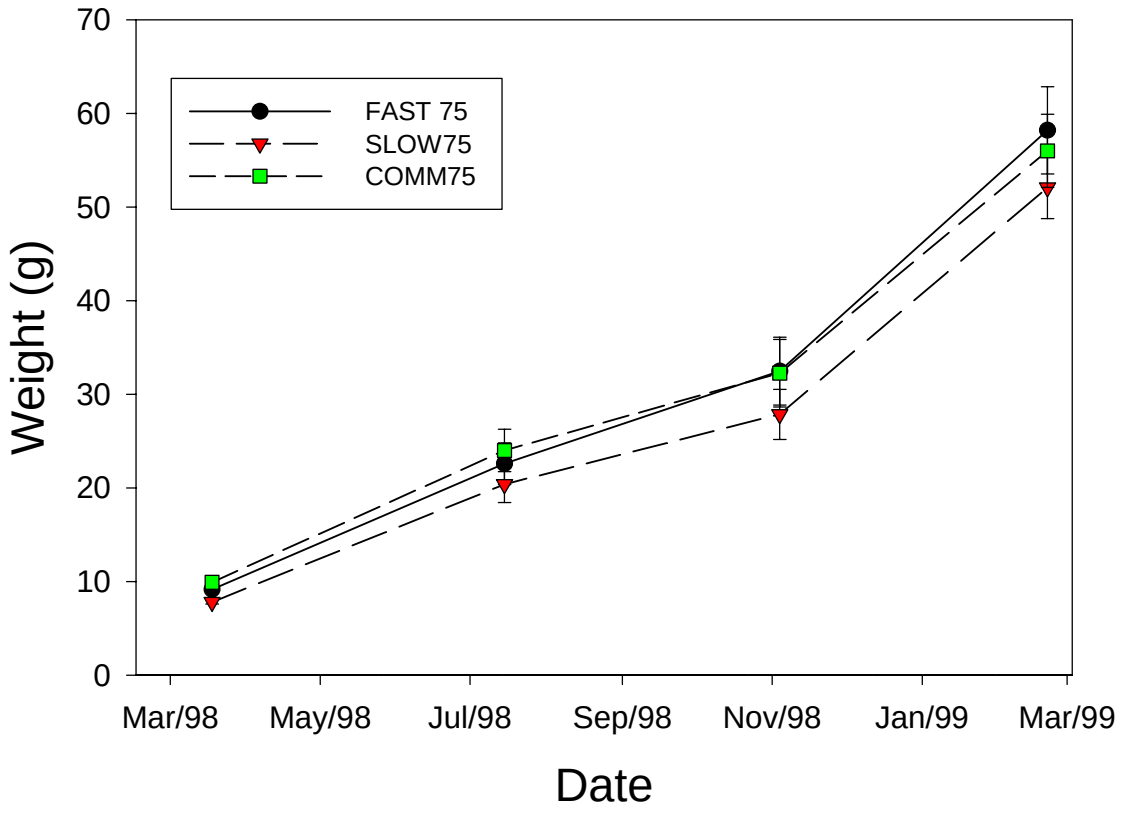

Fig. 4. Wet weight of whole oyster versus time for 3 different genetic lines of oysters. Means and standard deviations are shown, data are pooled for site.

Within size grades but pooled across farms, mean weight increase over one year (February 1998 - February 1999) varied from 33.9g for SLOW25, to 53.2g for the FAST75 line & grade (Figure 5).

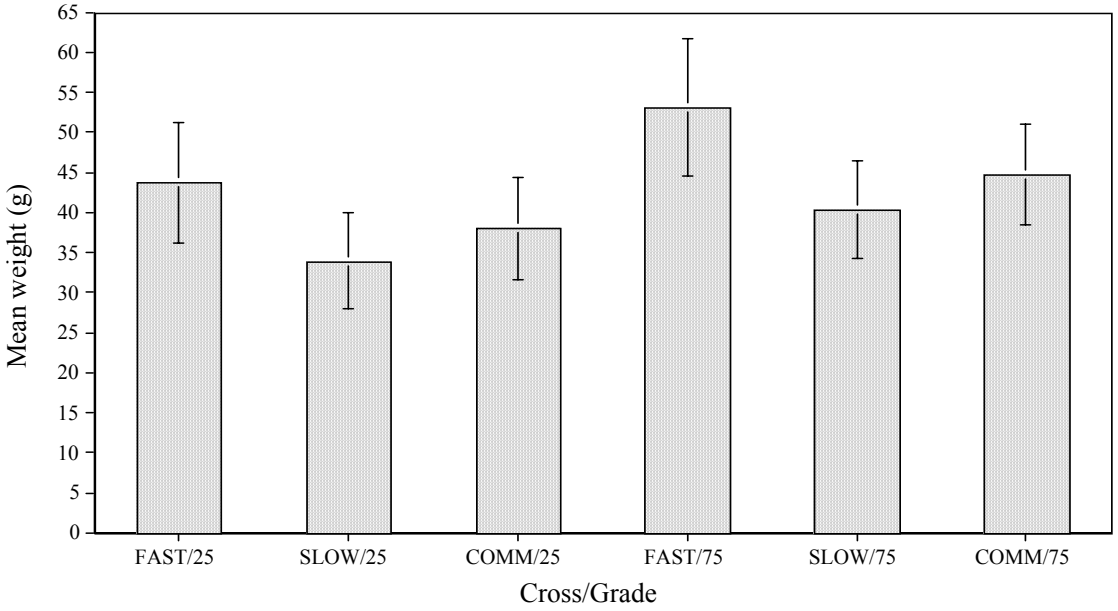

Figure 5. Mean weight increase over 12 months, of 6 groups of mass selected Pacific oysters (3 lines x 2 grades). Sites pooled. Bars are mean ± se (n=5).

Samples for total weight, shell weight, wet and dry meat weight were taken in November 1998. Ten randomly selected oysters per replicate were selected (maximum number ~ 270) and the mean of each set of ten observations was compared using a 2 way ANOVA (site by line & grade). Where ANOVA showed a significant difference the analysis was followed by Bonferroni *t*-tests to investigate the groups that were statistically different. For wet meat weight, dry meat weight and condition index COMM75 were not significantly different from FAST75 (Table 5).

Table 5. Oyster measurements November 1998 on first generation mass selected oysters and commercial control in two size grades, means and (standard errors of the means).

Line & Grade	Wet meat weight (g)	Dry meat weight (g)	Condition index total weight/(total weight-shell weight)
FAST 75	4.374 (0.212)	0.627 (0.0304)	37.845 (1.326)
FAST 25	3.328 (0.202)	0.463 (0.0319)	36.592 (1.390)
COMM 75	4.454 (0.212)	0.667 (0.0319)	42.669 (1.390)
SLOW 75	4.283 (0.202)	0.641 (0.0304)	45.258 (1.326)
SLOW 25	2.505 (0.202)	0.376 (0.0304)	42.624 (1.326)
COMM 25	2.945 (0.222)	0.445 (0.0333)	41.232 (1.452)

To a certain extent the differences between lines (8 – 20%) was masked by the larger differences in growth (up to 260%) that were observed between sites (Fig. 6, Table 6). When the data were separated by size grade and line it was very evident that site differences were substantial (Table 6, Fig. 6). Site (farm) differences were largely consistent across lines: e.g. a line that grew relatively slowly on one site tended to show similarly slow growth across all sites (Fig. 6). In Tasmania, growth appeared to be strongly correlated with chlorophyll *a* concentrations (a measure of algal densities), however this has not proven to be the case for the South Australian sites (data not shown). Subsequent work on this issue has shown winter growth in Tasmania is largely explained by temperature and chlorophyll *a* in the water (Lynch 2000).

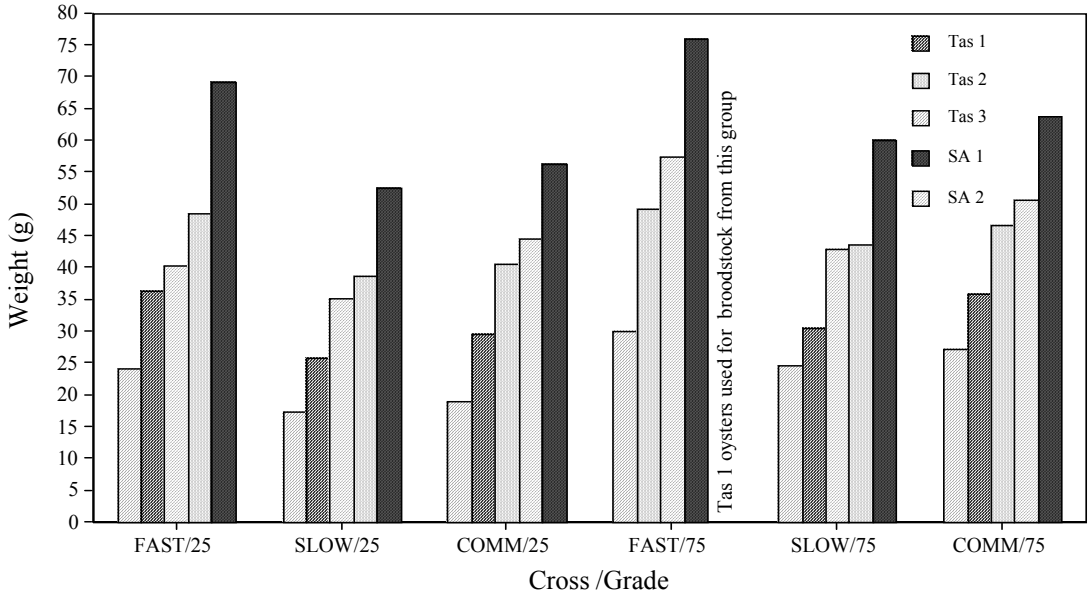

Figure 6. Mean weight increase (initial – final) over 12 months, of 6 groups of mass selected Pacific from oysters from 5 sites.

As site differences were the largest factor in determining oyster growth rates and these differences were largely consistent it is possible to pool the data over size and grade to examine the magnitude of the site effects. When considered over both size grades and all lines, some sites outperformed others (Table 6) with significant differences (Bonferroni *t*-tests) between the nominated control site (TAS 2) and all other sites. Growth was not temporally uniform across all sites, some sites enjoyed more substantial winter growth (e.g. SA 1, Fig. 7), while others had greater growth in summer (TAS 3, data not shown).

Table 6. Growth rates over entire grow-out period for mass selected oysters at different sites and pooled over size grades and lines.

Site	Mean (g/day)	SEM
SA 1	0.176	0.00296
SA 2	0.126	0.00384
TAS 3	0.128	0.00260
TAS 2	0.0900	0.00262
TAS 1	0.0671	0.00328

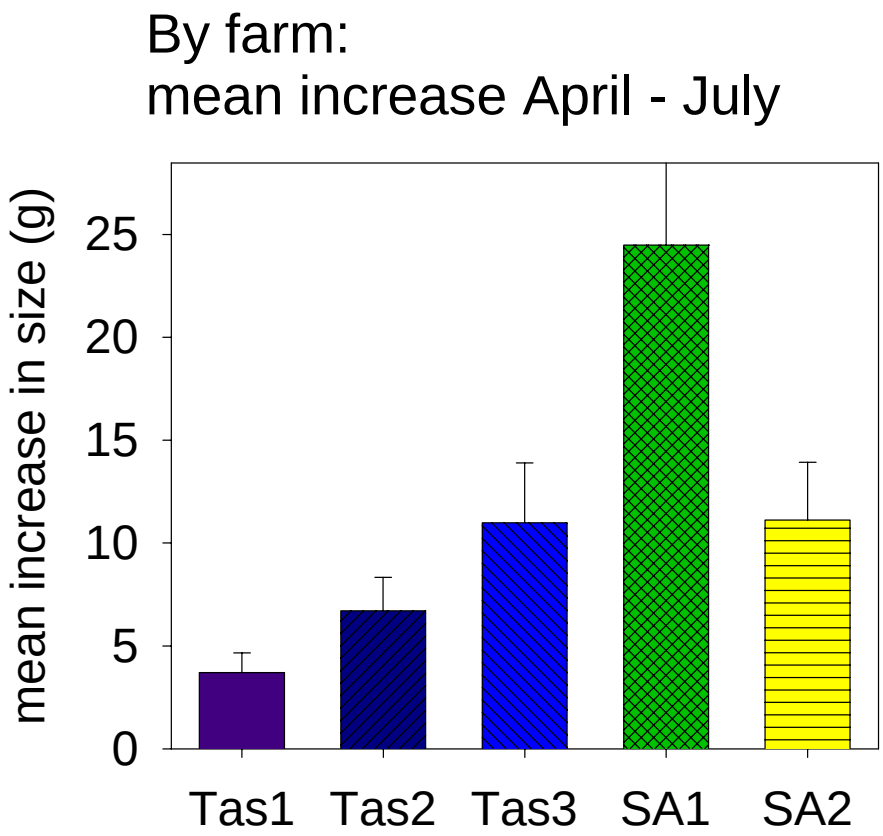

Fig. 7. Increase in mean size for all 'lines' and both size grades over the period April to November 1998 at each farm (mean ± SE).

The milestone for July 1998 was: "Complete spat production for 40 family groups (Generation F1)".

The 40+ F1 family lines spawned in February 1998 were held in upwellers at the University of Tasmania, Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratories (TAFI-MRL) until April 1998 when the individual oysters were all greater than 1.7 mm. Of these lines, 7 were in their second generation of selection (Table 6) based upon the 6 families spawned the previous year), but the grouping of family lines produced in 1997-1998 are referred to as F1. Once the oysters exceeded 1.7 mm they were transferred to nursery phase at a commercial farm (Bolduans Bay Oysters).

At Bolduans Bay the seed oysters were graded over 6000, 4000, 3360 and 2800 μm screens to give 5 groups. The mean weight of 100 oysters in each size fraction was determined and used to estimate the total number of oysters being held in each line. The oysters in each family line were then pooled and randomly thinned to approximately 5000 per family line on May 26th 1998. The various family lines and their numbers are listed in Table 7.

Table 7. Families lines held at Bolduans Bay Oysters in July 1998. Families were graded on 26-5-98 and ~ 5000 in each line was kept for the Project's needs.

		Retained	Discarded
1	(Y)A018	5000	23000
2	(Y)A013	5000	56000
3	(Y)A016	5000	37000
4	(R)B029	5000	29000
5	(R)B044	5000	8000
6	(R)B048	5000	42000
7	(R)B006	5000	49000
8	(R)B022	5000	47000
9	(W)A219	5000	23000
10	(Y)A011	5000	41000
11	(R)B042	5000	48000
12	(W)A225	5000	49000
13	(Y)A006	5000	34000
14	(R)BOO2	5000	45000
15	(R)B012	5000	55000
16	(R)B000	5000	51000
17	(W)A234	5000	5000
18	(W)A213	5000	59000
19	(W)A214	5000	21000
20	(R)B004	4200	0
21	(R)B011	5000	8000
22	(Y)B020	5000	29000
23	(W)A992*	5000	14000
24	(Y)A007	1700	0
25	(R)B032	5000	16000
26	CURLS	114	0
27	(W)A928*	5000	13000
28	(Y)A002	5000	17000
29	(Y)B008	5000	23000
30	(W)A979*	5000	24000
31	(W)A996*	5000	13000
32	(R)B015	5000	13000
33	(W)A980	5000	17000
34	(W)A993	5000	19000
35	M.COMM.	3700	0
36	(W)A921	<<5000	
37	(W)A925	<<5000	
38	(W)A930	<<5000	
39	(W)A981*	<<5000	
40	(W)A987*	<<5000	
41	(R)B003	<<5000	

*the 7 2nd generation lines.

The seed oysters were held at Bolduans Bay until February 1999 when they were large enough (> 6 mm) to be transferred to the other commercial farms around Tasmania and in SA. These > 6mm oysters were sorted by size into a 20-50% grade and a 50-80% grade. Where sufficient oysters were available (Table 8), two bags of each grade from each family line were distributed to all five farms. Some lines were entirely lost due to storm damage to equipment, including the commercial control (M.COMM, #35).

Table 8. Families at Bolduans Bay Oysters in February 1999. Families were graded and bags of 100 oysters were prepared for distribution to other farms.

		Number of bags in 20-50% grade	Number of bags in 50-80% grade
1	(Y)A018	10	10
2	(Y)A013	10	10
3	(Y)A016	none	none
4	(R)B029	10	10
5	(R)B044	0	10
6	(R)B048	10	10
7	(R)B006	10	10
8	(R)B022	10	10
9	(W)A219	9	0
10	(Y)A011	10	10
11	(R)B042	10	10
12	(W)A225	10	10
13	(Y)A006	10	10
14	(R)BOO2	10	10
15	(R)B012	10	10
16	(R)B000	10	10
17	(W)A234	10	0
18	(W)A213	10	10
19	(W)A214	10	10
20	(R)B004	10	9
21	(R)B011	none	none
22	(Y)B020	10	10
23	(W)A992*	10	10
24	(Y)A007	none	none
25	(R)B032	10	10
26	CURLS	1	0
27	(W)A928*	10	10
28	(Y)A002	10	10
29	(Y)B008	10	10
30	(W)A979*	10	10
31	(W)A996*	10	10
32	(R)B015	10	10
33	(W)A980	10	10
34	(W)A993	2	1
35	M.COMM.	none	none
36	(W)A921	10	10
37	(W)A925	6	6
38	(W)A930	8	9
39	(W)A981*	2	2
40	(W)A987*	4	4
41	(R)B003	10	9

The milestone for July 1 1999 was: "Complete spat production for second phase of mass selection trial (Generation M2)".

During the last week of December 1998 and during January-February 1999 we used the hatchery facilities of Marine Culture Pty Ltd at Bicheno. We set up ten 140 litre tanks for oyster propagation. The 47 individuals identified by the genetics team as having a 100% probability of being progeny of the M1-FAST parents were opened and their sex confirmed. These yielded 20 females and 27 males. They were randomly assigned to 2 groups (replicates) and eggs + sperm mixed to produce larvae for one new line M2-FAST1. The remaining 80+ animals were opened, sexed and mixed 50:50 with additional sperm and eggs from the first mass spawn and used to create another replicated line with ~ 130 parents (M2-FAST2). Finally the remaining eggs and sperm from these two lines were mixed 1/3, 1/3 and 1/3 with eggs and sperm from 43 (20 male, 23 female) oysters selected from the fastest growing family lines (1 year old oysters). These were used to produce the third mass selection line (M2-FAST3), also in duplicate.

We also produced a control cross consisting of 20 broodstock oysters that the hatchery had on hand for their own commercial production breeding program. These were mass spawned and then split into three groups (considered to be replicated 3 times). To complete the mass selection lines we also produced a SLOW line. Although a second generation SLOW line was not required by our FRDC project we had noticed that the previous SLOW lines tended to have better condition than FAST lines (at least at certain times of the year). Given that industry has a strong interest in meat weight as well as fast overall growth it was decided to propagate another generation of this line. In an effort to gain faster growth and improved condition we selected the faster growing individuals from the previous SLOW line and spawned 20 of these (7 males and 13 females) to continue the line (M2-SLOW).

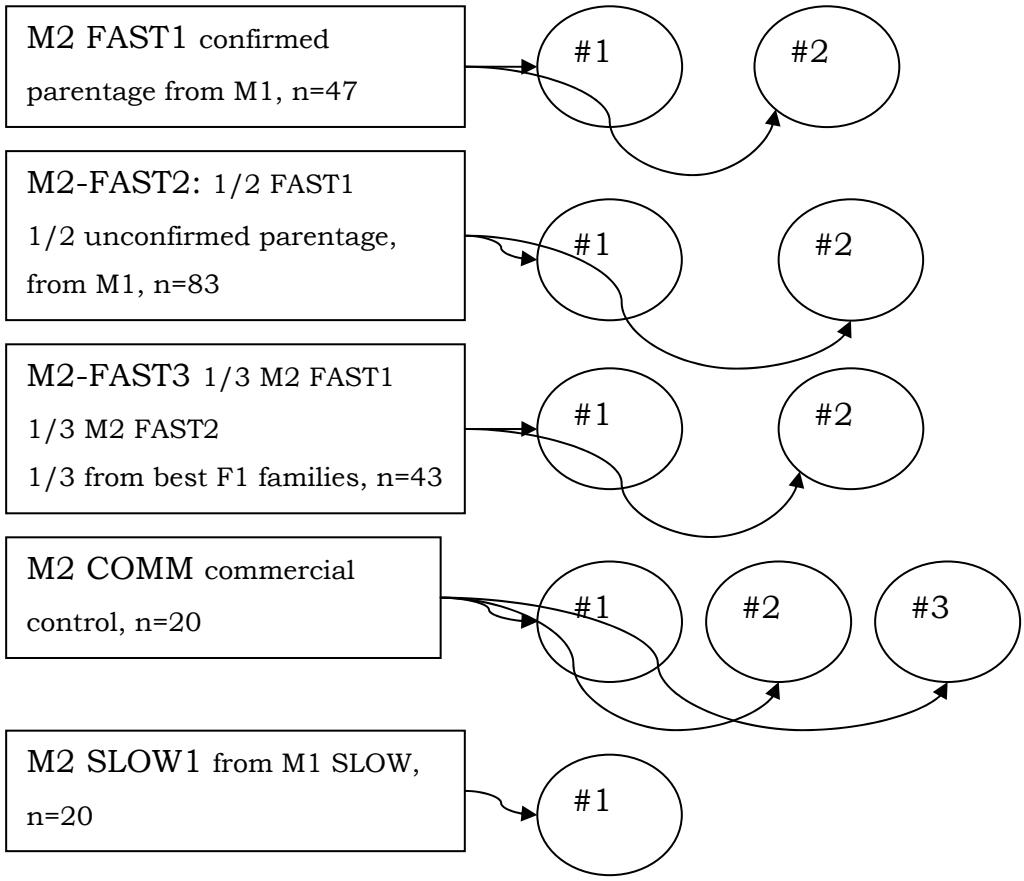

Figure 8. Schematic for production of M2 generation of mass selected oysters. Boxes represent the broodstock or parents, ovals are the new M2 lines.

During the first week of February 1999 the new mass selection lines under went metamorphosis and the larvae were allowed to "set" as single juveniles (onto very small scallop shell chips). These were maintained in downwellers in a hatchery/nursery system within the facilities of Marine Culture (at Harvey Farm Road site) until the 15th of February. On this day they were moved to TAFI-MRL at Taroona. There they were maintained in downwellers within two 4000 litre tanks, provided with supplementary algal food (grown at Taroona) and sand-filtered running seawater. On April 9th the juvenile oysters were thinned to approximately 5000 animals per line.

When all the oysters were sufficiently large to "sit" on 1.7 mm mesh (i.e. >1.7 mm) they were transferred to the outdoor nursery facility (contributed to the project by Jon

Poke's company, Bolduan Bay Oysters). This occurred on the May 6th, 1999. The oysters remained at the nursery facility of Bolduan Bay Oysters until early 2000 when they were large enough (> 6 mm) to be transferred to the five farms (3 in Tasmania and 2 in South Australia) participating in the project.

The milestone for January 1, 2000 was: "Complete grow out of 40 family groups (generation F1)".

From July 1, 1999 to Dec 31, 1999 we monitored the performance of the 40+ family lines at 5 farms. Trips to these farms were made in June-Jul 1999, Sept 1999 and December 1999. On each trip the oysters were assessed for performance. Densities were adjusted to maintain industry-accepted standards. Mortalities were noted and discarded.

On each sample date, performance was estimated from the whole weight of a known number of individuals (~100) in a minimum of 2 to a maximum of 4 replicates from each size grade in each family. During the September sampling 5 individual oysters were randomly selected from each replicate bag (2 to 4 bags x 5 oysters = 10 to 20 oysters per family line) for more detailed assessment of performance from each replicate. These samples were individually weighed, the body dissected out of the shell (shucked), the wet meat weight was recorded, the shells and meats were dried and dry weights recorded. The total number of animals measured for these parameters was ~ 4000 across all sites and families.

Results of F1 family lines

A 2 way ANOVA for growth rate as a function of site (farm) and family line was highly significant for both factors across both size grades of the oysters (Table 9). Multiple comparisons to test for significant differences between families were not attempted as the Bonferroni *t*-test reduces alpha for each comparison and 42x42 possible comparisons makes alpha impossibly small. For the following variety of reasons this 2 way ANOVA using site and family line as factors and examining size or growth rate was judged not the most appropriate tool to select lines for use in producing the second generation of family lines. Some F1 family lines were present only at one site due to low numbers (Table 8). A few lines were present in such low densities that they were raised at about half the density of the other lines of oysters (most notably some of the inbred lines WA981, CURLS). Also, the final weights for the various F1 lines were not obtained until March 8th, 2000, after the F2 family lines were produced. Estimates of the heritabilities for the various traits were not available at this time. They were eventually estimated from specialized experiments conducted as part of FRDC 2000/206. Finally the growth rates measured across all five farms ignores the first year of growth at the nursery site and can penalize oysters that start this phase at a larger size. Some ANOVA results are presented here but the ranking process described below was used to select lines for inclusion in the F2 generation.

Growth rates of the lines varied significantly (Table 9) from about 0.12 g/day (line WA 996) to 0.22 g/day (WA925), or slightly less than a factor of 2. This is noticeably greater than the 8 – 20% variation between the M1 mass selection lines.

Table 9. Two way ANOVAs for oyster growth rate as a function of family line and grow-out site (farm). The two size grades (20-50 and 50-80) are analysed separately.

Source of Variation	DF	SS	MS	F	P
Site (50-80 grade)	4	0.334	0.0835	92.0	<0.001
Line (50-80 grade)	37	0.202	0.00546	6.02	<0.001
Residual	262	0.238	0.000908		
Total	303	0.780	0.00257		
Site (20-50 grade)	4	0.343	0.0858	120.4	<0.001
Line (20-50 grade)	37	0.262	0.00707	9.92	<0.001
Residual	269	0.192	0.000713		
Total	310	0.813	0.00262		

Across Farms

It is clear that the differences between sites, reported previously for the mass selection lines, continued to be a large factor determining the performance of the family lines (Table 9). For any given family, the difference between mean size obtained on the farm with the fastest growth and that on the farm with the slowest growth was considerable. For example, across farms the family RB003 was a little as 28.6 grams per oyster (mean whole weight) on the slowest farm to almost three times that at 77.5 grams per oyster on the fastest farm (Fig. 9). Further assessment of family performance was undertaken by ranking the families on each site (farm) from "best" to "worst". These rankings were then compared across farms (sites). Note that due to the small numbers in some family lines not all families were distributed to all sites.

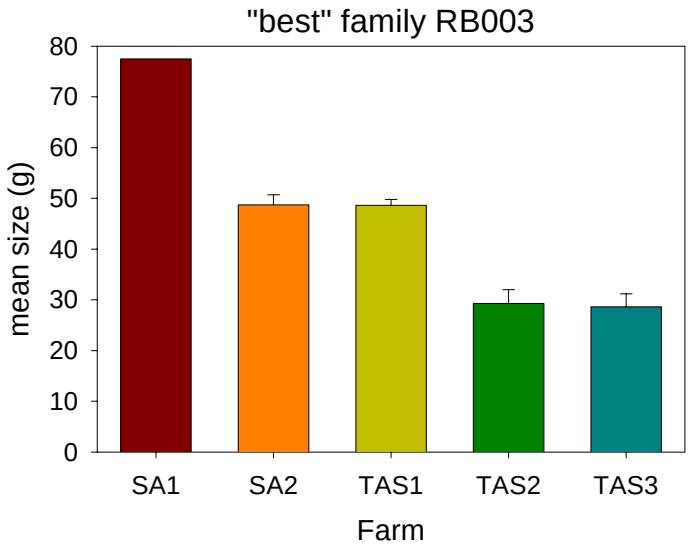

Fig.9. Mean weight (± s.e.) of the three replicates of family RB003 at the five grow out sites.

Across Families

Over all families, the range of family mean size was also quite large. For example, at the fastest growing farm (SA1) the heaviest family (RB003) had a mean whole weight of 77.5 (± 1.2) grams per oyster while the lightest family (WA996) had a mean whole weight of 32 (±0.8). In this example the minimum to maximum range was a factor of 2.5 or 4.7 standard deviations. The complete set of family rankings for another farm (TAS1) is shown in Fig. 10. At the time these data were collected (June 1999) the range from best (RB003) to worst (WA996 tied with YA 013) family on this site was a mean weight of 29.3 grams to a mean weight of 11.5 grams, also a factor of 2.5 and 4.7 standard deviations. Similar ranking were obtained for the families using wet meat weight (Fig. 11) and dry meat weight (data not shown). The correlation between mean whole oyster weight and mean wet meat weight across families at one site was high with $r^2 = 0.69$. The correlation between mean whole oyster weight and dry meat weight across all families at one site was even better at $r^2 = 0.89$.

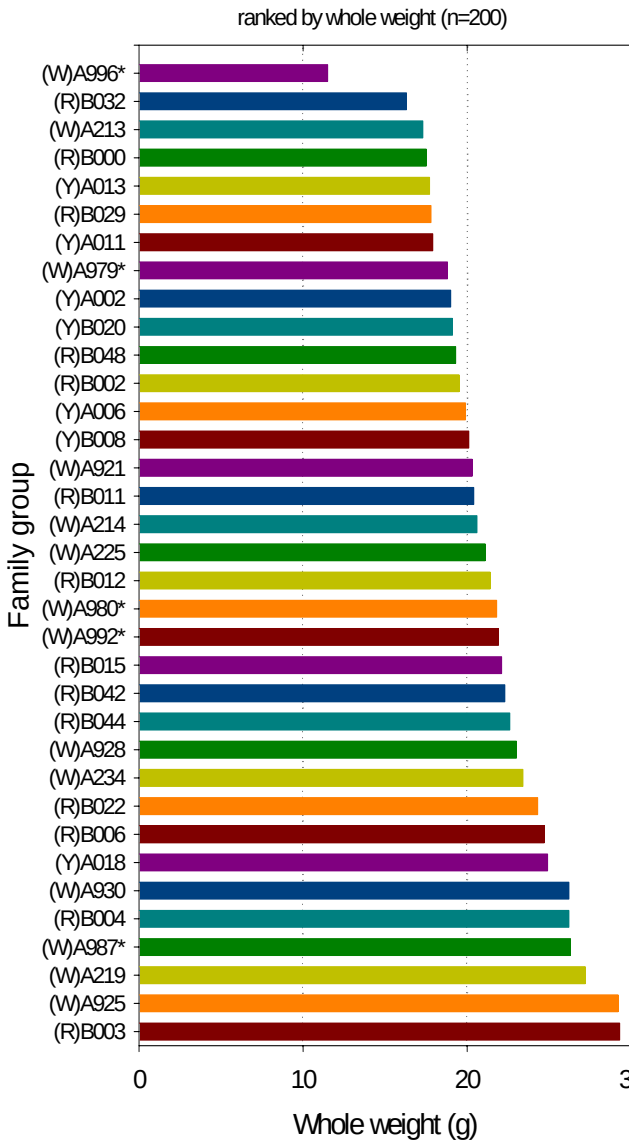

Fig. 10. Mean whole weights of oysters in F1 family lines at one site, showing variation among families.

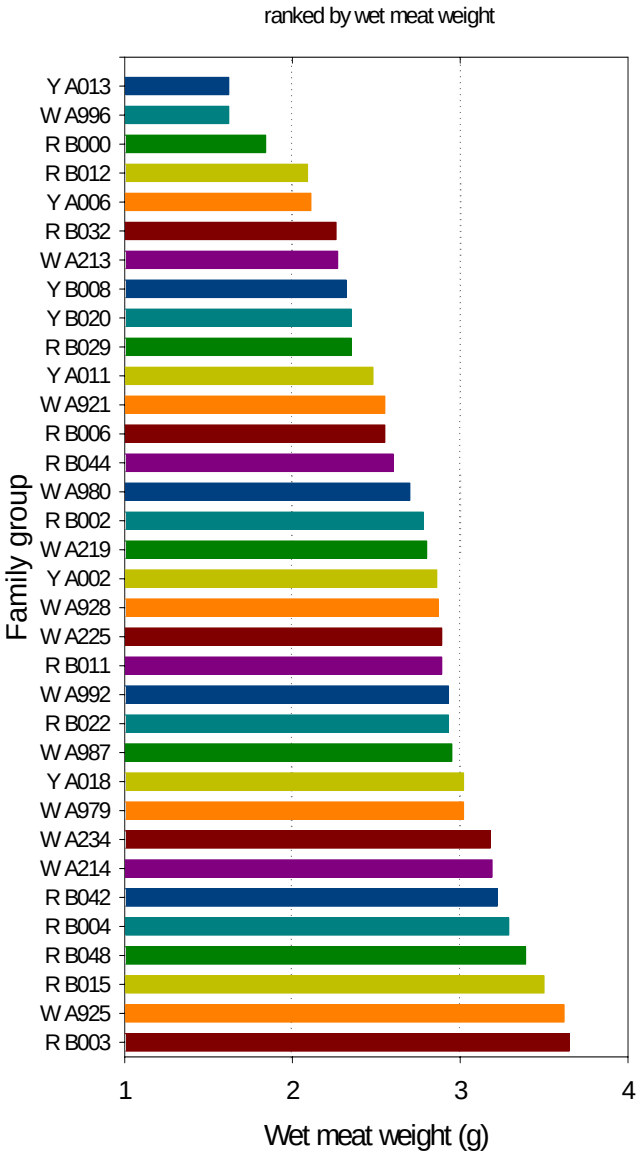

Fig. 11. Mean dry weights of oyster meats from F1 families from one site.

Concordance across sites

The rankings of the families were reasonably consistent across sites (Fig. 12). For the two intertidal sites within Tasmania the degree of correlation was 78% ($r^2 = 0.78$, for rankings based upon whole weight).

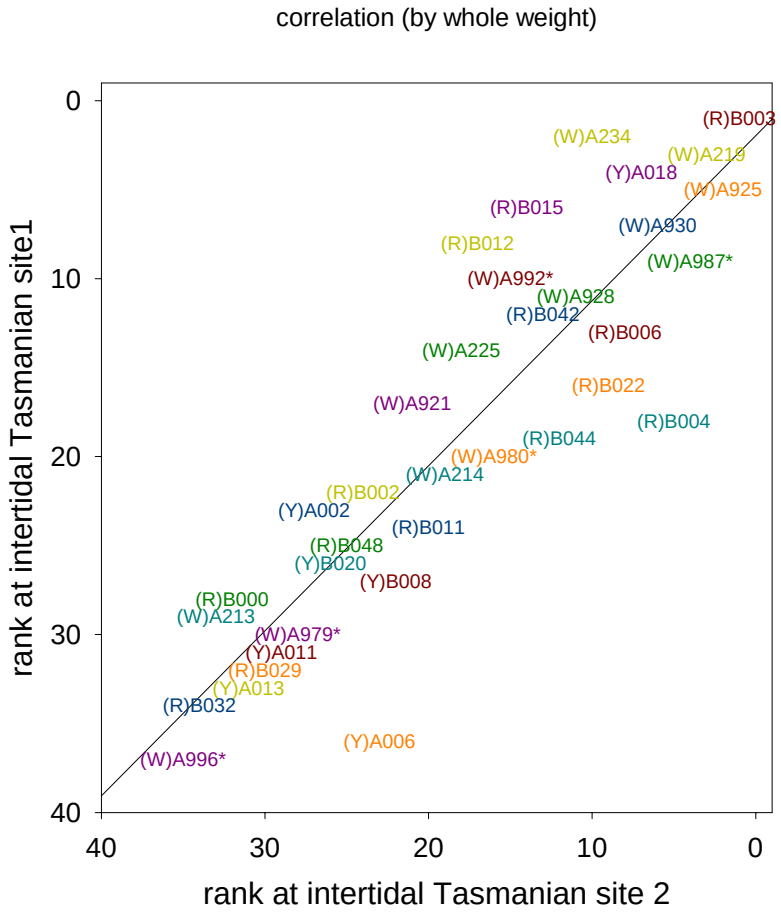

Fig. 12. Correlation between the rankings based on total oyster weight of F1 family lines across two inter-tidal sites in Tasmania.

Selecting the broodstock for F2 family lines

In general, a high performing family was high performing across all sites. For example, the family RB003 was the best performing family at all four intertidal sites (but only 16th of 40 families at the sub-tidal site). Some families were very good performers in South Australia but relatively weak in Tasmania. Finally some families were good

performers sub-tidally but not on intertidal farms. Efforts to rank families across all sites were complicated by this pattern of variation. The simplest approach of calculating a mean rank for all the families allowed them to be ranked over all sites (Table 10). This ranking was then checked to ensure that high performing families from any one site were also considered for the production of the F2 generation of families. Ten families were selected based upon the ranking over all farms. Several families were found to perform well at specific farms and these were also included in the plans for the F2 generation.

Table 10. F1 families ranked by performance of both grades and across all sites.

Family	*Overall Rank*	*Average Rank*
WA925	1	3.33
RB003	2	4.00
WA930	3	4.60
RB015	4	5.60
RB042	5	8.40
WA219	6	9.75
YA018	7	10.20
W A234	8=	10.40
RB004	8=	10.40
RB022	10	10.60
RB012	11	10.80
RB006	12	13.00
RB044	13	13.60
WA992	14	14.60
WA225	15=	14.80
WA928	15=	14.80
WA213	17	15.00
WA214	18	16.60
WA987	19	16.67
RB011	20	17.40

The major goal of ranking these families was to allow selection of the families from which the individual broodstock to produce the F2 generation of family lines could be selected. Analysis of the data from the 7 second generation family lines (WA979, WA980, WA992, WA993, WA981, WA987, WA996) by Dan McGoldrick, CRC-Aqua

Project D1, suggested that families at one site manifest genetic traits better than other sites, therefore broodstock were selected from this site (Tas 2). The broodstock consisted of the ten largest individuals from the selected family lines. These were removed from Tas 2 in October to prevent them from spawning and moved to holding system first at the University Of Tasmania and then at Shellfish Culture's hatchery facility at Bicheno. The basic plan was to crossbreed the top 10 families to produce the F2 generation by crossing those family lines indicated with an "x" (as illustrated in Table 11).

Table 11. Schematic of family rankings and planned crosses (*x*)

Rank	1	2	3	4	5	6	7	8	9	10
1	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*X*
2		*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*X*
3			*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*
4				*x*	*x*	*x*	*x*	*x*	*x*	*x*
5					*x*	*x*	*x*	*x*	*x*	*x*
6						*x*	*x*	*x*	*x*	*x*
7							*x*	*x*	*x*	*x*
8								*x*	*x*	*x*
9										*x*
10										

i.e. the best family would produce 9 new ones by cross breeding with the 2nd through 10th best families.

Producing the F2 family lines

The production of the F2 generation of families was commenced on November 29th, 1999 at the commercial hatchery of Shellfish Culture (in Bicheno). Due to logistic constraints the F2 generation was produced in two separate hatchery "runs" with the second run commenced on January 2nd, 2000. The first 24 crosses of the F2 generation of families are listed in Table 12 and the second set of crosses in Table 13.

Table 12. F2 generation family crosses produced November 29, 1999.

Cross #	Date of spawning	Female parent Family	Male parent Family	Notes
1	29/11/99	WA234	RB015	Failed
1N	1/12/99	RB015	WA234	
2	29/11/99	WA234	WA925	
3	29/11/99	RB003	RB015	failed - lost at set
4	29/11/99	RB003	WA234	
5	29/11/99	WA234	WA930	
6	29/11/99	YA018	WA234	
7*	29/11/99	WA234	WA219	failed - lost at set
8	29/11/99	RB015	WA219	
9	29/11/99	RB015	WA925	
10	29/11/99	RB042	WA234	
11	29/11/99	WA992	WA234	
12	29/11/99	WA925	WA930	
13	30/11/99	RB044	RB012	
14	30/11/99	WA213	RB022	
15	30/11/99	WA213	RB044	
16	30/11/99	WA987	WA987	Failed
17	30/11/99	RB015	WA930	
18	30/11/99	RB015	WA992	
19	30/11/99	YA018	RB015	failed - mixed with 20
20	30/11/99	RB042	RB015	failed - mixed with 19
21	30/11/99	WA925	WA992	
22	30/11/99	WA225	RB015	
23	30/11/99	WA925	WA219	
24	30/11/99	WA925	YA018	

*Cross 7 was not repeated, unlike the other failed crosses.

Table 13. F2 generation family crosses produced January 3, 2000

Cross #	Date of Spawning	Female parent Family	Male parent Family	Notes
3N	3/1/00	RB003	RB015	
16N	3/1/00	WA987	WA987	
19N	3/1/00	RB015	YA018	
20N	3/1/00	RB042	RB015	
25	3/1/00	RB042	WA925	
26	3/1/00	YA018	WA930	
27	3/1/00	WA992	WA930	
28	3/1/00	WA981	WA987	
30	3/1/00	WA992	YA018	
31	3/1/00	WA225	WA925	
32	3/1/00	RB042	WA930	
33	3/1/00	RB042	YA018	
34	3/1/00	RB003	WA987	Failed
34N	6/1/00	RB003	WA987	
35	3/1/00	RB042	WA992	
36	4/1/00	WA987	WA219	lost in nursery
37	4/1/00	WA987	WA930	
38	4/1/00	WA987	YA018	
39	4/1/00	WA987	WA992	
40	4/1/00	PHP*	RB015	
41	4/1/00	LST*	LST*	
42	4/1/00	WA987	RB003	
43	4/1/00	WA987	RB015	
44	4/1/00	WA987	WA925	
45	4/1/00	mass spawn	see below	
46	4/1/00	mass spawn	see below	
47	4/1/00	WA214	WA213	

45. mass spawning: WA925 F, WA219 F, WA987 F, YA018 F, WA214 F, WA234 M, RB015 M, RB003 M, WA930 M, WA992 M.
46. mass spawning: WA925 F, WA930 F, RB003 M, RB015 M. Similar to the commercial trial later carried out by Shellfish Culture (February 2000).
*PHP, Peter Hoare's Pets, a new line created from a grower's favourite oysters LST, a possibly low salt tolerant stock.

The basic breeding plan was to cross the best individuals from the 10 best families with each other. Exceptions from this basic plan were made for several reasons:

1. Exclusions for high performing families that were half sibs in the previous F1 generation

2. Exclusions where there was evidence of possible deleterious traits (such as the shell deformity "curl-back").
3. Inclusions for families that were high performing at specific sites.
4. Exclusions for lack of suitable broodstock (some lines were in poor condition when opened, others were 100% female [where a cross had been planned and both families were 100% female it had to be abandoned]).

The milestone for July 1, 2000 was "Produce spat from selection trials based on the 40 family lines (Generation F1)".

During the hatchery work carried out for the production of the F2 family lines a crude assessment of the relative fecundity and sex ratio of the F1 family lines was obtained from those selected to be used as the broodstock (adult F1 oysters). As can be seen in Table 14, there was substantial variation in fecundity between the various lines, with lines such as WA219, WA987 and RB042 showing considerably greater levels of fecundity than the other lines while several lines, RB044, RB015 and WA214 showed relatively low levels of fecundity. These results suggest that some family lines are late spawning and the oysters from these lines were not yet in peak spawning condition. Late spawning is of particular interest to industry (Appendix 3. Survey of oyster growers carried out by TORC). Late spawning lines may be useful in enabling farmers to have stock in good condition when the majority of their stock has spawned and is in poor, or un-saleable condition.

Table 14. Approximate fecundity (eggs/litre) and sex ratios for the broodstock utilised from F1 generation for the production of the F2 generation.

F1 Line	Fecundity (eggs/litre)	#Male/ #Female
RB003	2.23×10^6	1/14
RB011		10/0
RB015	1.28×10^6	3/5
RB022		10/0
RB042	3.61×10^6	0/11
RB044	1.20×10^6	1/1
WA213	3.50×10^6	0/2
WA214	1.40×10^6	0/2
WA219	5.10×10^6	3/1
WA225	1.50×10^6	0/11
WA234	3.07×10^6	1/3
WA925	2.56×10^6	3/8
WA930	7.60×10^6	9/1
WA981	2.60×10^6	0/1
WA987	4.49×10^6	2/6
WA992	2.43×10^6	2/3
YA018	1.58×10^6	3/5

The sex ratio data indicates some lines which appear to be mostly one sex (note: from a limited, non-random, sample) (Table 14), possibly all male, RB011 and RB022, or all female, RB042 and WA225. Single sex stocks are another characteristic considered important by the oyster industry, (Appendix 3; from TORC survey). One potential benefit of an all female line is that female oysters have a greater growth rate than male oysters, by up to 18% (Baghurst 2000); therefore an all-female fast-growing stock would be an added bonus for the oyster industry.

Due to logistic constraints the F2 generation was produced in two separate hatchery 'runs', with the first commencing in November 1999 (Table 12). Several of these crosses were lost due to mortality or mixing. These "lost" crosses were repeated along with the second set of crosses in January 2000 (Table 13).

After successful rearing through the larval stage and after set as single seed oysters in the hatchery the F2 family lines were transferred to the TAFI-MRL laboratories at Taroona, where they were on grown in a nursery system until large enough to be placed on a nursery farm site, (>1800 μm in diameter). The initial batches of F2 families

(Table 12) were transferred to Taroona on the January 14, 2000, with the remaining families being transferred on the February 1, 2000. The 47 F2 family lines were transferred to a commercial nursery site (Bolduans Bay Oysters) during April, 2000, where they were on-grown until large enough (> 6 mm) to be transferred to other commercial farms around Tasmania and South Australia. All the F2 families were graded regularly to reduce stocking densities and enhance growth rates (as per commercial practice). The treatment of each family was identical. They were graded on April 24, 2000 and June 6, 2000 and in late October, 2000, prior to being placed out onto the five farming sites in November, 2000. The number of oysters for each line held at Bolduans Bay, as of June 8th, 2000, is listed in Table 15.

Table 15. Approximate numbers of F2 Family Lines oysters on June 7th 2000 held at Bolduans Bay Oysters.

Cross #	Number Held
1	10000
2	10000
3	10000
4	10000
5	10000
6	10000
8	10000
9	10000
10	10000
11	10000
12	10000
13	10000
14	10000
15	10000
16	2000
17	10000
18	10000
19	2000
20	10000
21	10000
22	10000
23	10000
24	10000
25	10000
26	2000
27	10000
28	10000
30	10000
31	2000
32	10000
33	10000
34	10000
35	6000
36	10000
37	10000
38	10000
39	6000
40	10000
41	10000
42	10000
43	10000
44	6000
45	10000
46	6000
47	10000

The milestones for Jan 1, 2001 were:

"Assess performance of offspring from selection trials based on 40 family groups (Generation F2)."

"Make promising lines available to industry."

"Complete 2 year growth trial for second phase of the mass selection trial (Generation F2)"

Milestone #1. *Assess performance of offspring from selection trials based on 40 family groups (Generation F2).*

After successful rearing through the larval stage and after set (metamorphosis to juveniles) as single seed oysters in the hatchery, the F2 family lines were randomly thinned to approximately even out their density. It has been an issue of some concern (McGoldrick, pers. com.) that high stocking density may adversely affect growth thus confounding our assessment of genetic improvement. During the nursery phase the families were held in individual containers or "pots". Because there was a range in oyster density within a family's individual pot the issue of whether stocking density negatively influenced growth was investigated. We used a simple approach, that of plotting stocking density (estimated total number of individuals in a family) versus average size. The results indicate that there was no negative impact of stocking density on oyster growth over the ranges studied (Fig. 13). Rather surprisingly the data yielded a significant ($r^2 = 0.35$, $P<0.05$) positive correlation between growth and stocking density. It seems unlikely that this is a cause and effect relationship.

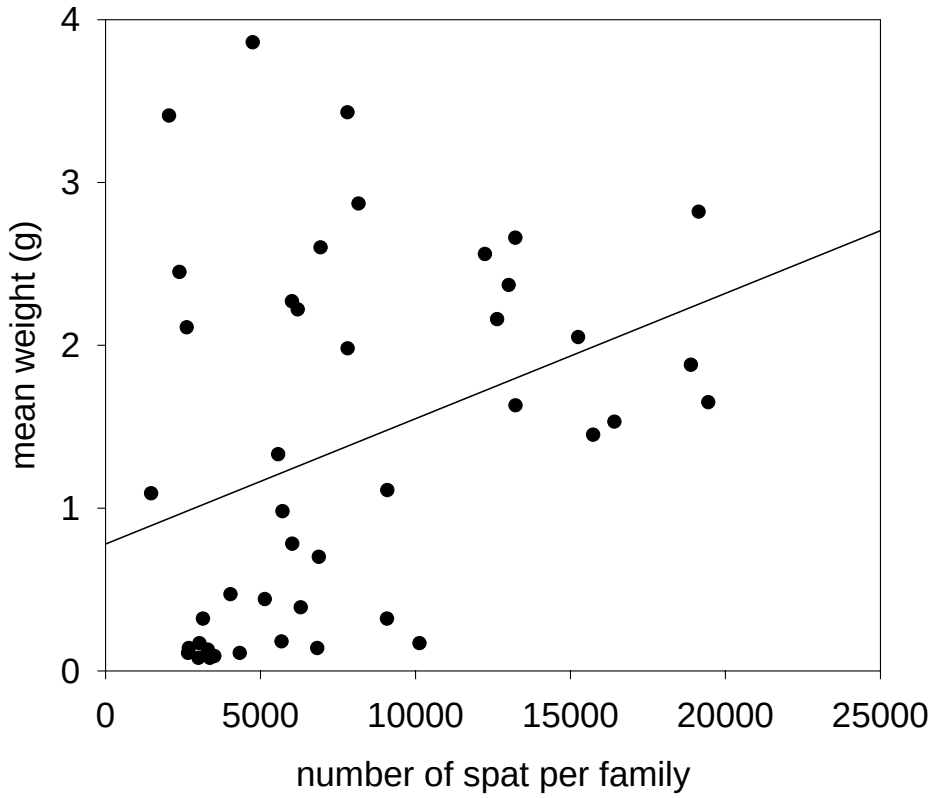

Fig. 13. Mean size (weight) as a function of stocking density for F2 families of oysters.

The juvenile oysters of the F2 family lines were nursery reared until large enough to be placed on a commercial farm site (>1800 μm). They were transferred to the commercial site (Bolduans Bay Oysters) during April, 2000, where they were on grown until large enough to be transferred to the other commercial farms around Tasmania and South Australia (November, 2000).

During grow-out at the Bolduan Bay site, the family lines were treated in a similar manner as normal commercial oysters, with the major difference being the need to keep all families separate. To facilitate separation they were raised in normal oyster seed trays but with an empty section between families. Wooden dividers compartmentalize seed trays. The lids have matching wooden dividers that are lag bolted to the lower dividers. Only new seed trays with extra lag bolts were used. Any oyster that somehow washed into the empty sections between families was discarded. A tag unique to its oyster family identified each tray section. In most cases, as the oysters grew, a single

family would eventually occupy its own seed tray (6 sections), and sometimes several seed trays. Normal commercial practice of increasing the mesh size of the trays was used. This was implemented in the normal manner by screening the oysters and those that were large enough to merit a larger size mesh were transferred to the larger mesh tray and those that were too small were left in the smaller mesh tray. This grading practice was implemented on all lines at the same time and executed several times during the winter-spring of 2000. The objective of this grading was to keep approximately equal biomass per unit surface area across all lines of oysters regardless of size so that any potential effects of crowding are held relatively constant across all oysters and family lines.

On October 17th, 18th, and 19th, 2000 the individuals from each of the F2 family lines were gathered together. All of the project's seed trays were brought in from the Bay. Each section was identified and the oysters from it added to a container unique to each family. When the entire family had been reunited from their different mesh trays the oysters within one family were thoroughly mixed. A random sample of 100 individuals was removed from each family and weighed for calculation of the mean weights. A random sub-sample of 20 individuals was selected for further measurements of wet meat weight, dry meat weight, shell length, width, depth and shape. Each family line was also judged qualitatively for various characteristics such as: colour of the left and right valves, degree of curl back, amount of frill on shell, shape, miscellaneous comments and ranked relative to each other on a scale of 1 to 10 (best). Input from the farm manger (Kerri Wells) was sought with regard to establishing the relative rankings, with his favourite family line being F2 line #16 (Table 16). From each family line 20 of the largest individuals were set aside as possible broodstock in the production of the F3 generation. Once these samples were selected the oysters were graded.

Following normal commercial practice the oysters were graded into 4 size grades (< 14 mm, 14-18 mm, 18-22 mm and >22 mm). The major difference between commercial practice and the research project was that each family was individually run through the grader. The number of oysters in each size grade was determined, either by direct count or estimated from a sub-sample of a fixed volume. From these four size grades the

correct numbers of each grade that were required to create two size classes for each family representing equal proportions of the overall size distribution were devised. The project team and industry representatives had previously agreed that we could split the family into two size classes with the divisions consisting of oysters in the size range 0 to 50% and 50 to 100% of the distribution for each family (or: smallest to the mean and mean to the largest).

For further grow-out it had also been agreed that we could assess only the 50 to 100% size grade in these F2 families (separating the F1s into 2 size grades had not given significantly more information than a single size grade). The oysters residual to this need (0-50% size range) were combined into a single bin (all families mixed) and turned over to Bolduan Bay farm. From the larger size grade 15 batches of 100 randomly selected individuals per family were bagged for subsequent delivery to the 5 farms participating in further grow-out trials (3 bags of 100 oysters from each family for each farm).

The data for the mean weights of 100 randomly selected individuals were used to establish relative growth rates for each family line (Table 17).

Table 16. Preliminary screening of the F2 families, carried out October 17, 18, 19, 2000. Observational data.

F1 Parents (F x M) F1 designations (e.g. WA XXX)		**F2 Family designations (1…45)**							
				Subjective descriptive information					
Female	***male***		***Colour*** ***Left valve***	***Right valve***	***Incidence of curlback***	***Amount of frill***	***Shape***	***Misc. comments***	***arbitrary, relative rank 1-10***
WA234	RB015	1	mixed, burgundy	pale		low	Variable		5
WA234	WA925	2	pale	pale	None	low	good	small	7
RB003	WA234	4	burgundy	pale	some	variable, low to heavy	good		5.5
WA234	WA930	5	burgundy, striped	burgundy, striped	very low		broad, deep	uniform size	7.5
YA018	WA234	6	burgundy	pale	low	moderate	broad, flat		4.5
RB015	WA219	8	burgundy (stripes)		some	low - moderate	broad, flat		6.5
RB015	WA925	9	burgundy	pale	very low	low	good	uniform size	9
RB042	WA234	10	burgundy	pale	very low	most smooth	good, variable		6
WA234	WA992	11	pale		some	high	wide, flat		3
WA925	WA930	12	pale		some	high		few good individuals	4
RB044	RB012	13	burgundy	white	some		good		6
WA213	RB022	14			some	low			5
WA213	RB044	15	burgundy		not evident	low	good, wide		8
WA987	WA987	16	dark burgundy	white	not evident		good	Kerri's favourites	10
RB015	WA930	17	variable	stripes	low		narrow		4
RB015	WA992	18	variable		none evident	low (a few moderate)	good		7
WA925	WA992	21	pale with burgundy stripes			low to moderate	good		6.5
WA225	RB015	22	burgundy	stripes	very HIGH				2

F1 Parents (F x M) F1 designations (e.g. WA XXX)		**F2 Family designations (1…45)**							
			Subjective descriptive information						
Female	***male***		***Colour Left valve***	***Right valve***	***Incidence of curlback***	***Amount of frill***	***Shape***	***Misc. comments***	***arbitrary, relative rank 1-10***
WA925	WA219	23	burgundy, striped	pale	some		some good		5
WA925	YA018	24	pale, some burgundy		moderate		twist in early shell growth	few with good shape	4
RB015	YA018	19	burgundy	pale		low	good		8.5
RB042	RB015	20	variable		some	low to moderate			6
RB003	RB015	3						too small for adequate assessment	
RB042	WA925	25		pale	low		some very good		6.5
YA018	WA930	26	burgundy	pale	very low	low	good		7
WA992	WA930	27	pale-red		none		round		
WA981	WA987	28	pale-red		none	moderate	broad and flat		7
WA992	YA018	30	pale		very low	moderate	very round and flat		6
WA225	WA925	31	mixed, burgundy & pale			low	very good		9
RB042	WA930	32	red-burgundy		none evident		good		8.5
RB042	YA018	33			some		broad	uniform size	5.5
RB003	WA987	34	burgundy		high				2
RB042	WA992	35	pale		not evident	very smooth	good		8
WA987	WA930	37	red		none	low		small? Lots of seed	6
WA987	YA018	38	variable		low		good		7
WA987	WA992	39	dark				very round	small?	6.5
PHP	RB015	40	very pale		some	very low		largest individuals look good	7.5
LST	LST	41	pale purple		some	high			4
WA987	RB003	42	variable		some		broad		7
WA987	RB015	43	dark		very low (absent?)		good		8.5
WA987	WA925	44	dark burgundy		none		good		8

F1 Parents (F x M) F1 designations (e.g. WA XXX)		**F2 Family designations (1…45)**							
				Subjective descriptive information					
Female	***male***		***Colour Left valve***	***Right valve***	***Incidence of curlback***	***Amount of frill***	***Shape***	***Misc. comments***	***arbitrary, relative rank 1-10***
Mass Spawn (a)	Mass Spawn (a)	45	pale		high				3
Mass Spawn (b)	Mass Spawn (b)	46			high				4
WA214	WA213	47	burgundy	burgundy stripe down center of valve	very low	low	small?		7

Table 17. Quantitative data on the performance (numbers in different size groups, estimated growth) of F2 families during early grow out (October 2000 ~ 10 to 11 months age).

Family ID 1…47	*Average Weight (g)*	*Size 1 < 14mm*	*Size 2 14 –18 mm*	*Size 3 18 –22 mm*	*Size 4 >22 mm*	*total #*	*age (days)*	*growth in mg/day*
first spawning								
1	1.11	3417	4500	1008	167	9092	324	3.43
2	1.53	6388	8113	1668	246	16415	324	4.72
4	2.60	1557	3008	1898	476	6939	324	8.02
5	3.43	221	2234	4323	1027	7805	324	10.59
6	2.05	1657	8900	3990	703	15250	324	6.33
8	2.16	4057	4602	3142	829	12630	324	6.67
9	2.22	1469	2872	1570	291	6202	324	6.85
10	2.82	2921	8884	5907	1425	19137	324	8.70
11	1.98	2209	3636	1592	372	7809	324	6.11
12	1.65	4650	10787	3310	699	19446	324	5.09
13	3.86	317	1044	2269	1119	4749	323	11.95
14	2.37	1388	6851	4212	555	13006	323	7.34
15	2.66	1157	5174	5971	919	13221	323	8.24
16	1.09	648	606	176	40	1470	323	3.37
17	1.88	4619	9510	3838	919	18886	323	5.82
18	1.45	4824	7012	3206	688	15730	323	4.49
21	2.87	859	3030	3354	919	8162	323	8.89
22	2.27	943	3055	1740	275	6013	323	7.03
23	2.56	1700	5529	3841	1170	12240	323	7.93
24	1.63	3274	7994	1711	245	13224	323	5.05

Table 17 continued. Quantitative data on the performance (numbers in different size groups, estimated growth) of F2 families during early grow out (October 2000 ~ 10 to 11 months age).

Family ID 1…47	*Average Weight (g)*	*Size 1 < 14mm*	*Size 2 14 –18 mm*	*Size 3 18 –22 mm*	*Size 4 >22 mm*	*total #*	*age (days)*	*growth in mg/day*
Second spawning								
19	2.45	100	948	1023	312	2383	289	8.48
20	0.70	3476	3184	195	28	6883	289	2.42
3	0.18	5088	550	39	0	5677	289	0.62
25	0.78	3838	1679	445	63	6025	289	2.70
26	2.11	286	1231	969	131	2617	289	7.30
27	0.17	9045	962	103	17	10127	289	0.59
28	1.33	1940	2840	715	73	5568	289	4.60
30	0.32	7686	1185	176	33	9080	289	1.11
31	3.41	76	414	923	639	2052	289	11.80
32	0.39	5445	800	52	0	6297	289	1.35
33	0.14	6440	372	17	0	6829	289	0.48
34	0.17	2540	437	51	0	3028	289	0.59
35	0.11	3933	400	0	0	4333	289	0.38
37	0.09	3383	131	0	0	3514	288	0.31
38	0.47	2976	1055	0	0	4031	288	1.63
39	0.08	3000	0	0	0	3000	288	0.28
40	0.14	2347	345	0	0	2692	288	0.49
41	0.98	2055	2915	643	95	5708	288	3.40
42	0.44	4566	534	40	0	5140	288	1.53
43	0.32	1962	1000	178	0	3140	288	1.11
44	0.11	2324	340	0	0	2664	288	0.38
45	0.13	NA	NA	NA	NA	NA	288	0.45
46	0.08	3245	122	0	0	3367	288	0.28
47	0.13	2659	640	0	0	3299	288	0.45

NA = not available at this time.

Milestone #2. Make promising lines available to industry

During 1999 - 2000 there were a number of discussions with various industry representatives regarding the possible use of improved broodstock. The most formal was the issuance of a letter (see attached copy in Appendix 4) as part of the new FRDC project 2000/206 to industry outlining the availability of these stocks and asking for expressions of interest. Previous to this formalization of our interaction with industry some improved stock were made available following a request by Shellfish Culture to Dan McGoldrick in 2000 and subsequent TORC approval. Project staff at the commercial facility of Shellfish Culture crossed these in February 2000 producing 2 distinct lines. One line consisted of an out-breeding of previously inbred parents thought to be free of the undesirable morphological (shell) trait known as "curl back". The other line consisted of 4 high performing F1 family lines spawned together. Both lines were produced in a manner to prevent inbreeding and to yield millions of offspring which the commercial facility made available for purchase to interested growers. More commercial trials occurred in 2001 when broodstock were made available to four hatcheries. A revised protocol for handling these trial commercial crosses was developed in consultation with industry during 2000. In 2000 many of the potential broodstock were too young, difficult to induce to spawn, producing only small numbers of eggs and with only limited numbers available strip spawning was not a sustainable option. It was a time consuming task to develop a suitable protocol that did not unduly restrict the commercial scale production while still allowing some quantitative estimation of the performance of these lines through out grow out.

Milestone #3. Complete 2 year growth trial for second phase of the mass selection trial (Generation M2)

The second generation of mass selected oysters was spawned in the summer of 1998/99. During their first year they were in the Shellfish Culture hatchery, followed by a nursery phase at TAFI-MRL and then to the commercial nursery site (Bolduans Bay

Oysters, Smithton, Tasmania). They were transferred to the five commercial grow-out sites in March 2000.

The line of oysters mass selected for rapid growth (FAST) continued to outperform their commercial controls (COMM) in the second generation. Growth over the autumn and winter periods (March – September, 2000) was relatively slow and the FAST oysters managed only a 5.5% (averaged over all farms) improvement over controls. By November, 2000, as the oysters approached commercial size, this had increased to an average 12.3% improvement in growth rate over the commercial controls (Fig. 14). The 12.3% increase in growth rate during grow-out is up substantially from the average 8.8% improvement in growth rate during the grow-out of the M1 generation of mass selected rapid growth animals.

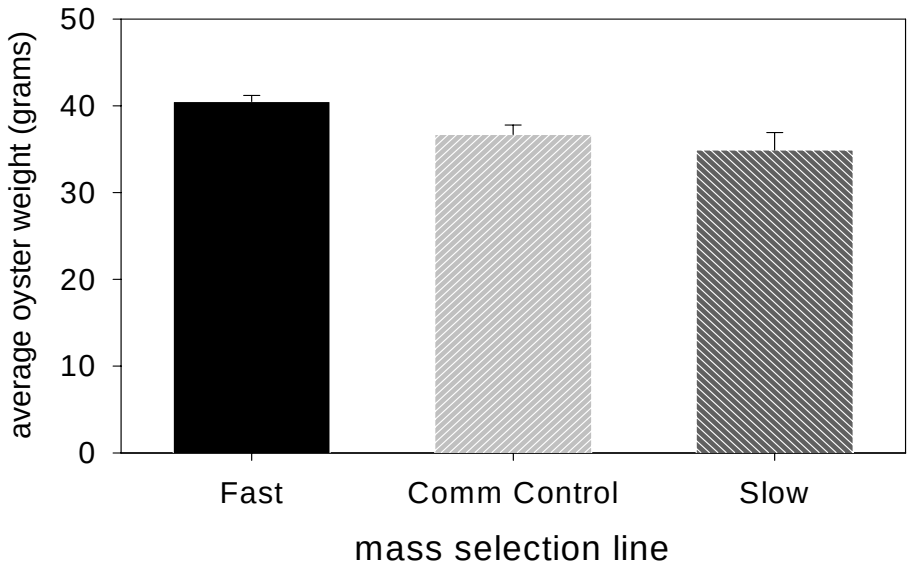

Fig. 14. Average weight of the three M2 mass selected lines (spawned in summer 1998-99) and measured in November 2000 after nursery phase and nine months of grow out at five different farms. The Fast and Slow lines are in their second generation.

Considering each bag of 100 oysters as an independent replicate for growth rate, differences in growth rate between lines (P = 0.0004) and differences between farms (P < 0.0001) were both statistically significant (Table 18). Growth rate showed no significant interaction between these two factors (P = 0.8897, line x farm). The FAST line performed a minimum of 6.2% better and a maximum of 20.1% better than the commercial controls depending upon the farm. The FAST line grew significantly faster and was heavier (by November 2000) than either the COMM controls or the SLOW line (ANOVA followed by Bonferroni adjusted multiple comparison technique).

Table 18. Statistical analysis of the results from grow out of the M2 lines of FAST and SLOW selected lines of oysters relative to their commercial controls as of November 2000.

Source of Variance	DF	SS	MS	F	P
Farm	4	5502	1375	64.5	<0.0001
Line	2	368	184	8.64	0.0004
farm x line	8	75.9	9.49	0.445	0.8897
Residual	73	1555	21.3		
Total	87	14213	163		

Farm differences remained the largest factor in the analysis of the mean growth (averaged over all lines) during the 9-month period. Growth at some farms was considerably more than at others (Fig. 15). The average weight (averaged over all three lines) gained during the nine month period at the farm with the slowest growth was 13 grams while at the farm with the greatest growth the average weight gain was 47 grams (equal to 360% faster growth).

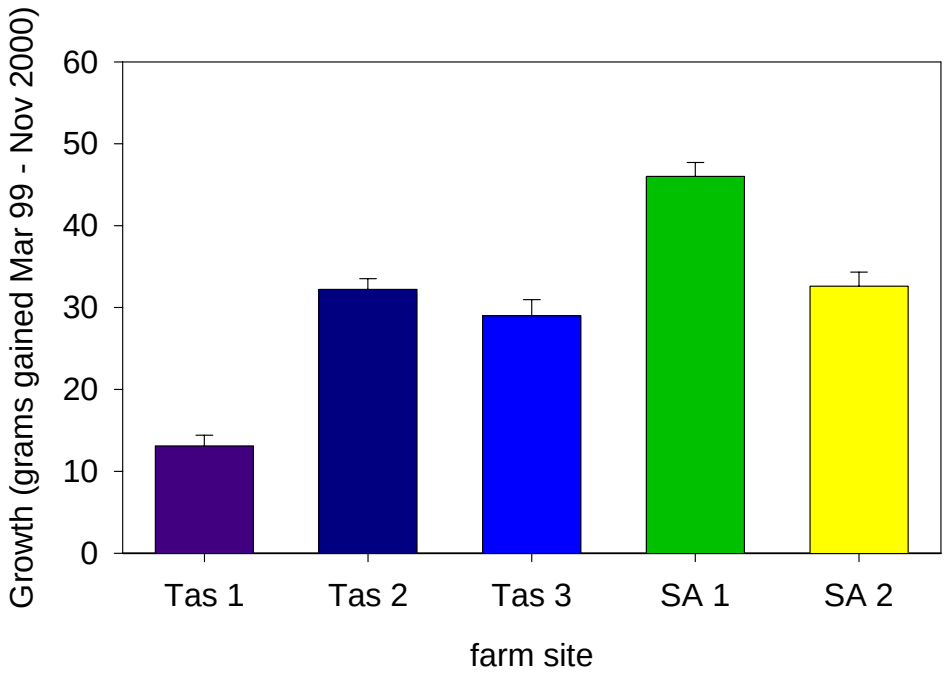

Fig. 15. Growth measured as average weight gain per oyster (averaged over all three lines of the second generation mass selected oyster lines) from initial placement at farms (March 2000) to measurement in November 2000 versus farm. Means + SEM are shown.

During sampling of the oysters in September, 2000, ten individuals were randomly selected from each batch of 100 oysters. These were returned to the laboratory and analysed for whole weight, wet meat weight, shell weight, shell shape and dry meat weight. Results were similar to those for whole weight measured on the 100 individuals per replicate (i.e. FAST>COMM>SLOW), but differences were not statistically significant between lines. Apparently it was not possible to obtain sufficient precision for a reliable statistical analysis from a sub-sample of 10 oysters that will resolve differences in the 10 to 20% range. The capacity to measure a significant difference between selected lines in meat was compromised, in part, by the early sampling. Oysters collected from the field in Tasmania during September are just starting to recover from winter. At less than 2 years of age they are not yet at market size nor was the older commercial crop in a very marketable condition at this time of year. While the strategy of sampling during September is not ideal we needed to have the data then to assist with the selection of broodstock (Family lines) and the Project staff were too busy with larval and seed rearing duties at other times. Differences between farms were still

statistically significant, for example in dry meat weight (Fig. 16) with the farm rankings being very similar to those derived from whole oyster weight.

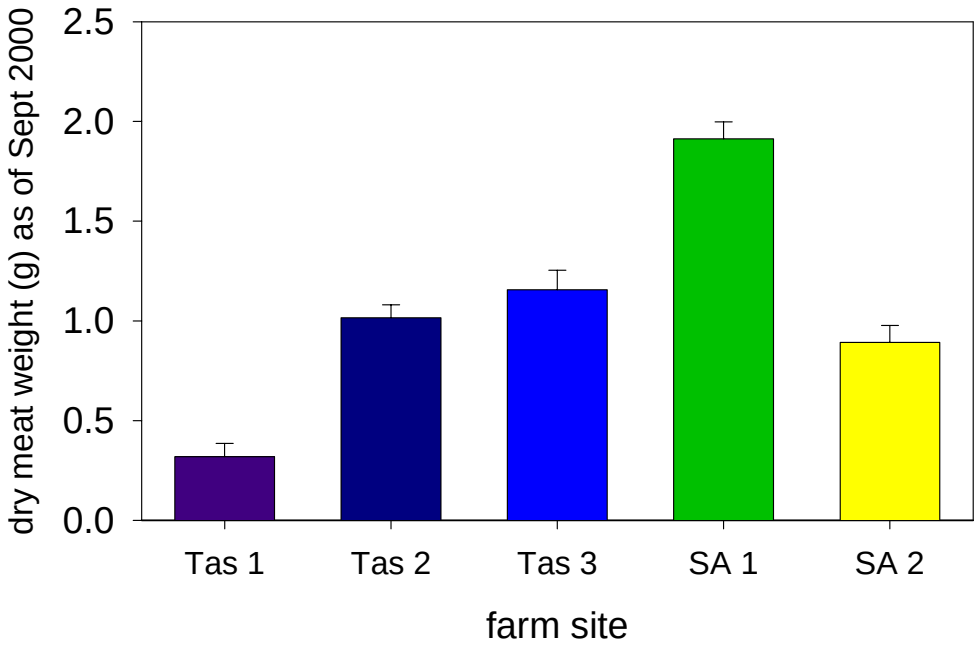

Fig. 16. Mean (averaged for all second generation mass selected lines) dry meat weight versus farm site. Means + SEM are shown.

Benefits

The domestication of the Pacific oyster should yield many benefits to the industry. These benefits will include improvements in the economics of oyster farming. We foresee benefits associated with faster growth, more uniform size, a reduction in mortality and a premium product for market although not all of these have been proven here. We can calculate a simple estimate of the benefit to the industry of 12.3% more rapid growth, or 12.3% more product for the same amount of effort. In a simplistic fashion this can estimated to be worth about 12.3% of ~ $25 million or about $3 million per year. Significantly greater improvements due to faster growth are available from specific family lines where the range of improvement was up to 250%. Given current trends in oyster production this benefit would be split 50:50 between Tasmania and South Australia. A more detailed analysis of the anticipated financial benefits is planned as part of a subsequent funding application.

Many other important benefits are more difficult to quantify. It is clear that specific lines free from shell deformity are a substantial benefit to hatcheries and nursery (seed producing) farmers. It is also clear that family selection produces oysters with a range of specific traits that may be a benefit to specific regions. For example, there are lines with very low extra shell (frill) that seem likely to provide less substrate for mud worm to settle and may be of benefit in those areas where mud worm is problematic. Size variation within a family line was small relative to mass spawned commercially produced lines such that considerable savings in labour are possible because of a reduced need for handling (grading and rumbling). Similar to other primary industries it seems reasonable that genetic selection and increased broodstock management will yield a range of benefits in the future.

Further development

The authors recognize that propagation of the benefits from this research project into the future will require successful commercialisation. As part of the new project on the selective breeding of the Pacific oyster (FRDC 2000/206) this process is well underway. There has been the incorporation of a joint venture company (Australian Seafood Industry Pty Ltd.) that brings together the two major industry bodies (Tasmania Oyster Research Council and the South Australian Oyster Growers Association [and associated Research Council]). There is a collaborative agreement between industry and the research providers (TAFI and CSIRO). Co-operative interaction between research providers and industry were critical during the early years to ensure the gestation of a successful joint venture company. A sense of shared commitment to the success of this new venture and a willingness to be flexible when necessary were required to successfully develop the ongoing selective breeding program now funded and managed by industry.

The FRDC project 2000/206 has seen the process of selective breeding through seven spawning cycles and 5 generations of mass and family selection. All of the commercial Pacific oyster hatcheries in Australia have enquired about using broodstock from the project and an increased number of commercial scale trials occurred in 2001. By 2001 different hatcheries were already selecting different F1 and F2 broodstock for trials in response to input from both the FRDC 2000/206 Project team and participating growers nominated as statewide Broodstock Managers, plus their assessment of the needs of their customers. Hatcheries have continued to specialized producing different lines from different broodstock for sale into the commercial market. Seed production from selected lines is increasing and seems destined to be increasingly tailored for specific growing regions. Specialized experiments conducted as part of FRDC project 2000/206 produced estimates of heritability for a range of traits and these are being used in a selection index to improve the future generations of selected oysters.

Planned outcomes

1. Oysters from the selected lines require less time to reach market size thus improving the competitive capability of the Pacific oyster industry.
2. Oysters from pedigreed selected lines are free from specific shell deformities significantly reducing the risk for hatcheries when selecting broodstock.
3. Growers with FRDC 1997/321 oysters on their farms are overwhelming enthusiastic about the selected lines. The on farm trials have proven to be very successful in demonstrating the benefits of the selected lines.
4. At the conclusion of FRDC 1997/321 the two peak bodies representing oyster growers in Tasmania and South Australia had begun the process of coming together to form a joint venture company (Australian Seafood Industries) to exploit the intellectual property developed by this FRDC funded project.
5. As of 2001 all the Pacific oyster hatcheries in Australia had formally expressed a desire to trial our selected lines of oysters as broodstock. Broodstock from the selected lines were supplied to all hatcheries producing commercial quantities of Pacific oysters in Tasmania and South Australia. The broodstock were young by industry standards but 10 to 20 million larvae were produced and ~ 5 million seed oysters were eventually offered as commercial product. As documented in FRDC 2000/206 the portion of commercial seed production from broodstock produced by this project continued to increase reaching 15-20% of the total market (in 2004) as the selected lines were continuously improved.
6. Genetic markers were developed by CRC-Aqua Project D1 and tested on the oyster lines produced in this project to enhance the long-term probability of success of the industry-based selective breeding project especially in terms of testing and maintaining genetic diversity in the mass selected lines.

Conclusion

This FRDC funded project has worked in close collaboration with other CRC Aquaculture funded oyster research to deliver a holistic approach to selective breeding of Pacific oysters. Well established techniques of selective breeding have been applied to the Pacific oyster in Australia resulting in significant and documented gains in a number of commercially valuable traits. The selective breeding program has provided new pedigreed lines and thus opportunities for molecular geneticists working in CRC-Aqua Project D1 to develop molecular markers for specific character traits. Market uptake has been very significant reaching 15-20% of market share by 2004 (Ward et al. 2005).

Improved mass spawned and family lines have been propagated through two generations. Each technique has delivered something of value to industry whether it is improved growth or a combination of factors. The merit of these lines has been rigorously tested over a range of farms in two States using both intertidal and sub-tidal grow-out techniques. Industry has provided a huge amount of logistic support for the project and they are keen to reap the benefits. Industry groups in both States have formed a joint venture company (Australian Seafood Industries or ASI) to oversee technology transfer to industry and monitor commercial trials, and have successfully taken over the continuation of the breeding program when FRDC support ended. ASI held its inaugural meeting at the beginning of April 2001. The research providers (CSIRO and UTas/TAFI) worked to establish a collaborative agreement with the company to ensure appropriate use of intellectual property gathered in past CRC and FRDC projects. The company may in the longer term also act as a vehicle for technology transfer to industry of other seafood research.

FRDC Project 1997/321 has demonstrated considerable gains in performance from both mass selection and family (combined) selection applied to the Pacific oyster. Experiments with half siblings, full siblings, family selection, combined selection and mass selection have shown that a range of traits from shell colour to growth rate are heritable. Determining the exact degree of these heritabilities is the goal of FRDC project 2000/206 (see next paragraph) but the authors of this report are confident that the evolving needs of the industry will benefit from the application of both mass and family selection.

Another FRDC project was funded to continue the Pacific oyster selective breeding work from November 2000 to November 2003. FRDC project 2000/206, Sustainable Genetic Improvement of Pacific oysters in Tasmania and South Australia, has six principal objectives:

1) Continued production of mass selection lines for growth rate and family lines for growth rate and other industry-desired traits.

2) Creation of crossbred family lines to assess the feasibility of combining desirable traits from different families into a single line.
3) Development of a multi-trait selection index.
4) Assessment of the performance of chosen lines in full-scale commercial trials.
5) Development of a breeding plan for sustainable genetic improvement.
6) Development of a commercialisation strategy (within 12 months of start).

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Appendix 1: Intellectual Property

1. Improved oysters, faster growing, greater meat, better shell shape, colour.
Documentation Source Notebooks in possession of Peter Thompson, Dept. of Aquaculture, University of Tasmania; now at CSIRO Marine and Atmospheric Research, Castray Esplanade, Hobart. Files in University of Tasmania computer asset # 00105714, now also in CSIRO Marine and Atmospheric Research computer, asset # CAF001488. Copies lodged with ASI.

2. Breeding plans for genetic improvement of oysters
Documentation Source Notebooks in possession of Peter Thompson, Dept. of Aquaculture, University of Tasmania; now at CSIRO Marine and Atmospheric Research, Castray Esplanade, Hobart. Files in University of Tasmania computer asset # 00105714, now also in CSIRO Marine and Atmospheric Research computer, asset # CAF001488. Copies lodged with ASI.

3. Tested methods of breeding oysters
Documentation Source Notebooks in possession of Peter Thompson, Dept. of Aquaculture, University of Tasmania; now at CSIRO Marine and Atmospheric Research, Castray Esplanade, Hobart. Files in University of Tasmania computer asset # 00105714, now also in CSIRO Marine and Atmospheric Research computer, asset # CAF001488. Copies lodged with ASI.

Appendix 2: Staff

Principal investigators

Peter Thompson (UTas)

Greg Maguire (UTas)

A large number of people have been employed directly by this project. Full time technical staff included:

Senior Technical Staff

Greg Kent (BSc UTas)	project inception to 1999
Matt Willis (BSc UTas)	1999 to end

Part time staff have assisted mostly in larval production or nursery rearing of the oysters. These junior technical staff included:

Bevan Hunter (BSc Honours UTas)

Alex Lynch (BSc Honours UTas)

Kate Turner (BSc Honours UTas)

Melannie Leef (B. Aqua, UTas)

Paul Armstrong (B. Aqua, UTas)

Heath Stafford (2nd year B. Aqua program, UTas)

James Wynne (2nd year B. Aqua program, UTas)

Michel Bermudes (PhD program, UTas)

Naomi Parker (PhD program, UTas)Greg Smith (PhD program, UTas)

Bronwyn Innes (CSIRO-CMR)

Appendix 3: TORC Survey

A survey conducted by the Tasmanian Oyster Research Council of Tasmanian oyster growers seeking information on desired traits in Pacific oysters (*Crassostrea gigas*).

Rank	Characteristic	Total score	No. farmers selecting
1	Meat yield	481	27
2	Total growth rate	406	24
3	Shell shape (L-W-D)	295	21
4	Disease resistance	265	23
5	Non-spawner	243	19
6	Glycogen content	202	16
7	Reduced curl-back	161	16
8	Late spawner	158	11
9	Improved shelf-life	125	13
10	Meat colour	105	14
11	Size of adductor muscle	94	14
12	Temperature tolerance	89	11
13	Mantle colour	85	11
14	Single sex stock	54	7
15	*Food conversion efficiency	50	1
16	*Uniform growth	45	3
17	Shell colour	40	6
18	Salinity tolerance	33	7

*Add ons to questionnaire

Appendix 4. Letter from Bob Ward to commercial oyster hatcheries

CSIRO Marine Research

GPO Box 1538 Hobart TAS 7001 Australia
Castray Esplanade Hobart Tasmania
Telephone (03) 6232 5222 Int +61 3 6232 5222
Facsimile (03) 6232 5000 Int +61 3 6232 5000
Web site: http://www.marine.csiro.au
Chief: Dr Nan Bray

To: Cameron of Tasmania Pty Ltd
Geordy River Aquaculture
Great Southern Oyster Company Pty Ltd
Shellfish Culture Ltd
South Australian Oyster Hatchery Pty Ltd

From: Bob Ward, CSIRO Marine Research

Date: November 22, 2000

cc: Peter Thompson, Barry Ryan, Mike Whillas, Vicki Wadley, Garry Zippel.

Commercial Trial of Genetically Improved Oysters

Summer 2000/2001 Hatchery Operation

The new FRDC project "Sustainable Genetic Improvement of Pacific oysters in Tasmania and South Australia" has as objective 4:

- Assessment of the performance of chosen lines in full-scale commercial trials.

Rationale:

The performance of some of the selected lines needs to be demonstrated and evaluated in full-scale commercial trials by standard hatchery production of millions of spat per chosen line and on-growing by any farm that wishes to purchase them.

Method:

1. Two lines of improved oysters plus a commercial control line (and by control line we mean any non-improved line that the hatchery wishes to spawn as part of normal operations) will be spawned each year. Broodstock will be made available to at least one hatchery in Tasmania and one hatchery in South

Australia. Spawning and rearing of spat will be the responsibility of the hatchery although the project team will provide information about which animals are to be crossed. These lines should be spawned as close together, temporally, as feasible.

2. At least some of the farms that buy the spat for on-growing will be requested to maintain these spat separate from other spat, so that growth can be monitored at six monthly intervals until point-of-sale.

We are now seeking expressions of interest from Tasmanian and South Australian hatcheries willing to carry out these three (two genetically improved, one control) spawning runs as part of their normal hatchery operation. It may be possible to supply additional lines to the hatchery if that is desired by the hatchery, but the minimum involvement sought is for the two improved lines plus a commercial control. It is anticipated that there will be no hatchery charge for these spawnings as spat will be sold by the hatchery.

The genetically improved broodstock will be supplied to participating hatcheries by the FRDC project team; the hatchery will be expected to supply commercial control broodstock.

Strip spawning will be deployed, with stripped animals returned to the project team for freezer storage and possible biochemical genetic analysis. Spawning will be such as to avoid inbreeding within families.

One condition of selling of this product to growers in Tasmania and South Australia is that the product be monitored during grow-out on some or all sites so that the relative performance of the three lines can be established.

According to the project Milestones, these commercial trials should be produced by 30 January 2001. We therefore need to establish which hatcheries wish to do this work soon, or else we will be too late for this year's spawning season.

Please direct any enquiries to either

- Bob Ward (CSIRO Marine Research, GPO Box 1538, Hobart, Tas 7001, phone 03 62325 370, fax 03 6325 000, email Bob.Ward@marine.csiro.au)

or

- Peter Thompson (Department of Aquaculture, University of Tasmania, PO Box 1214, Launceston, Tas 7050, phone 03 6324 3815, fax 03 6324 3804, email P.A.Thompson@UTas.edu.au).

Please forward expressions of interest to Bob Ward by December 15 latest.

Note that in future this request would be expected to come from the proposed company Australian Shellfish Industries Pty Ltd.