Sustainable Genetic Improvement of Pacific Oysters in Tasmania and South Australia

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Australian Government

Fisheries Research and Development Corporation

FRDC Project 2000/206

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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.

If the Joint Venture Company is not established by November 2000, we have three additional objectives:

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)

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

The project and its predecessor produced five generations of selectively bred oysters. Substantial improvements in the target trait, growth rate, were recorded. Currently c. 20% of industry production uses the selectively bred oyster lines, a percentage expected to increase. During the course of the project, the Tasmanian and South Australian oyster industries established a new company, Australian Seafood Industries Pty Ltd (ASI), to ensure the continuation of the breeding program and to facilitate technological transfer to industry. The main outcome is a more productive Pacific oyster industry.

The Pacific oyster breeding project initiated in FRDC 97/321 was continued. Both mass selection and family selection procedures were employed. The main trait of interest was growth rate, although shell shape and condition index were also recorded.

Families were monitored on five farms. Two were intertidal Tasmanian farms, one was a subtidal Tasmanian farm, and two were intertidal South Australian farms.

Growth rates among farms varied considerably, with the two South Australian farms generally producing higher growth rates than the three Tasmanian farms. However, rank performance of farms also varied from year to year, presumably due to environmental factors. Environmental variability from year to year makes inter-generational comparisons difficult, as any genetic gains may be confounded by environmental fluctuations. However, within any one generation, families generally performed with similar rank orders among farms, meaning that genotype by environment interactions were limited. That is, a good performing family at one farm usually also performed well at other farms.

There was a negative correlation between progeny numbers produced per family and weights at the end of nursery phase. This density dependent effect disappeared by the time oysters had reached two years of age.

Growth rates responded well to selection. The final generation of the project for which we have full data to the end of grow-out is generation four. In generation four, the mean of the mass selection lines and the mean of the family selection lines were both about 1.6x greater than the unselected commercial control line; while the three mass selection lines performed similarly, the fastest and slowest growing of the family selection lines had about 2.2x and 1.2x, respectively, the growth rate of the control line.

There was evidence in some but not all years of a negative correlation between growth rate and condition index. That is, the fastest growing families tended to have a lower condition index. However, condition index was estimated before animals reached market size, and there was some evidence that, at market size, differences in condition index might disappear. This needs further attention.

Attention was also paid to shape when selecting parents. In the fourth generation, virtually all oysters when individually measured showed acceptable shape. Indeed, using the ASI Shape Index, the mean shape scores of all selected families were lower than that of the control line, indicating superior shape for the selected families. There was some evidence that the heavier oysters were somewhat more elongate than lighter oysters, but the effect was very small.

There was also evidence from the fourth generation that the selected families showed less inter-individual variability for weight and condition index than the Control line; for shape variability there was little difference. Generally the selected lines gave more uniform product than the control line.

Full-scale commercial trials of some lines were undertaken. These were carried out to collect data from a much larger number of farms and also to expose these lines (and the project) to the wider community. Collecting reliable quantitative data from these lines proved impossible, but anecdotally these lines generally performed as well or better than control lines. These were necessarily early-generation lines and later lines would perform still better. Several lines have also been recently produced as standard commercial runs.

Heritability estimates indicated that additive genetic variance is present for most traits and that these could be exploited in any ongoing selective breeding program. Weight and length at both farms assessed (both Tasmanian) had high heritabilities, and at the better-sampled of these farms width and depth heritabilities were moderate to high. Overall there was no evidence for sire or dam (maternal) effects particularly in the parameter estimates from both farms. We observed very little evidence of genotype x environment interactions.

Genetic gains for the multi-traits identified as economically important (weight, width index and depth index) were estimated under different market scenarios and different genetic selection strategies. High weight gains, predicted to be 28%, were still possible when applying sufficient selection pressure on width index and depth index to maintain current shape. The strategy that produced the best gain was family/within family selection.

Molecular genetic research included developing new microsatellite DNA loci and comparing levels of genetic variation in mass selection lines with those in natural populations. The later generations of mass selection lines were shown to have reduced numbers of microsatellite alleles while allozyme alleles had been maintained. It was considered that because the allozyme diversity had been maintained, the microsatellite allele loss was not a great concern at this time. Census broodstock numbers in the mass selection lines examined were greater than the biologically effective broodstock numbers. Broodstock numbers in mass selection lines need to be maintained at reasonable numbers (ideally around 40-50 per generation and with equal numbers of males and females). It was shown that microsatellite DNA genotyping of individual larvae was achievable.

The project and its predecessor FRDC 97/321 produced five generations of selectively bred oysters before the program was handed over to Australian Seafood Industries Pty Ltd (ASI). ASI was established by the Tasmanian and South Australian industries during the course of the project to ensure the continuation of the breeding program at the end of the project and to facilitate technological transfer to industry.

KEYWORDS: Pacific oyster, *Crassostrea gigas*, growth rate, heritability, genetic selection, selection index, microsatellites.

ACKNOWLEDGEMENTS

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Many people have assisted this project over the years:

Greg Maguire (then of the University of Tasmania, now of WA Fisheries) was instrumental in its conception back in 1993 and was the initial Principal Investigator on FRDC 97/321.

Louise English, a CRC Aquaculture supported PhD student at CSIRO Marine Research/UTas Aquaculture, was responsible for much of the early allozyme diversity work.

Dan McGoldrick, a CRC Aquaculture supported post-doc at CSIRO Marine Research, was responsible for much of the earlier molecular genetic work including construction of marker and QTL maps, and the earliest estimates of heritability. This work is detailed in Ward and Thompson (2002)

Cam McPhee (Queensland Department of Primary Industry), Jordan Howarth (CSIRO Mathematics and Information Science), and Laurie Piper (CSIRO Livestock Industries) all provided quantitative genetics advice in the early stages of the project.

Greg Kent and Mat Willis (UTas Aquaculture) were senior technicians at TAFI/UTas who assisted with all hatchery and farm operations and collected most of the grow-out data.

Bronwyn Innes (CSIRO Marine Research) helped carry out the DNA laboratory work.

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

Colin Sumner, then Chair of TORC, provided highly valued input into the project in its formative years.

Barry Ryan, following Colin as Chair of TORC (and ASI), continued to support the on-going progress of the work and has always provided valuable comments to the research team.

Jon Poke kindly permitted the use of significant areas of his lease for nursery growth and was also one of the five grow-out farms used by the project. We also thank the remaining oyster farmers who provided space for our oysters on their farms: Di Murdoch/Tim Monigan (Pittwater), Sebastian Rainer (Coles Bay), Mike Whillas (Coffin Bay) and the Zippel brothers (Bruce, Gary and Ashley) (Smoky Bay).

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We thank Scott Parkinson (General Manager of ASI) for providing the final weight checks of the M4 and F4 animals and the end of nursery phase weight checks of the M5 and F5 animals; these were taken after the November 2003 when FRDC Project 2000/206 (except for the selection index time extension) stopped.

Scott Parkinson, Barry Ryan and Nick Elliott provided useful suggestions for improving earlier drafts of this Report.

CHAPTER 1. Introduction

1.1. Background

In Australia, production of Pacific oysters has been increasing, reaching about $20m in 1997/98 (and >$27m in 2002/03 ABARE 2004, Australian Fisheries Statistics 2003, Canberra). Most production is in Tasmania and South Australia, where the industry's complete reliance upon hatchery-produced seed makes it unusually well suited to gain benefits from selective breeding.

Before embarking on such a program, the industry wished to be assured that genetic variation remained in its source Tasmanian populations. A CRC-Aquaculture project (CRCA) established that little variation had been lost during or subsequent to the import of spat from Japan in the late 1940's and early 1950's (English *et al.*, 2000; Ward *et al.*, 2000; Ward and Thompson, 2001).

A selective breeding program was established by FRDC (97/321) and CRCA in 1997, with the full support of industry (Ward *et al.*, 2000; Ward and Thompson, 2001). Growth rate had been increased about 8% in the first generation of mass-selection. We anticipated that this rate of increase could be maintained for several generations to yield a commercial line up to 40% faster growing than current oysters. A second mass-selection generation was evaluated in that initial program with growth rates about 10% faster than control animals.

In 1997 we produced six full-sib families followed by a further 40 mostly half-sib families in 1998. Most of these lines were grown at five commercial oyster farms (three in Tasmania, two in South Australia). These lines had captured genetic variance for growth and other traits. At least one major growth gene, colour traits, and 'curlback' segregated within these families. The shell abnormality 'curl-back' can be a considerable impediment to oyster growers; it is recessive in nature, and we expected to be able to eliminate it from pedigreed lines.

Within the family lines we had data on oyster weight from F1 pair matings and some F2 inbred and outbred lines. There were some inter-farm differences in performance, indicating that specific lines of oysters might need to be developed that are better suited to specific areas. The data were analysed to determine how much of the variation in whole weight and meat yield among and within lines is genetic and can be captured in broodstock development. Initial estimates of these heritabilities by Dan McGoldrick were ~0.2 to 0.3.

A molecular genetics project, funded originally by the CRCA, ran alongside this initial phase of the selective breeding project. Microsatellite genetic markers enabled us to confirm (or refute) the pedigrees of particular animals. We produced a low resolution linkage map of the 10 oyster chromosomes and identified gene markers flanking a major growth QTL (quantitative trait loci) in one family. This work needs considerably more development, but could then be used in marker-assisted selection. This would allow us to choose better performing animals at a very early stage and would facilitate the transfer of valuable characteristics between lines.

This initial FRDC/CRCA program produced results that excited the Pacific oyster industry. At the end of that program a limited joint venture company was proposed, consisting of FRDC, CRCA and the two oyster associations TORC (Tasmanian Oyster Research Council) and SAOGA (South Australian Oyster Growers Association). The major assets of this company would be improved lines of oysters together with a breeding program.

In this Report we bring together data from this early work and data from the second phase of mass and family selection (FRDC project 2000/206). We also report on advances in commercialising the work.

1.2. Need

We demonstrated in FRDC 97/321 that oyster characteristics deemed valuable by industry could be improved by selective breeding. We and industry were convinced that substantial performance increases for commercial lines were achievable.

In FRDC 97/321 we concentrated our efforts on a single trait (growth). In FRDC 2000/206 we planned to continue the focus on growth while also assessing shape and condition index and eradicating or reducing the deleterious curl-back trait. This will yield a much improved commercial product.

The needs as specified in the FRDC 2000/206 project proposal were to:

1. continue the breeding program through at least three more generations, in both the mass selection and family lines, by producing, where possible, improved lines every year rather than every two years as in FRDC 97/321. Performance assessment would continue through to the second year.
2. develop a selection index which uses all information about genetic merit over several commercial traits. This is the sum of the commercial gains an individual can transmit weighted by commercial value.
3. monitor grow-out performance at one year of age and two years of age, to determine if crosses can be made at one year of age rather than two years. This would speed selective improvement. We need to assess whether performance at one year is a good indicator of performance at market size (currently ~2.5 years).

If the Joint Venture company (JVC) proposed to commercialise our work was not established, then we would need to:

4. work with industry to conduct trials of particular lines in both Tasmania and South Australia under full commercial conditions.
5. develop sophisticated long-term breeding plans which yield on-going performance improvements while avoiding the deleterious effects of inbreeding. These plans would be based on analysis of data collected during the project, and would require a major commitment from both technical staff and geneticists.
6. develop a commercialisation strategy (within 12 months of start) that included formation of an entity which provides for IP ownership, ongoing mass production of improved lines/families, industry funding and equity ownership.

Industry would take the majority of responsibility for developing this commercialisation strategy; FRDC would assist in facilitating this output.

FRDC funding was thus requested to complete the development program and, if the JVC were not established, to conduct the commercialisation trials and development of breeding plans. If the JVC was established, then we would provide it with broodstock for the trials but would expect it to develop its own long-term breeding strategy with input from and collaboration with our technical staff and geneticists.

1.3. General Introduction to this Report

Five generations of selectively bred Pacific oysters have now been produced with FRDC funding. The first two generations were produced in FRDC 1997/321 (with Greg Maguire / Peter Thompson as PIs); generations 3, 4 and 5 were produced in FRDC 2000/206. In November 2003 responsibility for continued production of the breeding lines was handed over to the newly-established Joint Venture Company *Australian Seafood Industries* who produced the generation 6 lines in January 2004.

Much of the information from the first two generations has been published in two previous reports, namely the CRC Aquaculture Final Report "Genetic Studies and Selective Breeding of Table Oysters" (Ward and Thompson, 2001) and the FRDC Final Report 1997/321 "Selective Breeding of Pacific Oysters" (Thompson, 2005).

Since the two FRDC projects were contiguous, so that, for example, the F2 generation produced in FRDC 1997/321 was monitored at the grow-out stage in FRDC 2000/206, we have decided to treat these two projects as essentially a single project in this Report. Spawning of the selection lines started in 1996/97, and the spawning each year (and growth of those spat into adults) is treated in a separate chapter. Both mass lines (using multiple parents) and family lines (using single-pair matings) have been produced. The various mass lines and family lines, and their inter-relationships, are summarised in Figure 1.1. After these chapters, there are chapters on specific topics such as commercial trials and commercialisation, selection index development and heritability estimation, and molecular genetics. Finally there is a summary chapter.

In each generation, oysters were spawned in a commercial hatchery (using project equipment) and then after settlement and at about 0.5mm spat were transferred to upwellers at TAFI facilities at Taroona. They were fed and maintained at TAFI for a period of several months until they were about 2mm in size and large enough to go to a farm nursery at Bolduans Bay oysters, Smithton. Some months later, at about 6 mm size, they were distributed to five grow-out farms for performance monitoring. Three of these farms were in Tasmania (intertidal farms at Smithton and Pittwater, a subtidal farm in Great Oyster Bay) and two were in South Australia (intertidal farms in Coffin Bay and in Smoky Bay). Three bags of 100 oysters per line (where available) were distributed to each farm[1]. Monitoring took place over approximately 18 months. At approximately three-monthly intervals, bags of oysters were weighed and numbers of oysters in each counted. At one of the assessment times, individual oysters were removed and assessed for individual size, shape and condition index.

[1] Except for the F1 lines, where two bags of juveniles of each of two size grades went to each farm.

The traits that formed the basis of the selection program were growth rate and meat yield. These traits were chosen following a survey of Tasmanian farmers by the Tasmanian Oyster Research Council. Sixteen 'desirable' characteristics were listed, and farmers asked to allot points according to their preferences (Table 1.1). The characteristic receiving the highest score was meat yield, followed by growth rate and by shell shape. These characteristics will be the ones focussed on in the selection programs. All are certainly highly variable in Tasmania, and while at the start of the program heritabilities for these traits had not been established in Tasmania, all were expected to be of the region of 0.2 to 0.3 (based on work elsewhere, e.g. Lannan, 1972; Sheridan, 1997).

Table 1.1. Desirable oyster traits (from TORC News No. 8, 1998)

Thirty-one Tasmanian farmers responded to the survey. Farmers were asked to allocate scores out of a total of 100 to the listed traits. Summed score is 2931, slightly less than the 3100 expected.

Rank	Characteristic	Total Score	Farms 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 by farms

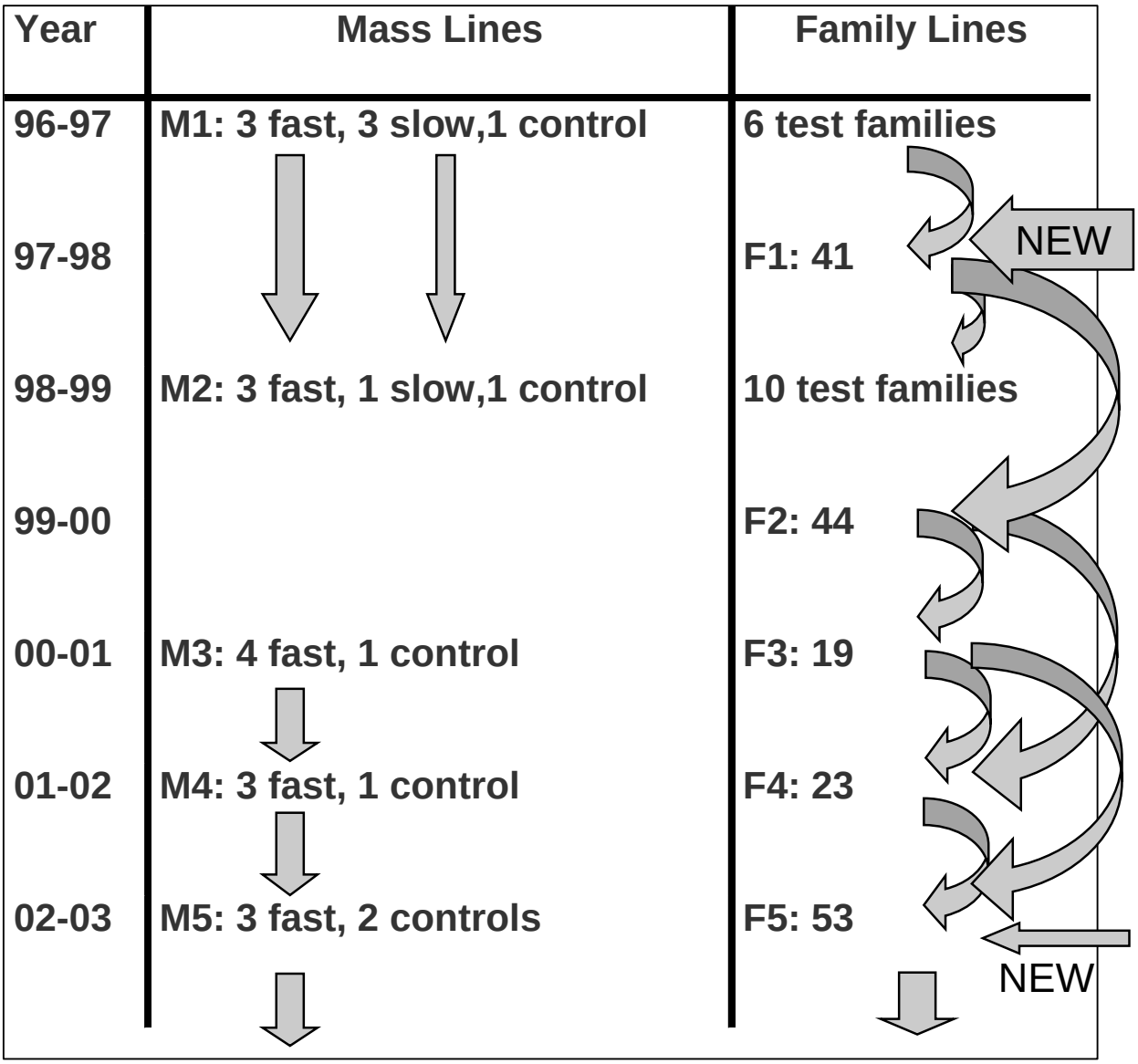


Fig 1.1. Outline of breeding program schedule. NEW indicates incorporation of animals from outside the breeding program.

CHAPTER 2. First Spawning Year 1996/7 – The Mass Selection M1 Lines plus 6 Test Families

2.1 The Six Test Families

The 6 test families (Table 2.1) were derived from single pairs of putatively unrelated or only distantly related commercial broodstock, and were created on 8 January 1997 at Marine Shellfish Hatcheries, Bicheno. On January 29 animals were transferred to TAFI (Taroona) and on May 8 to Bolduans Bay. These were subsequently used for various genetic molecular analyses (McGoldrick *et al.,* 2000, Ward and Thompson, 2001). These animals will not be discussed further here, other than to note that some of the progeny were used as parents in the first generation of pairwise matings in the selective breeding project, i.e. as F1 parents spawned in 1997/98.

2.2. The MI Lines

Marine Shellfish Hatchery was the hatchery used (19 February 1997), followed by spat rearing at TAFI (Taroona) (March 25-May 26) and nursery (overwintering) at Bouldans Bay (May 26 – March 1998).

The mass selection lines were derived from two separate groups of oysters that were spawned in the summer of 1993/4 and thus yielded two separate cohorts. In the summer of 1996/7 the upper and lower 20% size tails of each cohort (all of the same age within a cohort and therefore variation in size reflecting variation in growth rate) were selected to form the parents of high (fast-growing, F) and low (slow-growing, S) lines. The tails of each cohort were pooled as broodstock; about 70 oysters were used to found each of these lines. A control or commercial line (C) was also established. This was derived from 57 commercial broodstock animals that were unrelated to the mass selection parents. The hatchery operator chose oysters with broadly desirable characters, for example a 'good' shape. Arguably, it would have been preferable to have broodstock from the cohort from which the high/low lines were derived to act as a control, but available resources did not permit this. In any case, to have commercial value, selected lines must surpass normal hatchery lines; from this standpoint using a commercial spawning as a quasi-control was deemed appropriate. Animals were strip-spawned. Each line was subdivided to form three replicates, so that there were three fast-growing sub-lines, three slow-growing sub-lines and three control sub-lines.

Once animals had exceeded 6mm in the farm nursery they were graded as is standard commercial practice and two size grades were formed per replicate (25-50%, and 75-100%). One or more bags of each replicate and size grade went to each of the five grow-out farms in March 1998.

Bags were weighed and animals counted in March 1998, July 1998, October/November 1998 and February/March 1999. We present here only a summary of the data for the final weight checks (Table 2.2).

Several conclusions can be drawn from the mean oyster weights in Table 2.2. Firstly, the F lines consistently performed better than the commercial controls, with an average weight gain for both size grades of about 9-10%, and much better than the S lines, with an average weight gain of about 20-25%. In fact this final estimate of the Fast advantage will have been artificially lowered a little by removal of the largest

M1-F75 animals from one of the Tasmanian sites for use as broodstock in the production of the second generation of mass selection. Secondly, there was considerable performance variation among farms, with growth rates at the best performing farm about double those of the slowest farm. These site differences were quite consistent among lines. Thirdly, pooled across lines, the 75 grade grew about 20% faster than the 25 grade.

At the two last weight checks (Oct/Nov 98 and Feb/March 99), 10 randomly selected oysters per replicate were sacrificed and various measurements taken, including length (mm), width (mm), depth (mm), total wet weight (g), shell wet weight (g) and dry meat weight (g). We were particularly interested in determining the nutritive status of the different lines via estimation of a condition index, and whether condition index and meat yield were positively or negatively influenced by selection for rapid growth.

A.E. Hopkins is credited with defining the first quantitative condition index equation (as in Higgins 1938):

Condition index (CI) = [dry soft tissue wt (g) x 100]/internal shell volume (cm^3)

Lawrence and Scott (1982) presented a revision of this equation as:

CI = [dry soft tissue wt (g) x 100]/internal shell cavity capacity (g)

These two equations are effectively identical as the internal shell volume in cm^3 is very close to the weight of the flesh of the animal plus its surrounding fluids within the shell (the effective density of the flesh and surrounding fluids does not vary significantly from 1 g cm^3 , Lawrence and Scott 1982).

There are various other similar CI equations, some with a x1000 factor rather than x100.

We use here the formula recommended by Crosby and Gale (1990):

CI = [Dry soft tissue weight (g) x1000] / internal shell cavity capacity (g)

where dry soft tissue weight (g) is the dry weight of the flesh after overnight oven drying, and internal shell cavity capacity (g) is whole animal wet weight (g) minus the shell wet weight (g) measured after removing the flesh and fluid contents of the shell.

Animals were processed fresh, and were often held in recirculation tanks until measurements had been taken. This process took up to one week per farm.

Mean individual oyster weights (Table 2.3) accorded well with those derived from the bag estimates (Table 2.2). Condition index was highest at the Coffin Bay site (although fewer oysters could be measured here than elsewhere) and lowest at Coles Bay. There was little consistent difference in CI between the M1-F, M1-S and M1-control lines.

Table 2.1. Parental Phenotypes for Six Test Pair-Crosses 97-1 to 97-6.

Cross	Male	phenotype	Cohort		Female	phenotype	Cohort
97-1	1	large	dsar4	x	A	large	dsar4
97-2	2	medium	e231	x	B	large	e141
97-3	3	medium	dsar4	x	C	medium (golden)	mscx
97-4	4	medium	dsar4	x	D	small	e140
97-5	5	small	e91	x	E	small	e33
97-6	6	small	dsar4	x	F	medium	dsar4

Table 2.2. Final weight checks for M1 lines

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	Overall*
Date sampled	24/2/99	24/2/99	24/2/99	17/3/99	17/3/99	
Age (mths)	24	24	24	25	25	
Average oyster weight (mean of bag weights/oyster counts) with SD						
M1-F25	51.9±5.6	27.3±2.3	40.5±2.6	44.3±7.1	73.9±6.9	47.6±17.2
M1-S25	40.7±7.7	19.2±2.1	27.7±8.9	37.0±5.3	54.8±2.9	35.9±13.5
M1-C25	45.4±4.8	21.5±5.7	33.2±3.8	49.3±5.0	61.0±5.4	42.1±15.2
M1-F75	58.7±9.9	38.5±4.2	42.4±9.3	67.5±6.9	85.3±3.5	58.5±19.1
M1-S75	51.3±3.2	32.5±2.9	37.8±3.3	50.2±6.6	67.2±2.6	47.8±13.5
M1-C75	56.1±1.5	37.7±8.6	39.8±18.0	59.8±3.0	73.9±0.6	53.5±15.0
Overall**	50.7±7.5	29.4±8.2	36.9±5.5	51.3±1.6	69.4±2.2	
Total number of bags/oysters						
M1-F25	7/643	5/325	7/636	6/545	6/545	31/2694
M1-S25	9/778	8/587	8/712	7/595	7/633	39/3305
M1-C25	4/314	2/100	4/361	2/174	3/259	15/1208
M1-F75	6/555	5/324	6/403	5/417	5/448	27/2147
M1-S75	7/638	6/405	7/500	5/431	5/446	30/2420
M1-C75	4/358	2/157	4/212	3/257	3/263	16/1247

*overall mean (with SD) of the five farm means, or summation

** overall mean (with SD) of the six line means

Table 2.3. Average oyster weight and condition index of individually measured M1 oysters

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	Overall*
Date sampled	24/2/99	24/2/99	24/2/99	17/3/99	17/3/99	
Age (mths)	24	24	24	25	25	
Average oyster weight of individually measured oysters						
M1-F25	54.5±13.9	30.2±12.2	47.6±17.2	56.3±17.9	77.5±14.8	53.2±17.0
M1-S25	42.3±12.8	24.4±12.1	29.6±6.6	43.5±11.9	55.4±12.9	39.0±12.3
M1-C25	49.5±15.2	24.8±10.7	37.1±8.2	-	63.1±16.5	43.6±16.4
M1-F75	62.6±20.9	41.7±13.8	49.1±10.8	-	90.7±21.3	61.0±21.6
M1-S75	52.4±13.1	37.5±13.4	40.7±7.7	51.2±11.3	68.3±15.7	50.0±12.1
M1-C75	57.2±15.1	37.7±10.0	39.4±12.1	62.1±22.6	79.8±22.3	55.2±17.4
Overall	53.1±6.9	32.7±7.3	40.6±7.2	53.3±7.9	72.5±12.7	
Average condition index (CI)						
M1-F25	45.6±12.7	31.5±8.7	48.9±5.3	95.6±24.2	44.0±10.1	53.1±24.6
M1-S25	45.1±9.7	32.3±15.4	62.7±9.9	82.5±32.7	50.5±11.7	54.6±19.0
M1-C25	42.6±9.9	34.6±6.2	49.3±6.1	-	54.7±13.7	45.3±8.7
M1-F75	48.1±6.4	32.6±8.3	52.1±8.3	-	46.2±10.2	44.8±8.5
M1-S75	47.0±8.1	41.2±12.6	54.6±6.8	84.2±14.9	46.1±10.0	54.6±17.2
M1-C75	42.7±8.4	36.5±9.8	54.2±7.1	115.7±11.7	56.9±11.0	61.2±31.6
Overall**	45.2±2.2	34.8±3.6	53.6±5.0	94.5±15.3	49.7±5.2	
Total oysters measured						
M1-F25	30	30	30	18	30	138
M1-S25	30	30	30	14	30	134
M1-C25	30	16	28	0	30	104
M1-F75	30	28	30	0	30	118
M1-S75	30	29	30	12	30	131
M1-C75	30	20	30	3	30	113
overall	180	153	178	47	180	738

*overall mean (with SD) of the five farm means, or summation

** overall mean (with SD) of the six line means

CHAPTER 3. Second Spawning Year 1997/8 – The Family Selection F1 Lines

3.1. The F1 lines

These lines were spawned on December 4 1997 and January 2 1998 at Shellfish Culture, Bicheno. Forty single pair crosses plus one mass-spawned commercial control were established (Table 3.1).

For the single pair crosses, one male was mated to each of two separate females to allow the production of half-sib as well as full-sib families. This was to allow genetic parameter estimation, although in the final analyses these had very large standard errors and are not presented here. Instead, purpose designed experiments to estimate heritability were undertaken (Chapter 10). Most of the F1 crosses were true F1 families (with stock taken from the hatcheries of Shellfish Culture, Camerons and Geordy River, along with a few wild animals), but a few second generation F2 families were established from some of the single pair crosses (97-1 to 97-6) made in 96/97 (Table 2.1). Three of the seven latter were inbred lines produced by full sib mating. Spat rearing was at the TAFI facility at Taroona . Spat were moved to nursery grounds at Bouldans Bay Oysters and graded in May 1998, retaining a maximum of 5000 animals per family. Ten of the 41 families (Table 3.1) did not produce this many survivors. A small and a large size grade were made for each family. These were animals in the 20-50% and 50-80% size ranges, respectively. When large enough, animals were transferred to the five on-growing farms in November and December 1999. Generally, each family was represented by two replicates of 100 oysters each of each of the two size grades (where numbers permitted). Not all families/size grades were represented at each site where there were small numbers.

Animals were assessed in June/July 99, August/September 99, December 99, and March/April 00**.** On each visit performance was estimated from the whole weight of a known number of individuals per bag (usually about 100, but in the latter stages sometimes the 100 oysters were separated into two bags of 50 oysters). In June/July 1999, families were ranked for growth performance and a mean rank estimated (Table 3.6). This formed the basis for selection of parents for the F2 lines (section 5.1)

Final weights (derived from bag estimates, grades pooled) and oyster numbers are presented in Tables 3.2 and 3.3 respectively. Three families were lost (MCOMM, YA007 and YA016) leaving 38 families with data. Pooling the grades here is reasonable as, overall, about equal numbers of the two grades were weighed (Table 3.3) and it simplifies presentation of the results.

There is an approximately two-fold difference in weight between the best-performing (RB015) and worst-performing (WA996) families. WA996 was an inbred family, it and the other two inbred families (WA981 and WA 987) made up three of the bottom four families. WA996 deserves its position as the slowest-growing family; it was present at four of the five sites and at each site it was by an appreciable margin the slowest family. On the other hand, WA981 and WA987 were only present in Tasmania (at two and one site, respectively), and where present grew only slightly less than the overall mean for these sites. Because they were absent from the two

higher-performing South Australian sites, their overall unweighted means are lower than they would otherwise be.

Generally speaking, families that performed well at one site performed well at another, and those that performed poorly at one site tended to perform poorly at another. In other words, any genotype by environment interaction was not high. This effect was analysed using the ASReml computer package (Gilmour *et al.,* 2002). The following model was fitted:

weight = mean ± site ± family ± family*site

with family and family*site being treated as random effects, and site as a fixed effect. In this generation, grade and site*grade were also included as fixed effects. It is difficult to test hypotheses about fixed effects in a mixed model like this, because the error degrees of freedom are unknown. However, the family*site variance ratio, or genotype by environment interaction, was estimated at 0.19 ± 0.04. This is significant, but not large.

The family correlations between pairwise combinations of farms are shown in Figure 3.1. These graphs were created in the statistical package "R" (R Development Core team, 2004) using the results from the ASReml analysis (Gilmour *et al*., 2002). Lines are smoothed regressions and the numbers in the upper panel are simple product moment correlations between each set of family means. Correlations range from 0.66 to 0.91 (all $P < 0.001$).

There is again a very large site-difference, with the two South Australian sites performing better than the three Tasmanian sites, and with growth rates at Smoky Bay being about double that at Pittwater. Note that at the final weight check, relatively few families were present at Coles Bay, and none of these were present in replicates. Coles Bay in fact started off with about the same number of animals as Pittwater and Smithton, but more than half the bags were lost when the module in which they were contained broke away and sank to the bottom of the bay. In the M1 mass selection lines, spawned a year earlier, Coles Bay had the slowest growing oysters – in these F1 family lines, Pittwater had the slowest growing animals. Such apparent inconsistencies may relate to differing environmental conditions between sites and years, or perhaps to possibly differing locations within leases between years.

Differences between the two size grades were very marked at the first weight checks (Table 3.4), with the larger 80 grade being about 30% larger than the smaller 20 grade. This was about 13 months post-grading, when the animals were about 19 months old. At the final weight check, the 80 grade animals remained larger than the 20 grades, but only by about 12%. These performance differences of the grades were quite consistent among farms. Also, the performance differences of the farms were consistent over time, with Smoky Bay doing best at both first and final checks and Pittwater doing least well.

Individual measurements of weight and dry meat weight were taken on 17 August 1999 (Coles Bay), 26 August (Pittwater), 9 September (Smithton) and 23 September (Coffin Bay). No such measurements were taken for Smoky Bay because they perished in transit and putrified. Oysters collected from the field in Tasmania in

August and September are just starting to recover from winter. At less than two years of age they are not yet at market size nor is the older commercial crop in a very marketable condition at this time of year. While the strategy of taking individual measurements at this time of year was not ideal, we needed the data then to assist with the selection of broodstock and the project staff were too busy with larval and spat rearing duties at other times. These caveats need to be born in mind when assessing condition index data. We were more interested in differences among lines within farms than with differences among farms.

Data, pooling the 20 and 80 grades, are summarised in Table 3.5. This gives the mean wet weight and mean condition index per family per farm. There are some gaps in the data. Condition index was on average highest at Coffin Bay and lowest at Pittwater. At each farm, and overall, when mean family weights and condition index were cross-correlated, the correlation was negative. This negative correlation was statistically significant ($P < 0.05$) for two of the four farms and for the overall data (where $r = -0.388$, see Table 3.5). In other words, those families with higher wet weights tended to have lower condition index, and vice versa. While this trend was virtually non-existent at Coffin Bay which had the highest growth rates, the selection program will have to bear this unfavourable correlation in mind.

Table 3.1. The first generation of family lines. Three families of uncertain origin were eliminated (NOTAG, YAO16XX and X1). Females mated once, many males mated twice. Animals all from hatchery stock except when indicated as 'wild'. SFC=Shellfish Culture

Family Number / Female parent	Male parent	Gen.	Inbreeding coefficient	Half-sib family	Number retained	Female source	Male source
Spawn Dec 4 1997							
WA214	RB010	F1	0.0	3	5000	Cameron	SFC
WA225	RB010	F1	0.0	3	5000	Cameron	..
RB000	RB018	F1	0.0	4	5000	SFC	SFC
YB020	RB018	F1	0.0	4	5000	Geordy River	..
RB048	RB023	F1	0.0		5000	St Helens wild	Geordy River
RB004	RB024	F1	0.0	5	4200	SFC	Geordy River
YA018	RB024	F1	0.0	5	5000	SFC	..
RB002	RB033	F1	0.0	6	5000	SFC	Geordy River
RB011	RB033	F1	0.0	6	5000	SFC	..
RB044	RB039	F1	0.0	7	5000	St Helens wild	unknown
YA011	RB039	F1	0.0	7	5000	SFC	..
RB006	RB046	F1	0.0	8	5000	SFC	St Helens wild
WA219	RB046	F1	0.0	8	5000	Cameron	..
RB042	RB050	F1	0.0	9	5000	St Helens wild	unknown
YA016	RB050	F1	0.0	9	5000	SFC	..
RB029	YB021	F1	0.0	13	5000	Geordy River	Cameron
YA013	YB021	F1	0.0	13	5000	SFC	..
RB012	YB022	F1	0.0	14	5000	SFC	Cameron
WA213	YB022	F1	0.0	14	5000	Cameron	..
RB022	YB033	F1	0.0	15	5000	Geordy River	Cameron
YA006	YB033	F1	0.0	15	5000	SFC	..
Spawn Jan 2 1998							
WA979	WA978	F2	0.0	1	5000	97-2	97-1
WA980	WA978	F2	0.0	1	5000	97-2	97-1
WA992	WA981	F2	0.0	2	5000	97-5	97-6
WA993	WA981	F2	0.0	2	5000	97-5	97-6
WA981	WA982	F2	0.250		<<5000	97-3 (gold)	97-3 (inbred)
WA987	WA988	F2	0.250		<<5000	97-2 (red)	97-2 (inbred)
WA996	WA997	F2	0.250		5000	97-5	97-5 (inbred)
CURLS	CURL1	F1	0.0		114	curlback	curlback
MCOMM	MCOMM	G1	0.0		3700	mass animals	mass animals
WA928	WA919	F1	0.0		5000	Cameron	Bridport wild
YB008	RB014	F1	0.0		5000	Cameron	SFC
YA007	WA919	F1	0.0		1700	unknown	unknown
RB015	WA924	F1	0.0	10	5000	SFC	Cameron
YA002	WA924	F1	0.0	10	5000	Cameron	..
RB003	WA931	F1	0.0	11	<<5000	SFC	Cameron
WA925	WA931	F1	0.0	11	<<5000	Cameron	..
RB032	YA001	F1	0.0	12	5000	Geordy River	Cameron
WA921	YA001	F1	0.0	12	<<5000	Cameron	..
WA234	YA243	F1	0.0		5000	Cameron	golden
WA930	YB007	F1	0.0		<<5000	Cameron	Cameron

Table 3.2 Final weight checks for F1 lines (ranked in order, bags weighed and animals counted, size grades pooled, mean weight (g) per animal given)

Date sampled Age (mths)*	Pittwater 10/3/00 28 mean±SD	Coles Bay 9/3/00 28 mean±SD	Smithton 8/3/00 28 mean±SD	Coffin Bay 4/4/00 29 mean±SD	Smoky Bay 6/4/00 29 mean±SD	Unweighted mean±SD
RB015	55.62±2.58		86.12±6.91	93.71	127.97±7.67	90.86±29.72
RB012	49.30±6.46		83.18±15.39	103.47±6.52	122.44±2.64	89.60±31.28
RB042	50.11±3.50		79.88±5.94	104.07±3.80	115.90±7.80	87.49±29.08
WA213	47.24±3.62		72.99±3.65	100.21±16.75	122.93±9.02	85.84±32.85
RB003	55.06±11.64	70.11	91.89±10.83	85.04±2.16	123.86±18.42	85.19±25.86
RB006	53.57±5.26		80.46±2.12	94.68±4.01	111.41±1,51	85.03±24.49
WA225	55.69±5.03		81.35±6.37	78.51±12.42	122.92±8.36	84.62±28.00
YA002	49.20±5.07		74.78±7.07	89.05±8.22	121.87±6.95	83.73±30.31
WA214	53.46±5.78		76.53±5.22	93.84±5.44	109.73±16.31	83.39±24.12
RB044	49.31±4.12	83.81	69.21±4.75	89.62±1.80	123.73±5.00	83.14±27.52
WA930	52.66±5.97		71.83±6.10	86.12±13.80	112.80	80.85±25.33
WA219	48.75±5.90	88.31	75.46±7.16		110.76±14.20	80.82±25.89
YA018	50.40±4.79	73.07	71.42±8.09	93.37±2.18	108.43±6.19	79.34±22.27
CURL			78.8±8.52			78.80
WA928	51.48±4.33	82.06	76.55±8.98	78.65	104.92±10.56	78.73±19.00
WA234	53.52±5.04	72.50	79.38±9.21	79.65±12.65	107.93±9.64	78.59±19.55
WA925	51.10±13.01	86.56	81.44±9.83	93.83		78.23±18.79
YA006	44.65±2.65	94.30	60.95±11.16	80.39±10.68	104.34±7.63	76.92±24.30
WA993			74.66±9.47			74.66
WA992	46.84±4.69	70.12	76.24±8.18	70.62±7.94	109.16±7.87	74.59±22.38
RB029	39.62±8.51	77.56	64.53±8.09	78.48±13.38	103.99±9.30	72.84±23.44
YA013	46.17±3.53	59.26	65.72±7.76	92.83±7.57	99.81±9.56	72.75±22.77
YB008	49.95±1.55	63.15	65.22±5.60	78.55±5.88	106.72±9.19	72.72±21.54
RB022	47.91±5.41		62.8±11.31	75.56±9.34	104.58±8.52	72.71±24.07
WA921	43.07±6.24	76.50	68.69±12.62	75.00	97.80±6.98	72.21±19.64
RB004	42.95±8.41		61.04±16.66	84.16±5.46	91.40±16.01	69.89±22.14
RB000	38.02±3.02		62.72±9.48	80.28±14.45	95.33±11.91	69.09±24.63
YB020	43.84±3.56	60.30	63.19±6.64	80.64±7.11	93.59±10.71	68.31±19.24
WA979	43.69±8.43	57.41	61.62±8.07	74.80±10.75	100.67±7.89	67.64±21.54
RB032	41.20±4.73		55.93±8.26	74.35±9.40	98.46±14.15	67.49±24.71
YA011	39.50±8.32	67.27	62.07±5.05	77.59±4.89	89.30±10.79	67.15±18.64
RB002	44.71±2.41		63.31±9.05	70.35±5.37	86.77±6.51	66.28±17.42
WA980	47.31±6.05	48.79	64.53±13.32	64.57	103.20±9.09	65.68±22.54
RB011	40.13±6.91	62.74	56.58±8.39	81.35±19.64	85.10±6.21	65.18±18.48
WA981			63.53±17.91			63.53
RB048	37.42±6.47		55.15±11.44	64.08±1.30	85.31±8.74	60.49±19.91
WA987	46.33±13.16		63.18±21.23			54.75±11.92
WA996	27.78±3.56		49.93±7.07	57.25±14.17	57.17±6.25	48.03±13.93
Mean	46.79±6.08	71.88	69.81±9.64	82.57±11.20	104.86±14.78	74.66±9.63

Table 3.3. Numbers of oysters present at each site at the final weight check, separated by grade. Family order as in Table 3.2

	Pittwater			Coles Bay			Smithton			Coffin Bay			Smoky Bay			Overall		
	N20	N80	Total	N20	N80	Total	N20	N80	Total	N20	N80	Total	N20	N80	Total	N20	N80	TOTAL
RB015	193	182	375				169	139	308		70	70	101	99	200	463	490	953
RB012	194	170	364				175	171	346	70	52	122	99	101	200	538	494	1032
RB042	95	191	286				115	181	296		146	146	100	49	149	310	567	877
WA213	189	170	359				181	180	361	74	151	225	101	51	152	545	552	1097
RB003	92	177	269	46		46	126	134	260	144	95	239	99	50	149	507	456	963
RB006	186	97	283				133	181	314	140	137	277	100	99	199	559	514	1073
WA225	94	85	179				182	191	373	148	147	295	99	100	199	523	523	1046
YA002	192	95	287				127	182	309	74	74	148	94	100	194	487	451	938
WA214	186	190	376				181	177	358	150	100	250	98	101	199	615	568	1183
RB044	190	95	285	47		47	180	180	360	74	158	232	100	50	150	591	483	1074
WA930	192	91	283				130	179	309	66	139	205		50	50	388	459	847
WA219	180	180	360	49		49	167	179	346		74	74	49	100	149	445	533	978
YA018	95	181	276	95		95	87	178	265	70	145	215	100	98	198	447	602	1049
CURL							50	44	94							50	44	94
WA928	186	95	281		48	48	139	178	317	74	73	147	99	99	198	498	493	991
WA234	92	179	271		50	50	179	138	317	150	73	223	99	100	199	520	540	1060
WA925	186	95	281	43		43	173	134	307		81	81				402	310	712
YA006	186	177	363		30	30	179	132	311	144	71	215	100	92	192	609	502	1111
WA993							164	91	255							164	91	255
WA992	184	181	365	50		50	179	134	313	145		145	98	101	199	656	416	1072
RB029	149	182	331		48	48	184	161	345	74	151	225	98	100	198	505	642	1147
YA013	184	181	365	47		47	172	184	356	74	148	222	101	100	201	578	613	1191
YB008	189	178	367	46		46	180	181	361	149	76	225	46	100	146	610	535	1145
RB022	185	177	362				174	180	354	146		146	98	100	198	603	457	1060
WA921	182	187	369		48	48	184	189	373	66	72	138	100	100	200	532	596	1128
RB004	91	181	272				178	179	357	66	76	142	100	51	151	435	487	922
RB000	190	88	278				163	134	297	74	144	218	50	100	150	477	466	943
YB020	193	184	377	43		43	173	181	354	73	67	140	102	100	202	584	532	1116
WA979	184	87	271	49		49	130	180	310	75	75	150	50	100	150	488	442	930
RB032	192	182	374				182	182	364	76	121	197	101	50	151	551	535	1086
YA011	184	180	364		48	48	81	181	262		151	151	100	100	200	365	660	1025
RB002	188	178	366				183	183	366	147	70	217	101	100	201	619	531	1150
WA980	160	179	339	38	80	118	194	179	373	70		70	98	100	198	560	538	1098
RB011	190	180	370	50		50	181	181	362	149	63	212	100	100	200	670	524	1194
WA981							130	76	206							130	76	206
RB048	191	86	277				175	183	358	68	76	144	99	101	200	533	446	979
WA987	97	80	177				172	141	313							269	221	490
WA996	175	171	346				174	180	354	47	44	91	99	100	199	495	495	990
Sum 20	5835			603			6026			2877			2979			18321		
Sum 80		5312			352			6158			3120			2942			17884	
Total			11148			955			12184			5997			5921			36205

Table 3.4. Comparison of the overall performance of different size grades at the first and the final bag weight checks of the F1 lines.

	Pittwater mean±SD	Coles Bay mean±SD	Smithton mean±SD	Coffin Bay mean±SD	Smoky Bay mean±SD	Unweighted mean±SD
First check						
Date sampled	29/6/99	22/6/99	23/6/99	5/7/99	6/7/99	
Age (mths)*	19	19	19	20	20	
20 grade	12.94±2.58	15.63±3.99	14.75±3.67	28.51±4.62	33.01±7.33	20.27±3.91
80 grade	19.04±3.51	21.40±4.54	26.16±4.72	37.27±5.03	45.34±8.37	29.52±4.10
overall	15.99±4.33	18.52±5.14	20.62±7.12	32.89±6.52	39.27±9.98	24.93±3.66
Final check						
Date sampled	10/3/00	9/3/00	8/3/00	4/4/00	6/4/00	
Age (mths)*	28	28	28	29	29	
20 grade	43.06±6.57	69.74±11.04	63.06±11.28	76.44±11.01	98.39±15.78	68.56±10.94
80 grade	51.02±6.37	72.26±18.23	76.13±9.89	88.59±10.92	111.34±15.26	80.58±8.66
overall	47.04±7.57	70.67±13.67	69.59±12.42	82.72±12.47	104.97±16.72	74.57±9.42

Overall weights of final checks very slightly different from those in Table 3.2 due to different pooling procedures.

Table 3.5. Summary of individual mean weight and condition index, averaged over F1 families. Per family data given in Appendix 3. Correlations of wet weight with condition index across families shown together with probability (*P* values). N is the number of families.

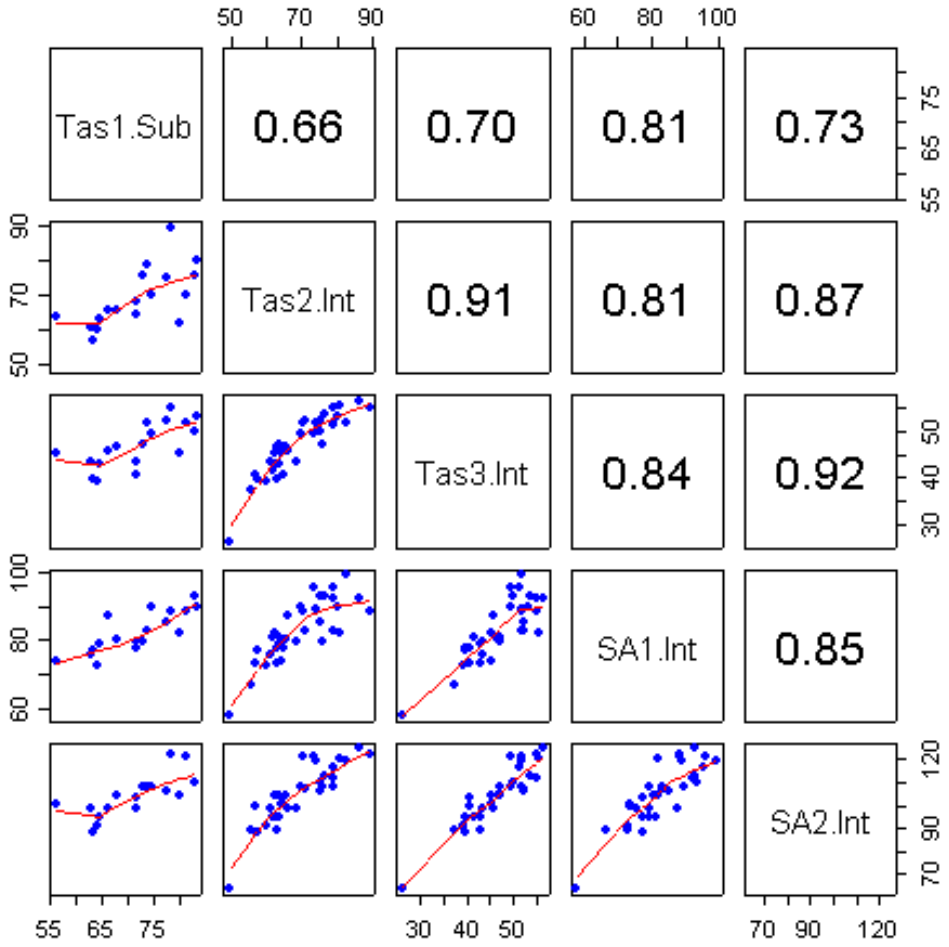
	Pittwater	Coles Bay	Smithton	Coffin Bay	Overall
Date sampled	26/8/99	17/8/99	9/9/99	23/9/99	
Age (mths)	21	21	22	22	
Mean wet weight (g)	19.34±3.71	28.24±6.52	31.52±5.27	41.43±8.99	30.24±4.54
Mean condition index	36.40±2.65	45.62±4.5	51.01±4.61	56.19±16.56	46.92±5.78
N – wet wt	35	34	37	33	37
N – condition index	35	34	34	31	36
correlation	-0.493	-0.562	-0.311	-0.097	-0.388
d.f.	33	32	32	29	34
P	0.003	<0.001	n.s.	n.s.	0.019

Table 3.6. Ranks over sites for top 20 families, as of June 1999. For example, RB003 ranked 1st at the 4 intertidal sites but only 16th at the subtidal site, giving an average rank of 20/5=4.

Family	Overall Rank	Average Rank	F2 usage
WA925	1	3.33	9
RB003	2	4.00	4
WA930	3	4.60	7
RB015	4	5.60	11
RB042	5	8.40	6
WA219	6	9.75	2
YA018	7	10.20	7
WA234	8=	10.40	7
RB004	8=	10.40	
RB022	10	10.60	1
RB012	11	10.80	1
RB006	12	13.00	
RB044	13	13.60	2
WA992	14	14.60	7
WA225	15=	14.80	2
WA928	15=	14.80	
WA213	17	15.00	3
WA214	18	16.60	1
WA987	19	16.67	10
RB011	20	17.40	

In addition, WA981 was used once in F2 generation

Figure 3.1. Correlations among pairwise combinations of farms for the F1 families at their final weight checks. Axes are mean weights per family. Correlations of family weights also shown. Tas1.sub = Coles Bay (9/3/00), Tas2.int = Smithton (8/3/00), Tas3.int = Pittwater (10/3/00), SA1.int = Coffin Bay (4/4/00), SA2.int = Smoky Bay (6/4/00).



CHAPTER 4. Third Spawning Year 1998/99 – The Mass Selection M2 Lines plus Test-crosses and Tetraploid-crosses

4.1. The Test-crosses and Tetraploid Crosses

The intention was to produce a set of diallel crosses from WA981, WA987 and WA996 (Table 4.1). This was part of the CRC Aquaculture project rather than the selective breeding program. The nature of some of these crosses (highly inbred) and the relative immaturity of some of the broodstock (i.e. less then 25mm shell length) meant that not all crosses survived to set with sufficient numbers. Four lines were set as single seed and small numbers of several lines were set on large shell chips.

Ten tetraploid x tetraploid crosses, five diploid male (DM) x tetraploid female (TF) and five tetraploid male (TM) x diploid female (DF) crosses were also made. This also was part of the CRC Aquaculture project rather than the selective breeding program. The ploidy status of all oysters was confirmed by flow cytometry from tissue samples prior to commencing the experiment. Subsamples of sperm and eggs were collected from the experimental animals on the day the crosses were made and subsequently tested for ploidy by flow cytometer. All but the tetraploid male and diploid female crosses had high failure rates in the hatchery. Surviving to set were 4/5 TMxDF crosses, one TxT and one TFxDM. Subsequent testing for ploidy did not confirm the triploidy of the TFxDM cross, nor was the surviving TxT cross tetraploid. Mosaic cells are considered responsible for the positive testing of the parent stock as tetraploid while they produced haploid gametes. Triploidy was confirmed for the progeny of the DMxTM lines.

These lines will not be discussed further in this report.

4.2. The M2 Mass Selection Lines

During December 1998 to February 1999, the next generation of mass selection lines were established. 130 of the largest animals from M1-FAST were chosen in November 1998 and conditioned**.** Various mass selection lines were produced in a spawning at Shellfish Culture in the last week of December 1998:

1. M2-FAST1. Two independent replicates established, each from about 13 males and 10 females (total of 27 males and 20 females), which had been confirmed by microsatellite genotyping of bioassays as being the progeny of the M1-FAST parents.
2. M2-FAST2. The remaining 80± animals were spawned. Two lines were produced (each with 6 or 7 males and about 13 females) and each was mixed with equal numbers of sperm and eggs from M2-FAST1. Thus two replicated lines produced.
3. M2-FAST3. Remaining eggs and sperm from these two replicated lines (M2-FAST1 and M2-FAST2) were mixed 1:1:1 with eggs and sperm from 43 (20 male and 23 female) oysters selected from the fastest growing F1 family lines. Eggs were mixed and then split into two, and each fertilised with mixed sperm. This gave two pseudoreplicates.

4. M2-SLOW. Because there was a suggestion that the slow growth line M1-SLOW had better condition than the M1-FAST lines, and given that the industry has a strong interest in meat weight as well as growth rate, it was decided to propagate another generation of this line. Twenty of the fastest growing M1-SLOW animals (7 males, 13 females) were spawned for M2-SLOW.
5. CONTROL. Twenty broodstock oysters the hatchery had on hand for their own production were mass spawned and then split into three groups or replicates. Each of these replicates was subdivided, making a total of six groups per line per farm.

Spat were moved to TAFI in February 1999 and thinned to about 5000 per line (where possible) in April and moved to nursery (Bouldans Bay) in May 1999. Only one grade was taken; these were from the largest 75-100% size grade, supplemented with the 50-75% size grade where necessary. They were transferred to the five commercial growout sites in March 2000. Each of the Fast and Control replicates was subdivided, making a total of four or six groups respectively per line per farm. The Slow line was not subdivided. Sometimes numbers of animals were not sufficient to make all groups available to each farm.

Bags of M2 animals were weighed in March, June, September/October and November 2000, and in February/March 2001. Smithton animals were not weighed in February/March 2001, as they had in December 2000 formed the basis for the M3 spawnings (see section 6.1). The three replicated lines of oysters mass selected for rapid growth (M2-FAST) continued to outperform their commercial controls (COMM) in the second generation. At every farm at the final bag weight checks (Table 4.2), each of the FAST lines (only two of the three Fast lines were present at Coffin Bay) was larger at the final weight check than the Control line. On average, the Fast lines were about 10% heavier than the Control line. Relative performance appeared to vary a little among Fast lines and farms, from about 20% improvement (M2F1 at Coffin Bay) to only 2% (M2F2 at Smithton). In fact, the performance of the M2 Fast lines relative to the Control was quite similar to the M1 Fast lines relative to its Control, so there was little evidence of any significant genetic gain here. Note though that the Control lines themselves may have varied in quality between these two spawnings, making the comparison problematic.

The Slow line was at each farm the worst-performing line, although on average it was only about 4% lighter than the control line. This line had in fact been promulgated from the fastest growing of the M1 Slow animals, so its only slightly-depressed performance is not very surprising.

Farms varied, as usual, in their relative growth rates, with the South Australian sites performing better than the Tasmanian counterparts. Note that final measurements from the Smithton site were of younger animals than the other sites.

Individual oysters were weighed and measured at about 21 months post-spawning. The target was to assess 10 animals per group per line per farm, giving an intended maximum per line of 40 (for each Fast line), 20 (for the Slow line) and 60 (for the Control line) animals. Actual numbers assessed together with weights and condition index data are given in Table 4.3.

The average weight of the Fast lines was only about 5% greater than the Control line, with the Slow line being about 10% lighter than the Control. These differences are in the same direction as those shown in the final bag weights, but the absolute

percentages vary. The growth advantages of the Fast lines became more marked during spring and summer 2000/2001.

The weight differences between the farms was not as marked as in the final bag weight checks (Table 4.2). The two South Australian sites were only a little heavier than one of the Tasmanian sites, so the marked final differences between the sites reflected much greater spring/summer growth in 2000/2001 in South Australia than in Tasmania.

Condition index varied little among lines within farms, although it does vary substantially among farms. Condition index for these M2 lines was greatest at Coffin Bay, as it was previously for the M1 and F1 lines. Note though the caveats presented in Chapter 3 – we are more interested in any differences among lines than differences among farms.

Table 4.1. The pedigree lines produced in 1998/1999 spawnings

Female family	Male family WA981	WA987	WA996
WA981 (inbred 97-3; gold)	CBI (F3 inbred)	CBII	CBIII
WA987 (inbred 97-2; red)	CBIV	CBV (F3 inbred)	CBVI
WA996 (inbred 97-5)	not done*	not done*	not done*

*No female WA996 were found in the conditioned sample from this family

Table 4.2. Final weight checks for M2 lines

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	Overall
Date sampled	22/2/01	20/2/01	22/11/00	7/3/01	6/3/01	
Age (mths)	26	26	23	27	27	
Average oyster weight (mean of bag weights/oyster counts) with SD						
M2F1	45.82±1.73	59.56±0.61	42.12±5.21	100.19±31.35	81.91±6.16	65.92±24.70
M2F2	47.53±2.10	57.34±2.73	39.95±2.54	98.05	80.40±8.88	64.65±24.09
M2F3	44.27±5.30	63.82±3.32	40.95±1.57	-	79.97±4.16	(57.25±18.20)
M2S	39.67	50.73	37.65±1.94	-	74.14	(50.55±16.75)
M2Control	40.59±5.30	54.88±4.09	38.28±3.73	80.89±11.03	75.82±9.56	58.09±19.64
overall	43.48±3.37	57.27±4.92	39.79±1..85	(93.04±10.58)	78.45±3.39	
Total number of bags/oysters						
M2F1	4/360	2/220	4/400	2/158	4/364	16/1502
M2F2	4/361	3/223	4/399	1/79	4/360	16/1422
M2F3	4/368	2/182	4/400	0/0	4/363	14/1313
M2S	1/86	1/89	2/200	0/0	1/91	5/466
M2Control	6/559	2/169	6/600	3/227	6/526	23/2081
overall	19/1734	10/883	20/1999	6/464	19/1704	

Table 4.3. Average oyster weight and condition index of individually measured M2 oysters

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	Overall*
Date sampled	11/10/00	19/9/00	4/9/00	21/9/00	21/9/00	
Age (mths)	22	21	21	21	21	
Average oyster weight of individually measured oysters						
M2F1	22.09±5.87	46.59±15.92	40.03±8.68	47.82±14.61	43.09±9.62	39.29±10.43
M2F2	20.04±3.82	39.16±10.24	41.54±9.46	53.86±13.22	47.64±10.91	40.45±12.76
M2F3	19.46±4.72	48.81±11.10	37.87±7.45	51.77±13.29	44.86±11.19	40.55±12.89
M2S	18.61±4.15	37.35±12.30	34.12±7.71	41.21±9.34	36.94±7.70	33.65±8.78
M2Control	20.80±4.97	40.44±13.55	38.20±8.35	49.74±15.38	40.73±8.45	37.98±10.57
overall	20.20±1.33	42.47±4.96	37.93±3.03	48.88±4.84	42.65±4.07	
Average condition index (CI)						
M2F1	39.56±13.06	60.27±13.73	60.38±11.30	91.57±23.04	44.61±13.17	59.28±20.30
M2F2	39.75±9.85	61.32±11.84	58.09±9.47	80.54±16.54	44.35±6.88	56.81±16.06
M2F3	38.78±11.06	63.54±12.79	59.67±10.69	93.53±18.54	46.91±10.79	60.48±20.96
M2S	37.40±8.55	61.51±14.43	68.19±13.42	95.18±8.79	48.78±9.70	62.21±21.90
M2Control	39.33±9.09	62.11±12.36	64.95±11.32	93.02±22.69	46.65±9.62	61.21±20.72
overall	38.96±0.94	61.75±1.20	62.73±4.68	90.77±5.86	46.26±1.82	
Total oysters measured						
M2F1	40	20	40	40	36	176
M2F2	37	30	40	40	36	183
M2F3	40	20	40	38	39	177
M2S	20	10	20	10	10	70
M2Control	60	20	60	60	59	259
overall	197	100	200	188	180	865

*overall mean (with SD) of the five farm means, or summation

** overall mean (with SD) of the five line means

CHAPTER 5. Fourth Spawning Year 1999/00 – The Family Selection F2 Lines

5.1. Selection of Parents and Spawning Details for F2 Generation

The F1 families provided the parents for the F2 families. Families were ranked for performance at each site in June 1999, and then a mean rank estimated (Table 3.6). Families were selected from the best twenty performing families at that time for F2 production. Broodstock were removed from Pittwater in October 1999 to prevent them from spawning and moved to a holding system first at the University of Tasmania and then to Shellfish Culture's Bicheno facility. Broodstock consisted of the ten (sometimes a few more) largest individuals from the chosen family lines.

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 few animals selected to be used as the broodstock (adult F1 animals). As can be seen in Table 5.1, there was substantial variation in fecundity between the various lines, with lines such as WA219, WA987 and RB042 showing considerably higher levels of fecundity than other lines. Lines RB044, RB015 and WA214, with relatively low levels of fecundity, may consist of late-spawning animals not yet in peak spawning condition. This is of particular interest to industry as late spawning was considered an important characteristic (Table 1.1). Late spawning animals may enable farmers to have some stock in good condition when the majority of their stock has spawned and therefore in poor, un-saleable condition.

The sex ratios of the F1 show some intriguing lines which appear to be all one sex, admittedly from a limited sample of ten or so per line (Table 5.1). The samples of RB011 and RB022 are all male, those of RB042 and WA225, all female. Single sex stocks is another characteristic considered important by the oyster industry (Table 1.1). Female oysters have a greater growth rate than male oysters, up to 16-18% (Baghurst and Mitchell 2002), therefore an all-female fast growing stock would be a added bonus for the oyster industry. Section 5.3 considers the sex ratios of the F2 lines.

Due to logistic constraints the F2 generation was produced in two separate hatchery 'runs', with the first commencing in November 1999 (Table 5.2) with 19 families produced and the second in January 2000 (Table 5.3) with 25 families produced. Crosses 1, 3, 16, 19 and 20 were lost due to mortality or mixing and were repeated (crosses 1N in Table 28 and 3N, 16N, 19N and 20N in Table 29). Cross 7 was lost but not repeated.

The relationships of the F2 families are shown in Table 5.4. Note that instead of intercrossing the top ranking 10 F1 family lines, as had been the original intention, it was decided to intercross as many as possible of the top 8 families, and add in some additional families. There are 28 possible pairwise combinations of 8 families; we successfully produced 19 of these combinations. Family WA987 was also used extensively. This an inbred family that, in early work carried out as part of the preceding CRC Aquaculture project, was thought to carry a high growth gene and which we wished to test in outbred combinations. Two massed spawn families

(numbers 45 and 46) were also produced using a number of the better F1 families (Table 3.6), with steps taken to ensure that inbreeding within contributing families was prevented.

5.2. F2 Performance

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 laboratories at Taroona, the first set on 4/1/00, the second set on 1/2/00. The 44 F2 family lines were transferred to the commercial nursery site (Bouldans Bay Oysters) during April 2000 and went to grow- out sites in November 2000.

In October 2000, when all the juveniles were still at Bolduans Bay, all animals of the F2 family lines were gathered together and assessed. A random subsample 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 curlback evident, amount of frill on shell, shape, miscellaneous comments and subjectively 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 (Table 5.5). The data for the mean weights of 100 randomly selected individuals were used to establish relative growth rates for each family line (Table 5.6). The inbred family, number 16N, was an average growing family in the second spawning, but was visually an attractive oyster.

The range in growth rates was high, with a 3.5x factor in the first spawning and a 42.1 x factor in the second spawning. In the second spawning, family 31 was outstanding, with high growth and a visually attractive oyster. Both numbers per family and growth rates were higher for the 1st spawning (the latter as expected since they had been growing longer). In each spawning, there was an indication that family growth rate was negatively correlated with total number (density) (Figure 5.1); correlations were –0.443 (P = 0.058) for spawning 1 and –0.307 (P = 0.154) for spawning 2. Although these negative correlations look high, they are in fact non-significant (although only just so for spawning 1). The combined P value for the two tests is 0.051, hovering on significance.

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 (Table 5.6). Following normal commercial practice the oysters were graded into 4 size grades (< 14mm, 14-18mm, 18-22mm and >22mm). The major difference between commercial practice and this phase of 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 subsample 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 growout it had also been agreed that we need assess only the 50 to 100% 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 Bouldan Bay farm. From the larger size grade, 15 batches of 100 randomly selected individuals per family were bagged (November 2000) for subsequent delivery to the five farms participating in further growout trials (3 bags of 100 oysters from each family for each farm wherever possible).

Bags were weighed in November 2000, February/March 2001, June/July 2001 and October/November/December 2001. Mean weights per oyster per family per farm (Table 5.7) were estimated from the final bag weights and counts per bag. The total numbers of contributing oysters is given in Table 5.8.

There was a greater than two-fold difference in mean oyster weights between the best performing (number 13) and worst performing (number 16N) families (Table 5.7). Family 16N – an inbred line – was earlier characterized by Kerri Wells at Bolduan's Bay as perhaps the best looking line. The two massed spawned lines in spawning 2, produced using parents from the faster growing F1 families, performed no better than average among the F2 2nd spawned families. This was perhaps to be expected, given that all F2 families were produced from superior F1 families.

Final overall performance figures are at least partly confounded by whether the family was produced in spawning 1 (end of November 1999) or spawning 2 (start of January 2000). In the final weight checks, about 23 months post-spawning, the average weight of spawning 1 families was about 15% greater than spawning 2 families (difference highly significant, $P < 0.001$), and the spawning 1 families are mostly clustered in the top-ranking families and spawning 2 families mostly in the lower ranking families (Table 5.9). The one month delay in spawning has produced significantly smaller animals about 23 months post-spawning. In November 2000, about 12 months post-spawning, the relative difference between the 1st spawned and 2nd spawned animals was much greater, nearly 300% (average weight of 3.00 and 1.14 g, respectively, full data not shown).

Reml (restricted maximum likelihood) analysis of family rankings from the final F2 growout data (taken October-November 2001) are provided in Table 5.10. These are not simple averages across those farms that had families (as in Table 5.7), but account for the fact that some families are missing from some farms. Rankings are generally similar to the earlier rankings, but there are some exceptions. For example, family 2 is ranked 3rd in Table 5.7 but only 22nd in Table 5.10; this is because family 2 was absent from the farm with the lowest overall weight, and had it been present there, its overall rank in Table 5.7 would have fallen substantially.

As for the F1 families, families that performed well at one site tended to perform well at another, and those that performed poorly at one site tended to perform poorly at another Figure 5.2). Inter-farm family correlations ranged from 0.49 to 0.88. The family*site variance ratio, or genotype by environment interaction, was estimated at 0.20 ± 0.05. This is significant, but not large, and almost identical to the value noted earlier for the F1 generation. There is, as usual, a very large site-difference, with the two South Australian sites performing better than the three Tasmanian sites.

Individual measurements were taken on animals from the Pittwater site only (Table 5.11). These animals were removed on August 7 2001, with 10 animals being removed at random from each replicate, wherever possible (a maximum of 3 x 10 = 30 animals per family). 42 families were available at Pittwater (F2 families 22 and 34 of those successfully spawned were not represented there).

Mean weights for these about 20 month old animals ranged from 35.55 g (family 10) to 13.30 g (family 16N), a factor of 2.7. Condition index was much less variable, ranging from 93.28 (family 18) to 62.09 (family 3), a factor of 1.5. There was not a significant relationship between mean family weight and mean condition index ($r = 0.049$), so that faster growing families did not have a significantly higher (nor lower) condition index than slower growing families.

5.3. Sex Ratio Analysis of F2 Lines

Sex ratios in most F2 lines were measured twice, once in November 2002 and once in January 2003 (Table 5.12). At this time the oysters were ~ 2 years old (i.e. 2 years post fertilization). We used χ^2 tests to test for deviations from a 1:1 sex ratio at each sampling period and then across sampling periods combined. Here we focus on the combined datasets. A total of 39 F2 families were tested, and only those with an overall deviation probability (final column of Table 5.2) of less than 0.05/39 = 0.0013 were accepted as truly deviant. This Bonferroni probability correction was made to minimise the probability of a type II error (accepting that a line has a biased sex ratio when it really does not). After this correction, eight of the 39 family lines were still significantly biased, five towards females (lines 2, 25,38, 41 & 43), with an average female proportion of 81.22%, and three towards males (lines 13, 14 & 21), with an average male proportion of 88.50%. The fact that most of these single-pair families, of the same age, had roughly equal sex ratios, but some were very highly skewed towards females or towards males, suggests that sex ratio in oysters is under genetic control (Haley, 1977, Guo *et al.*, 1988). The differing sex ratios might reflect differential segregation in families of sex determining genes.

Table 5.1. Approximate fecundity (eggs/litre) and sex ratios for the broodstock utilised from F1 generation for the production of the F2 generation. Only animals assessed for spawning were sexed.

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

Table 5.2. F2 generation family crosses produced November 29, 1999.

Cross #	Date of spawn	Female parent Family	Male parent Family	Inbreeding coefficient	Notes
1	29/11/99	WA234	RB015	0	failed
1N	1/12/99	RB015	WA234	0	
2	29/11/99	WA234	WA925	0	
3	29/11/99	RB003	RB015	0	failed - lost at set
4	29/11/99	RB003	WA234	0	
5	29/11/99	WA234	WA930	0	
6	29/11/99	YA018	WA234	0	
7*	29/11/99	WA234	WA219	0	failed - lost at set
8	29/11/99	RB015	WA219	0	
9	29/11/99	RB015	WA925	0	
10	29/11/99	RB042	WA234	0	
11	29/11/99	WA992	WA234	0	
12	29/11/99	WA925	WA930	0	
13	30/11/99	RB044	RB012	0	
14	30/11/99	WA213	RB022	0	
15	30/11/99	WA213	RB044	0	
16	30/11/99	WA987	WA987	0.375	failed
17	30/11/99	RB015	WA930	0	
18	30/11/99	RB015	WA992	0	
19	30/11/99	YA018	RB015	0	failed - mixed with 20
20	30/11/99	RB042	RB015	0	failed - mixed with 19
21	30/11/99	WA925	WA992	0	
22	30/11/99	WA225	RB015	0	
23	30/11/99	WA925	WA219	0	
24	30/11/99	WA925	YA018	0	

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

Table 5.3. F2 generation family crosses produced January 3, 2000.

Cross #	Date of spawn	Female parent Family	Male parent Family	Inbreeding coefficient	Notes
3N	3/1/00	RB003	RB015	0.0	
16N	3/1/00	WA987	WA987	0.375	
19N	3/1/00	RB015	YA018	0.0	
20N	3/1/00	RB042	RB015	0.0	
25	3/1/00	RB042	WA925	0.0	
26	3/1/00	YA018	WA930	0.0	
27	3/1/00	WA992	WA930	0.0	
28	3/1/00	WA981	WA987	0.0	
30	3/1/00	WA992	YA018	0.0	
31	3/1/00	WA225	WA925	0.0	
32	3/1/00	RB042	WA930	0.0	
33	3/1/00	RB042	YA018	0.0	
34	3/1/00	RB003	WA987	0.0	failed
34N	6/1/00	RB003	WA987	0.0	
35	3/1/00	RB042	WA992	0.0	
36	4/1/00	WA987	WA219	0.0	lost in nursery
37	4/1/00	WA987	WA930	0.0	
38	4/1/00	WA987	YA018	0.0	
39	4/1/00	WA987	WA992	0.0	
40	4/1/00	PHP*	RB015	0.0	
41	4/1/00	LST*	LST*	0.0	
42	4/1/00	WA987	RB003	0.0	
43	4/1/00	WA987	RB015	0.0	
44	4/1/00	WA987	WA925	0.0	
45	4/1/00	mass spawn	see below	0.0	
46	4/1/00	mass spawn	see below	0.0	
47	4/1/00	WA214	WA213	0.0	

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, an available hatchery stock from Hastings Bay with a distinctive gold shell colouration; LST, a very frilly possibly low salt tolerant stock from Lune River/Ida Bay; both southern Tasmania.

Table 5.4. Summary of the families successfully produced in the 1999/2000 spawning season. Cross-reference to Tables 5.2 and 5.3.

Rank		1	2	3	4	5	6	7	8=	8=	10	11	12	13	14	15=	15=	17	18	19	20		new	new
		WA925	RB003	WA930	RB015	RB042	WA219	YA018	WA234	RB004	RB022	RB012	RB006	RB044	WA992	WA225	WA928	WA213	WA214	WA987	RB011	WA981	PHP	LST
1	WA925			12	9	25	23	24	2						21	31				44				
2	RB003				3N				4											34N,42				
3	WA930				17	32		26	5						27					37				
4	RB015					20N	8	19N	1N						18	22				43			40	
5	RB042							33	10						35									
6	WA219																							
7	YA018								6						30					38				
8=	WA234														11									
8=	RB004																							
10	RB022																	14						
11	RB012													13										
12	RB006																							
13	RB044																	15						
14	WA992																			39				
15=	WA225																							
15=	WA928																							
17	WA213																		47					
18	WA214																							
19	WA987																			16N		28		
20	RB011																							
	WA981																							
new	PHP																							
new	LST																							41

Table 5.5. Preliminary screening of the F2 families, carried out October 17-19, 2000. Observational data.

F2 Family	F1 Female	F1 Male	Valve colour Left	Valve colour Right	Curlback	Frill	Shape	Comments	Rank 1-10
1N	RB015	WA234	mixed, burgundy	pale		low	variable		5
2	WA234	WA925	pale	pale	none	low	good	small	7
3N	RB003	RB015						too small for adequate assessment	
4	RB003	WA234	burgundy	pale	some	variable, low to heavy	good		5.5
5	WA234	WA930	burgundy, striped	burgundy, striped	very low		broad, deep	uniform size	7.5
6	YA018	WA234	burgundy	pale	low	moderate	broad, flat		4.5
8	RB015	WA219	burgundy (stripes)		some	low - moderate	broad, flat		6.5
9	RB015	WA925	burgundy	pale	very low	low	good	uniform size	9
10	RB042	WA234	burgundy	pale	very low	most smooth	good, variable		6
11	WA992	WA234	pale		some	high	wide, flat		3
12	WA925	WA930	pale		some	high		few good individuals	4
13	RB044	RB012	burgundy	white	some		good		6
14	WA213	RB022			some	low			5
15	WA213	RB044	burgundy		not evident	low	good, wide		8
16N	WA987	WA987	dark burgundy	white	not evident		good	Kerri's favourites	10
17	RB015	WA930	variable	stripes	low		narrow		4
18	RB015	WA992	variable		none evident	low (a few moderate)	good		7
19N	RB015	YA018	burgundy	pale		low	good		8.5
20N	RB042	RB015	variable		some	low to moderate			6
21	WA925	WA992	pale with burgundy stripes			low to moderate	good		6.5
22	WA225	RB015	burgundy	stripes	very high				2
23	WA925	WA219	burgundy, striped	pale	some		some good		5
24	WA925	YA018	pale, some burgundy		moderate		twist in early shell growth	few with good shape	4
25	RB042	WA925		pale	low		some very good		6.5
26	YA018	WA930	burgundy	pale	very low	low	good		7
27	WA992	WA930	pale-red		none		round		
28	WA981	WA987	pale-red		none	moderate	broad and flat		7
30	WA992	YA018	pale		very low	moderate	very round and flat		6
31	WA225	WA925	mixed, burgundy & pale			low	very good		9
32	RB042	WA930	red-burgundy		none evident		good		8.5
33	RB042	YA018			some		broad	uniform size	5.5
34N	RB003	WA987	burgundy		high				2
35	RB042	WA992	pale		not evident	very smooth	good		8
37	WA987	WA930	red		none	low		small? Lots of seed	6
38	WA987	YA018	variable		low		good		7
39	WA987	WA992	dark				very round	small?	6.5
40	PHP	RB015	very pale		some	very low		largest individuals look good	7.5

F2 Family	F1 Female	F1 Male	Valve colour		Curlback	Frill	Shape	Comments	Rank 1-10
			Left	Right					
41	LST	LST	pale purple		some	high			4
42	WA987	RB003	variable		some		broad		7
43	WA987	RB015	dark		very low (absent?)		good		8.5
44	WA987	WA925	dark burgundy		none		good		8
45	Mass Spawn (a)	Mass Spawn (a)	pale		high				3
46	Mass Spawn (b)	Mass Spawn (b)			high				4
47	WA214	WA213	burgundy	central burgundy stripe	very low	low	small?		7

Table 5.6. Quantitative data on the performance of F2 families during nursery phase. See text.

F2 Family	Average Weight (g)	Size 1 < 14mm	Size 2 14-18mm	Size 3 18-22mm	Size 4 >22mm	total surviving	age (days)	growth (mg/day)
First spawning								
1N	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
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
Second spawning								
3N	0.18	5088	550	39	0	5677	289	0.62
16N	1.09	648	606	176	40	1470	289	3.37
19N	2.45	100	948	1023	312	2383	289	8.48
20N	0.70	3476	3184	195	28	6883	289	2.42
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
34N	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.

Table 5.7. Final weights per individual (g) for the 44 F2 lines (bags weighed, animals counted)

F2 Date Age (mths)*	Pittwater 15/11/01 23 mean±SD	Coles Bay 6/12/01 24 mean±SD	Smithton 25/10/01 22 mean±SD	Coffin Bay 19/11/01 23 mean±SD	Smoky Bay 2/11/01 23 mean±SD	Unweighted mean±SD	Rank
1N	38.22±2.77	36.92±1.56	52.59±0.30	112.69±7.11	77.97±4.75	63.68±31.99	19
2	41.85±1.94		53.90±2.80	97.52±4.42	86.68±9.11	69.99±26.38	3
3N	32.48±2.76	26.07±10.25		89.85±9.21	66.19±5.45	53.65±29.87	35
4	38.30±4.91	42.65±12.15	51.75±0.04	106.64±16.08	70.73±0.48	62.02±27.88	24
5	46.87±2.31	36.40±3.40	60.09±5.69	108.30±11.17	94.36±6.50	69.20±30.90	4
6	37.91±3.34	41.88±5.63	56.94±6.44	105.65±6.82	76.36±3.96	63.75±27.87	18
8	41.67±0.16	22.73±1.12	63.74±1.32	113.34±8.88	77.38	63.77±34.70	17
9	40.88±2.54	44.98±7.00	60.13±2.42	97.56±6.25	89.25±6.48	66.56±25.70	12
10	48.67±2.32	43.04±6.05	63.92±5.55	100.86±13.67	78.14±4.87	66.93±23.40	9
11	32.60±2.53	33.06±4.92	51.57±0.97		73.68±1.50	47.73±19.43	40
12	37.51±1.38	41.03±0.90	55.50±0.78	113.15±1.09	72.63±5.13	63.97±30.79	15
13	46.07±4.94	41.74±1.56	64.03±5.21	131.28±5.61	89.73±8.15	74.57±36.90	1
14	45.34±4.71	37.00±0.36	59.60±2.69	98.13±2.65	93.14±2.69	66.64±27.73	10
15	42.46±4.89	42.53±7.44	53.02±0.76	101.78±9.46	91.96±7.26	66.35±28.40	13
16N	12.67±3.11	12.29±2.78			62.10±31.61	29.02±28.65	44
17	39.63±4.92	30.69±7.38	59.10±3.54	112.89±13.73	97.77±3.92	68.02±35.98	6
18	41.06±5.99	27.70±2.69	56.87±0.96	106.22±11.92	86.50±5.15	63.67±32.34	20
19N	44.22±9.32	34.83±2.18	57.42±1.63	107.15±0.10	89.50±7.68	66.63±30.67	11
20N	42.44±13.83	41.25±6.99	61.03	105.14±15.35	86.38±4.00	67.25±27.98	8
21	44.54±1.84	41.74±10.14	57.63±2.24	97.42±17.65	95.85±8.19	67.44±27.33	7
22			61.51±1.09			61.51	26
23	47.61±4.75	34.86±5.48	65.62±1.22	113.73±7.30	95.24±5.15	71.41±32.77	2
24	39.24±5.13	27.39±5.82	58.55±1.91	110.33±9.79	82.82±2.70	63.67±33.48	21
25	41.17±3.46	38.07±2.59	60.74±9.63	103.41±9.95	78.75±1.66	64.43±27.27	14
26	39.56±2.70	37.02±4.09	50.30±0.44	97.23±*.38	74.53±0.98	59.73±25.67	28
27	33.28±4.01	22.91±6.23		86.01	71.89±19.33	53.52±30.33	36
28	38.25±0.66	43.24±5.47	59.10±3.64	80.00	79.34±8.15	59.99±19.55	27
30	28.93±2.84	30.01±4.08	49.57	81.05	65.67±1.30	51.04±22.63	38
31	45.81±4.34	36.41±9.02	57.54±2.67		89.27±3.01	57.26±23.03	30
32	35.26±1.99	26.76±5.66		78.75	70.61±5.28	52.85±25.66	37
33	29.93±7.68	37.60±2.72				33.76±5.43	42
34N		31.82±18.78				31.82	43
35	34.52±3.67	35.52±2.55	50.61	89.71	71.68±5.45	56.41±23.93	32
37	30.03±5.07	28.60±6.16		104.00	91.48±6.16	63.53±39.84	22
38	33.66±8.65	29.28±4.93	56.88±7.45	92.41±9.07	78.44±3.69	58.13±27.48	29
39	28.69±2.71	21.06±7.16		90.00±0.00	60.77±1.09	50.13±31.66	39
40	35.34±7.24	22.14±8.92			71.37±3.52	42.95±25.48	41
41	37.38±2.94	29.17±5.78	55.44±0.73	87.85±0.22	74.80±10.64	56.93±24.65	31
42	34.30±2.16	27.71±0.91		113.64±7.51	79.99±1.36	63.91±40.49	16
43	37.95±5.02	29.49±4.52	59.14±2.21	120.20±18.58	93.56±4.12	68.07±38.22	5
44	31.93±3.24	37.42±6.56		88.33±2.36	58.99±3.84	54.17±25.60	33
45	36.78±7.07	35.70±8.04		102.12±3.00	78.35±4.40	63.24±32.65	23
46	34.57±4.28	24.66±8.01		90.00	66.74±3.76	53.99±29.98	34
47	35.10±4.79	27.95±6.18		111.55±11.19	72.34±5.75	61.73±38.49	25
Mean	37.73±6.60	33.17±7.48	57.46±4.36	101.24±11.91	79.58±10.55	59.43±10.14	

Table 5.8. Final counts per family for F2 lines (counts across bags)

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	Total
Date sampled	15/11/01	6/12/01	25/10/01	19/11/01	2/11/01	
Age (mths)*	23	24	22	23	23	
1	269	298	197	82	255	1101
2	270	0	196	81	262	809
3N	226	131	0	25	108	490
4	179	280	274	153	263	1149
5	268	286	264	92	218	1128
6	260	298	271	124	267	1220
8	180	291	250	86	84	891
9	271	292	260	64	218	1105
10	270	296	262	135	279	1242
11	259	287	194	0	272	1012
12	258	295	274	98	273	1198
13	270	297	291	103	250	1211
14	253	297	269	31	155	1005
15	268	293	255	30	169	1015
16N	208	247	0	0	139	594
17	270	267	187	63	149	936
18	241	291	261	43	153	989
19N	240	291	250	84	249	1114
20N	180	281	97	29	243	830
21	269	296	274	122	244	1205
22	0	0	276	0	0	276
23	243	287	256	46	200	1032
24	259	230	257	64	227	1037
25	259	288	247	65	226	1085
26	270	248	271	126	253	1168
27	157	299	0	60	191	707
28	168	295	272	1	276	1012
30	264	288	92	19	168	831
31	244	235	232	0	219	930
32	260	290	0	8	206	764
33	260	300	0	0	0	560
34N	0	299	0	0	0	299
35	270	293	98	35	233	929
37	257	238	0	30	155	680
38	235	267	121	27	221	871
39	238	300	0	16	71	625
40	177	293	0	0	208	678
41	265	286	251	56	271	1129
42	270	193	0	31	177	671
43	263	290	185	29	191	958
44	244	297	0	31	183	755
45	180	291	0	46	148	665
46	234	264	0	8	138	644
47	244	289	0	48	221	802
Total	10170	11674	6884	2191	8433	39352

Table 5.9. Partitioning the F2 families by spawning according to performance in final weight checks

Rank	Family number		Weight (g)
	1st Spawning	2nd Spawning	
1	13		74.57
2	23		71.41
3	2		69.99
4	5		69.20
5		43	68.07
6	17		68.02
7	21		67.44
8		20N	67.25
9	10		66.93
10	14		66.64
11		19N	66.63
12	9		66.56
13	15		66.35
14		25	64.43
15	12		63.97
16		42	63.91
17	8		63.77
18	6		63.75
19	1N		63.68
20	18		63.67
21	24		63.67
22		37	63.53
23		45	63.24
24	4		62.02
25		47	61.73
26	22		61.51
27		28	59.99
28		26	59.73
29		38	58.13
30		31	57.26
31		41	56.93
32		35	56.41
33		44	54.17
34		46	53.99
35		3N	53.65
36		27	53.52
37		32	52.85
38		30	51.04
39		39	50.13
40	11		47.73
41		40	42.95
42		33	33.76
43		34N	31.82
44		16N	29.02
Mean weight (g)	65.31±5.41	54.97±10.68	59.43±10.14

Table 5.10. REML (restricted maximum likelihood) analysis of family rankings from the final F2 growout data (taken October-November 2001), together with the number of times progeny from each family had been used in F3 and F4 crosses. Means are mean weights per individual, in gm.

			# parents used in						# parents used in		
Rank	Family	Mean	F3	F4	Total	Rank	Family	Mean	F3	F4	Total
1	13	73.61	7	1	8	23	42	62.01			
2	23	70.54	1	4	5	24	4	61.96	2		2
3	5	68.61	5	1	6	25	28	61.95			
4	43	67.17		4	4	26	45	61.66			
5	17	67.14		3	3	27	37	61.64			
6	21	66.99	5	2	7	28	34	60.62			
7	20N	66.76		2	2	29	33	60.13			
8	31	66.58	7		7	30	26	59.85			
9	10	66.52	4	4	8	31	47	59.84			
10	14	66.51	1	1	2	32	38	58.43			
11	9	66.18		1	1	33	11	58.14			
12	19N	66.10	3		3	34	35	57.56			
13	15	65.98	2	2	4	35	41	57.56			
14	22	64.89				36	40	55.22			
15	25	64.20		1	1	37	32	53.98			
16	6	63.57				38	46	53.89			
17	8	63.56				39	44	53.82			
18	24	63.50				40	3N	53.27			
19	18	63.47				41	30	52.91			
20	12	63.35				42	27	52.88			
21	1N	63.33				43	39	50.39			
22	2	63.08				44	16N	43.78			

Table 5.11. Individual measurements of F2 oysters from Pittwater. Families 22 and 34 absent from that farm

F2 Family	Weight Mean±sd	Condition index Mean±sd	Number measured
1	23.78±15.85	68.16±10.04	30
2	28.85±18.33	76.44±17.35	30
3	21.36±13.42	62.09±21.59	30
4	19.35±13.11	73.36±8.67	30
5	34.19±22.27	87.17±21.60	30
6	24.23±17.18	73.46±7.31	29
8	31.59±21.98	75.60±21.85	30
9	23.89±15.82	66.71±7.67	30
10	35.55±24.85	79.01±18.59	30
11	25.54±15.95	78.19±8.00	30
12	24.51±15.70	82.18±23.82	30
13	27.37±20.16	64.15±4.93	30
14	27.31±17.70	76.99±28.16	30
15	27.66±17.89	72.03±6.87	30
16	13.30±9.71	86.84±28.33	30
17	26.81±16.83	79.06±18.93	30
18	24.98±16.67	93.28±21.82	30
19	26.30±18.50	77.25±15.51	30
20	28.00±19.22	65.43±6.72	30
21	26.49±18.71	68.39±5.86	30
23	31.92±21.66	67.18±20.66	29
24	30.69±20.63	80.07±19.23	30
25	23.12±16.11	78.80±22.12	30
26	26.85±16.94	83.13±19.30	30
27	18.40±14.83	70.61±19.90	20
28	25.27±18.79	67.57±6.88	30
30	18.47±13.48	70.12±12.33	30
31	31.47±20.35	69.21±5.70	30
32	23.29±16.91	69.99±6.72	30
33	19.05±13.20	74.56±11.15	30
35	24.94±16.79	84.90±33.42	30
37	19.75±12.51	70.07±6.20	30
38	22.17±14.34	70.13±5.92	30
39	16.81±11.50	73.44±9.49	30
40	18.19±10.16	83.16±17.74	30
41	28.06±19.19	70.79±4.05	30
42	23.84±17.25	71.37±14.67	30
43	22.67±15.80	65.84±4.34	30
44	19.35±13.76	73.89±8.69	30
45	22.54±14.83	81.27±36.52	27
46	21.66±13.85	64.02±35.70	30
47	19.21±13.51	76.69±24.31	30
Overall	24.49±4.85	74.35±7.12	1245

Table 5.12. Sex ratios in F2 families sampled in November 2002 and January 2003. Numbers of females, males, and indeterminate ('?') given. Probabilities of fit to 1:1 male:female ratio are given; n.s. indicates a non-significant deviation.

	F2 lines sampled in Nov-02					F2 lines sampled in Jan-03					F2 overall
Line	No. female	No. male	?	% male	P	No. female	No. male	?	% male	P	P
1	12	18	0	60.0	ns	15	15	0	50.0	ns	ns
2	26	4	1	13.3	<0.001	26	2	0	7.1	<0.001	<0.001
3	8	18	4	69.2	<0.05	14	16	0	53.3	ns	ns
4	12	13	5	52.0	ns	12	18	0	60.0	ns	ns
5	21	9	0	30.0	<0.05	13	15	0	53.6	ns	ns
6	21	9	0	30.0	<0.05						<0.05
8	21	9	0	30.0	<0.05						<0.05
9	15	12	3	44.4	ns						ns
10	13	17	0	56.7	ns	18	7	0	28.0	<0.05	ns
11	17	12	1	41.4	ns	22	7	0	24.1	<0.01	<0.01
12	18	10	2	35.7	ns	17	12	0	41.4	ns	ns
13	0	30	0	100.0	<0.001	2	18	0	90.0	<0.001	<0.001
14	0	30	0	100.0	<0.001	1	28	0	96.6	<0.001	<0.001
15	10	10	0	50.0	ns	12	18	0	60.0	ns	ns
16	11	8	7	42.1	ns						ns
17	14	8	7	36.4	ns	14	16	0	53.3	ns	ns
18	16	14	0	46.7	ns	14	15	0	51.7	ns	ns
19	10	20	0	66.7	ns	9	18	0	66.7	ns	<0.05
20	17	13	0	43.3	ns						ns
21	8	22	0	73.3	<0.05	9	20	0	69.0	<0.05	<0.001
23	16	14	0	46.7	ns						ns
24	12	17	0	58.6	ns	13	17	0	56.7	ns	ns
25	19	5	1	20.8	<0.01	18	10	2	35.7	ns	<0.001
26	9	21	0	70.0	<0.05	13	17	0	56.7	ns	<0.05
30	15	11	4	42.3	ns	15	15	0	50.0	ns	ns
31	18	12	0	40.0	ns	14	16	0	53.3	ns	ns
32	15	15	0	50.0	ns						ns
33	14	16	0	53.3	ns						ns
35	12	13	5	52.0	ns	17	12	1	41.4	ns	ns
37	15	14	1	48.3	ns	17	12	0	41.4	ns	ns
38	25	5	0	16.7	<0.001	27	3	0	10.0	<0.001	<0.001
39	9	20	1	69.0	<0.05	13	17	0	56.7	ns	ns
41	28	2	0	6.7	<0.001	20	6	0	23.1	<0.01	<0.001
42	19	11	0	36.7	ns						ns
43	25	5	0	16.7	<0.001	18	11	1	37.9	ns	<0.001
44	15	15	0	50.0	ns	15	10	0	40.0	ns	ns
45	18	11	1	37.9	ns						ns
46	11	19	0	63.3	ns	12	17	0	58.6	ns	ns
47	14	12	4	46.2	ns	15	13	0	46.4	ns	ns

Figure 5.1. Growth rates plotted against spat number for each spawning of the F2 families

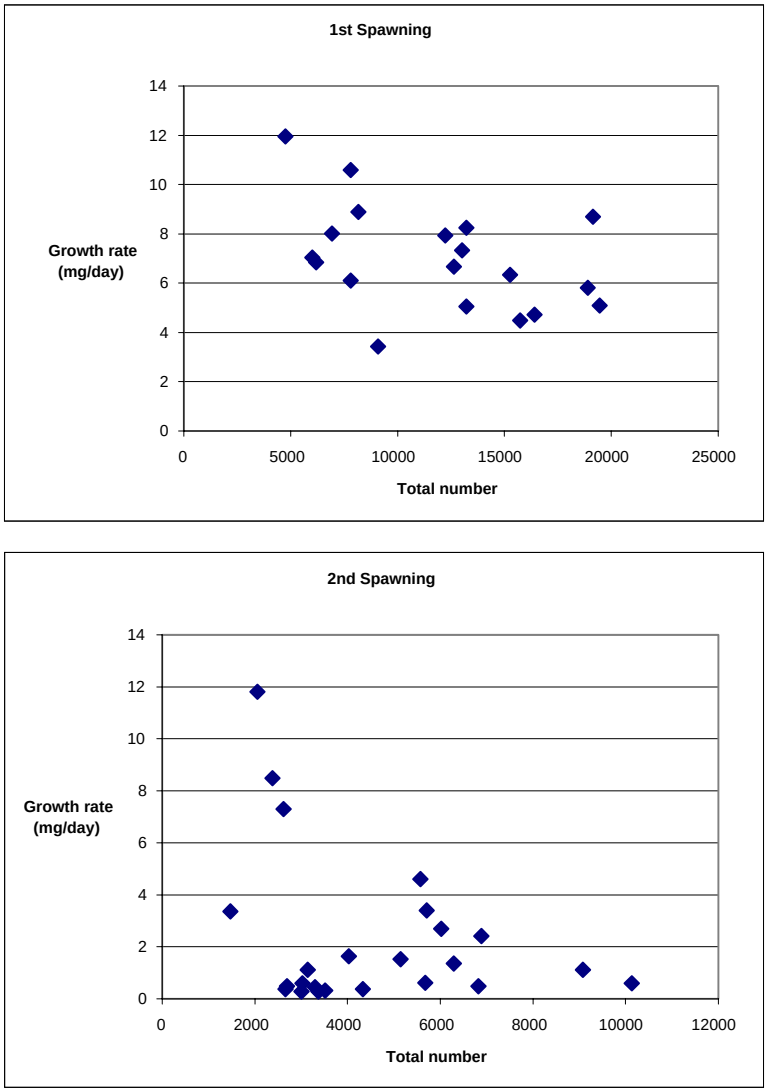
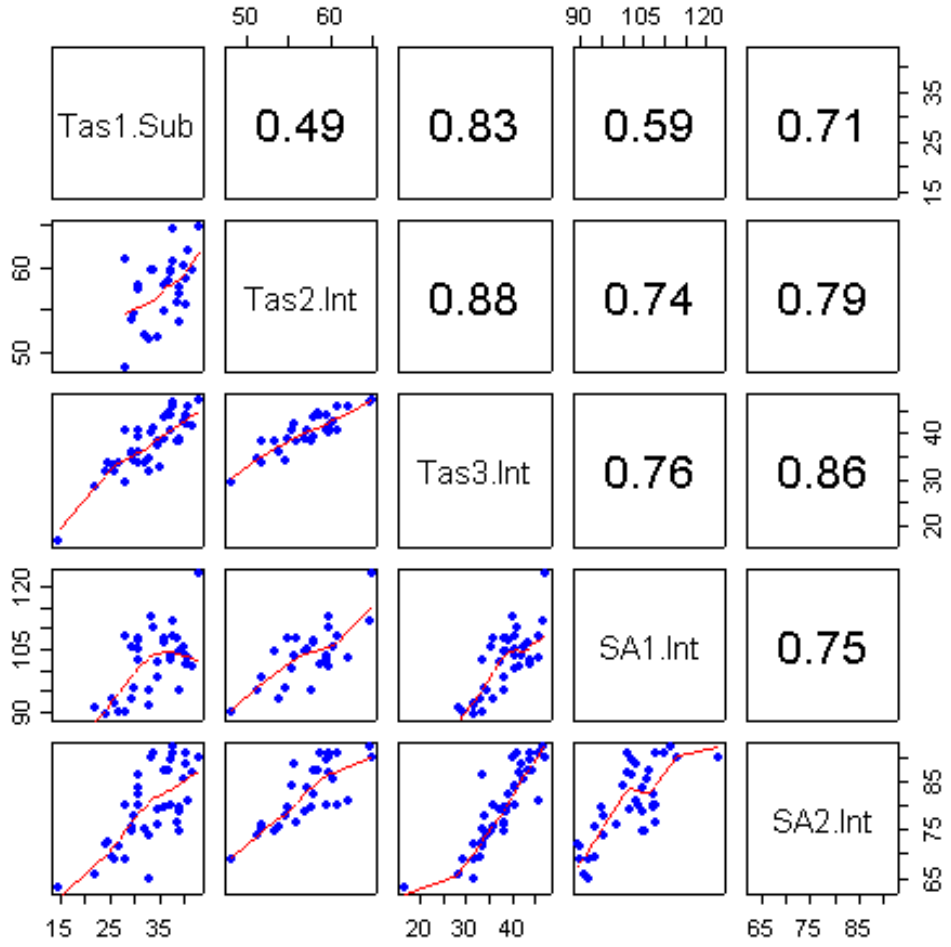


Figure 5.2. Correlations among pairwise combinations of farms for the F2 families at their final weight checks. Axes are mean weights per family. Correlations of family weights also shown. Tas1.sub = Coles Bay (6/12/01), Tas2.int = Smithton (25/10/01), Tas3.int = Pittwater (15/11/01), SA1.int = Coffin Bay (9/11/01), SA2.int = Smoky Bay (21/11/01).



CHAPTER 6. Fifth Spawning Year 2000/01 – The Mass Selection M3 Lines plus the Family Selection F3 Lines

6.1. The Mass Selection M3 Lines

Third generation mass selection lines were established on December 6 2000 at Shellfish Culture. Five lines were made, a control and two replicates of two lines, M2-FAST1 and M2-FAST2. M2-FAST3, which was a combination of high selected animals plus the best of the family lines, was not propagated following advice from Laurie Piper, a quantitative geneticist. The M2-SLOW line was also not promulgated as it had no significant advantages in, for example, condition index, and also grew more slowly than the Fast lines. The parents for these lines (two years old) came from Smithton, meaning that the final weights for the M2 lines were taken at Smithton in November 2000 rather than February 2001 (section 4.2). The M3 parents were selected as the largest mature animals from the 400 M2-FAST 1 and 400 M2-FAST2. The five new lines are:

Control Random selection of 49 (21 females, 28 males) oysters from M2-FAST1. Average weight = 43.7, SD=9.2

M3-FAST1,1 33 females from M2-FAST1 stripped, eggs combined. 14 males stripped and put in separate beakers. Equal portions of eggs added to each beaker. After 30 min, $2x10^6$ fertilized eggs added to a larval tank. Average weight = 48.0, SD=8.1

M3-FAST1,2 36 females from M2-FAST1 stripped, eggs combined. 14 males stripped and put in separate beakers. Equal portions of eggs added to each beaker. After 30 min, $2x10^6$ fertilized eggs added to a larval tank. Average weight = 49.5, SD=8.0

M3-FAST2,1 36 females from M2-FAST2 stripped, eggs combined. 14 males stripped and put in separate beakers. Equal portions of eggs added to each beaker. After 30 min, $2x10^6$ fertilized eggs added to a larval tank. Average weight = 55.9, SD=7.2

M3-FAST2,2 32 females from M2-FAST2 stripped, eggs combined. 16 males stripped and put in separate beakers. Equal portions of eggs added to each beaker. After 30 min, $2x10^6$ fertilized eggs added to a larval tank. Average weight = 51.3, SD=6.8

These M3 (and the F3) families were transferred to TAFI (Taroona) on January 5 2001, and from there to Bolduan's Bay on March 14, 2001.

M3 lines were weighed at the end of their nursery phase at Bolduan's Bay at the end of October 2001. At that time, 100 of the largest oysters per line were removed and put aside as putative parents for the M4 generation. Of the remaining animals, random selection of 100 oysters were made and weighed, prior to dispatch in bags to the five grow-out farms. Each farm received three replicates of each line. At this time, the four

M3 lines were very similar to one another and had about twice the average weight of the Control group of unselected, random animals from M2-FAST1 (Table 6.1). Clearly the mass selection process had effected some considerable change at this early stage.

Bags were weighed October 2001, February/March, April/May, July/August, October/December 2002 and (Tasmanian sites only) January/March 2003.

About midway through grow-out (April-May 2002), the mass selection M3 lines were still performing better than the CONTROL line, except at Pittwater where the CONTROL line was marginally better than one of the four mass selection lines (Table 6.2). The average weight of an M3 oyster (19.0 gm) was now about 30% greater than that of a CONTROL oyster (14.7 gm).

At the final bag weight checks, it was observed that 20 of the initial 75 bags had been lost. This could be due to tag losses (leading to discarding of untagged bags), or to physical bag loss in storm or similar events. Such losses were quite commonly observed throughout this project. Final bag weight checks were taken at quite variable dates and with the oysters between 23 and 27 months of age (Table 6.3), the average weight of an M3 oyster was about 10% greater than that of a CONTROL oyster. All four M3 selected lines performed better than the CONTROL except at Coffin Bay, where the CONTROL line was a little heavier than the M3-2a line. Remembering that the CONTROL line was derived from unselected animals taken from M2-FAST1, it is clear that the selection process has had a significant effect on this mass-selected generation, and will have built on earlier gains.

Individual measurements were made on oysters removed from Pittwater on August 7, 2002. Ten oysters were removed from each M3 bag remaining at that time (3 bags per selected line, 1 bag for the CONTROL line), and assessed (Table 6.4). Unexpectedly, and in contrast with all bag-derived weights, the mean weight of the CONTROL line (32.81 g) was slightly higher than the overall mean weight of the four selected lines (30.39) and higher than the mean weight of three of the four selected lines . This may have been an artefact generated by only having one replicate sample of 10 control oysters to measure, whereas each selected line had the full suite of 30 animals. The condition index of each selected line, and therefore the overall mean of the selected lines (73.21) was slightly higher than that of the CONTROL line (70.57).

6.2. The Family Selection F3 Lines

6.2.1. Selection of Parents and Spawning Details for F3 Generation

The twenty best individuals from each of the F2 families had been put aside from their grading at Bolduans Bay farm in October 2000, and conditioned at the UTAS Department of Aquaculture in Launceston followed by Shellfish Culture Ltd. The F2 families selected to provide parents for the F3 were the best performing F2 families assessed late October 2000 from bag weights at Smithton. Pre-eminent among these 44 families were 5, 10, 13 and 21 of the first spawning run, and 19N and 31 of the second spawning run; these families provided the majority of the parents of the F3 generation. Note that some F2 families that subsequently proved to be good

performers at the final F2 weight checks in a REML analysis (Table 6.5) and not used to generate F3 families were subsequently used in F4 production.

Nineteen F3 families were established on December 2nd and 3rd 2000 at the Shellfish Culture hatchery in Bicheno. These families are listed in Table 6.6.

6.2.2. Performance of F3 Generation

These families (as for the M3 lines reared at the same time) were weighed at the end of their nursery phase at Bolduan's Bay at the end of October 2001. At that time, 40 of the largest oysters per family were removed and put aside as putative parents for the F4 generation. Of the remaining animals, random selection of 100 oysters were made and weighed (smaller numbers when 100 not available), prior to dispatch in bags to the five grow-out farms. Each farm received three replicates of each line where numbers permitted (see Table 6.7).

At the end of nursery grow-out, the F3 family lines varied considerably in average weight per individual, ranging from 4.26 to 0.54 gm (Table 6.7). Weight was to some extent negatively correlated with family size ($r = -0.591$, $P < 0.01$, see also Chapter 5 for similar results for the F2 families). Family 56 with only 62 animals had the highest average weight (4.26 gm), although family 54 with only 87 animals had an average weight (1.62 gm) very close to the mean of 1.63 gm. The slowest growing family, 53, only consisted of 600 animals, much less than the average number of 2380. Correcting for density had an appreciable effect on the rank of some but not all F3 lines. It is worth noting that the mass selection M3 lines did not compare favourably with the F3 lines. In fact all four FAST selection lines were smaller (average weights per animal of 0.98 to 1.16 gm) than both the mean (1.63 gm) and median (1.36) of the 19 F3 families (Tables 6.1 and 6.7). This implies, of course, that our family selection procedures are having more effect than mass selection (although the latter, as discussed earlier for the M3, above, is still effective).

Bags were weighed October 2001, February/March, April/May, July/August, October/December 2002 and (Tasmanian sites only) January/March 2003.

At the final grow-out bag weight checks, oysters ranged from about 23 (Coffin Bay, Smoky Bay) to about 27 (Coles Bay, Smithton) months of age. Unweighted mean weights per family ranged from about 80 g (family 52) to about 54 g (families 53 and 56). This is a smaller performance range than that observed for the F2, but there were more F2 families and the F2 families included an inbred slow-growing line. The heaviest animals at final bag weights were from Coles Bay, but these animals were four months older than those in the final weight checks from South Australia: had the animals been of the same age then no doubt (and as previously) the South Australian animals would have been heavier. Animals from Smoky Bay were larger than those from Coffin Bay, in contradistinction to results from some earlier generations.

As for the F1 and F2 families, families that performed well at one site tended to perform well at another, and those that performed poorly at one site tended to perform poorly at another (Figure 6.1). Inter-farm family correlations were all high and ranged from 0.76 to 0.97. The family*site variance ratio, or genotype by environment interaction, was estimated at 0.08± 0.07. This is very low and essentially non-

significant – there was little or no genotype by environment interaction in this data set.

Individual measurements were made on Pittwater animals collected on August 7 2002. As for the F2 generation (and the M3 lines), 10 animals were removed at random from each replicate, wherever possible (a maximum of 3 x 10 = 30 animals per family). 16 families were available at Pittwater (families 53, 54 and 56 of those successfully spawned were not represented there – subsequently families 50, 58, 64 and 66 were lost from Pittwater due to a storm prior to final bag weight checks).

Mean weights for these about 20 month old animals ranged from about 45.5 g (families 52, 57 and 59 – also top performers in the final bag weight checks, Table 6.8) to about 23.0 g (family 64), a factor of about 2.0. Condition index was much less variable, ranging from about 100 (family 63) to about 67 (families 59 and 68), a factor of about 1.5. There was a negative but non-significant relationship between mean family weight and mean condition index ($r = -0.280$), so that as for the F2 generation, faster growing families did not have a significantly higher (nor lower) condition index than slower growing families.

6.3. Comparison of F3 and M3 Animals

The average weight of the F3 individuals was slightly greater at final bag weight checks than that of the M3 individuals (and larger than the M3 Control animals) (Table 6.11). The top-performing F3 animals considerably out-performed the M3 Fast selection lines (compare Tables 6.7 and 6.3) – the number one ranking F3 family (number 52) grew about 25% heavier than the number one ranking M3 fast line (M3-2a).

The individually measured F3 animals removed from Pittwater on August 7 2002 had a higher average weight than the individually measured M3 Fast animals (36.11 g versus 30.39 g). They also had a slightly higher average condition index (77.15 versus 73.21) (compare Tables 6.4 and 6.10).

6.4. Sex Ratio Analysis of F3 and M3 Families

The F3 lines were examined once, in April 2003 (Table 6.12). All eleven single pair families inspected showed significantly deviant sex ratios, even after Bonferroni correction. One had a female bias, with 93.9% females, and ten had a male bias, with an average male proportion of 92.81%. This male bias extended outside those lines which had F2 parents from male dominated family lines and included the two mass selection lines examined. Perhaps the majority of F3 oysters were simply late making the protandric transition from apparent male to female, a supposition that gains some support from the predominance of males in the mass selection lines as well as the F3 lines. Because of the bias in the mass selection lines we would hypothesize that environmental factors had delayed the normal transition of many protandric males to female. This hypothesis needs further investigation. It has been suggested that sex ratio might be able to be manipulated for commercial gain, as females tend to grow faster than males (Baghurst and Mitchell, 2002).

Table 6.1. Weights of M3 oysters at the end of their nursery phase at Bolduan's Bay, October 2001. Animals weighed in lots of 100 and weights given are average weights per animal in gm with SD.

	Average animal weight (g)	Number per family*
M3-1a	0.98±0.07	5242
M3-1b	1.06±0.06	2858
M3-2a	1.16±0.04	2401
M3-2b	1.14±0.05	2423
CONTROL	0.49±0.02	3351

*after removal of 100 of the largest oysters per line as putative parents for M4

Table 6.2. Mid-way (April/May 2002) weights per individual (g) with SD for the M3 lines (bags weighed, animals counted)

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	
Date sampled	14/5/02	9/4/02	8/5/02	1/5/02	30/4/02	Overall
Age (mths)	17	16	17	17	17	unweighted
Average oyster weight (mean of bag weights/oyster counts) with SD						
M3-1a	21.01±3.20	7.75±2.46	16.63±0.97	18.56±2.70	26.45±7.27	18.08±6.85
M3-1b	22.95±1.74	6.57±0.56	19.02±0.79	19.29±4.67	26.69±0.25	18.90±7..57
M3-2a	24.32±1.67	7.05±0.50	18.65±0.95	19.89±1.82	25.18±2.52	19.02±7.25
M3-2b	25.50±2.39	5.99±0.60	18.72±1.80	20.93±4.97	29.00±2.49	20.03±8.80
CONTROL	21.10±3.59	4.98±1.76	11.88±0.73	13.32±3.26	22.01±0.33	14.66±7.05

Table 6.3. Final weights per individual (g) for the M3 lines (bags weighed, animals counted)

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	
Date sampled	28/1/03	6/3/03	19/2/03	24/10/02	23/10/02	Overall
Age (mths)	26	27	26	23	23	unweighted
Average oyster weight (mean of bag weights/oyster counts) with SD						
M3-1a	51.36±4.17	80.79±9.46	45.76±4.08	54.72	68.09±1.75	60.14±14.17
M3-1b	51.21±3.7	78.20±10.18	53.49±1.29	46.14	64.95±0.78	58.80±12.85
M3-2a	53.87±2.64	101.45±2.99	53.01±2.07	41.91	62.67±3.65	62.58±22.94
M3-2b	-	73.24±8.86	52.15±4.19	47.88	68.63±1.02	60.47±12.34
CONTROL	48.89	77.18±3.63	40.39±0.37	42.08±5.37	60.29±1.16	53.77±15.25
Total number of bags/oysters*						
M3-1a	2/179	3/88	3/300	1/72	3/243	12/882
M3-1b	2/163	2/55	3/300	2/166	3/271	12/955
M3-2a	2/180	2/60	3/300	1/94	3/264	11/898
M3-2b	-	2/72	3/300	1/99	3/288	9/759
CONTROL	1/90	2/67	3/250	2/173	3/271	11/851

*note that 10 oysters were removed from each Pittwater bag on 7/8/02 for individual (and destructive) measurements-these not accounted for in the above numbers.

Table 6.4. Individual measurements of M3 oysters from Pittwater on August 7, 2002

M3 line	Weight Mean±sd	Condition index Mean±sd	Number measured
M3-1a	28.85±8.51	73.42±5.85	30
M3-1b	28.51±8.92	72.74±7.74	30
M3-2a	35.08±11.08	75.63±7.1	30
M3-2b	29.11±10.90	71.04±5.96	30
CONTROL	32.81±13.92	70.57±4.24	10

Table 6.5. REML (restricted maximum likelihood) analysis of family rankings from the final F2 growout data (taken October-November 2001), together with the number of times progeny from each family had been used in F3 and F4 crosses. Means are mean weights per individual, in gm.

			# parents used in						# parents used in		
Rank	Family	Mean	F3	F4	Total	Rank	Family	Mean	F3	F4	Total
1	13	73.61	7	1	8	23	42	62.01			
2	23	70.54	1	4	5	24	4	61.96	2		2
3	5	68.61	5	1	6	25	28	61.95			
4	43	67.17		4	4	26	45	61.66			
5	17	67.14		3	3	27	37	61.64			
6	21	66.99	5	2	7	28	34	60.62			
7	20N	66.76		2	2	29	33	60.13			
8	31	66.58	7		7	30	26	59.85			
9	10	66.52	4	4	8	31	47	59.84			
10	14	66.51	1	1	2	32	38	58.43			
11	9	66.18		1	1	33	11	58.14			
12	19N	66.10	3		3	34	35	57.56			
13	15	65.98	2	2	4	35	41	57.56			
14	22	64.89				36	40	55.22			
15	25	64.20		1	1	37	32	53.98			
16	6	63.57				38	46	53.89			
17	8	63.56				39	44	53.82			
18	24	63.50				40	3N	53.27			
19	18	63.47				41	30	52.91			
20	12	63.35				42	27	52.88			
21	1N	63.33				43	39	50.39			
22	2	63.08				44	16N	43.78			

Table 6.6. Parents of the F3 generation, families 50 to 68.
Parentage removed from this public access version of the Report, for commercial-in-confidence reasons.

Date made	F3 number	Parents female F2	Parents male F2	Female grandparents female F1	Female grandparents male F1	Male grandparents female F1	Male grandparents male F1	Inbreeding coefficient
Saturday	50							0.0
2/12/00	51							0.0
	52							0.0
	53							0.0
	54							0.0
	55							0.0
	56							0.0625
Sunday	57							0.0625
3/12/00	58							0.0
	59							0.0
	60							0.0
	61							0.0
	62							0.0
	63							0.0
	64							0.0
	65							0.03125
	66							0.0
	67							0.125
	68							0.0
	55R**							0.0

*intended to be a 31 female from F2 but no 31 females were left
**replacement for 55 where fertilization was weak

Table 6.7. Weights and ranks of F3 oysters at the end of their nursery phase at Bolduan's Bay, October 2001. Animals were usually weighed in lots of 100. Where less than 100 animals were available, number used is given. Weights given are average weights per animal in gm. The family lines are ranked by weight, both uncorrected and corrected for number (density).

F3 families	Average animal weight	Number per family*	Family rank	Density corrected Rank	Number of times used in F4	Number of bags of 100 distributed to farms				
						Pittw	Coles Bay	Smithton.	Coffin Bay	Smoky Bay
50	1.44	1858	9	14	1	3	3	3	3	3
51	1.36	3494	10	6	-	3	3	3	3	3
52	3.45	1144	3	3	3	3	3	3	-	2+(44)
53	0.54	600	19	19	-	-	3	3	-	-
54	1.62	87	6	18	1	-	-	(50)	-	-
55R	0.96	3873	12	8	-	3	3	3	3	3
56	4.26	62	1	1	4	-	-	(50)	-	-
57	2.42	1790	4	5	3	3	3	3	3	3
58	0.82	3694	18	12	-	3	3	3	3	3
59	3.56	1421	2	2	4	3	3	3	2+(21)	3
60	1.01	3650	11	9	-	3	3	3	3	3
61	1.56	2979	8	7	2	3	3	3	3	3
62	1.84	3358	5	4	1	3	3	3	3	3
63	0.89	3578	17	10	-	3	3	3	3	3
64	0.93	2695	15	17	-	3	3	3	3	3
65	0.91	2842	16	16	-	3	3	3	3	3
66	0.96	2921	13	15	-	3	3	3	3	3
67	1.57	1749	7	13	1	3	3	3	3	3
68	0.95	3426	14	11	-	3	3	3	3	3

*after removal of 40 animals as putative parents for F4

Table 6.8. Final weights per individual (g) for the 19 F3 lines (bags weighed, animals counted)

F3 Date Age (mths)*	Pittwater 28/1/03 26 mean±SD	Coles Bay 6/3/03 27 mean±SD	Smithton 19/2/03 27 mean±SD	Coffin Bay 24/10/02 23 mean±SD	Smoky Bay 23/10/02 23 mean±SD	Unweighted mean±SD	Rank
50		74.49±15.47	45.15±13.74	52.17±8.66	78.41±1.52	62.55±16.37	8
51	54.79±1.62	73.93±8.53	54.02±3.09	57.58	71.61±2.89	62.38±9.61	9
52	67.37±3.39	91.97±7.36	66.53±2.91		95.01±11.08	80.22±15.38	1
53		61.22±19.16	48.24±14.06			54.73±9.18	18
54			58.61			58.61	14
55R	40.42	75.40±15.70	47.34±5.40	52.28±1.67	65.15±1.79	56.12±14.07	17
56			53.66			53.66	19
57	62.17±2.93	78.80±11.51	58.15±3.18	70.83±20.69	78.39±6.99	69.67±9.35	3
58		102.33±15.44	46.85±9.69	46.23±2.54	69.87±0.79	66.32±26.41	6
59	67.14±7.52	81.83±11.89	59.11±6.46	66.34	81.84±4.59	71.25±10.16	2
60	51.54	77.75±20.15	41.24±3.35	61.45±12.61	71.84±2.02	60.77±14.81	11
61	51.86±1.04	80.57±14.34	43.36±8.30	57.88	67.44±3.95	60.22±14.37	12
62	55.33	99.51±22.21	46.28±5.27	64.82±8.93	70.29±0.65	67.25±20.23	4
63	46.20	85.69±19.90	45.19±0.97	51.25	70.94±2.31	59.85±17.79	13
64		73.77±12.90	40.14±1.63	58.21±13.84	60.31±4.01	58.10±13.82	15
65	60.81±18.03	76.67±15.87	45.11±5.72	59.57	65.34±1.00	61.50±11.38	10
66		84.93±20.43	52.36±4.47	54.62±6.08	74.56±7.05	66.62±15.76	5
67	48.11±5.06	92.57±19.44	43.79±4.49	61.14±10.49	67.73±4.85	62.67±19.30	7
68	45.21	73.02±18.33	47.81±5.42	49.41±14.61	70.27±1.75	57.14±13.36	16
Overall	54.25±8.71	81.44±10.46	49.63±7.05	57.58±6.75	72.44±8.13	61.89±6.06	

SD = Standard deviation of mean weights per individual/bag

Table 6.9. Final counts per family for F3 families (counts across bags)

F3 Family	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	Total
Date sampled	28/1/03	6/3/03	19/2/03	24/10/02	23/10/02	
Age (mths)*	26	27	27	23	23	
50	0	108	255	235	236	834
51	179	110	298	99	258	944
52	180	113	291	0	240	824
53	0	110	153	0	0	263
54	0	0	59	0	0	59
55R	90	89	280	184	280	923
56	0	0	50	0	0	50
57	268	144	300	181	276	1169
58	0	83	212	289	239	823
59	178	74	288	101	268	909
60	90	84	300	165	250	889
61	178	89	300	113	269	949
62	90	81	298	299	282	1050
63	90	49	298	96	253	786
64	0	105	300	190	256	851
65	180	107	200	93	262	842
66	0	61	297	186	280	824
67	180	89	299	255	263	1086
68	90	84	280	270	230	954
Overall	1793	1580	4758	2756	4142	15029

Table 6.10. Individual measurements of F3 oysters from Pittwater on August 7, 2002. Families 53, 54 and 56 absent.

F3 Family	Weight Mean±sd	Condition index Mean±sd	Number measured
50	35.10±12.28	78.2±7.73	30
51	37.54±12.77	84.10±8.49	29
52	45.56±11.61	75.61±17.43	28
53			
54			
55	31.85±8.00	76.93±9.16	20
56			
57	45.84±12.66	72.15±5.10	30
58	34.26±11/30	76.95±6.03	30
59	45.64±16.11	67.76±7.51	29
60	38.41±14.49	78.24±9.79	30
61	33.51±10.73	74.91±10.15	30
62	36.05±8.35	78.67±14.09	30
63	32.56±13.22	100.36±28.90	30
64	22.96±9.53	74.56±7.01	30
65	32.61±11.26	77.79±5.31	29
66	35.89±7.83	77.40±10.05	20
67	32.85±11.94	73.83±13.56	30
68	37.17±12.70	66.93±5.11	27
Overall	36.11±5.90	77.15±7.46	452

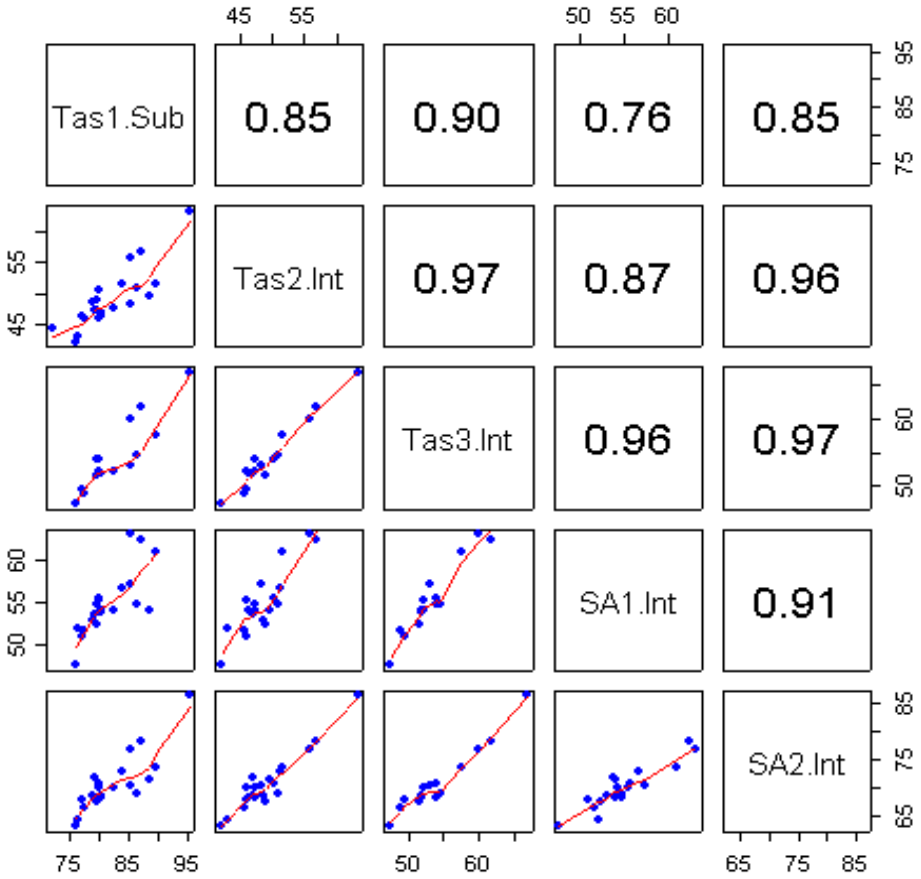
Table 6.11. Comparison of F3 (19 families) and M3 (3 Fast lines) Performance at Final Bag Weight Checks (SD is among families)

F3 Family Date sampled Age (mths)*	Pittwater 28/1/03 26 mean±SD	Coles Bay 6/3/03 27 mean±SD	Smithton 19/2/03 27 mean±SD	Coffin Bay 24/10/02 23 mean±SD	Smoky Bay 23/10/02 23 mean±SD	Unweighted mean±SD
F3	54.25±8.71	81.44±10.46	49.63±7.05	57.58±6.75	72.44±8.13	62.61±6.47
M3 Fast	52.15±1.49	83.42±12.42	51.10±3.61	47.67±5.32	66.08±2.79	60.50±1.57
M3 Control	48.89	77.18	40.39	42.08	60.29	53.77

Table 6.12. Sex ratios in F3 and M3 families sampled in April 2003. Numbers of females, males, and indeterminate ('?') given. Probabilities of fit to 1:1 male:female ratio are given.
Parentage removed from this public access version of the Report, for commercial-in-confidence reasons.

	2003 data for F3 lines					F2 parent family	
Line	# female	# male	?	% male	P	female	male
50	3	19	9	86.4	<0.001		
51	1	29	0	96.7	<0.001		
52	not sampled						
53	31	2	0	6.1	<0.001		
54	not sampled						
55	0	30	0	100.0	<0.001		
56	not sampled						
57	not sampled						
58	4	28	0	87.5	<0.001		
59	not sampled						
60	1	28	1	96.6	<0.001		
61	not sampled						
62	not sampled						
63	6	21	3	77.8	<0.01		
64	not sampled						
65	4	58	7	93.5	<0.001		
66	2	26	2	92.9	<0.001		
67	0	27	3	100.0	<0.001		
68	1	29	0	96.7	<0.001		
M3-1a	0	35	0	100.0	<0.001		
M3-2b	1	29	0	96.7	<0.001		

Figure 6.1 Correlations among pairwise combinations of farms for the F3 families at their final weight checks. Axes are mean weights per family. Correlations of family weights also shown. Tas1.sub = Coles Bay (6/3/03), Tas2.int = Smithton (19/2/03), Tas3.int = Pittwater (28/1/03), SA1.int = Coffin Bay (24/10/02), SA2.int = Smoky Bay (23/10/02).



CHAPTER 7. Sixth Spawning Year 2001/02 – The Mass Selection M4 Lines plus the Family Selection F4 Lines

7.1. The Mass Selection M4 Lines

Three M4 mass-selection lines were made, on January 5 2002, at Shellfish Culture. Five M3 mass-selection lines had been made, and the intention had been to propagate these forward. These five lines were M3-1a, M3-1b, M3-2a, M3-2b and a CONTROL line (from M2-FAST1). These animals were, like the F3 animals, only one year old when we attempted to use them as parents for the M4 lines. The 100 largest animals from each M3 line were taken (like the F3 parents) at the end of their nursery assessment in October 2001 (Table 6.1). They were small and, when opened for spawning on January 6, 2002, most were still immature:

M3-1a, n=100, 2 females, 33 males, 65 no gonad
M3-1b, n=100, 4 females, 25 males, 71 no gonad
M3-2a, n=100, 7 females, 41 males, 52 no gonad
M3-2b, n=100, 15 females, 29 males, 16 no gonad (the remaining 40 not opened)
CONTROL n=100 (only 10 animals of a large enough size to warrant opening were found), 2 males, 0 females, 8 with no gonad, too small to persevere with.

As a consequence, only three M4 lines could be made:

M4-1a/b: 1 mass cross with 6 females and 58 males from M3-1a and M3-1b
M4-2a: 1 mass cross with 7 females and 41 males of M3-2a
M4-2b: 1 mass cross with 15 females and 29 males of M3-2b
It was not possible to continue on the mass-selection control line.

An overall control line for the mass-selection and family lines, termed CONTROL4, was made from the PD11 commercial line spawned by Shellfish Culture on January 25, 2002.

M4 spat were transferred to upwellers at the TAFI Taroona laboratory on February 1 2002, and transferred to Smithton and put in seed trays on May 8 2002. Animals were weighed at the end of their nursery phase at Smithton on December 11 2003, and transferred to the grow-out farms on the following dates: Smithton 11/12/02, Pittwater 20/12/02, Coles Bay 15/1/03, Coffin Bay 21/1/03 and Smoky Bay 22/1/03.

At the end of the nursery phase, the M4 selected animals were more than twice as heavy, on average, as the CONTROL4 animals (Table 7.1). Only a very small part of this difference can be attributed to the CONTROL4 animals being two weeks younger. About mid-way through grow-out, the M4 selected animals were about twice as heavy as the CONTROL4 animals, both at individual farms and across farms (Table 7.2).

Bag weights were taken in December 2002, February/March, May/July, August/October 2003, and February/April 2004.

At the time of final bag weights (taken by Scott Parkinson of ASI, see section 12.1), selection lines M4-a/b and M4-2b were performing similarly, with M4-2a slightly behind; all three mass selection lines were growing much faster than the CONTROL4 line. At the four farms with M4 and CONTROL4 lines, the average of the M4 growth rates was greater than that of the CONTROL4 line by factors of 1.5 (Coffin Bay and Smoky Bay), 1.6 (Smithton) and 1.9 (Coles Bay) – there was no CONTROL4 line at Pittwater.

Individual measurements were taken on animals removed from Smithton on 7 August 2003 at 19 months of age (Table 7.4). The two M4 selected lines examined had average weights (about 33 g) that were still about twice that of the CONTROL4 line (about 17 g), but the CONTROL4 line had an average condition index markedly greater (about 120) than those of the M4 selected lines (about 80). However, the range of condition index values noted for the CONTROL4 line was extremely high (from an abnormally low value of 23.26 to an abnormally high value of 251.74, SD = 50.71). This range may be compared with those from M4-1a/b (range 67.1 to 96.3, SD = 6.47) and M4-2b (range 36.16 to 152.28, SD = 16.23). Either some of the CONTROL4 values were erroneous, or this line has an extremely high degree of condition index variation.

7.2. The Family Selection F4 Lines

7.2.1. Selection of Parents and Spawning Details for F4 Generation

The selection of F4 families was generated by:

(1) analysing the final F2 set of grow-out data and making sure that all good families were propagated onwards (note that the F3 families had been made based on early F2 grow-out information – all that was available at the time). Table 6.5 shows, for example, that while we had already selected from the 12 month F2 growth data most of the good-performing F2 families to make F3s, some (e.g. families 17 and 43) had been missed. One cross, female from 23 and male from 43, was done twice (families 102 & 103) to check on repeatability.
(2) by analysing the very early F3 data and choosing individuals from promising families (Table 6.7), and
(3) by minimising inbreeding (all families had inbreeding coefficients of zero or close to zero with one exception, family 115, made to propagate onwards a particularly desired shell-shape, that of flat and wide).

Sixteen single-pair F4 families were spawned on January 5 2002 at Shellfish Culture. Two of these resulted in low numbers due to technical mishaps, and were discarded. Nine single-pair F4 families were spawned on January 6, including the two replacements for the failures on day 1 (together with the three M4 mass-selection lines). 23 F4 family lines were thus spawned, although six failed at set. Thus 17 F4 family lines were generated, ten producing more than 2000 spat (Table 7.5).

Note that only 4 of the crosses (108, 118. 120, 121) are true F4 families – 7 are further crosses of F2 families, and 12 are F2 x F3 crosses. Strictly, these are the fourth set of

spawnings, but we retain the term F4 for convenience. The F3 animals used as parents came from 40 of each F3 family taken at the end of the nursery phase assessment in October 2001, and held for less than two months first at Bolduan's Bay and then at TAFI.

On 2 February 2002 at Shellfish Culture, we planned to inbreed families 14, 15, 16, WA981 and WA 996. Three of these inbred families were successfully established: In15 (15 x 15), In981 (WA981 x WA981) and In996 (WA996 x WA996). Two attempts to inbreed family 16 (itself a double inbreeding of 97-2 through WA987) failed, and the broodstock set aside to inbreed family 14 was entirely male. Note that we have now rationalised our notation for inbred families, where inbred families are those made from full-sib crosses. The notation will now be XX/Y, where XX is the original ancestral family, and Y is the generation of inbreeding. In15 was thus subsequently renumbered 15/1 to indicate it had had one generation of inbreeding, and In981 and In996 were subsequently numbered 97-3/2 and 97-5/2 to indicate two generations of inbreeding from the original ancestral families of 97-2 and 97-5 respectively.

7.2.2. The Performance of the F4 Families

The F4 (and M4) spat were transferred to upwellers at the TAFI laboratory at Taroona on February 1, with the inbreds transferred March 5. Initial weight checks were made on 16 April 2002, but at this age weight is very highly correlated with density (spat number) and is not a reliable indicator of future growth. On May 8 all spat were transferred to Smithton and put into seed trays. They were checked and thinned out on July 23.

Weight checks were taken at the end of the F4 nursery stage on December 10/11 2002 (Table 7.6). Larger animals were set aside as potential broodstock (Table 7.7). Animals were transferred to the grow out farms on the following dates: Pittwater 20/12/02, Coles Bay15/1/03, Smithton 11/12/02, Coffin Bay 21/1/03 and Smoky Bay 22/1/03.

At the end of the nursery stage there was a strong density (number) effect. This can be seen in Figure 7.1 which plots average weight of a family (F4, M4, CONTROL and inbreds, weights including the bags sent to farms plus the oysters set aside as potential broodstock, compiled from Tables 7.6 and 7.7) against number produced (Table 7.6). Figure 7.1 also shows that the three highly inbred families have, as expected, performed poorly: the average sizes of their progeny are small despite not many animals being produced. The (unselected) control line is also substantially below the average line, indicating that it has not performed as well as the F4 and M4 families. Finally, once the density factor is taken into account, there is not a great deal of variation in the performance of the selected F4 and M4 families: all lie quite close to the line. The correlation is negative (-0.453) and statistically significant ($P < 0.05$, $n = 24$); it becomes more negative (-0.587) and more statistically significant ($P < 0.01$, $n = 21$) when the three inbred families are excluded.

Bag weights were taken in December 2002, February/March, May/July, August/October 2003, and February/April 2004.

Mid-way through grow-out, when the animals are between 16 and 18 months of age, at each farm each family line was growing faster than the control (Table 7.8). The best performing families (100, 107R and 118) were growing close to three times faster than the control. It seems likely that the Control4 line was in fact a particularly poorly growing line – even the three inbred lines were about on a par with the control. At this time, the above high and significant correlations between progeny numbers and average weight described above had fallen substantially, to non-significant values: from –0.453 to –0.181 ($P > 0.05$, $n = 23$) for all families, and from –0.587 to –0.387 ($P > 0.05$, $n = 20$) when inbreds are excluded. The original strong density effect on growth rate had therefore decreased substantially as the oysters aged and other factors, including genetics, came into play. Furthermore, once animals are sent to the nursery at Smithton, and also subsequently in the on-growing period, numbers (densities) are managed more uniformly than they are at the very early spat stage at TAFI.

The final F4 weight checks were made by Scott Parkinson of ASI when the oysters were about 25 months of age (Tables 7.9 and 7.10). It is very clear that the selected families were heavier than the Control animals, sometimes very markedly so. Lines 107R, 118 and 100 were ranked 1st, 3rd and 4th respectively. Line 110 was now ranked 2nd overall, but it was only at one site (Smithton, where it ranked 1st but with only 85 animals). Lines 107R and 118 were respectively about 2 x and 1.7 x the size of Control4 at each of the three sites where they were grown. The correlations between progeny numbers and average overall weights remained non-significant ($r = 0.159$ with all lines, $r = 0.048$ without the inbreds, note the correlations are positive now).

Mean performance across the sites showed similar trends to previous generations, with large variability, and higher growth rates at the South Australian sites relative to the Tasmanian sites. Like the F1 and F2 (but unlike F3) there was a small but significant G*E interaction, with an estimated ratio of family*site variance to total variance of 0.25±0.08. Nevertheless, the rankings of families were relatively consistent across sites (Figure 7.2), with correlations between families at the intertidal sites ranging from 0.52 to 0.94 (mean of 0.802). Correlations between the sub-tidal site and other sites were lower, ranging from 0.44 to 0.75 (mean of 0.630). The abnormally low correlation of 0.52 between Pittwater and Coffin Bay was largely due to family 114, which was the top-performing family at Pittwater but only an average performer at Coffin Bay. Note that these analyses, unlike previous similar analyses, also include the mass-selection lines. This was because there were relatively few single-pair family lines produced this generation and we included the M4 lines to increase the amount of data available for analysis. Across generation patterns in correlation variation are considered in Chapter 13.

Oysters for individual measurements were removed from Smithton on August 7, 2003. Mean weight per family of these 19 month oysters ranged from a low of 16.95 (Control4) to 44.13 (#110) and 44.23 (#107R), a range of 2.6, and condition index ranged from a low of 68.44 (#107R) to 119.82 (Control4), a range of 1.75 (Table 7.11). The F4, inbred and control families, a total of 19 lines, showed a significant negative correlation between mean weight and mean condition index ($r = -0.629$, $P < 0.01$) – those families with smaller slower growing oysters tended to have higher condition indices. This correlation had also been negative and significant in the F1 families (section 3.1, $r = -0.388$, $0.05 > P > 0.01$, just positive but non-significant in

the F2 families (section 5.2, r = +0.049, P > 0.05), and negative but non-significant in the F3 families (section 6.2, r = -0.280, P > 0.05).

These generally negative correlations between growth rate and condition factor are a concern. However, it must be noted that these assessments of condition factor in this project are taken when the oysters are quite young and before they reach market size. It is interesting that later assessments of condition index for line 118 (which had a mean index of only 70.75 in Table 7.11) and the Control line (mean index of 119.82 in Table 7.11), using oysters of two years of age, showed that the two lines were now very similar (mean indices of 18.45 and 16.59, respectively, using a different method of estimating condition index, Table 7.15).

7.3. Comparison of F4, M4, Inbred and Control Performance

Final weight checks may be assessed with the most rigor at the Smithton site, as here all 16 F4 family selection lines, all three mass selection lines, the two inbred lines, and the control line are all present (Fig 7.3). Here, the average of the family selection lines was 57.33 g (minimum = 42.61 g, maximum = 78.82 g), the average of the mass selection lines was 57.12, the average of the inbred lines was 41.33, and the control line was 35.08. The family lines and the mass selection lines had very similar means (both about 1.6x the growth rate of the control), but the former were more variable among lines. Line 107R was the fastest growing, at 2.0x the control rate; line 120 was the slowest, at 1.2x the control rate. The control line was clearly inferior to all other lines, including, surprisingly, the inbred lines.

7.4. Shape Measurements of F4 and M4 Oysters

Individual oysters were measured for length, depth and width. These were the same oysters removed from Smithton on August 7, 2003 and assessed for condition index. The data were examined to see if the selection procedure had changed shape indices. i.e. to see if the shapes of the F4 family oysters differed from the Control4 line. Three shape indices were used.

The first was one suggested by ASI (Scott Parkinson). This is:

$$SI\ (ASI) = 100 * \frac{\max(length/3, width/2, depth) - \min(length/3, width/2, depth)}{\max(length, width, depth) - \min(length, width, depth)}$$

This assesses the extent of deviation from a suggested optimal ratio of 3:2:1 for length:width:depth. An optimal oyster will have a score of 0, so the closer the score to 0, the closer the oyster to the optimum. Note that the factor of 100 emphasises any deviations from 0.

The second one was proposed by Crozier (1914) and adopted by Galtsoff (1964). This is:

$$SI\ (Galtsoff) = (length + depth) / width$$

Gary Zippel has suggested that scores < 3 indicate a good shape, about 3 average shape, and >3 poor shape.

Note that what we call length (the measurement from umbo to the extreme end of the shell) Galtsoff termed height, what we call width (the maximum measurement across the shell perpendicular to our length measurement) Galtsoff termed length, and what we call depth (the depth or vertical height of the two shells) Galtsoff termed width. In Galtsoff's terms, the shape index is (height + width) / length. The two versions of the equation are identical once the differing terminologies are understood.

The third one is a simple elongation index, derived by Ward (unpublished). This is:

EI = (2 x length) / (3 x width)

An oyster with a length:width of 3: 2 will have a value of 1; values greater than 1 indicate an elongate oyster, values less than 1 a rounder oyster.

All F4/M4/inbred families have an average SI-ASI less than the Control4 line (Table 7.12), and (after removing three outliers, one from each of three families), the standard deviations of all the selected families are less than that of Control4. Thus the selected families on average have a better shape and show less variation than the Control.

Virtually all oysters had a "good" shape using the SI-Galtsoff formula (SI-Galtsoff<3). Only two oysters (one in family 111, and one in 112) had SI-Galtsoff values greater than 3, indicating a poor shape.

Most oysters accorded closely to the 3:2 ratio of length:width, with EI values averaging close to 1. The two most elongate oysters (with EI values of 1.90 and 1.64) were the same oysters that had SI-Galtsoff values greater than 3. These two oysters also had high SI-ASI scores (21.69 and 17.40).

Line 115, originally spawned to be flat and wide (section 7.2.1), had low values for all three shape indices, indicating that the breeding objective for this family had been achieved.

There was no significant relationship between SI-ASI score and the wet weight of individual oysters ($r = -0.037$, $n = 1564$, Figure 7.4). However there was a small but significant positive correlation between EI score and wet weight ($r = 0.084$, $P < 0.01$, $n = 1564$). In other words, the heavier oysters tended to be somewhat more elongate than lighter oysters, but the effect (at least in this data set) is very small (Figure 7.5).

Measurements of individual F4 oysters at Smithton were also made by Scott Parkinson five months later when the oysters had reached two years of age (post-spawning). Twenty random oysters per family were removed and measured (Table 7.13). Scott compared his results for one of the standout families, 118, with those from CONTROL4. Survival rate for 118 was estimated at 92% compared with 84% for CONTROL4. Mean weight of 118 was about 50% greater than that of CONTROL4 (64.95 g versus 42.55 g). The mean score of SI-ASI for 118 was about

half that of CONTROL4 (Table 7.11), and the standard deviation of 118 was much reduced. Thus family 118 showed a better and more uniform shape than CONTROL4. Scott noted that other desirable characteristics of family 118 included a dark shell (top and bottom), smooth shell and a wide body for sitting flat on the plate (however, EI for 118 is only a little greater than for CONTROL4, and not significantly so). All shape indices had increased somewhat for both families from those recorded five months earlier, SI-ASI from 6.51 to 8.23 for family 118 and 10.95 to 19.06 for CONTROL4, SI-Galtsoff from 2.11 to 2.20 for family 118 and 1.89 to 2.23 for CONTROL4, and EI from 1.08 to 1.14 for family 118 and 0.92 to 1.07 for CONTROL4. The oysters had become somewhat more elongate in the interval between the two sets of measurements.

7.5. Product Uniformity

Oysters growers would like to raise a product that grows more uniformly. This would allow them to reduce the amount of handling needed on the farm and would reduce labour costs. This characteristic was not among the traits surveyed for grower importance by TORC in its initial questionnaire back in 1998. In this survey, several farms identified it as important and thus it appeared in the final ranked list, as an add-on, at number 16 (Table 1.1). Had it been identified as one of the top priority traits at the time, more attention would have been lavished on it in the selective breeding program. If a new survey were to be carried out, growth uniformity might well appear at or close to the top of the list. Despite not being a focus of the breeding program, it is reasonable to expect that increased uniformity would result from the breeding program. This is because genetic diversity within families spawned in the program is much less than genetic diversity among unrelated spawners. Variability within family lines is expected to be less than in control lines spawned from unrelated individuals. Indeed, as the breeding program progresses and genetic factors become better controlled, product uniformity is expected to improve each generation.

Is this observed in practise? Variability within families needs to be assessed from measurements of the individuals within families. It is not possible to assess uniformity from a single mass weighing of 50 or 100 individuals; we can only use weight data available from measurements of individuals. Of course, characteristics such as shape and condition index can only be assessed on individuals anyway.

In this generation, we have this information for 16 family lines, two mass selection lines, two inbred lines and one commercial control line. Table 7.14 (see also Tables 7.4 and 7.11) gives summary data for total weight, condition index and ASI shell shape. Means per individual per family are given, together with coefficient of variances (CV). The CV is simply the standard deviation expressed as a percentage of the mean, and is used to compare the variation of populations independent of the magnitude of their means.

With respect to weight, the Control line has a higher CV than any other line: it is the most variable. The CV of the Control line is about 50% higher than the mean of the 20 selected lines.

With respect to condition index, the Control line has the second highest CV – it is just pipped by inbred line 97-5/2. The CV of the Control line is about 300% higher than the mean of the 20 selected lines.

With respect to shape (using the ASI formula), the Control line has the 7th highest CV. The Control line has a CV about equal to the mean of the 20 selected lines. Three selected lines have very high CVs, lines 101, 114 and M41ab with values of 108.60, 150.50 and 121.77 respectively. In each case these are due to single aberrant individuals. If these are removed, CVs fall to 53.99, 62.53 and 44.99 respectively. Then the CV of the Control line is about 16% higher than the mean of the selected lines.

There is thus evidence from this analysis that the selected lines are indeed more uniform for total weight and condition index, but there is little evidence for any change in the shape CV. Not too much weight should be drawn from these conclusions, with only one control line being examined, but generally the results are roughly in accord with expectations. Shape appears to be less responsive than weight and condition index; it is possible that this trait is less genetically and more environmentally influenced than the other two traits.

These animals were about 19 months old when examined.

At two years of age, Scott Parkinson examined line 118 and the Control line again (Table 7.15, see also Table 7.13). The Control line had double the CV of line 118 for total weight, about the same CV for condition index (assessed simply as wet meat weight as percentage of total weight), and about double the CV for shape. While somewhat different from the more extensive data collected at 19 months of age, especially with respect to the shape trait, these data again confirm that the Control line was generally more variable than at least this one selected line.

Table 7.1. Weights of M4 oysters at the end of their nursery phase at Bolduan's Bay, 11 December 2002. Animals weighed in lots of 100 and weights given are average weights per animal in gm. Also given are total numbers of oysters produced and numbers of bags (of 100 oysters each) distributed to each farm.

	Average animal weight (g)*	Number per family	Numbers of bags distributed				
			Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay
M4-1a/b	1.33±0.06	2742	3	3	3	3	3
M4-2a	1.07±0.05	1200	3	1	3	0	3
M4-2b	1.17±0.06	7712	3	3	3	3	3
CONTROL4	0.36±0.03	1400	3	3	3	2	3

*after removal of 100 of the largest oysters per M4 selected line as putative parents for M4. Mean sizes of these potential broodstock were: M4-1a/b 2.65 g, M4-2a 2.41 g and M4-2b 1.17 g.

Table 7.2. Mid-way (May/July 2003) weights per individual (g) for the M4 lines (bags weighed, animals counted)

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	
Date sampled	7/7/03	21/5/03	19/5/03	24/6/03	25/6/03	Overall
Age (mths)	18	16	16	17	17	unweighted
Average oyster weight (mean of bag weights/oyster counts) with SD						
M4-1a/b	13.29±3.67	15.73±1.64	18.07±0.42	33.81±1.48	30.45±1.32	22.27±9.23
M4-2a	-	13.20	13.60±3.36	-	25.72±2.65	17.50±7.12
M4-2b	13.78±1.23	14.58±1.64	17.83±0.91	29.15±4.75	28.48±1.58	20.76±7.50
CONTROL4	-	4.46±1.03	8.83±0.47	12.51±1.68	18.32±4.22	11.03±5.87

Table 7.3. Final weights per individual (g) for the M4 lines (bags weighed, animals counted). Data collected by Scott Parkinson (ASI).

	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	
Date sampled	3/3/04	4/2/04	22/2/04	29/3/04	1/4/04	Overall
Age (mths)	26	25	26	27	27	unweighted
Average oyster weight (mean of bag weights/oyster counts) with SD						
M4-1a/b	56.83±2.60	50.72±9.82	59.38±5.42	131.43±4.53	88.64±2.58	77.40±33.56
M4-2a	-	45.21	54.40±2.35	-	77.01±1.87	58.87±16.37
M4-2b	52.81±2.61	52.10±7.41	57.59±2.00	119.31±7.34	83.70±0.98	73.10±28.90
CONTROL4	-	26.51±5.70	35.08±8.94	82.84±6.59	55.76±9.18	50.05±25.07
Total number of bags/oysters*						
M4-1a/b	3/293	3/283	3/246	3/270	3/287	15/1379
M4-2a	0	1/73	3/255	0	3/266	7/594
M4-2b	3/273	3/269	3/249	3/287	3/294	15/1372
CONTROL4	0	3/220	3/219	2/145	3/219	11/803

*note that 10 oysters were removed from each Pittwater bag on 7/8/02 for individual (and destructive) measurements-these not accounted for in the above numbers.

Table 7.4. Individual measurements of M4 oysters from Smithton on September 1, 2003

M4 line	Weight Mean±sd	Condition index Mean±sd	Number measured
M4-1a/b	33.45±5.40	75.91±6.47	40
M4-2a	-	-	0
M4-2b	33.46±7.13	82.81±16.23	40
CONTROL4	16.95±5.59	119.82±50.71	39

Table 7.5. Parents of the F4 generations, families 100 to 122, plus CONTROL4 plus inbred families

Parentage removed from this public access version of the Report, for commercial-in-confidence reasons.

Date made	Family number	Parents* Female	Parents* Male	Nature of cross Female	Nature of cross Male	Inbreeding coefficient	Number at settlement
5/1/02	100			F2	F3	0	500
	101			F2	F2	0	100
	102			F2	F2	0	2500
	103			F2	F2	0	600
	104			F2	F3	0	6000
	105			F2	F2	0	0
	106			F2	F2	0.063	150
	107- discard			F2	F3	0	discarded
	108			F3	F3	0	0
	109- discard			F2	F3	0	discarded
	110			F2	F3	0	250
	111			F3	F2	0	6000
	112			F2	F3	0	6000
	113			F2	F3	0.031	0
	114			F2	F3	0	2500
	115			F2	F2	0.125	6000
6/1/02	116			F2	F3	0	2500
	117			F2	F2	0	2200
	118			F3	F3	0	2400
	119			F2	F3	0	0
	120			F3	F3	0	6000
	121			F3	F3	0	0
	122			F2	F3	0.031	8
	107R			F2	F3	0	750
	109R			F2	F3	0	0
25/1/02	CONTROL4	Cross of Shellfish Culture				low	3000
2/2/02	15/1			F2	F2	0.250	1000
	97-3/2			F1	F1	0.375	3000
	97-5/2			F1	F1	0.375	300
	97-2/3			F2	F2	0.500	0

*no parent was used twice, i.e. there are no half-sib lines. 107R and 109R are repeat matings of matings that failed the first time.

Table 7.6. Weights of F4 oysters at the end of their nursery phase at Bolduan's Bay, 11 December 2002. Animals weighed in lots of 100 (unless otherwise stated) and weights given are average weights per animal in gm. Animals set aside as broodstock not included (see Table 7.7). Also given are total numbers of oysters produced and numbers of bags (of 100 oysters each unless otherwise specified) distributed to each farm.

			Numbers of bags distributed				
F4 Family	Average animal weight (g)*	Number per family	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay
100	2.64±0.14	426	-	-	3	-	(76)
101	2.92	125	-	-	1	-	-
102	1.14±0.04	2331	3	3	3	3	3
103	3.81	170	-	-	1+(50)	-	-
104	0.31±0.07	8191	3	3	3	3	3
106	2.79	111	-	-	(91)	-	-
107R	3.26±0.20	1038	3	(38)	3	-	3
110	4.88	157	-	-	1+(37)	-	-
111	0.58±0.06	5091	3	3	3	3	3
112	1.50±0.01	1486	3	3	3	1+(86)	3
114	1.24±0.17	3803	3	3	3	3	3
115	1.02±0.06	1772	3	3	3	3	3
116	1.61±0.05	2004	3	3	3	3	3
117	1.81±0.12	1185	3	2+(35)	3	-	3
118	2.21±0.02	1703	3	3	3	3	3
120	0.39±0.04	4227	3	1	3	-	3
122	-	8	-	-	-	-	-
CONTROL4	0.36±0.03	1400	3	3	3	2	3
15/1	0.87±0.07	825	(67)	-	3	-	-
97-3/2	-	22	-	-	-	-	-
97-5/2	0.47±0.04	300	-	-	3	-	-

*after removal of broodstock. See Table 7.7

Table 7.7. Numbers and weights of F4 oysters set aside as potential broodstock.

F4 Family	Average broodstock weight (g)	Number of broodstock	F4 Family	Average broodstock weight (g)	Number of broodstock
100	4.90	50	114	2.62	100
101	6.30	20	115	2.10	100
102	2.30	100	116	3.43	100
103	6.55	20	117	3.46	50
104	1.44	100	118	4.67	100
106	5.45	20	120	1.85	100
107R	5.67	100	122	8.87	8
110	7.55	20	15/1	2.12	25
111	2.41	100	97-3/2	0.27	22
112	3.10	100	97-5/2	-	0

Table 7.8. Mid-way (May/July 2003) weights per individual (g) for the F4 and inbred lines (bags weighed, animals counted)

Date sampled	Pittwater 7/7/03	Coles Bay 21/5/03	Smithton 19/5/03	Coffin Bay 24/6/03	Smoky Bay 25/6/03	Overall
Age (mths)	18	16	16	17	17	unweighted
Average oyster weight (mean of bag weights/oyster counts) with SD						
100			24.27±1.40		37.12	30.69±9.09
101			20.57			20.57
102	14.38±1.55	10.78±3.58	18.13±1.10	29.05±1.97	28.94±0.91	20.26±8.39
103			20.15±0.35			20.15
104		3.35±0.56	9.20±2.50	27.68±6.37	20.61±0.86	15.21±10.98
106			18.68			18.68
107R		28.37	25.40±0.40		37.08±5.05	30.28±6.07
110			25.53±0.94			25.53
111	9.97±1.02	6.58±1.22	14.67±1.68	22.22±4.60	24.20±3.43	15.53±7.61
112	17.41±1.90	13.65±0.55	21.13±0.12	38.99±2.46	31.06±3.59	24.45±10.40
114	14.64±0.46	10.36±2.58	18.37±0.25	29.47±2.34	30.17±1.04	20.60±8.88
115	10.54±1.33	8.50±0.83	16.63±0.91	19.41±1.68	23.32±1.51	15.68±6.15
116	16.81±.52	13.73±2.21	20.80±1.31	29.70±1.53	29.99±3.79	22.20±7.41
117	18.58±2.26	16.45±7.96	25.50±3.81		32.35±4.89	23.22±7.21
118	18.44±3.10	15.75±2.82	24.13±0.42	36.63±1.78	39.70±2.47	26.93±10.75
120	7.74±1.75	5.44±0.96	10.67±1.92	16.67±2.47	19.81±3.15	12.06±6.04
122						
CONTROL4	-	4.46±1.03	8.83±0.47	12.51±1.68	18.32±4.22	11.03±5.87
15/1	10.34		15.00±1.20		22.98±0.90	16.11±6.39
97-3/2			10.89			10.89
97-5/2	8.99		10.33±1.45		17.55±0.81	12.29±4.60

Table 7.9. Final (February/March 2004) weights per individual (g) for the F4 and inbred lines (bags weighed, animals counted). Data supplied by Scott Parkinson, ASI

F4 Family Sample date Age (mths)	Pittwater 3/3/04 25	Coles Bay 4/2/04 24	Smithton 22/2/04 25	Coffin Bay 29/3/04 26	Smoky Bay 1/4/04 26	Overall unweighted	Rank
100			63.83±3.53		86.53	75.18±16.05	4
101			59.11			59.11	10
102	46.66±2.84	39.74±9.47	57.34±1.76	110.45±14.14	85.97±8.10	68.03±29.55	5
103			54.04			54.04	15
104	34.15	37.11±19.62	49.50±7.95	100.41±13.63	70.14±4.11	58.26±27.49	11
106			46.97			46.97	18
107R		88.57	71.33±2.28		100.63±10.88	86.85±14.73	1
110			78.82			78.82	2
111	45.11±1.34	23.98±1.98	48.93±5.13	89.29±10.90	79.01±0.41	57.26±26.57	13
112	60.71±3.37	35.64±2.96	64.10±4.30		89.67±5.55	62.53±22.10	9
114	69.26±2.28	38.23±8.10	51.39±1.07	93.10±19.69	84.78±9.08	67.35±22.77	6
115	42.85±0.23	31.18±1.42	44.72±0.33	100.61±11.17	69.88±2.76	57.85±27.76	12
116	49.42±1.42	41.98±6.30	59.01±0.96	98.09±5.04	70.15±7.96	63.73±21.92	8
117	61.12±2.28	34.28±5.85	65.60±2.62		98.92±6.15	64.98±26.52	7
118	56.53	45.56±8.07	60.03±3.65	131.25±6.96	98.16±3.70	78.31±35.63	3
120	47.94	43.34±9.11	42.61±3.08	70.84±11.62	68.11±4.84	54.57±13.79	14
122							
Average*	51.37±10.41	41.78±16.64	57.33±9.96	99.26±17.31	83.22±12.61	63.91±10.31	
CONTROL4		26.51±5.70	35.08±8.94	82.84±6.59	55.76±9.18	50.05±25.07	16
15/1	34.05		44.71±2.56		65.29±1.00	48.02±15.88	17
97-3/2							
97-5/2	39.65		37.95±4.01		54.80±0.32	44.13±9.28	19

*Average of selected lines, i.e those above this row.

Table 7.10. Final counts per family for F4 families (counts across bags). Data from Scott Parkinson, ASI.

F4 family	Pittwater	Coles Bay	Smithton	Coffin Bay	Smoky Bay	
Sample date	3/3/04	4/2/04	22/2/04	29/3/04	1/4/04	Total
Age (mths)	25	24	25	26	26	count
100	0	0	246	0	75	321
101	0	0	90	0	0	90
102	289	253	232	245	276	1295
103	0	0	89	0	0	89
104	82	138	167	204	250	841
106	0	0	66	0	0	66
107R	0	35	232	0	221	488
110	0	0	85	0	0	85
111	282	256	250	256	293	1337
112	290	280	255	167	247	1239
114	285	257	243	287	273	1345
115	180	262	161	248	288	1139
116	196	271	255	243	181	1146
117	288	301*	155	0	229	973
118	295	278	239	274	275	1361
120	202	176	210	198	264	1050
122	0	0	0	0	0	0
Control4	0	220	219	145	219	803
15/1	84	0	242	0	290	616
97-3/2	0	0	0	0	0	0
97-5/2	85	0	205	0	283	573

*Note that this total is larger than the number of spat originally thought to have been allocated to this family (c. 235, Table 7.6). The discrepancy is in the numbers in replicate 3, but its source is unclear.

Table 7.11. Individual measurements of F4 and inbred oysters from Smithton on August 7, 2003

F4 family	Weight Mean±sd	Condition index Mean±sd	Number measured
100	38.48±7.51	79.50±17.70	39
101	37.74±6.88	75.95±7.39	20
102	33.38±6.30	71.00±6.08	39
103	37.13±6.29	77.15±13.83	39
104	26.93±5.90	79.35±17.63	37
106	29.87±6.89	101.83±21.32	19
107R	44.23±8.83	68.44±7.74	39
110	44.13±10.24	79.17±10.18	30
111	23.71±5.89	81.64±9.52	39
112	35.97±7.88	69.14±6.16	40
114	29.78±5.18	103.48±30.77	40
115	25.25±5.10	79.09±7.24	39
116	38.84±8.22	72.02±8.16	38
117	36.60±8.44	72.50±6.72	39
118	35.94±8.04	70.75±5.96	38
120	21.94±6.09	77.52±6.80	40
CONTROL4	16.95±5.59	119.82±50.71	39
15/1	24.16±5.17	81.13±6.96	40
97-5/2	22.54±5.3	103.96±44.64	39
Mean	32.56±7.27	80.31±11.29	693

Table 7.12. Summary of shape indices of individual F4, M4 and inbred oysters from Smithton of August 7 2003. 19 months of age.

		SI-ASI		SI-Galtsoff		EI	
F4/M4 line	N	Mean±sd	Range	Mean±sd	Range	Mean±sd	Range
100	39	7.88±3.83	1.61-18.61	2.07±0.25	1.64-2.53	1.05±0.14	0.77-1.34
101	40	8.60±9.34	0.10-61.04	2.17±0.22	1.71-2.60	1.08±0.13	0.72-1.36
101*	39	7.26±3.92	0.10-18.58	2.18±0.21	1.76-2.60	1.09±0.12	0.88-1.36
102	39	9.21±2.98	3.20-14.55	2.27±0.18	1.89-2.80	1.19±0.10	0.95-1.47
103	40	7.48±5.17	1.31-21.82	1.88±0.19	1.47-2.33	0.93±0.11	0.71-1.17
104	38	6.58±3.95	1.28-21.09	2.00±0.19	1.46-2.39	1.00±0.10	0.75-1.25
106	19	10.91±5.96	1.82-23.68	2.13±0.19	1.67-2.49	1.03±0.10	0.80-1.19
107R	39	8.90±2.86	3.86-15.26	2.22±0.18	1.75-2.72	1.16±0.11	0.89-1.46
110	30	7.65±4.64	2.18-24.66	1.88±0.13	1.54-2.22	0.93±0.08	0.74-1.11
111	39	10.41±3.53	1.18-21.69	2.43±0.29	1.96-3.62	1.26±0.17	0.94-1.90
112	40	10.27±3.34	4.79-20.04	2.34±0.23	1.90-3.08	1.23±0.13	0.99-1.64
114	40	10.93±16.45	1.31-107.14	2.19±0.28	1.22-2.71	1.09±0.14	0.65-1.35
114*	39	8.46±5.29	1.31-33.57	2.17±0.28	1.22-2.64	1.09±0.14	0.65-1.35
115	39	5.76±3.46	0.82-16.51	1.97±0.17	1.60-2.35	1.00±0.09	0.83-1.20
116	38	8.58±3.30	2.11-15.50	2.31±0.21	1.88-2.78	1.18±0.11	1.00-1.45
117	39	7.73±3.82	1.46-18.58	2.22±0.22	1.78-2.73	1.12±0.12	0.93-1.40
118	38	6.51±3.27	0.60-16.07	2.11±0.19	1.78-2.59	1.08±0.10	0.87-1.32
120	40	8.58±4.28	0.86-18.75	2.24±0.23	1.82-2.92	1.11±0.13	0.88-1.50
122 CONTROL4	38	10.95±6.33	1.58-29.63	1.89±0.18	1.59-2.27	0.92±0.10	0.76-1.11
15/1	39	7.83±4.05	1.29-19.11	1.89±0.18	1.54-2.28	0.97±0.10	0.79-1.18
97-3/2 97-5/2	39	7.69±5.01	2.50-31.13	2.23±0.20	1.79-2.96	1.14±0.10	0.98-1.53
M41ab	40	8.68±10.57	0.37-70.91	2.10±0.20	1.73-2.69	1.06±0.09	0.87-1.24
M41ab*	39	7.09±3.19	0.37-14.44	2.08±0.18	1.73-2.49	1.06±0.09	0.87-1.24
M42b	40	8.05±3.33	2.09-15.11	2.15±0.22	1.71-2.63	1.10±0.11	0.89-1.42

*after removing one oyster with an outlying SI-ASI score

Table 7.13. Summary of shape indices of individual family 118 and CONTROL4 oysters from Smithton at 2 years of age. Mean weights of 118 and CONTROL4 are 64.95±9.48 and 42.55±11.53 g respectively. Data from Scott Parkinson (ASI newsletter insert, volume 1, issue 2 2004)

		SI-ASI		SI-Galtsoff		EI	
F4/M4 line	N	Mean±sd	Range	Mean±sd	Range	Mean±sd	Range
118	20	8.23±3.40	0.00-13.60	2.20±0.23	1.78-2.71	1.14±0.13	0.91-1.45
CONTROL4	20	19.06±17.32	3.26-74.60	2.23±0.19	1.85-2.53	1.07±0.13	0.86-1.37
CONTROL4*	19	16.14±11.67	3.26-47.22	2.23±0.20	1.85-2.53	1.07±0.13	0.86-1.37

*after removing one oyster with an outlying SI-ASI score

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Table 7.14. Means and coefficients of variance for total weight, condition index and ASI shape index for the 4th generation families at 19 months of age. Individual measurements of oysters from Smithton on August 7, 2003.

F4 family	Total weight		Condition index		ASI Shape index	
	mean	CV	mean	CV	mean	CV
100	38.48	19.51	79.50	22.26	7.88	48.60
101	37.74	18.23	75.95	9.73	8.60	108.60
102	33.38	18.87	71.00	8.56	9.21	32.36
103	37.13	16.94	79.02	9.15	7.48	69.12
104	26.93	21.91	79.35	22.22	6.58	60.03
106	29.87	23.07	101.83	20.94	10.91	54.63
107	44.23	19.96	68.44	11.31	8.90	32.13
110	44.13	23.20	79.17	12.86	7.65	60.65
111	23.71	24.84	81.64	11.66	10.41	33.91
112	35.97	21.91	69.14	8.91	10.27	32.52
114	29.28	17.69	103.48	29.74	10.93	150.50
115	25.25	20.20	79.09	9.15	5.76	60.07
116	38.84	21.16	72.02	11.33	8.58	38.46
117	36.60	23.06	72.50	9.27	7.73	49.42
118	35.94	22.37	70.75	8.42	6.51	50.23
120	21.94	27.76	77.52	8.77	8.58	49.88
M41ab	33.45	16.14	75.91	8.52	8.68	121.77
M42b	33.46	21.31	82.81	19.60	8.05	41.37
15/1	24.16	21.40	81.13	8.58	7.83	51.72
97-5/2	22.54	22.76	103.96	42.94	7.69	65.15
Mean*	32.65	21.12	80.21	14.70	8.41	60.56
Control	16.95	32.98	119.82	42.32	10.95	57.81

*Mean of the lines above

Table 7.15. Means and coefficients of variance for total weight, condition index and ASI shape index for the F4 control line and F4 family 118 at 2 years of age. Individual measurements of oysters from Smithton by Scott Parkinson (ASI)..

F4 family	Total weight		Condition index*		ASI Shape index	
	mean	CV	mean	CV	mean	CV
118	64.95	14.60	18.45	13.71	8.23	41.31
Control	42.55	27.09	16.59	14.35	19.06	90.87

*assessed as % wet meat to total weight

Figure 7.1. Average weight of individuals at end of nursery phase plotted against numbers of individuals produced. The three inbred lines, single control line and three mass selection lines (M4) are identified: all other families are F4 family lines.

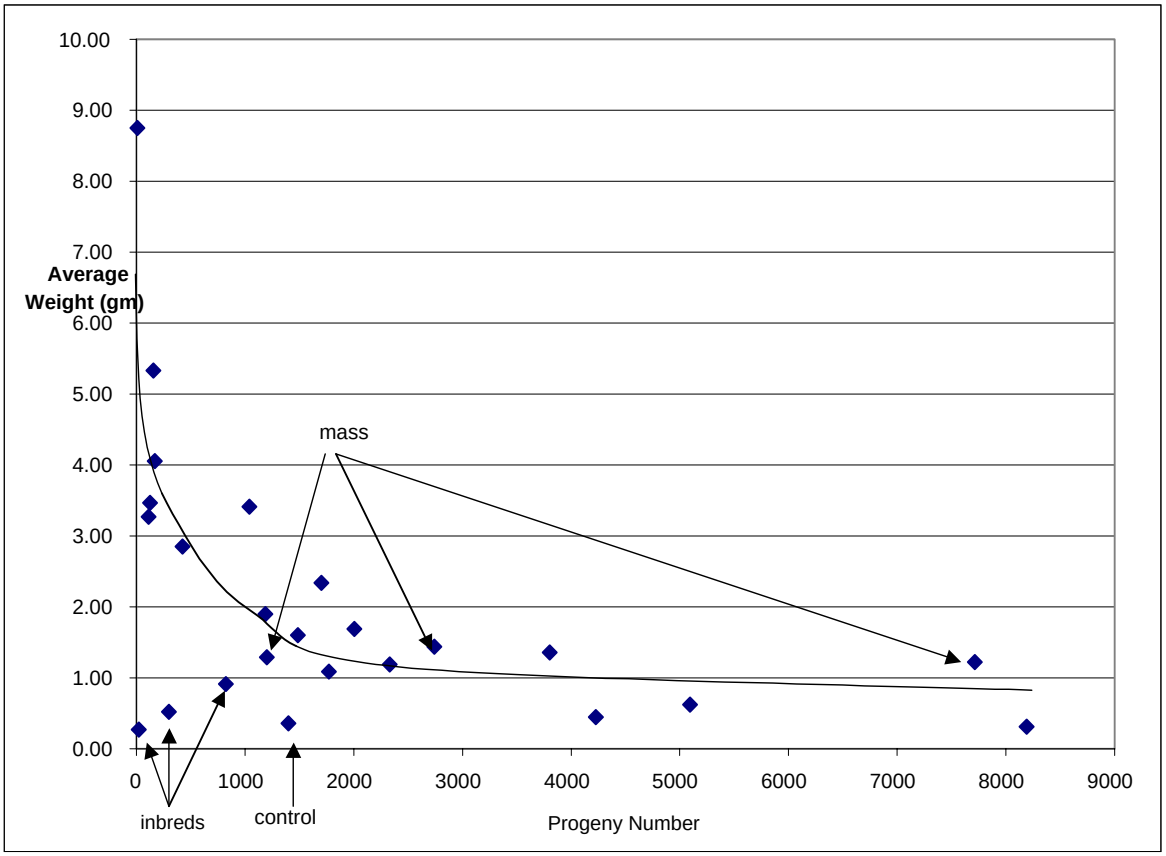


Figure 7.2. Correlations among pairwise combinations of farms for all F4 and M4 families and at their final weight checks. Axes are mean weights per family. Correlations of family weights also shown. Tas1.sub = Coles Bay (4/2/04), Tas2.int = Smithton (22/2/04), Tas3.int = Pittwater (3/3/04), SA1.int = Coffin Bay (29/3/04), SA2.int = Smoky Bay (1/4/04).

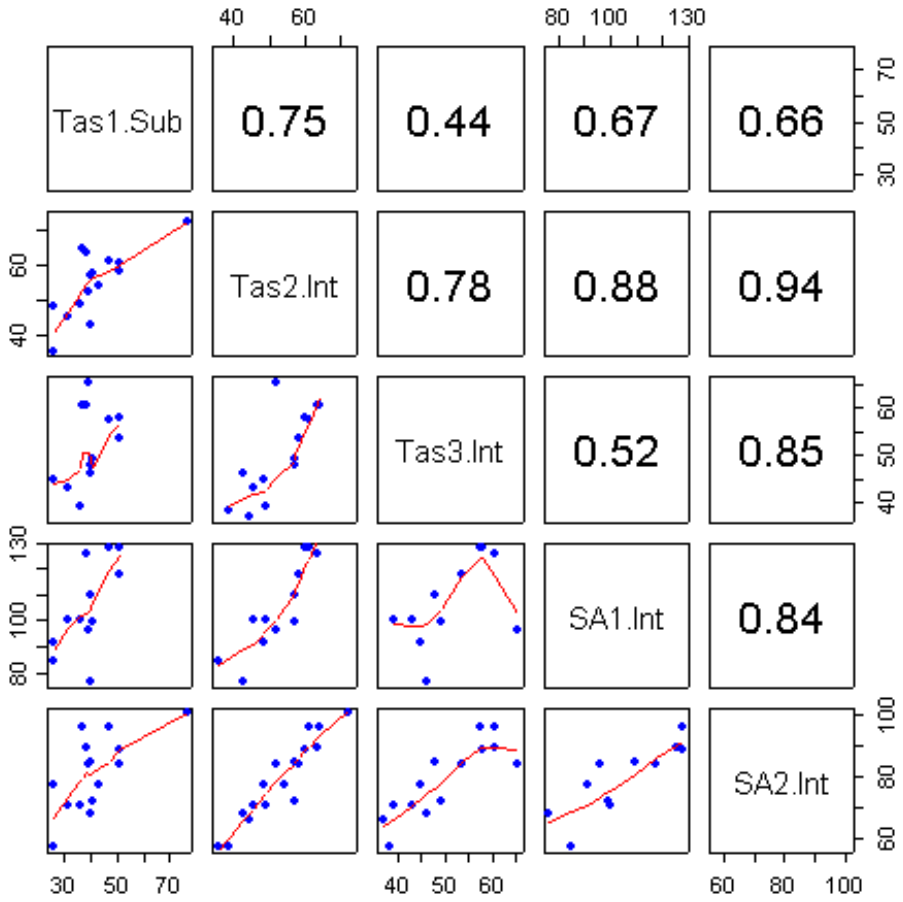


Figure 7.3. Performance of all F4 lines at Smithton at final weight checks (age 25 months). Lines 1-16 are the family selection lines, line 18 is the Control line, lines 20-22 are the mass selection lines, and lines 24 & 25 are the inbred lines.

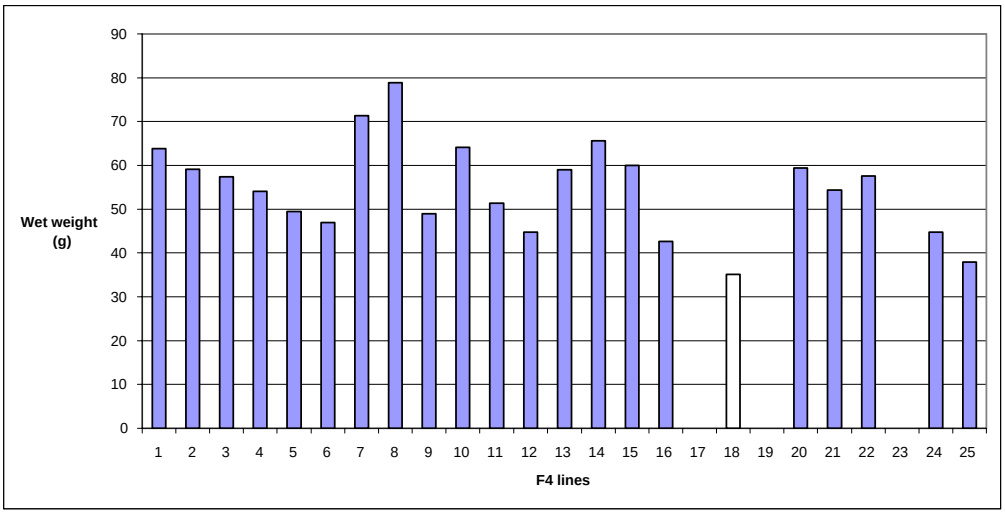


Fig 7.4. ASI Shape Index plotted against weight of individual oysters. The three outliers referred to in the text can be clearly seen.

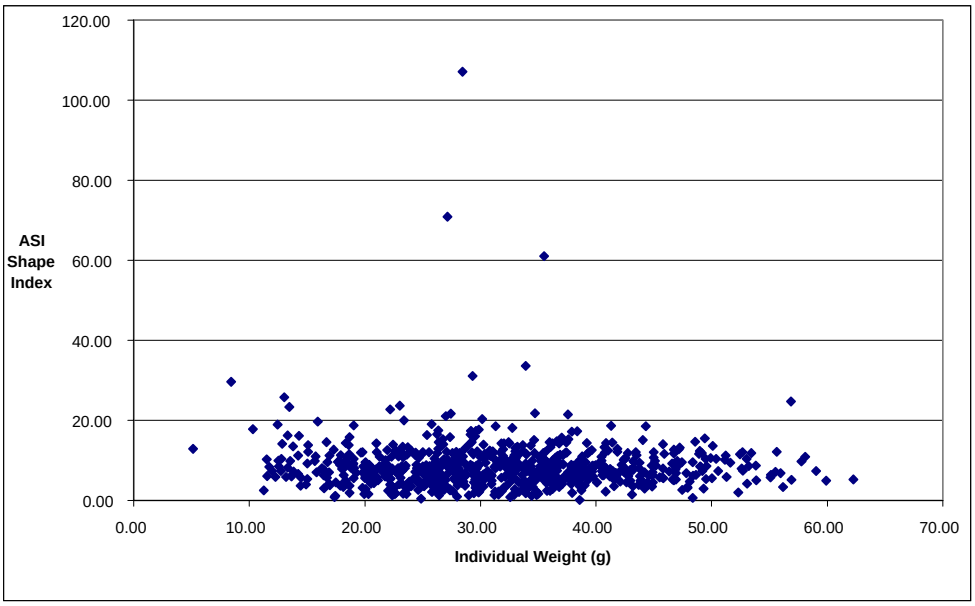
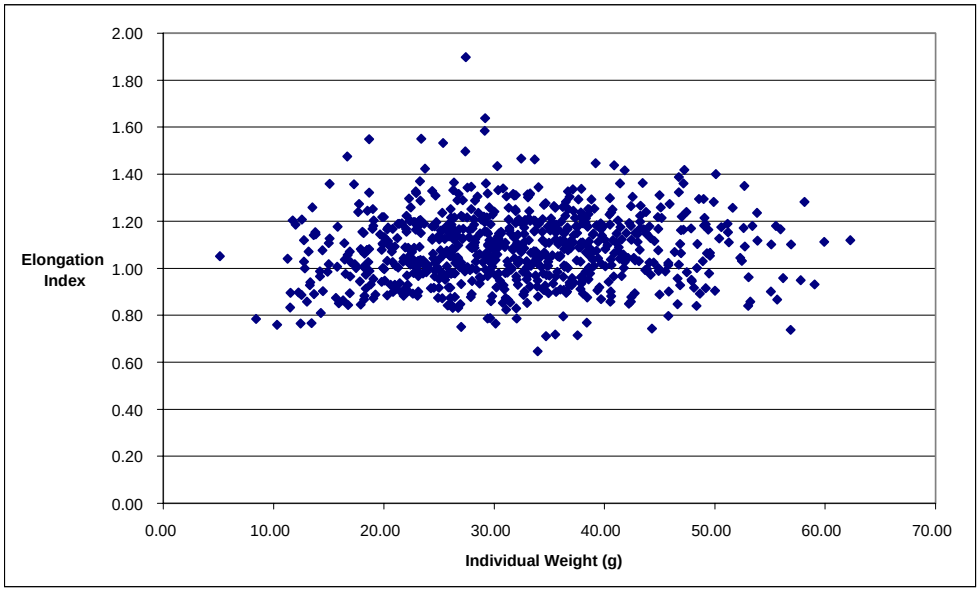


Fig 7.5. Elongation Index plotted against weight of individual oysters



CHAPTER 8. Seventh Spawning Year 2002/03 – The Mass Selection M5 Lines plus the Family Selection F5 Lines

8.1. The Mass Selection M5 Lines

Three M5 mass-selection lines were made at Shellfish Culture, on January 5, 2003. These represented continuations of the earlier mass-selection lines, together with one mass-selection control line. The 200 largest animals had been set aside (rather than the 100 for the M4 lines) as potential broodstock from each of the three M4 lines at the end of their nursery period.

The M5 lines were made from the following animals:

M5A: 1 mass cross with 14 females and 45 males from M4-1a/b,
M5B: 1 mass cross with 16 females and 34 males from M4-2a,
M5C: 1 mass cross with 19 females and 32 males from M4-1a/b,
M5control: 1 mass cross with 11 females and 22 males (unselected for size) from the M3 control line. Note that there was no M4control line as a year ago the M3 control line was then too small to breed from.

An overall control line for the mass (and family) selection lines, termed CONTROL5, was taken from a commercial line, PE10, spawned by Shellfish Culture on January 8, 2003.

8.2. The Family Selection F5 Lines

8.2.1. Selecting the Parents for the F5 Lines

Selection procedures for the 5th generation of family lines were discussed at several meetings including a meeting of the Project Team (December 20, 2002). We now had several generations of animals available, and considerable quantitative data. We wanted to use this data in our selection of breeding animals, and in this generation chose to do this in an optimised approach taking into account breeding values and inbreeding levels.

In a breeding program, inbreeding increases as related animals are chosen as parents. The rate of increase in inbreeding depends on factors such as population size, heritability of the traits under selection, and the method used to estimate the genetic merit of selection candidates (methods which use pedigree information lead to higher rates of inbreeding because related animals are more likely to be selected). We have, as yet, little data on the issue of heritabilities of selection traits. Nonetheless, computer algorithms have been developed which seek to optimise selection response and inbreeding and which include performance data. We chose to use the algorithm developed by Meuwissen (1997). This method maximises selection response for a given rate of change in inbreeding between generations, and we chose an average rate of inbreeding of not more than 1% per generation for the F5 matings. This rate is low enough to be sustainable for the foreseeable future (see Appendix 4).

The main inputs required by the method are the population pedigree (to allow calculation of inbreeding coefficients) and estimated breeding values (EBV) for animals which are selection candidates. Because the selection candidates in this case came from several different generations, EBV were derived using "BLUP" (best linear unbiased prediction) methodology, which is in widespread use in many livestock industries, such as dairy, beef, and pigs. This is a statistical model which through the use of pedigree information allows comparison of animals across generations, even though their performance data is recorded at different times. A BLUP model was applied to oyster growth rate generating EBV for the F1 - F4 generations. This was not the standard application of this model because the data used were family means rather than individual animal records. However, it is a reasonable approximation at this stage. The EBVs were then used in the Meuwissen optimisation procedure, which outputs a list of families which are to be used as parents in the F5 generation, and the number of matings in which each family should be used. This list was then used to form a list of mating pairs with minimised average inbreeding. The optimised mating list was used as the basis for the F5 matings.

The inbreeding coefficients and EBVs for the chosen matings are given in Tables 1 and 2. Note that the EBVs are the mean of the EBVs of the two contributing families. Therefore, in those few families where a parent is inbred, and because inbred families typically perform very poorly, EBVs are very low or negative. Examples here are families 171 (987/2 x 118), and 183 (987/2 x 15) where family 987/2 is inbred. In such situations, EBVs are not expected to be a good indicator of expected performance: outbreeding or heterotic effects are not fitted by the BLUP model described above.

The first set of crosses (Table 8.1), spawned January 4 & 5, included 25 family crosses, selected as above with the exception of family 171 (an inbred x family mating), together with the mass-selection and mass selection control lines.

An overall control line for the family (and mass) selection lines, termed CONTROL5, was taken from a commercial line, PE10, spawned by Shellfish Culture on January 8, 2003.

The second set of crosses (also in Table 8.1), spawned February 1 & 2, produced 28 additional families. These consisted of further inbred lines, an outbred cross between two inbred lines, further F5 families chosen using the Meuwissen algorithm, and new families introducing new genetic diversity into the breeding program.

Family 97-2/2 (which used to be known as family 16) was full-sib inbred for a further generation to make family 97-2/3. This inbreeding failed twice last year, but this time all three attempts produced viable larvae; the one with fewest mortalities was continued. Family 14 was inbred for the first time to make family 14/1: last year's attempt to do this failed as all set-aside broodstock had been male. Family 21 was inbred for the first time to generate family 21/1.

An outbred cross between two inbred families, 97-2/2 and 15/1 (made last year), was planned, but none of the set-aside individuals of 15/1 was mature. Instead, a cross

(#183) was made of 97-2/2 and 15. This cross was made twice, with the better being retained.

Eighteen additional F5 families were made, most using the Meuwissen algorithm to select parents.

Six 'new' families were made. These crosses were carried out to introduce some new genetic diversity into the program, in order to assist its long-term sustainability. In each case, one parent came from outside the breeding program and the other from a good-performing F4 or F5 family. Camerons of Tasmania kindly donated some animals that they considered had good shapes and growth rates, and Tasman Isle Seafoods (one of our grow-out farms) also kindly donated some 'pets' – oysters that had good shapes and had been set-aside at Pittwater for several years. These 'new' oysters were allocated an EBV of zero.

In addition, on February 5, some 'selfed' crosses were attempted. Sperm had earlier (February 2002) been extracted from 128 male oysters from a total of 31 F2 families and stored cryogenically in liquid nitrogen. Some males switch to females in subsequent seasons, and the intention was to use this sperm to self-fertilise eggs produced by males that had switched to females. This would generate an inbreeding coefficient of 0.5, equivalent to three generations of full-sib crossing. The frozen sperm, in cryovials (with cryopreservant), was taken to Shellfish Culture in a dewar flask. Approximately 50 sperm-contributing oysters were opened, twelve of which had become females. Four of these had already spawned and contained remnants of eggs and re-absorbed eggs. The remaining eight were strip spawned and eggs mixed with their sperm. One cross, from a family 12 individual, showed signs of fertilisation after two hours. This was placed in one larval tank and designated family s12 ('s' for selfed). The other seven families appeared frozen at the stage of polar body release. Since there was at that time only one spare larval tank, these remaining fertilised eggs from the seven families were carefully washed, to eliminate any remaining sperm and prevent cross-fertilisation, and combined into that one tank. We plan to biopsy and microsatellite genotype any oysters that might develop to determine whether in fact they are true selfed progeny.

8.2.2. Performance of F5 Families

F5 went to TAFI for initial raising. They were transferred to Smithton on 14 April 2003 and then by ASI to grow-out farms on November 21, 2003.

The breeding component of the FRDC project terminated November 2003 – only the selection index work was extended (to the beginning of 2005) – so that only the initial weights taken at the end of the nursery period (18 November 2003) at Bolduans Bay are given here (Table 8.2). These were taken by Scott Parkinson of ASI.

A strong relationship between average juvenile weight and numbers of progeny per family (estimated from the number weighed) is again evident (Figure 8.1). Families with more progeny tend to have lower average weights. The correlation is –0.600 ($P < 0.01$) across all the 48 F5, M5 and inbred families and –0.643 ($P < 0.01$) when the three inbred families are excluded. The inbred families and the two CONTROL lines

fall below the plotted curve, indicating that these lines perform less well (for their progeny numbers) than the great majority of other lines.

After allowing for this density effect, seven families can be identified as high-performers. These all lie substantially above the plotted line in Fig 8.1 and are: 150, 155, 163, 166, 167, 170 and 176 (note that families 150 and 170 are at the same point in Fig 8.1). These are also the seven top ranking families in families of more than 500 progeny (Table 8.2). The average weight of these families is nearly six times that of Control5.

The correlation between EBV and juvenile weight of the 40 non-inbred F5 lines at this time was non-significant ($r = -0.061$). This is not surprising given the strong density-dependent relationship just described. The seven top-ranking families had a mean EBV of 3.09, a little higher (but not significantly so, $P = 0.082$) than the mean of the remaining 33 non-inbred lines, which was 2.33. Therefore at this early stage of growth, the EBV does not appear to be a good predictor of growth rate.

Table 8.1. F5 and M5 crosses made on January 4 & 5, 2003. No cross indicates that family number was not used on that date (but was used subsequently). EBV is estimated breeding value, estimated from the mean of the contributing parent families.
Parentage removed from this public access version of the Report, for commercial-in-confidence reasons.

Date made	Family Number	Parent Female	Parent Male	Parent Generation Female	Parent Generation Male	Inbreeding coefficient	Family EBV	Estimated numbers 27/02/03
4/1/03	150			F3	F4	0	2.86	2500
	151			F4	F4	0.035	1.92	3000
	152			F3	F4	0.020	2.91	3000±
	153			F3	F3	0.039	2.98	2000
	154			F3	F3	0	4.12	200
	155			F3	F4	0	1.59	400
	156	no cross						
	157			F3	F3	0	3.96	3000±
	158	no cross						
	159	no cross						
	160	no cross						
	161			F4	F4	0.043	2.19	3000
	162			F4	F4	0.008	3.33	1500
	163			F3	F4	0.020	3.43	3000±
	164	no cross						
	165	no cross						
	166			F3	F4	0.008	3.73	3000±
	167			F3	F3	0	3.57	400
	168			F3	F3	0	3.76	30
	169	no cross						
	170			F3	F4	0	3.56	500
	171			F2	F4	0	-5.34	1500
5/1/03	172			F3	F4	0	2.68	3000±
	173							
	174			F3	F4	0	2.85	2000
	175			F3	F3	0.008	3.28	1000
	176			F3	F3	0.008	2.88	2500
	177			F3	F3	0	3.07	30
	178			F3	F3	0.016	1.81	2000
	179			F3	F4	0	0.32	3000
	180			F3	F4	0.020	3.43	30
	181			F3	F4	0.016	3.20	3000±
	182			F3	F4	0.008	2.47	50
	M5A	M41a/b	M41a/b	M4	M4	-	-	2000
	M5B	M42a	M42a	M4	M4	-	-	3000
	M5C	M42b	M42b	M4	M4	-	-	20
	M5control	M3control	M3control	M3	M3	-	-	3000±
8/1/03	Control5	Cross of Shellfish Culture				-	-	3000±
1/2/03	97-2/3			F2	F2	0.500	-14.29	3000
	14/1			F2	F2	0.250	3.30	500
	21/1			F2	F2	0.250	4.82	2500
	183			F2	F2	0	-4.85	2500
	156			F3	F4	0.039	2.66	3000±
	159			F3	F4	0	2.85	3000
	160			F3	F4	0	3.55	2500

Date made	Family Number	Parent Female	Parent Male	Parent Generation Female	Parent Generation Male	Inbreeding coefficient	Family EBV	Estimated numbers 27/02/03
	164			F4	F4	0.008	1.97	1500
	184			F3	F4	0.016	4.41	0
	185			F4	F3	0	2.12	3000±
	186			F4	F3	0.016	3.16	1000
	189			F3	F4	0	2.50	3000±
	192			F3	F4	0.008	2.10	
2/2/03	158			New	F3	0	2.64	3000±
	165			New	F3	0	3.85	2500
	169			New	F3	0	1.77	3000
	173			New	F3	0	2.47	3000
	187			F4	F3	0.125	4.72	500
	188			F3	F4	0.031	1.99	3000±
	190			F2	F2	0.031	3.62	3000
	191			F3	F4	0	1.12	500
	193			F3	F4	0.012	2.67	3000
	194			F4	F4	0	-0.54	3000
	195			F2	F4	0	0.85	3000±
	196			F2	F4	0.008	0.33	1000
	197			F4	F2	0	0.59	25
	198			F3	New	0	1.48	5
	199			New	F4	0	2.08	1500
5/2/03	Selfs	selfed crosses (see text)						6

Table 8.2. Weights of 36 lines of F5 and M5 oysters at the end of their nursery phase at Bolduan's Bay, 18 November 2003 (data supplied by Scott Parkinson, ASI). Animals weighed in lots of 100 (unless otherwise stated) and weights given are average weights per animal in gm. Also given are numbers of bags (of 100 oysters each unless otherwise specified) distributed to each farm. Coles Bay not used as a grow-out farm

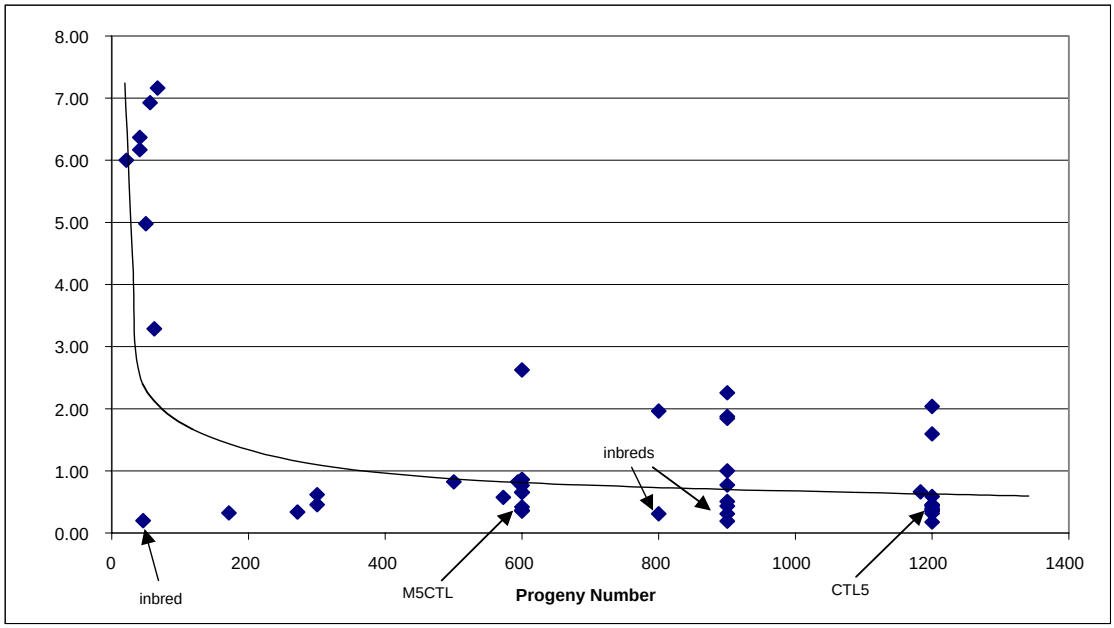
F5 and M5	Average animal weight (g)*	Overall ranking (n=48)	Ranking of 'good' families** (n=34)	Numbers of bags distributed			
				Pittwater	Smithton	Coffin Bay	Smoky Bay
150	1.84±0.32	12	6	3	3	-	3
151	lost						
152	0.86±0.04	17	10	-	3	-	3
153	0.42±0.02	35	25	-	3	-	3
154	4.98	6		-	(50)	-	-
155	2.63±0.47	9	1	-	3	-	3
156	0.32±0.05	45	32	3	3	3	3
157	0.45±0.01	30	22	3	3	3	3
158	0.19±0.01	46	33	3	3	-	3
159	lost						
160	0.65±0.05	23	17	-	3	-	3
161	0.62	26		-	3	-	-
162	0.83±0.05	18	11	-	3	-	2 (+94)
163	1.60±0.27	11	7	3	3	3	3
164	0.57±0.06	27	19	-	3	-	2 (+73)
165	lost						
166	2.03±0.14	10	3	3	3	3	3
167	2.26±0.38	13	2	3	3	-	3
168	6.93	2		-	(56)	-	-
169	0.51±0.03	28	20	3	3	-	3
170	1.88±0.029	14	5	3	3	-	3
171	1.00±0.23	15	8	3	3	-	3
172	0.44±0.03	36	23	3	3	3	3
173	0.46±0.04	29	21	3	3	3	3
174	0.77±0.04	20	13	3	3	-	3
175	0.86±0.33	16	9	-	3	-	3
176	1.96±0.64	8	4	2	3	-	3
177	6.37	3		-	(41)	-	-
178	lost						
179	0.35±0.05	43	31	-	3	-	3
180	lost						
181	6.00	5		-	(21)	-	-
182	6.17	4		-	(41)	-	-
183	lost						
184	no progeny						
185	0.43±0.03	30	24	3	3	-	3
186	0.77±0.06	21	14	-	3	-	3
187	0.82±0.04	19	12	-	3	-	2
188	0.38±0.06	33	27	3	3	3	3
189	0.59±0.06	25	18	3	3	3	3

F5 and M5	Average animal weight (g)*	Overall ranking (n=48)	Ranking of 'good' families** (n=34)	Numbers of bags distributed			
				Pittwater	Smithton	Coffin Bay	Smoky Bay
190	0.18±0.04	48	34	3	3	3	3
191	0.67±0.01	24	15	3	3	2 (+83)	3
192	0.32	42		-	1 (+71)	-	-
193	0.34	41		-	2 (+72)	-	-
194	0.40±0.02	37	26	3	3	3	3
195	lost						
196	lost						
197	3.29	7		-	(62)	-	-
198	lost						
199	0.36±0.07	34	29	3	3	3	3
CTL	0.35±0.04	38	30	3	3	3	3
M5CTL	0.37±0.01	39	28	-	3	-	3
M5A	0.45	32		-	3	-	-
M5B	0.66±0.05	22	16	-	3	-	3
M5C	7.16	1		-	(67)	-	-
97-2/3	0.31±0.04	44		3	3	-	2
14/1	0.20	47		-	(46)	-	-
21/1	0.31±0.05	40		3	3	-	3
selfs	Not weighed			-	(7)	-	-

*Mean and SD of the average weights per farm

** "Good" families exclude inbreds and families where total number of progeny are less than 500

Fig 8.1. Relationship between average juvenile weight (g) and estimated numbers of progeny per 5th generation family



CHAPTER 9. The Commercial Trial and Commercial Production Runs

9.1. Commercial Trials

The selective breeding component of this project was assessing growth and performance on just five farm sites, with a maximum of 300 oysters per family being assessed at any one site. The sites had been chosen to provide a variety of habitats but clearly good performance on these sites does not necessarily extrapolate to good performance on all farms. Furthermore, the project needed only small numbers of juveniles as only small numbers of oysters were being tested at each site, and procedures being used from spawning to final grow-out assessment differed somewhat from normal commercial practise. It was therefore decided that some larger-scale trials of some of the lines should be carried out. These were termed commercial trials and involved production of specified families by hatcheries using standard commercial practise and release of offspring to farms at normal commercial rates. No supplement or royalty was to be charged for these offspring. These oysters were to be grown alongside non-selected, or control, stock. Data were to be collected to assess how well the selected lines performed compared with the control.

9.2. First Commercial Trial

Shellfish Culture Ltd contacted the project team in late 1999 requesting that Shellfish Culture undertake a full-scale commercial trial of genetically improved oysters. The project team and the TORC Board agreed to this request. The project team proposed the production of two batches:

1. Putative Rapid Growth Line (RGL). We recommended use of the four fastest-growing F1 families (as off June 1999) as parents, i.e. WA925, RB003, WA930 and RB015 (Table 3.6). Males from family RB015 and RB0003 were used to fertilize eggs from females from WA925 and WA930, and conversely females from RB015 and RB003 were crossed with males from family WA925 and WA930. In this way we avoid crossing males and females within the same family and generating inbred product. The resulting two sets were combined post fertilization and stocked to a commercial tank.

2. Non-Curl Back Line (NCL). In the second batch we recommended use of the two inbred families (Table 3.1): WA987 (burgundy) and WA981 (wild type colour morph, selected from the non-gold animals in this line). Curl-back is an undesirable morphological shell trait, and these families had been curl-back free. To avoid further inbreeding, and to promote heterosis, males and females of the two families were separated, and males from one family were crossed with females of the second family as before.

The two batches were spawned on February 22, 2000. At the same time the hatchery spawned a commercial control line, B7. Approximately 2.5 million RGL and 3.8 million NCB spat were produced. From spat distributions in grading trays in October 2000, the estimated average size of RGL was, at 2.42 mm, about 3.5% larger than that

of NCL. However, the hatchery manager preferred the shape of the NCL line. By April 2001 about 1.3 million RGL and 1.4 million NCB had been sold by Shellfish Culture.

Some early data grow-out data were available from one South Australian farm (Table 9.1). At a grading in July 2001, the RGL and NCL oysters were on average similar in size to one another and about 3.6% larger than line 1 oysters (which we assume to be the control line B7 produced by Shellfish Culture). The farmer told us in November 2001 that he considered the performance of the RGL and NCL lines to be good.

It should be noted that RGL included the same parental families as our F2 family 46, spawned at about the same time as the Shellfish Culture line. F2 family 46 performed poorly in our tests, ranking 41st of 44 families in June 2001, and 38th in October/November 2001 (Table 5.10). This particular combination of fast growing F1 families did not yield a fast-growing product. NCL included the same parental families as our F2 family 28. Family 28 performed somewhat better in our tests, ranking 7th in June 2001 although slipping to 25th in October/November 2001. Clearly, both of these families were substantially out-performed by many other F2 families in our tests. However, we did not of course have this information at the time of recommending these trials to Shellfish Culture.

Subsequent data on the progress of these trials proved hard to come by. Comments from South Australia growers that received these lines were summarised in the SAORC Newsletter of May 2002. Although we have not seen the relevant data, and the comments are somewhat anecdotal and inconsistent, they are interesting:

Cowell grower: RGL "a lot quicker with normal shape". NCL and Control "even in growth", NCL has a "really good shape".
Streaky Bay grower: Control line "way in front". Disappointed with RGL and NCL, which are "similar in growth rates and both have long tails".
Denial Bay grower: Control "slightly ahead" of NCL, which are "slightly ahead" of RGL, "but not a lot of difference".
Smoky Bay grower: RGL and NCL "similar in growth, both ahead of the control". RGL "appear to have the best shape", with NCL with "more frill than the other lines".

Bolduans Bay Oysters received NCL and RGL spat on 31/1/01. All were successfully on-sold to other farms by the end of June 2002. Both lines were sold on average 15.3 months after arrival. The average NCL size at point of sale was c. 51.6 mm (n = 211,309) and average RGL size was 50.5 mm (n = 173,677). No information on the Control line was made available.

9.3. Second Set of Trials

The Joint Venture Company Australian Seafood Industries (ASI, see Chapter 12) did not become operational until April 2001 and this meant that no commercial trials were initiated over the summer 2000/2001 period. Protocols agreed by ASI and the research providers regarding the establishment by the FRDC Project of commercial trials were circulated to hatcheries on July 19 & 20, 2001. The establishment of these protocols was made more difficult because clear guidelines for collaboration between ASI and the research providers had not been finally negotiated.

The three hatcheries in Tasmania and the one hatchery in South Australia all expressed an interest in carrying out commercial trials of improved broodstock in 2001. These are Shellfish Culture Ltd of Bicheno (Tasmania), Geordy River Aquaculture of St Helens (Tasmania), Camerons of Tasmania, and the South Australian Oyster Hatchery.

Geordy River was sent five animals of each of F1 families WA234, RB012, RB042 and RB044. These were sent in April 2001. We heard no more from the hatchery but we understand that an attempt to naturally spawn these animals failed.

South Australia Oyster Hatchery was sent five animals of each of F1 families WA992, WA925, YA018 and RB015 in April 2001. These were strip spawned shortly afterwards in Mat Willis's presence but all were males except for one hermaphrodite – no larvae were produced.

Cameron of Tasmania was sent 100 of each of F2 families 10, 13, 28 and 31 in September 2001. Families 10, 13 and 28 were rejected for use and returned; only family 31 was kept. These apparently grew rapidly under conditioning, but were not used. These F2 animals were probably at least one year younger than normal broodstock used by Cameron.

Shellfish Culture was sent 19 animals of F1 family WA987, 20 of F1 family WA981 and 10 of F1 family RB015. These were used to produce two commercial trial lines, by natural spawning. NCL was reproduced again (WA987 x WA981, spawned 12 October 2001), although now termed 28 (after our F2 notation). A different RGL line was produced (WA987 x RB015, spawned 24 January 2002), termed 43 (equivalent to our F2 family 43, 4th of the 44 F2 families, see Table 5.10). Eight million #28 and 21 million #43 spat were recovered. The hatchery manager commented very favourably on the shape and uniformity of growth of #28.

It was a condition of sale of these genetically improved spat to farms, as part of the commercial trial process, that a specific protocol be followed by the farm. This was designed to enable the project to obtain better information on performance than was possible with the 1999/2000 commercial trial. The protocol (see Appendix 5) was supplied to each receiving farm. Essentially, any improved line was to be distributed to growers together with a control (unimproved) line spawned at about the same time, and the grower was to put aside 50 oysters from each line and maintain these ungraded and unmixed with other oysters for measurement by the project.

Some spat were subsequently lost through mortality through grading, and large numbers of the late-spawned RGL stock were additionally lost through over-winter mortality, possibly through inadequate algal productivity. The NCL and RGL stocks were sold to farmers from 28/1/02 and 17/10/02, respectively. 14 farms bought NCL stock, a total of 2.7m oysters and 11 bought RGL stock, a total of 1.3m oysters (see Table 9.2).

The protocol for assessing performance of commercial trial animals in grow out required that standard hatchery produced oysters spawned at a similar time (the control line) be purchased together with the commercial trial animals, in order for useful comparative data to be collected. It was also suggested that a small number of

animals (50 was suggested) from each of the trial and control lines be set aside for more detailed examination. Several different control lines were distributed, with ages ranging from 4 to 15 months post-fertilisation.

Only three farms set aside the small numbers of animals for more detailed examination (Table 9.3). The weights taken showed little difference in growth performance between the trial line and the control line; indeed, at one farm (Bolduans Bay) the trial line was less than the control line. However, these were early measurements and the animals in all cases were quite some way from point-of-sale. Also noteworthy was the rapid growth at Tasmanian Clean Water Oysters (St Helens) of both trial and control lines, nearly twice that of Barilla Bay oysters. Meaningful comparisons are difficult, as grading and sorting of spat prior to sale, together with different spawning dates of trial and control lines, makes it hard to ensure that the trial and control lines have had similar treatments.

The majority of the 21 farms that had taken commercial trial stock were contacted; 13 had kept the commercial trial and control stocks separated. Ten of these 13 answered a simple questionnaire that was sent to them to assess how the farmers felt about the commercial trial stock. Six major questions were asked:

On a score of 1 to 10, with the control animals given 5, how would you rate the trial oysters for:

1. growth performance
2. uniformity
3. shape
4. shell colour
5. an overall score

The sixth question was: would you buy the trial product again?

The results of the first five questions are summarised for the two trial lines in Table 9.4.

Some farmers commented that it was a little early to comment definitively on the performance of the animals, as they had not yet reached market size.

The responses to the first five questions reveal that the these two commercial trial families did on average perform better than the commercial control line. Nine of the 10 average scores were greater than the default value of 5 allocated to the control, and the one that was less was only marginally less than the default. Generally there was rather little difference between the two families, with no or minor differences in perceived average growth rates, uniformity and overall score. The trait with the largest range of scores, and for both families, was shape. Either shape varies markedly between farms, or there is a large subjective element to shape scores: both possibilities are likely. Family 28, the non-curlback line, had been selected for good shape and on that attribute it performed substantially better than family 43, the rapid growth line. Family 43 scored somewhat higher than family 28 for shell colour – apparently farmers liked the dark shell colour associated with this family.

The responses to question 6 – would you buy the product again – are clearly important. For family 28, three of the five respondents said they would buy the product again, even at premium price; two said they would not buy the product again. For family 43, four of six respondents said they would buy the product again, two at a premium price, while two said they would not buy the product again. It is not clear why those farmers that said they would not buy the product said so – on all scores the commercial trial families did at least as well as the control family. Possibly the control line was thought to be an inferior line to a typical hatchery line – we did not ask that question.

Overall, this seems to be a reasonably good scorecard. It must be remembered that these are only F2 products and that more recent F3 commercial offerings can be expected to perform better. Improvements will compound with generations, given care with the breeding program.

9.4. Third Set of Trials: 2002/03

January 24, 2003. Shellfish Culture spawned two commercial trials. One was a cross of families 21 and 31. This was our F3 family 57, released as 3H. This was a good performer in our trials (Table 6.8). The other was a mass spawning of individuals from the mass selection line M3-2b, released as M4C. At the same time a commercial control PE11 was spawned. ASI will try to collect information from these trials.

9.5. Commercial Production

Camerons requested approval from ASI to reproduce F2 family #21, and this was given subject to agreed protocols being followed.. On June 25 2002, Bolduans Bay dispatched 40 oysters from WA925 and 16 oysters from WA992 to Camerons for conditioning; they were spawned on September 9 2002. Since this was a commercial production run rather than a commercial trial, no specific protocols had to be adhered to by purchasing farmers. This was released as 2T. Gary Zippel writing in the first ASI newsletter (volume 1, issue 1, June 2003) says: “this line has experienced very good growth and the feedback I have had has been positive. Camerons inform me that the grower feedback they have received is also positive. Zippel Enterprises has some of this stock. Our experience has been that it is rapid growing with probably the best uniformity in growth across the batch I have seen. Because of our poor growing season the previous year, it is in danger of catching the previous year’s stock. It is impressive”.

Camerons later reproduced F3 family 59. This good performing F3 line (see Table 6.8) uses parents from F2 families 21 and 10. It was spawned September 2 2003, and released as line 3J. Scott Parkinson in the second ASI newsletter (volume 1 issue 2) says “Mike Cameron has advised me that this line has performed well through the hatchery and nursery stages and has a very good shape”.

Table 9.1. Performance of first commercial trials after 6 months at one South Australian farm

Line	July 12, 2001 Size grade (mm)	Number of units
RGL	24	38
	19	64
	12	74
	7	3
	Mean = 16.97	
NCL	24	47
	19	77
	12	94
	7	2
	Mean=16.97	
1 (control, B7?)	24	51
	19	67
	12	113
	7	8
	Mean=16.36	

Table 9.2. List of farms receiving commercial trial oysters spawned by Shellfish Culture in 2001/02

Date of sale	Farm	Trial line	Trial Line Quantity (and size at sale, in mm)	Control Line Quantity
28/1/02	West Eyre Shellfish	28	237,000 (2.2)	763,000
20/3/02	Coal River Oysters	28	150,000 (4.0) 3,336 (5.0)	150,000
25/3/02	Tas. Oyster Company	28	120,596 (4.0)	131,091
9/4/02	Bolduans Bay Oysters	28	180,587 (4.0)	120,197 699,216
22/4/02	Bolduans Bay Oysters	28	272,519 (4.0)	657,232 117,577
2/5/02	Clifton Pacific Oysters	28	100,000	78,000
20/5/02	Bolduans Bay Oysters	28	275,218 (4.0)	341,427 383,355
23/5/02	Elf Plumbing Oysters	28	50,000 (4.0)	50,000
27/5/02	Tasman Isle Seafoods	28	150,000 (4.0)	200,000 150,000
4/6/02	Tasmanian Clean Water Oysters	28	84,747 (4.0)	415,253
20/6/02	Ceduna Clearwater Oysters	28	203,993	296,007
19/08/02	Cremorne Pacific Oysters	28	236,488	863,512
14/12/02	Southern Cross Marine Culture	28	108,987	641,851 786,570
17/10/02	Fulham Aquaculture	43	51,336	204,960
21/10/02	South Sea Oysters	28	110,075	180,400
		43	81,486	

Date of sale	Farm	Trial line	Trial Line Quantity (and size at sale, in mm)	Control Line Quantity
4/11/02	Bing-I-Oysters	28	304,589	115,124
		43	195,540	
15/11/02	Ceduna Clearwater Oysters	28	103,684	344,996
		43	73,920	
15/11/02	Holmes Oysters	43	109,472	403,139
25/11/02	S & D Evans	43	128,600	127,797
2/12/02	Shoalwater Oysters	43	211,032	301,496
9/12/02	Barilla Bay Oysters	28	40,716	364,026
		43	116,632	
24/12/02	Peninsula Shellfish	43	102,613	205,000
6/1/03	G & Y Montgomerie	43	59,555	43,044
13/1/03	Cowell Oysters	43	102,859	410,072
30/1/03	Barilla Bay Oysters	43	80,296	430,332

Table 9.3. Weight data for family 28 on three farms and family 43 on one farm.

Farm	Number per line	Trial			Control		
		Line	Approx age (mths)	Total weight (g)	Line	Approx age (mths)	Total weight (g)
Bolduans Bay Oysters[1]	50	28	19	440	PD mix	20	620
Tasmanian Clean Water Oysters[2]	100	28	20	4218	PD mix	21	4037
Barilla Bay Oysters[3]	150	28	20	2288	PD10	17	2124
		43	16	1960			

Dates sampled: [1] 19/5/03, [2] 5/6/03, [3] 3/6/03

Table 9.4. Questionnaire results.

Question	Average score and range*	
	Family 28 (5 respondents)	Family 43 (6 respondents)
1 - growth	6.0 (6-6)	6.0 (5-8)
2 - uniformity	5.4 (5-7)	5.5 (4-8)
3 - shape	6.0 (3-9)	4.8 (2-8)
4 - shell colour	5.6 (5-7)	6.3 (5-8)
5 - overall score	5.75 (4-8)	5.8 (4-8)

*Note that control animals were assigned a score of 5

CHAPTER 10. Genetic Parameters and Predicted Genetic Gains under Different Selection Strategies

10.1. Introduction

A successful selective breeding program requires, firstly, an analysis of the objectives and, secondly, a well planned process of how to reach those objectives. Defining the processes by which the breeding objectives are achieved requires knowledge of the genetics of the population in question. In this chapter we aim to develop an understanding of the genetics of the Pacific oyster population which has been the basis of selection described in previous chapters.

In this chapter we have two main areas of study. The first involves the establishment and analysis of an experiment to measure the genetic parameters in our Pacific oyster population. We estimate the heritabilities and genetic correlations of traits identified as being economically important to the Australian industry and, where possible, compare our estimates to those made elsewhere. The second area of study involves exploring the genetic gains with different multi-trait selection strategies in our Pacific oyster population. We use the population generated from the first part of this study (the genetic parameters study) to test the gains with differnt economic weights on each trait, and with different selection strategies.

10.1.1. Importance of Genetic Parameters

In quantitative genetics, the relative importance of heredity in determining an individual's phenotypic (or observed) value is the heritability of the trait (Falconer and Mackay, 1996). We use heritabilities to predict the rate of response to selection and they determine the degree of resemblance between relatives (Gjedrem, 1983; Tave, 1993; Falconer and Mackay, 1996; Kinghorn *et al*., 1999; Bourdon, 2000). The larger the trait's heritability, the easier it is to change the trait mean in selective breeding programs. Likewise, offspring will show a high resemblance to the parents for traits with high heritability (Bourdon, 2000).

The proportion of additive genetic variance that is transmitted to the next generation and observed in the phenotype variance is heritability (h^2)

> $h^2 = V_A/V_P$, where V_A is additive genetic variance or breeding value and V_P is total phenotypic variance.

We can estimate trait heritability using a variety of techniques. One of the most commomly used, and the method used in this study, is sib analysis and a typical design involves mating a number of random males to a number of random females. Variation is then apportioned to differences between family and within family groups. These variance components are now generally calculated with residual maximum likelihood (REML) methods (e.g., ASReml, Gilmour *et al*., 2002).

The estimation of heritability and other genetic parameters such as genetic correlations are critical to the ongoing success of any breeding program so that rates

of response can be prediced (Tave, 1993). Keeping this in mind, Sheridan (1988) highlighted that parameter estimates (particularly those with large associated errors, as in some oyster studies) may not however translate into reliable prediction tools for selection responses even if the estimates came from the population in question. Lannan (1972) also stated that heritability values are only applicable to the exact environmental conditions, species, and populations that they were measured from. It is therefore essential that such parameter estimates are reliable and accurate as this leads to more accurate selection and increased genetic improvement (Kinghorn *et al*., 1999).

10.1.2. Heritability Estimates in Oysters

The estimation of genetic parameters in oysters (not just *C. gigas*) is largely restricted to those for larval and juvenile stages (Lannan, 1972; Newkirk *et al*., 1977; Mallet and Haley, 1984; D. Ruzzante pers. comm. 1986; Toro and Newkirk, 1990; Boudry *et al*., 2004). Not surprisingly, many authors have concluded that these larval and juvenile trait heritabilities are not generally useful for making selection decisions in latter stages of the oyster life cycle (Newkirk, 1980; Haley and Newkirk, 1982; Toro and Newkirk, 1990).

From the literature, heritability estimates for larval and juvenile growth in oysters (*C. gigas*, *C. virginica* and *Ostrea edulis*) have ranged from 0.09 to 1.17 (based on full and half sib families) (Lannan, 1972; Newkirk *et al*., 1977; Losee, 1979; D. Ruzzante pers. comm. 1986). Lannan (1972) reported heritability estimates for larval traits in *C. gigas*. Heritability for larval survival (11 to 15 full-sib families) was 0.31 ± 0.06 and for setting rate heritability was 0.09 ± 0.08. However, these estimates were based on full sib families and included non-additive genetic effects. Lannan (1972) also reported heritabilities of juvenile Pacific oysters at 18 months of age. The values for total weight, shell weight, and wet meat weight were, respectively, 0.33 ± 0.19, 0.32 ± 0.30 and 0.37 ± 0.20. Toro and Newkirk (1990) reported heritability estimates for live weight and shell height in *O. edulis* (at 18 months of age) as 0.11 and 0.24 respectively. These estimates were based on full sib analysis. In *O. edulis*, heritability estimates for whole live weight, shell length and shell width for six month old juveniles were 0.09, 0.05 and 0.10 respectively (D. Ruzzante pers. comm. 1986).

In older oysters, Jarayabhand and Thavornyutikarn (1995) reported a realised heritability estimate of 0.28 from one generation of mass selection in *Saccostrea cucullata* while Toro *et al*., (1995) estimated realised heritabilities for live weight between 0.43 and 0.70 and shell length between 0.24 and 0.36 in two year old *O. chilensis*. Heritability for shell width in three year old *Pinctada fucata martensi* was estimated at 0.47 by Wada (1986). Toro and Newkirk (1991) estimated realised heritability in shell height in 30 month old *O. chilensis* at 0.34 ± 0.12.

Of the limited heritabilities reported in *C. gigas* at later life stages, those reported by Hedgecock *et al*. (1991) & (1993) are the most encouraging. They demonstrated the heritability of individual wet meat weight at harvest in Pacific oysters to be approximately 0.2, although the study was based on a small number of sires. Additionally, there is some evidence to suggest that shell shape is not highly heritable (see Hedgecock *et al*., 1997 and references within). Langdon *et al*. (2003) also reported on encouraging heritability results for *C. gigas* based on realised heritability

of average family live weight. Heritability was estimated up to 0.50 for family bags after the bag weights had been adjusted for within family selection.

Heritability information for almost all other traits (particularly in *C. gigas*) is lacking. Growth rate in adult oysters at least is considered to be a trait of low to moderate heritability (depending on species) (Toro and Newkirk, 1990). The relatively few heritability estimates of oyster production traits (other than larval traits as outlined above) do indicate however that selection in oyster populations for increased growth should be favourable (Sheridan, 1997).

10.1.3. Selecting for Multiple Traits

Most breeding programs involve selecting for more than one trait. Oyster breeding is no exception, with up to 16 'desirable' traits being identified by the industry (See Table 1.1). An effective breeding program needs to be able to combine these traits into a single index value so that selections can be made objectively. Typically, an index has the following form:

$$\boldsymbol{I} = EBV_1.W_1 + EBV_2.W_2 + \ldots + EBV_n.W_n$$

Where I is the index value, $EBV_{1\ldots n}$ are the estimated breeding values for the traits in the index, and $W_{1\ldots n}$ are the economic weights for those traits.

The EBV is an estimate of the genetic value of a trait – it is not the phenotypic value. At its simplest, an EBV can be the phenotypic value multiplied by the heritability. This then gives a measure of the amount of genetic change that is expected for a trait. A more sophisticated way of estimating an EBV is using Smith-Hazel indices (see Cotterill and Dean, 1990 for details) which have the advantage of using genetic correlations between traits, as well as heritabilities. However, the best method of calculating EBV's is Best Linear Unbiased Prediction, or BLUP. These methods calculate an EBV using information from all relatives (e.g. siblings, parents) and, in a multivariate analysis, information across all different traits. BLUP EBV's are based on the premise that related individuals have genes in common, and therefore data from an individual's relatives is used to estimate the EBV for that individual. BLUP's are now the method of choice for EBV estimation and software is widely available to undertake these analyses (e.g. ASReml, see Gilmour *et al*., 2002).

An economic weight is a measure of the dollar value of a change in a genetic trait. For example, the economic weight for growth rate would measure the value of a change in producing larger oysters at harvest, and the economic weight for width index would measure the value of a unit change in that index. When combined with an EBV's in a selection index (such as that shown above), economic weights allow the value of a particular genotype to be objectively measured and this, in turn, provides a means by which animals with the highest economic value can be selected.

10.2. Methods for Producing Families

The mating design we used in this study was to mate each female in the experiment to two males, and each male to two females. In a matrix of parents, females by males, the matings were those along the diagonal. Our aim was for minimal grading and to select unrelated parents to sample as much genetic diversity in the population as

possible. Measurements were then made on the progeny, which have known genetic relationships to one another. This is a powerful way of extracting useful information on heritabilities when the number of crosses that can be made is limited.

At a breeding program meeting at CSIRO Marine Research in June 2002, it was decided that measurements should be made at 12 months and at 24 months post-spawning. Traits to be considered for parameter estimation at 12 months were: whole weight, length/depth/width, possibly volume (displacement), extent of frill (photo), colour (photo) and curl back (presence or absence). Traits to be considered for parameter estimation at 24 months were: whole weight, meat weight, shell weight, a (subjective) condition score, length/depth/width, extent of frill (photo), colour (photo), glycogen level, gonad development, curl back (presence or absence) and shelf life (time to gape). There was also some discussion about whether it would be sensible to set up taste panel trials on these oysters but this deemed impractical given our resources.

10.2.1. The First Attempt– January 2001 Spawnings

The crossing plan for this spawning was to have 30 females and 30 males, with each female crossed with two males and each male with two females, giving a total of 60 families (Table 10.1). Spawnings were carried out on 6th and 7th January 2001 at Shellfish Culture Ltd. Since we did not need large numbers of offspring, but we needed to make large numbers of crosses, a new small-scale spawning system was developed. Lines were reared in 20L plastic buckets, each with an airline and heater, and transferred every two days to clean buckets. Spat were transferred to TAFI, Taroona, on 2nd February 2001.

In total, 52 crosses were attempted. The 26 males were all wild-collected feral animals. Eighteen of the 25 females were wild-caught; seven came from the breeding program. Only 20 crosses produced >1000 spat. The crosses were transferred directly to the three chosen grow-out farms on 8th May (one farm) and 10th May (the remaining two farms) 2001. Two of these farms were intertidal, one was subtidal; they were the Tasmanian farms used in the selective breeding program. Each of the 20 sizeable families was present as two replicates (each of 100 animals), at each farm. A further 11 families only produced 100 or less animals; these were allocated to one of the intertidal farms. Animals were initially grown on the farms in specially adapted seed trays but when larger the intention was to transfer them to mesh bags.

Unfortunately, the subtidal animals were lost quite early due to equipment failure. On one of the intertidal farms, a check in October 2001 revealed that families had become mixed due to faulty equipment design. At about that time, only one of the two replicates remained on the other intertidal farm, the other replicate having broken away and been lost. It was clear that this heritability experiment would not yield sufficient data to be useful, and we decided to repeat the experiment the following year.

10.2.2. The Second Attempt – February 2002 Spawnings

We expected that with the improvements we made to our equipment as a result of the experience in the first heritability experiment, we could make more than the 20

sizeable families we had produced in 2001. We also expected to be able to follow them through grow-out more successfully. We made 62 crosses following the same matrix breeding design in January 2001 (Table 10.2). The oysters used were commercial oysters from Barilla Bay (Pittwater), and feral oysters from the north end of the Tamar River (near George Town), Esperance and Hastings River.

Sixty-two families were created with good larval quality in the first 3-4 days of the larval cycle. However, growth then stopped for five days although algal and water quality appeared to be good. Growth similarly stopped at that time in Shellfish Culture's commercial tanks. The larvae stayed at ca.100 microns, about the same size as their ciliate predators, and could not be size-screened from them. Predation together with normal mortality resulted in some families failing to survive to metamorphosis. The no-growth period was followed by a slow-growth period for another 5-7 days. The normal 14-20 day period from fertilisation to metamorphosis extended to 26-28 days, providing opportunities for increased predation. Only one of the 62 crosses survived to set, and the experiment was abandoned.

A major contributing factor in this failure was the 20 L size of the tanks used to rear the larvae. Three out of four inbred families established at the same time in our 140 L tanks survived satisfactorily, despite the fact that they were inbred crosses. In fact, the reason the fourth inbred family failed might be because its larvae had been transferred out of the 140 L tank to a 20 L tank where they died.

10.2.3. The Third Attempt – November-December 2002 Spawnings

Our first two attempts at deriving families for heritability assessments and selection index development had failed. Both attempts had found it difficult to satisfactorily produce oyster families to set in the 20 L tanks. For this, our third attempt, we decided to use our larger 140 L tanks which we routinely and successfully use for our standard family production. However, this meant we could set up no more than about 30 families at one time, due to space and equipment restrictions. Therefore, we established 30 families on the 2nd and 3rd of November 2002. We allowed them to set, cleaned the tanks, and established a further 30 families in the same tanks on 30th November and 1st December 2002 (Table 10.3). A mixture of farmed and feral animals was used. In total, 29 males (25 of feral origin, 4 from the breeding program) and 29 females (24 feral, 5 breeding program) were used.

Having two separate runs one month apart is clearly not ideal as significant time (and potential environmental) factors are introduced. It was suggested that a genetic link between the two runs would be useful, as this would enable an estimate of the size and effect of this time factor. Therefore, one female (N) and one male (15) from the first run were drilled, the gametes removed to make crosses, the hole plugged, and the animals were then strip spawned one month later in run two (as female X and male 24) to make further crosses. Thus cross 15N is identical to cross 24X. Unfortunately, these linkage crosses largely failed to survive in the first run.

Settled animals were sent to the TAFI lab on 27/11/02 (first spawning) and 27/12/02 (second spawning), and kept and fed in upwellers.

At the beginning of February 2003, approximately 100 animals (where possible) from each family were placed into each of six mesh bags (960 micron mesh) and labelled. The animals from the first spawning, being approximately one month older, were significantly larger than those from the second spawning. Two bags per family (where possible) were sent to three of our growout farms: Smithton (Bolduans Bay Oysters, sent 18/2/03), Pittwater (Tasman Isle Seafoods, sent 20/2/03)**,** and Coles Bay (sent 24/2/03) The spat were very small when taken to the growout farms, some still sitting on 1000 micron screens rather than the 1800 micron screens they normally are when transferred from TAFI to Bolduans Bay for nursery rearing. The major reason for this was that we wanted the spat to experience different environments from an early stage, and, more pragmatically, we also had to release equipment at TAFI for incoming F5 spat.

Bags were serviced on 14/4/03, 19/5/03, and 9/7/03 at Smithton, on 16/4/03 and 18/6/03 at Coles Bay and on 9/4/03, 15/5/03, and 18/6/03 at Pittwater.

Surviving animals were counted on 9/7/03 at Smithton, on 18/6/03 at Coles Bay, and 15/5/03 at Pittwater. These numbers are given in Table 10.4. The most obvious feature of this table is that progeny survived well from the first spawning, but nearly all of the progeny from the second spawning died. The progeny from the second spawning were a month younger than those of the first spawning, and therefore appreciably smaller when put out on the farms. It seems they were unable to cope with the rigors of intertidal farm life, but the cause of the mass mortality is unknown. The majority of spat from the first spawning at Smithton also died, leaving only small sample sizes at that farm. It was therefore decided to focus on the selection index animals at Pittwater and Coles Bay, where enough animals survived in enough families to allow estimates to be made of trait heritability.

On 25th July 2003, a request for an extension of time to April 2005 was made to FRDC to enable the heritability and subsequent selection index work to be completed after the main part of the FRDC project terminated in November 2003. This request was approved on 10th August 2003.

On 26th August 2003, the heritability animals were moved from Pittwater to TAFI for cleaning and counting. Most unfortunately, they met a tragic end there. All died or were lost. This meant that the only surviving heritability trial animals were at Coles Bay. It was decided that when these animals at Coles Bay were next counted (September 2003), those bags with large enough spat numbers should be split in two, with one half returning to Coles Bay and the other going to Pittwater. This was to guard against the possibility of storm or other loss of animals at Coles Bay.

10.2.4. Measurements of Family Line Oysters at 12 and 24 Months

After discussion (January 2004), the traits for parameter estimation at 12 months were revised to: whole weight (g), length, depth, and width (mm) while general information on the extent of frill (photo) and shell colour (photo) were also collected. Traits to be considered for parameter estimation at 24 months (following revision and discussions with ASI, 26/10/04) were: whole weight (g), length, depth, width (mm), wet meat weight (g), wet shell weight (g), dry meat weight (g), lid shape and sex. Following this, three other parameters would be calculated;

Condition index CI = {[Dry soft tissue (g) ×1000]/internal shell cavity capacity (g)} (see Crosby and Gale, 1990).

Width index = (width/length) (mm) & Depth index = (depth/length) (mm) (pers. comm. Scott Parkinson 2005)

10.3. Assessments and Analysis of the Genetic Parameter Study

10.3.1. Preliminary Data Analysis at 13 Months

The oysters were collected from Pittwater on 6th January 2004 and Coles Bay on 16th January 2004, for tagging and measuring. Small plastic tags numbered 01 to 99 (excluding 06, 09, 60, 66, 90, 99 to prevent confusion) were super glued to each oyster. The length, width and depth of each animal was measured using digital callipers and weight recorded using an electronic balance. Each family was photographed as a group for the top and bottom shell colour and shape. General colour and pattern trends were noted for each family. This information was recorded in an excel spreadsheet and backed up on CMR's server. This sampling time, approximately 13 months after fertilisation, is considered Time =1. While they were being assessed, animals were maintained alive and fed in a three tank re-circulating system at CSIRO. Each family was placed in a new 3 mm mesh bag. Bags were returned to Pittwater on 13th January 2004 and Coles Bay on 25th January 2004. Both sites were intertidal.

Our vacation scholar, Angela Holmes was responsible for the initial oyster tagging and preliminary measurements. With the help of project staff, Angela undertook preliminary data analysis on these first measurements and prepared a brief report titled "Heritability estimates and trait correlations for the Pacific oyster, *Crassostrea gigas*, Thunberg, at 12 months of age". The following are excerpts from Angela's 2004 report.

'Average and standard deviations were calculated for each trait in each family to demonstrate the differences between replicates and farms. Phenotypic lengths to weight correlations were calculated in excel for both farms and length to width and width to weight for Pittwater. ASReml calculated genetic and phenotypic correlations for all trait combinations for both farms combined. The program R v. 1.8.1 (2003) was used to generate descriptive statistics, to test for normal distribution of traits, run linear models and to calculate heritabilities. ASReml was used to produce heritability values for each trait and phenotypic and genetic correlations between each trait using pedigree information and the animal model.

The descriptive statistics showed broad differences between farms. Coles Bay had a wider range for minimum and maximum values of all traits and higher means for all traits (Table 10.5). The length histogram showed an approximate normal distribution with slightly more values on the minimum side of the peak. Width showed a normal distribution with a wider range of values than length. Depth showed an approximate normal distribution with slightly more values on the maximum side of the peak. The weight distribution was skewed heavily to the left.

Narrow sense heritabilities were estimated using ASReml and broad sense heritabilities were estimated using R (Table 10.6). Weight was not transformed for R but was log transformed in ASReml. Both estimates were almost identical, suggesting that all genetic variance is additive (i.e. there is very little dominance variation). Early length and depth traits showed moderate heritability estimates while width and weight show lower heritability estimates. The heritability of weight was almost doubled when the data was log transformed (the distribution was improved after log transformation but was still not normal).'

10.3.2. Trait Assessments at 22 Months

In February 2004, flooding occurred in numerous river systems along the east coast of Tasmania. We undertook a census of the family lines at both Pittwater and Coles Bay so as to ascertain if the probable high levels of fresh water had impacted on our animals. At this time, there were several reports in the local media of oyster farmers facing large scale losses through mortalities. The census at Coles Bay was undertaken in March 2004 and at Pittwater in April 2004. At these times, we also checked the 3 mm bags; bags were replaced if badly damaged or knots were tied in both ends to prevent escapes.

Table 10.7 shows the number of survivors at each site. As can be seen, we lost approximately 37% of our individuals at the Coles Bay site. The sample size in some families (and replicates) was severely impacted and indeed we lost some replicates altogether. Our oysters at Pittwater were less affected, with only 8% of all animals either dead or missing. Following the census, oyster bags were returned to the racks.

Families were checked in September 2004, approximately 22 months after fertilisation. Bags were brought in from the intertidal racks and length (mm) (using digital callipers) and total weight (g) (using an electronic balance) were measured for each individual oyster (Time = 2). Bags were replaced where necessary and the replicates were returned to the oyster racks. Data were entered into our spreadsheets and backed up on CMR's server. Table 10.8 shows the number of oysters that had measurements recorded and also the distribution of remaining families across the two sites.

10.3.3. Final Measurements at 25 Months

In January 2005, the families from Pittwater were brought into the CMR laboratories and a number of traits were measured on all survivors. A team of 5 people (including James Burke from ASI) measured total wet weight (g), length, width and depth of the whole oyster (mm), wet meat weight (g), wet shell weight (g), sex and characterised lid shape (as either flat, convex, undulating, concave). Shells and meats from each oyster were placed into individual plastic bags and frozen at -20^0C. Dry meat weights (g) were then measured on each meat after desiccation in the freeze dryer for two days. The dry meats and shells from each Pittwater oyster are stored in the -20^0C freezer.

In April 2005, the families at Coles Bay were also measured. Due to their small size, only non-destructive measurements were taken (i.e. whole weight, length, width and depth). A sub-sample of these families was put back onto the lease at Coles Bay, with

the rest of the animals discarded. Individual data from the Pittwater and Coles Bay families were recorded in excel spreadsheets and backed up on CMR servers.

We then considered the key traits for our heritability analyses and trait correlation estimations as weight, width index, depth index, length, width and depth (at time Time = 3) as these measurements were taken (or could be calculated) on oysters from both farms. We also calculated condition index, wet meat and wet shell heritability from the Pittwater animals. While we have data on the other traits (e.g., lid shape, sex), due to time and resource limitations, we concentrated only on the above traits.

10.3.4. Data Analysis of Final Measurements

The individual oyster data were examined first using R to calculate simple statistics, test for normality, remove anomalies and perform initial analysis using linear models without pedigree information.

ASReml (Gilmour *et al*., 2002) was then used to estimate fixed and random effects, variance components, trait heritabilities and genetic correlations (univariate and bivariate models). Maximum likelihood (ML) procedures accommodate any structure of genetic relationship within the data. ML evaluates the probability that the observed data could have been obtained given the values of the parameters. ASReml uses iterative computer algorithms to find the combination of parameters at which the 'likelihood' is maximised.

The models fitted included the fixed effects of farm while animal, dam and sire were fitted as random effects. As each oyster had been individually tagged and family records were available, we were able to fit an animal (random) model as the pedigree was present. Covariances among full and half-sibs enabled division of phenotypic variance into genetic and environmental components of variance.

All traits were analysed with single trait animal models. The animal variance component provided the estimate of the additive genetic variance (σ^2_A) and the phenotypic variance (σ^2_P) was estimated from the sum of all variance components. Up to three random models were applied to the data (interaction of fixed and random effects is a random effect model). The fixed effect of Farm was always fitted. ANOVAs in ASReml were calculated from individual data (and using pedigree information) on each farm (and a combined Pittwater & Coles Bay analysis) for weight, width index, depth index, length, width and depth. ANOVAs were also undertaken on individual data from the Pittwater oysters for condition index, wet meat and wet shell.

The three fitted models are shown below where μ is the overall mean, Farm_i is the fixed effect of ith farm, Sire_j is the random effect of jth Sire, Dam_k is the random effect of kth Dam, Family_l is the random effect of lth Family, A_m is the additive genetic effect of mth animal and ε_{ijklm} is the residual random effect. The Sire × Dam effect represents the dominance variation and the Family × Farm effect represents the genotype × environment interaction. The distributions of the random effects A and ε were assumed normal with zero mean. Heritability was estimated as the proportion of the additive genetic variance to the total variance ($h^2 = \sigma^2_A / (\sigma^2_A + \sigma^2_P)$).

Model	
I	Trait = $Y_{im} = \mu + Farm_i + A_m + \varepsilon_{im}$; $\varepsilon_{im} \sim (0,\sigma^2)$
II	Trait = $Y_{ijkm} = \mu + Farm_i + (Sire_j \times Dam_k) + A_m + \varepsilon_{ijkm}$; $\varepsilon_{ijkm} \sim (0,\sigma^2)$
III	Trait = $Y_{ijklm} = \mu + Farm_i + (Sire_j \times Dam_k) + (Family_l \times Farm_i) + A_m + \varepsilon_{ijklm}$; $\varepsilon_{ijklm} \sim (0,\sigma^2)$

10.4. Results of Genetic Parameter Study

10.4.1. Descriptive Statistics and Fixed Effects

Following the final trait assessments at Time = 3, and with removal of anomalies and dead oysters, 503 animals at Pittwater (across 19 families) and 137 animals at Coles Bay (across 20 families) were analysed (Table 10.10). Pittwater oysters had been assessed for both non-destructive and destructive measurements while due to small size, only the non-destructive traits were measured at Coles Bay. A total of 640 individuals were used for trait measurement across the farms. At Pittwater, 6 dams and 3 sires were represented in half sib families resulting in 14 half sib and 5 full sib families. At Coles Bay, 5 dams and 3 sires were represented in half sib families resulting in 13 half and 7 full sib families. Table 10.8 shows the distribution of the families across the two farms.

Means and standard errors of difference for the traits that were measured on live oysters is shown in Table 10.9 (width index and depth index are functions based on measurements of length, width and depth). We did not adjust for growing densities within each bag (i.e. replicate), and this ranged from n = 1 to 49 per bag.

As can be seen from Table 10.9, the mean for weight at Pittwater ranged from 24.21 to 48.33 g (across the 19 families) and from 11.63 to 26.17 g at Coles Bay (across 20 families). Pittwater animals consistently weighed more than those at Coles Bay. Not unexpectedly, these trends were also seen in the other three non-destructive measurements. The mean length, width and depth at Pittwater ranged from 54.06 to 70.05, 44.17 to 52.70 and 22.87 to 29.60 mm respectively. At Coles Bay, the same traits ranged from 36.73 to 59.96, 31.83 to 46.28 and 16.08 to 22.79 mm.

From the boxplots produced in R (graphs not shown), Pittwater oysters had a broader range for weight, length and depth than Coles Bay oysters while the width range at Coles Bay was greater. Normality of the trait distributions was assessed using a Shapiro-Wilk normality test and qqnorm plots in R. Of the four traits across both farms, weight was consistently skewed (Shapiro-Wilk W = 0.984, $P < 0.001$) and the corresponding qqnorm plots were not straight. Length, width and depth were generally distributed normally (W = 0.992, $P = 0.002$; W = 0.98, $P = 0.426$; W = 0.993, $P = 0.006$). Log transforming weight however did not improve the distribution.

Table 10.11 shows the statistical significance for the fixed effects of family, farm and replicate for weight, length, width and depth. The initial linear models undertaken in R showed that replicate was not significant. The other two main effects were statistically significant ($P < 0.001$).

10.4.2. Analyses Using ASReml

Heritabilities, genetic and phenotypic correlations were estimated for nine traits at Pittwater, six traits at Coles Bay and, therefore, six traits across both farms. The traits measured across both farms were weight, width index, depth index, length, width, depth. The estimates (including standard errors) of variance components and heritability are shown in Table 10.12. Genetic and phenotypic correlations between traits are shown in Table 10.13.

Most traits have significant additive genetic variation. Moderate to high heritabilities ($h^2 = 0.22 – 0.57$) were observed at Pittwater. Heritability ranged from 0.00 (depth) to 0.81 (length) at Coles Bay however, the standard errors of these estimates were also very high. For some traits at Coles Bay, the data should be treated with caution due to the very small sample sizes. Despite the high standard errors for some estimates, our study indicates that a large portion of the overall variance in these traits is genetic. Weight has a consistently high heritability, both on a single farm and across combined farms. Length and depth also displayed high heritabilities, while at Pittwater, wet meat was only moderately heritable yet wet shell was highly heritable.

We found no significant dominance effects when the data was combined across both farms (Table 10.12). When farms were assessed individually, no significant dominance effect was seen at Pittwater however at Coles Bay, width appeared to have some dominance effects. Both width and depth did however show genotype × environment interactions.

The bivariate analyses in ASReml indicated weight was positively correlated with length, width and depth (both genetic and phenotypic) at each farm and when the farms were considered together (Table 10.13). Length, width and depth were also positively correlated between each other in all instances. The function traits (i.e., width, depth and condition index) however were negatively correlated (r_g) with the other traits. The phenotypic correlations for these functional traits were always positive but they were much smaller than those for weight, length, width and depth.

10.5. Discussion on the Genetic Parameter Study

10.5.1. Esimated Heritability Values

The estimation of heritability for economically important traits is imperative for the ongoing oyster selective breeding program. Our study is the first in Australia to estimate Pacific oyster trait heritabilities at harvest age.

Our heritability estimates indicated that additive genetic variance is present for most traits and these could be improved in a selective breeding program. We observed high estimates for weight and length (0.56, 0.58 respectively) at both Pittwater and Coles Bay. Width and depth were also considered moderate to highly heritable at Pittwater. Condition index at Pittwater was also quite high although we are uncertain what this condition index is actually measuring and if it is applicable for the current industry. Perhaps surprisingly, the heritability estimate for wet shell (at Pittwater) was very high yet the wet meat estimate was quite low (cf., 0.62 & 0.22). These estimates need

to be further explored and the genetic correlations between the two traits estimated so that we can be sure we would not be selecting just for increased wet shell.

The trait heritabilities at Coles Bay were also very high (e.g. length $h^2 = 0.81$) but with very high standard errors due to the small number of sample per family. Width and depth effectively showed little to no additive genetic variance at this site.

Brake *et al*. (2003) advised that absolute measures such as shell length and width may be less useful in categorizing oysters due to their variable 'shape' and culture environments which effect shell shape. They suggest, as we have done, using descriptors to characterise shell shape (we used depth to length and width to length). The heritability estimates of our indices (width, depth and condition) are moderate to high, with the depth index more heritable than width. As such, width will be harder to improve in comparison to weight and depth. The culture environment obviously plays a greater role in determining width in our Pacific oysters and it is well known that stocking density has a significant impact on overall shape (e.g. Jarayabhand and Thavornyutikarn, 1995) While width may not be as important as depth in determining quality (Brake *et al*., 2003) selection on depth without consideration of width could result in deep, narrow oysters. In our study, width consistently showed the lowest heritability, particularly across both farms.

The condition index is a point of concern for us. While we have used the model proposed by Crosby and Gale (1990), we are not confident in the parameters this index is measuring. Crosby and Gale (1990) themselves highlight the problems in comparing condition index between studies as there is a lack of accepted condition index formulae. Indeed, our index is not currently used by the oyster industry in Tasmania. We need to ascertain the parameters that a condition index should be measuring – is it meat condition or gonad coverage and at what time in the life cycle of the oyster should we be assessing condition. Condition indices have previously been used to follow seasonal changes in nutrient reserves, assess meat yields, monitor pollutants and disease, and assess accumulation of glycogen reserves (Crosby and Gale, 1990). The condition index we used is greatly affected by volume displacement (see also Crosby and Gale, 1990) and in many cases, we were seeing large losses of water when we opened the oysters; the loss of internal water can greatly affect the overall result. A further caveat on the condition index is that it was only estimated from animals at one site and at one point in time. We would recommend undertaking additional work on condition and the index measurement across multiple sites.

In general, we consider that we have obtained reasonably reliable heritability estimates as we used an animal model that accounted for effects common to full sibs other than just additive effects (see also Gitterle *et al*., 2005). Of course these estimates would have been more robust had the number of half sib families been higher, particularly at Coles Bay (as full sibs are not the preferred option as maternal effects confound the estimation (Willis, 1991)). The precision of our estimates of heritability (given by the standard errors) varied and in some instances was very large probably caused by the small sample sizes in some families as a result of variable juvenile and grow-out success. The estimates might also be more accurate if the number of offspring per family was higher. Newkirk *et al*., (1977) stated that optimally the best estimates of heritability should come from a large number of families. Willis (1991) also suggests a minimum of 10 sires and 5 offspring should be

used; not all of our families were represented by this number. Unbalanced family sizes may also influence the estimation of genetic parameters (Norris and Cunningham 2004).

However, while the estimation of heritability based on half-sibs (which uses progeny from different sires and dams) is the preferred option (Lannan, 1972; Newkirk *et al*., 1977; Davis and Hetzel, 2000), we need to remember that the correlation between half sibs is effectively quadrupled so that any errors in our heritability estimates are also magnified by the same order (Willis, 1991; Falconer and Mackay, 1996). Irrespective, narrow sense heritability from half sib analyses is the most useful because only additive genetic variance can be used in selection programs. Due to the presence of some full-sib families in the analyses, we might not have always separated the dominance effects from the additive effects (Quinton *et al*., 2005) and there may be some additional covariance between the full sib groups as compared to the half sibs (see also Newkirk *et al*., 1977). This may cause an upward bias in some of our heritability estimates.

Overall, we did not see evidence for sire or dam (maternal) effects particularly in the parameter estimates from across both farms. These 1st generation animals were spawned from essentially wild stock. We also observed very little evidence of genotype × environment interactions. Previously, Toro and Newkirk (1991) reported that maternal effects might be of importance for *O. chilensis* (particularly for traits that are measured at an early age as *O. chilensis* broods the larvae until they are ready to set). The traits in our oysters were measured at a later life stage (two years of age) which could perhaps account for why we did not see any dominance effects.

10.5.2. Estimated Correlation Coefficients from the Current Study

Correlations between traits can be assessed on either a phenotypic or genetic basis. The phenotypic correlations are made up of both genetic and environmental components ($r_P = r_G + r_E$). Genetic correlations are a measure of strength of the relationship between breeding values for one trait and breeding values for another (see also Bourdon, 2000). By estimating the genetic correlations, we were able to assess the changes that occur in one trait when making genetic selecitons on another.

Genetic correlations were high and positive between weight, length, width and depth and selection for one of these traits would produce a positive response in the other traits. Therefore, when selecting for weight a correlated response in shape occurs and there is a risk that adverse changes in shape may occur if appropriate weightings are not applied to shape traits when selecting for weight. The nature of these responses in explored further in Section 10.6.

Although phenotypic correlations maybe high and positive this does not always give an indication to the underlying genetic correlation. Selection would be hampered if positive phenotypic correlations are only used without considering genetic correlations particularly if underlying genetic correlations are negative (see also Willis, 1991).

Our phenotypic correlations are similar to those found in *O. chilensis* for weight and shell length (r = 0.94) (Toro *et al*., 1995). High genetic correlations similar to ours

were reported for live weight and shell length in *O. edulis* (r_g = 0.99) (Toro and Newkirk, 1990). Shell height was also highly correlated with total wet weight in *C. virginica* (Haley and Newkirk, 1978).

10.5.3. Wider Application of Genetic Parameters

Lannan (1972) reminds us that the estimate of heritability for any trait will not be universally applicable even within the particular species that it was assessed. Heritability estimates are only valid for the conditions of environment in which the oysters were grown and measured.

Ideally, a commercial breeding program should estimate genetic parameters under commercial grow-out conditions. This ensures that the traits selected for use in the breeding program are the same traits that would be expected under the commercial conditions. Our grow-out system and stocking densities were not the same as those used in the commercial farms in Tasmania due to experimental difficulties and indeed the environment in Pittwater and Coles Bay in Tasmania may be different to that experienced elsewhere. We note that the number of individuals per family was not always consistent and in some cases was quite small. We also experienced an uncharacteristic event in Coles Bay during the summer of 2004.

As such our genetic parameters are not applicable to all Pacific oyster grow-out conditions or breeding applications. Our heritability and correlation estimates are illustrative and alternative results may be obtained from different populations of oysters. The demonstrated levels of additive genetic variation in most traits shown in this study however indicate that these traits could be exploited in the oyster selective breeding program.

10.6. Methods for Estimating Genetic Gains with Multi-Trait Selection

Gains from multi-trait selection were estimated using an index of breeding values and economic weights (see section 10.1.3 for the form of this index).

We estimated EBV's of both individuals and families using a BLUP analysis. Although 16 traits were initially listed as desirable, we used only three in this study. These were weight (or growth rate), width (measured using a width index) and depth (measure using a depth index). The heritability of these traits was discussed in section 10.4.

Calculating economic weights involves a thorough analysis of the production system for an enterprise. All factors which contribute to costs and returns need to be identified, and the impacts of these factors on enterprise profit then needs to be measured. However, such an analysis is a major undertaking and is beyond the scope of this study. Therefore, we assumed a range of market scenarios and approximated a set of economic weights for each scenario. Our simulation illustrates the range of selection outcomes possible and demonstrates the importance of having true economic weights as the basis for selection.

Genetic gains for the traits identified as being economically important (which were weight, width index, and depth index) were estimated under different market

scenarios and under different genetic selection strategies. We assumed that a positive change in each of these traits is favourable and a negative change adverse. A large increase in both the width index and depth index would produce an excessively round and very deep oyster and this would not be desirable. However, given that only small changes appear possible for shape and that the shape indices have negative genetic correlations with growth (explained further in Results section); it is likely that genetic selection will always be aimed at increasing both the width index and depth index.

We evaluated five different market scenarios and, for each, different economic weights were used in the selection index. Each market scenario and the selection indices appropriate to that scenario are described below. The economic weights we used are shown in Table 10.14 and are described below.

1. Value is realised through larger or faster growing oysters, and shape has no economic value. Selections are made with a 'growth only' index.
2. Value is realised through faster growing or larger oysters. An improvement in shape has no economic value, but a decline in shape will result in a loss in value or increase in growing costs. Selections are made with a 'growth emphasis, but no shape change' index.
3. Value is realised equally from larger/faster growing oysters and improved shape. Selections are made with an 'equal emphasis on growth and shape' index.
4. Value is realised through improved shape. Producing larger or faster growing oysters has no economic value, but smaller or slower growing oysters will cause value loss. Selections are made with a 'shape emphasis, but no growth change' index.
5. Value is realised through improved shape only and changes in size at harvest or grow-out time have no economic value. Selections are made with a 'shape only' index.

We evaluated three different genetic selection strategies. These were:

A. Family selection, where the best 50% of families were selected (10 out of 20), but no selection was made within families.
B. Family/within family selection, which was a two-stage approach where the best 50% of families were selected (stage 1), and then the best 4 individuals selected within each family (stage 2) to give a total of 40 selections.
C. Within family selection, where the best two individuals were selected from every family (20 families) to give a total of 40 selections.

Individual animal or family (depending on the selection strategy) index values were calculated for each selection strategy as:

$$\boldsymbol{I} = EBV_{wt}.W_{wt}/\sigma_{wt} + EBV_{wi}.W_{wi}/\sigma_{wi} + EBV_{di}.W_{di}/\sigma_{di}$$

Where $\boldsymbol{I}$ is a unit less index value, EBV is the individual animal or family estimated breeding value for each trait (see Table 10.15 for definition of subscripts), σ is the standard deviation for the breeding values; and W is the economic weight for each trait. The economic weights used for each index are shown in Table 10.14 and the summary statistics for the EBV's are given in Table 10.15. EBV's were estimated by

fitting Model I (section 10.3.4) with ASReml. This model estimated both progeny and parental EBV's, and family breeding values were calculated as the average effect of the parental EBV's. EBV's are weighted by standard deviations so that economic weights can be expressed in standard deviation units. For example, economic weights of 1:1:1 for growth, width index and depth index (selection index 3 in Table 10.14) places equal value on a single standard deviation shift in each selection trait.

We estimated the gain for each trait by calculating the average breeding value of the selected group. This was done separately for each market scenario and for each selection strategy. Gains were expressed as a percentage change of the population mean.

For each market scenario, we estimated the assortments of oyster product grades. The industry currently grades oysters into six classes by length and these grades and their definitions are shown in Table 10.16. The assortments of these product grades were estimated using the predicted lengths and by assuming a typical normally distributed population with a coefficient of variation of 15%.[2] For the base case, a mean length of 70 mm was assumed and for other selection scenarios the mean was adjusted by the predicted gain in length. The percentages of the harvest in each class were calculated using the cumulative probabilities of the standard normal distribution.

10.7. Results of Multi-Trait Selection Studies

10.7.1. Genetic Gains with Different Selection Indices

The genetic gains that are expected when using different selection indices (for different market scenarios) are shown in Table 10.17. In this table, all gains are based upon a family and within family selection strategy.

When selecting for weight only, large increases in the growth rate are expected. The predicted weight increase is 36% (Table 10.17, growth only index). Length, width and depth all increase, but length increases at a much greater rate than the other dimensions. Consequently, this growth increase is accompanied by an adverse change in the width index and depth index meaning the oysters would become longer and flatter. Shape can, however, be maintained with a comparatively small sacrifice in growth gains (Table 10.17, growth emphasis with no shape change index). High weight gains, predicted to be 28%, are still possible when applying sufficient selection pressure on width index and depth index so that current shape is maintained.

Gains in shape are small in comparison to those for growth rate. At best, and when selecting for shape alone, increases of 8% for the width index and 9% for the depth index are possible (Table 10.17, shape only index). Seeking maximal changes in shape is accompanied with a decline in the growth rate, with a 6% decrease in weight. This weight decrease can, however, be avoided by applying a small selection pressure to growth (Table 10.17, shape emphasis with no growth change index). Under this selection strategy, the gain in the width index and depth index are, respectively, 6% and 8% whilst the change in growth rate is very close to zero. Genetic selection is not

[2] The coefficient of variation is standard deviation divided by the mean. The value of 15% was calculated from individual animal measurements done on the F3 and F4 generations generated as part of this project.

a powerful tool to manipulate shape. For a standard sized oyster of typical shape,[3] changes in the width index and depth index of these magnitudes (that is 6 to 8%) represent approximately a 3 mm width increase and a 2 mm depth increase.

10.7.2. Genetic Gains with Different Selection Strategies

The predicted genetic gains when using different genetic selection strategies are shown in Table 10.18. The strategy that gives the best gain is family/within family selection, with gains for this strategy being up to two times larger for the targeted traits. Within family selection tends to give better gains than family selection, and presumably this reflects the large genetic variation that occurs within families. Family selection alone (that is, without applying selection to individuals within a family) gave the least gain. Family selection is more important than individual selection when heritabilities are lower. This is because an individual's phenotypic value is a poorer predictor of the EBV as heritability decreases. In this study, the traits had moderate to high heritabilities, and therefore individual phenotypic values were probably reasonable predictors of genetic value.

The gains predicted in this study were not based on large population sizes. This population consisted of only 20 families and approximately 700 individuals and higher gains would be possible with higher selection intensities. For example, doubling the population size would mean that the selection intensity could be increased by about 25% (Falconer and Mackay 1996, Appendix Tables) and, since genetic gain is directly proportional to selection intensity, genetic gains would be expected to also increase by the same proportion.

10.7.3. Impact of Changes in Growth Rate

Selections to improve growth rate could provide benefits in two ways – either by decreasing the grow-out time required to produce a desired product size, or resulting in larger harvest volumes being produced in a given grow-out time.

If larger harvest volumes are produced in a fixed grow-out time then the distribution of product grades will change. These changes have been predicted in Table 10.19. Importantly, significant reductions in the proportions in the smaller product grades are predicted when high economic weights are placed on growth. For example, 47% of oysters meet the small product grades (Bistro or Buffet) in the 'base case', but only 26% of oysters meet these grades when selecting using a 'growth emphasis no shape change index'. Therefore, selecting for improved growth rate may have important consequences with regard to the type of product produced. Consequently, we advise that the grower needs to be clear about what markets are intended before doing genetic selections for growth.

[3] Assumed length, width index and depth index is 75 mm, 0.66 and 0.33 respectively.

Table 10.1. Breeding plan for 1st heritability trials. Shaded squares failed. Numbers in parentheses are numbers of spat. (Oyster source: BB=Batman Bridge, W=Windermere, others are animals from the breeding program.)

	Males	A	B	C	D	E	F	G	H	I	J	K	L	M
	origin	BB	BB	BB	BB	W	W	W	W	W	W	W	BB	BB
Females														
	1 BB	1A	1B											
	2 BB	2A	2B											
	3 BB		3B	3C (100)										
	4 BB			4C (75)	4D (3000)									
	5 BB				5D	5E (30)								
	6 BB					6E	6F							
	7 BB						7F (50)	7G (1000)						
	8 BB							8G (2000)	8H (2000)	8I (2000)				
	9 W								9H	9I				
	10 CBIII										10J	10K (3000)		
	11 RxC										11J (100)	11K (2000)		
	12 BB											12K (3000)	12L (3000)	
	13 gold												13L (3000)	13M (3000)
		M	N	O	P	Q	R	S	T	U	V	W	X	Y
		BB	BB	BB	BB	BB	W	W	BB	W	W	BB	BB	BB
	14 W	14M	14N (2)											
	15 CBI		15N (100)	15O										
	16 CBI			16O (100)	16P									
	17 CBII				17P (3000)	17Q (3000)								
	18 BB					18Q (3000)	18R							
	19 BB						19R	19S (30)						
	20 BB							20S (1000)	20T					
	21 W								21T	21U (50)				
	22 BB									22U (30)	22V (30)			
	23 BB										23V (2000)	23W (3000)		
	24 gold											24W (3000)	24X (3000)	24Y

Table 10.2 Breeding plan for 2nd heritability trials (a) Crosses made Saturday February 2, 2002, (b) Crosses made Sunday February 3, 2002

(a)	Males																		
Females	A	B	C	D	E	F	G	H	I	J	K								
1	1A	1B																	
2	2A	2B																	
3		3B	3C																
4			4C	4D															
5				5D	5E														
6					6E	6F													
7						7F	7G												
8							8G	8H											
9								9H	9I										
10									10I	10J									
11										11J	11K								
12										12J	12K								
(b)	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
13	13L	13M																	
14	14L	14M	14N																
15			15N	15O															
16				16O	16P														
17					17P	17Q													
18						18Q	18R												
19							19R	19S											
20								20S	20T										
21									21T	21U									
22										22U	22V								
23											23V	23W							
24												24W	24X						
25													25X	25Y					
26														26Y	26AA				
27															27AA	27AB			
28																28AB	28AC		
29																	29AC	29AD	
30																		30AD	30AE
31																		31AD	31AE

Table 10.3. Breeding plan for the 3rd heritability trials. Crosses in bold are the intended genetic linking crosses between the two sets of matings made at the two different times. 15N and 24X are identical. See text. Numbers are approximate spat numbers retained. Identities given underneath table (b).

(a) The pair matings established on November 2 and 3.

		November 2							November 3							
		MALE														
Origin		P	BB	W	BB	BB	BB	SC	LS	O	BB	O	O	BB	BB	LS
	FEMALE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15(24)
BB	A	1A 2000	2A 2000													
BB	B		2B 2000	3B 2000												
P	C			3C 2000	4C 2000											
K	D				4D 300	5D 50										
SC	E					5E 2000	6E 2000									
BB	F						6F 500	7F 2000								
K	G	1G 500						7G 100								
O	H								8H 2000	9H 30						
BB	I									9I 2000	10I 2000					
LS	J										10J 30	11J 0				
LS	K											11K 6	12K 0			
BB	L												12L 2000	13L 2000		
LS	M													13M 10	14M 15	
LS	N(X)														**14N** 0	***15N*** 0
LS	O								8O 6							**15O** 10

(a) The pair matings established on November 30 and December 1.

		November 30							December 1							
		MALE														
Origin		O	LR	61	W	W	W	M1	59	LS	K	O	W	W	52	LR
	FEMALE	16	17	18	19	20	21	22	23	24(15)	25	26	27	28	29	30
59	P	16P	17P													
		1000	500													
O	Q		17Q	18Q												
			2000	200												
LS	R			18R	19R											
				2000	2000											
61	S				19S	20S										
					2000	2000										
LS	T					20T	21T									
						2000	2000									
K	U						21U	22U								
							2000	2000								
57	V	16V						22V								
		2000						2000								
O	W								23W	**24W**						
									2000	0						
LS	X(N)									***24X***	**25X**					
										2000	20					
LS	Y										25Y	26Y				
											500	2000				
52	Z											26Z	27Z			
												750	200			
K	AA												27AA	28AA		
													500	300		
M1	AB													28AB	29AB	
														1000	1000	
LS	AC														29AC	30AC
															500	2000
LS	AD								23AD							30AD
									2000							2000

BB=Bolduans Bay oysters (farmed), K=Kettering (d'Entrecasteaux Channel) (feral), LR=Leven River (Ulverston) (feral), LS=Little Swanport (Marine Culture Ltd) (farmed), M1=mass selection 1F (breeding program), O=Orford (East coast Tasmania) (feral), P=Pittwater (Tasman Isle Seafoods) (farmed), SC=Shellfish Culture Ltd (farmed), W=Woodbridge (d'Entrecasteaux Channel) (feral), 52, 57, 59, 61= from the respective families in the breeding program.

Table 10.4. 3rd heritability trials. Surviving numbers of SI animals. '-' indicates zero survival, 'N/A' indicates not available (insufficient spat to be allocated when sent to farm), 'lost' indicates missing bag.

November spawning							December spawning						
	Pittwater		Coles Bay		Smithton			Pittwater		Coles Bay		Smithton	
Family	R1	R2	R1	R2	R1	R2	Family	R1	R2	R1	R2	R1	R2
1A	49	68	lost	81	18	-	16P	-	-	18	-	-	-
2A	14	18	57	38	8	-	17P	-	-	-	10	-	-
2B	74	70	81	72	26	1	17Q	-	-	17	-	-	-
3B	50	17	61	65	-	1	18Q	-	-	N/A	N/A	N/A	N/A
3C	37	48	76	79	18	3	18R	-	-	28	26	-	-
4C	63	23	lost	49	11	6	19R	-	-	-	23	-	-
4D	85	93	81	14	N/A	N/A	19S	-	-	-	-	-	-
5D	80	16	N/A	N/A	N/A	N/A	20S	-	-	-	-	-	-
5E	45	39	73	65	23	8	20T	-	-	-	50	-	-
6E	60	59	73	79	35	9	21T	-	-	12	-	-	-
6F	70	49	63	76	-	29	21U	-	3	-	-	-	-
7F	61	53	83	83	22	15	22U	-	-	-	-	-	-
7G	76	N/A	N/A	N/A	N/A	N/A	22V	-	-	-	38	-	-
1G	81	67	73	52	N/A	N/A	16V	-	-	-	-	N/A	-
8H	13	28	30	66	N/A	4	23W	-	-	-	-	-	-
9H	48	N/A	N/A	N/A	N/A	N/A	24X	-	-	-	7	-	-
9I	28	1	59	41	12	3	25X	N/A	N/A	N/A	N/A	N/A	N/A
10I	36	28	58	62	10	5	25Y	-	-	-	-	-	-
10J	lost	N/A	N/A	N/A	N/A	N/A	26Y	-	-	-	-	-	-
11K	lost	N/A	N/A	N/A	N/A	N/A	26Z	-	-	-	-	-	-
12L	57	49	82	60	1	8	27Z	-	-	10	-	-	-
13L	42	13	61	37	4	-	27AA	-	-	-	-	-	-
13M	7	N/A	N/A	N/A	N/A	N/A	28AA	1	-	lost	-	-	-
14M	10	N/A	N/A	N/A	N/A	N/A	28AB	-	-	-	-	-	-
15O	7	N/A	N/A	N/A	N/A	N/A	29AB	-	-	-	21	-	-
8O	5	N/A	N/A	N/A	N/A	N/A	29AC	2	-	-	-	-	-
							30AC	-	-	17	-	-	-
							23AD	-	-	-	-	-	-

Table 10.5. Mean value of oyster traits measured at each farm at thirteen months (Time = 1)

Trait	Pittwater	Coles Bay	Total
length[a]	16.82	18.78	17.70
width[a]	14.54	16.73	15.53
depth[a]	8.17	8.44	8.29
weight[b]	1.24	1.34	1.31

[a]mm; [b]gm

Table 10.6. Heritabilities at 13 months calculated from linear mixed models fitted by R and ASReml

Trait	h^2 estimate from R	h^2 estimate from ASReml (se +/-)
length	0.38	0.38 (0.11)
width	0.23	0.23 (0.08)
depth	0.45	0.45 (0.12)
weight	0.26	0.24 (0.08)
log weight		0.50 (0.14)

Table 10.7. Census count of individuals at Pittwater and Coles Bay (March-April 2004)

Category	Pittwater	Coles Bay	Total
Alive	558 (92.0%)	314 (62.5%)	872
Dead[a]	30 (4.9%)	166 (33.1%)	196
No data[b]	19 (3.1%)	22 (4.4%)	41
Total	607	502	1109

[a]oyster shells gaping and no contents
[b]oyster missing from bag

Table 10.8. Count of live oysters at Pittwater and Coles Bay during September 2004 at age 22 months (Time = 2).

Family	Pittwater	Coles Bay
A1	27	5
A2	25	11
AB29	NA	6
AC30	NA	2
B2	42	21
B3	33	20
C3*	84	21
C4	11	4
D4	36	19
E5	32	8
E6	27	16
F6	42	17
F7	38	12
G1	26	20
H8	18	7
I10	26	15
I9	10	3
L12	37	22
L13	25	13
P16	NA	4
Q17	NA	1
R19	NA	3
T20	NA	1
V22	NA	3
Z27	NA	3
Total	539	257

*this group was subsequently split into three families (AD31, AE32, AF33) for ASReml coding due to a mix up in family tags and bags across the replicates

10.9. Family means and standard errors of difference (SED) for weight (gm), width index , depth index , length (mm), width (mm) and depth (mm) at Pittwater and Coles Bay at 25 months (Time = 3)

Farm	Family	Weight		Width index		Depth index		Length		Width		Depth	
		Mean	SED	Mean	SED	Mean	SED	Mean	SED	Mean	SED	Mean	SED
Pittwater	A1	41.65	10.75	0.83	0.09	0.45	0.04	63.02	7.07	51.88	6.67	28.24	2.77
	B2	30.26	9.16	0.80	0.08	0.43	0.04	59.08	8.11	47.10	5.50	25.09	2.63
	AD31	40.15	8.61	0.72	0.05	0.42	0.06	67.63	6.12	48.46	4.55	28.61	3.39
	D4	31.13	10.90	0.76	0.06	0.39	0.04	63.57	8.30	48.42	6.57	24.89	3.29
	E5	27.17	6.45	0.79	0.07	0.43	0.05	55.98	5.71	44.17	6.01	23.83	2.50
	E6	34.33	7.69	0.79	0.10	0.41	0.04	61.03	5.15	48.07	7.26	24.87	1.75
	F6	36.84	7.96	0.70	0.06	0.38	0.04	69.49	7.48	47.92	4.74	26.33	3.24
	F7	32.07	8.64	0.72	0.07	0.42	0.05	62.54	8.38	44.86	5.18	25.98	2.76
	G1	42.08	13.20	0.71	0.06	0.39	0.05	70.55	10.73	49.50	7.31	27.13	3.68
	H8	24.21	5.29	0.82	0.11	0.43	0.06	54.06	4.94	44.20	5.93	22.87	2.75
	I9	39.24	6.54	0.80	0.10	0.45	0.04	64.03	4.08	51.01	6.41	28.48	2.43
	I10	30.50	7.53	0.78	0.08	0.44	0.03	59.84	6.53	46.34	5.46	26.21	2.49
	L12	33.32	7.14	0.76	0.08	0.45	0.05	60.05	6.36	45.24	5.22	26.75	2.83
	L13	32.71	8.70	0.80	0.11	0.47	0.08	56.56	8.05	45.00	5.55	27.06	3.32
	A1	45.96	13.61	0.77	0.07	0.40	0.04	68.39	7.80	52.70	5.77	27.54	3.51
	B3	30.53	9.59	0.77	0.09	0.39	0.04	61.60	9.40	47.18	6.29	23.80	2.96
	C4	36.78	11.69	0.76	0.06	0.46	0.05	64.34	6.47	48.66	4.81	29.60	4.19
	AE32	48.33	11.10	0.77	0.07	0.42	0.04	68.19	6.24	52.26	3.88	28.68	2.38
	AF33	38.12	9.42	0.79	0.10	0.40	0.04	66.62	8.06	52.58	7.69	26.24	2.61
Coles Bay	A2	16.39	5.23	0.92	0.07	0.46	0.05	43.42	6.35	39.82	5.77	19.79	1.64
	B2	15.15	3.31	0.95	0.08	0.45	0.03	43.68	3.35	41.56	4.23	20.01	2.30
	AD31	24.31	5.48	0.83	0.01	0.44	0.03	52.57	7.64	43.16	3.57	22.79	1.85
	D4	14.27	4.13	0.83	0.10	0.45	0.04	43.19	5.25	35.78	6.07	19.37	2.18
	E5	12.84	1.81	0.84	0.14	0.46	0.03	40.03	4.89	34.00	8.09	18.29	1.42
	E6	16.74	3.28	0.78	0.07	0.41	0.04	47.19	4.04	36.78	4.72	19.21	1.21
	F6	19.13	4.80	0.89	0.08	0.41	0.03	50.27	6.92	44.61	7.03	20.44	1.44
	F7	13.96	2.38	0.88	0.15	0.43	0.04	45.19	5.53	38.96	3.13	19.11	2.51
	G1	21.15	5.29	0.77	0.06	0.42	0.03	50.89	5.46	39.31	4.69	21.05	2.62
	H8	11.79	4.19	0.93	0.11	0.47	0.05	38.54	5.45	35.69	4.72	18.08	1.70
	I9	14.24	0.00	0.90	0.00	0.55	0.00	36.73	0.00	32.92	0.00	20.16	0.00
	I10	12.90	3.66	0.87	0.09	0.44	0.03	41.27	3.85	35.99	6.05	18.11	1.50
	L12	15.72	6.19	0.87	0.09	0.47	0.04	43.05	6.53	37.02	5.12	19.91	2.99
	L13	18.03	6.47	0.91	0.08	0.47	0.04	44.68	5.99	40.45	4.84	20.76	2.86
	AC30	15.73	0.00	1.03	0.00	0.39	0.00	41.68	0.00	43.04	0.00	16.08	0.00
	B3	14.42	2.07	0.93	0.15	0.44	0.03	41.71	4.38	38.39	4.37	18.06	1.67
	C4	17.42	3.50	0.93	0.10	0.48	0.06	44.73	6.42	40.97	2.17	21.10	1.63
	R19	11.63	3.74	0.85	0.09	0.49	0.06	37.98	8.29	31.83	3.56	18.25	1.66
	V22	26.17	6.22	0.77	0.09	0.35	0.02	59.96	4.23	46.28	2.55	20.77	3.04
	Z27	14.37	1.79	0.69	0.12	0.39	0.01	49.31	4.98	33.51	2.95	19.16	1.76

10.10. Means and standard errors of difference for traits at 25 months (Time = 3) in Pacific oyster family lines, given across farms (SEDs given in parenthesis).

Farm	Weight (gm)	Width index (mm)	Depth index (mm)	Condition index* (gm)	Wet meat* (gm)	Wet shell* (gm)	Length (mm)	Width (mm)	Depth (mm)
Pittwater n = 503	34.90 (10.68)	0.76 (0.08)	0.42 (0.05)	70.45 (12.69)	5.26 (1.67)	22.55 (7.00)	63.05 (8.69)	47.80 (6.28)	26.26 (3.38)
Coles Bay n = 137	16.12 (5.35)	0.87 (0.10)	0.44 (0.05)	_	_	_	44.60 (6.61)	38.34 (5.60)	19.63 (2.33)
Combined n = 640	31.03	0.79	0.42	see Pittwater	see Pittwater	see Pittwater	59.25	45.85	24.89

*only non-destructive traits were measured on Coles Bay oysters due to small size

Table 10.11. ANOVA (from R) for fixed effects at 25 months for a combined site analysis

Trait	Effect	F value	*P* value
Weight	Farm	385.77	< 0.001
	Family	15.81	< 0.001
	Replicate	0.37	0.694
Length	Farm	556.29	< 0.001
	Family	17.93	< 0.001
	Replicate	0.36	0.698
Width	Farm	219.96	< 0.001
	Family	8.78	< 0.001
	Replicate	0.83	0.435
Depth	Farm	460.13	< 0.001
	Family	13.99	< 0.001
	Replicate	1.19	0.304

10.12. Variance component estimates for oyster traits at 25 months as estimated by various single-trait animal models fitted to family line data (from ASReml)

Farm	Trait*	$\sigma^2_A \pm$ se	$\sigma^2_{S\times D} \pm$ se	$\sigma^2_{G\times E}$** $\pm$ se	$\sigma^2_E \pm$ se	$h^2 \pm$ se
Pittwater	Weight^{II}	68.56 ± 26.57	0.00 ± 0.00		51.46 ± 14.49	0.57 ± 0.16
	Width index^{II}	0.22 ± 0.10	0.00 ± 0.00		0.67 ± 0.07	0.25 ± 0.10
	Depth index^{II}	0.13 ± 0.05	0.00 ± 0.00		0.15 ± 0.03	0.47 ± 0.15
	Condition index^{II}	87.42 ± 34.55	0.00 ± 0.00		81.43 ± 19.25	0.52 ± 0.15
	Wet meat^{I}	0.63 ± 0.33			2.28 ± 0.25	0.22 ± 0.10
	Wet shell^{II}	33.01 ± 12.75	0.00 ± 0.00		20.12 ± 6.84	0.62 ± 0.17
	Length^{II}	40.85 ± 15.83	0.00 ± 0.00		36.09 ± 8.78	0.53 ± 0.15
	Width^{II}	13.12 ± 5.63	0.00 ± 0.00		27.15 ± 3.61	0.33 ± 0.12
	Depth^{II}	6.43 ± 2.57	0.00 ± 0.00		5.82 ± 1.43	0.52 ± 0.16
Coles Bay	Weight^{II}	20.54 ± 10.12	0.00 ± 0.00		10.97 ± 6.10	0.65 ± 0.23
	Width index^{II}	0.61 ± 0.33	0.00 ± 0.00		0.59 ± 0.22	0.51 ± 0.22
	Depth index^{II}	0.17 ± 0.08	0.00 ± 0.00		0.07 ± 0.05	0.70 ± 0.24
	Length^{II}	41.20 ± 18.31	0.00 ± 0.00		9.45 ± 10.50	0.81 ± 0.23
	Width^{II}	2.72 ± 18.13	4.20 ± 9.12		25.67 ± 9.95	0.08 ± 0.56
	Depth^{II}	0.00 ± 0.00	0.70 ± 0.51		4.83 ± 0.64	0.00 ± 0.00
Combined	Weight^{III}	56.79 ± 20.72	0.00 ± 0.00	0.00 ± 0.00	44.88 ± 11.28	0.56 ± 0.15
	Width index^{III}	0.17 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.58 ± 0.06	0.30 ± 0.12
	Depth index^{III}	0.14 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.03	0.49 ± 0.16
	Length^{III}	41.89 ± 15.46	0.00 ± 0.00	0.00 ± 0.00	30.47 ± 8.35	0.58 ± 0.15
	Width^{III}	10.00 ± 5.81	0.00 ± 0.00	1.61 ± 1.59	27.50 ± 3.46	0.26 ± 0.14
	Depth^{III}	4.62 ± 1.93	0.00 ± 0.00	0.14 ± 0.28	5.87 ± 1.07	0.43 ± 0.15

*Model I, II, III as per data analysis section
**genotype × environment factors only estimated in the combined data sets

10.13. Genetic (r_g) and phenotypic (r_p) trait correlations at 25 months (Time = 3) as calculated by ASReml and Excel (Pearson's product moment correlation coefficient); r_g above diagonal (± standard error), r_p below (* $P < 0.01$)

Farm	Trait	Weight	Width index	Depth index	Condition index	Length	Width	Depth
Pittwater	Weight	*****	-0.30 ± 0.29	-0.22 ± 0.40	-0.74 ± 0.16	0.82 ± 0.09	0.87 ± 0.08	0.83 ± 0.09
	Width index	0.30*	*****	0.48 ± 0.24	0.67 ± 0.20	-0.70 ± 0.16	-0.07 ± 0.31	-0.28 ± 0.28
	Depth index	0.38*	0.49*	*****	0.15 ± 0.28	-0.51 ± 0.21	-0.28 ± 0.27	0.41 ± 0.23
	Condition index	0.65*	0.50*	0.52*	*****	-0.78 ± 0.14	-0.48 ± 0.24	-0.73 ± 0.16
	Length	0.91*	0.33*	0.40*	0.80*	*****	0.77 ± 0.13	0.57 ± 0.19
	Width	0.89*	0.56*	0.49*	0.84*	0.96*	*****	0.59 ± 0.20
	Depth	0.89*	0.41*	0.64*	0.82*	0.95*	0.94*	*****

		Weight	Width index	Depth index	Length	Width	Depth
Coles Bay	Weight	*****	-0.45 ± 0.32	-0.70 ± 0.22	0.92 ± 0.06	0.82 ± 0.14	0.93 ± 0.09
	Width index	0.76*	*****	0.86 ± 0.15	-0.70 ± 0.21	-0.02 ± 0.49	-0.24 ± 0.43
	Depth index	0.78*	0.85*	*****	-0.92 ± 0.08	-0.43 ± 0.34	-0.46 ± 0.40
	Length	0.97*	0.81*	0.84*	*****	0.72 ± 0.19	0.76 ± 0.20
	Width	0.96*	0.88*	0.87*	0.99*	*****	0.80 ± 0.21
	Depth	0.96*	0.83*	0.90*	0.99*	0.99*	*****

		Weight	Width index	Depth index	Length	Width	Depth
Combined	Weight	*****	-0.28 ± 0.26	-0.19 ± 0.26	0.82 ± 0.09	0.90 ± 0.06	0.82 ± 0.09
	Width index	0.43*	*****	0.68 ± 0.16	-0.72 ± 0.15	-0.20 ± 0.30	-0.21 ± 0.27
	Depth index	0.57*	0.68*	*****	-0.64 ± 0.16	-0.31 ± 0.26	0.23 ± 0.26
	Length	0.95*	0.52*	0.64*	*****	0.78 ± 0.11	0.60 ± 0.17
	Width	0.92*	0.66*	0.70*	0.98*	*****	0.66 ± 0.17
	Depth	0.93*	0.57*	0.76*	0.98*	0.98*	*****

Table 10.14. Details of the selection strategies and selection indices used for genetic gain estimates

Selection index	Economic weights [a]			Market scenario
	weight	width index	depth index	
1. Growth only	1	0	0	Value realised from weight only
2. Growth emphasis, no shape change	3	1	1	Value from weight, current shape adequate
3. Equal emphasis, growth and shape	1	1	1	Value equally from weight & shape
4. Shape emphasis, no growth change	3	1	1	Value from shape, current weight adequate
5. Shape only	0	1	1	Value realised from shape only

a) Economic weights give the relative importance of a one standard deviation shift in each trait

Table 10.15. Summary statistics for Estimated Breeding Values used in the evaluation of selection strategies

Trait	unit	Individual animal EBV's					Family EBV's				
		n	mean	max.	min.	sd	n	mean	max.	min.	sd
Weight (wt)	g	637	0	-12	22	6	20	0	-8	11	5
Width index (wi)	width/length	637	0	-0.11	0.09	0.04	20	0	-0.06	0.05	0.03
Depth index (di)	depth/length	637	0	-0.07	0.07	0.03	20	0	-0.05	0.04	0.02

Table 10.16. Definitions of oyster product classes

Product name	Top shell length (mm)
Bistro	50-60
Buffet	60-70
Standard	70-85
Large	85-100
Jumbo (and Super Jumbo)	>100

Table 10.17. Genetic gains (% of mean) when using different selection indices. Selections are made under a strategy of family and within family selection

Selection index	Relative weights	Weight	Width Index	Depth Index	Length	Width	Depth
Base case	0 : 0 : 0	0	0	0	0	0	0
Growth only	1 : 0 : 0	36	-4	-4	13	5	8
Growth emphasis, with no shape change	3 : 1 : 1	28	1	3	7	5	8
Equal emphasis growth & shape	1 : 1 : 1	13	6	6	0	3	5
Shape emphasis, with no growth change	1 : 3 : 3	1	7	8	-6	0	2
Shape only	0 : 1 : 1	-6	8	9	-8	0	1

Table 10.18. Genetic gains (% of mean) when using different selection strategies. Selections are made using an index that gives equal emphasis to weight, width index and depth index

Selection strategy	Relative weights	Weight	Width Index	Depth Index	Length	Width	Depth
Growth emphasis, no shape change							
Family selection	3 : 1 : 1	12	1	2	3	2	4
Family/within family selection	3 : 1 : 1	28	1	3	7	5	8
Within family selection	3 : 1 : 1	19	1	0	6	3	4
Equal emphasis growth & shape							
Family selection	1 : 1 : 1	5	3	3	-1	2	2
Family/within family selection	1 : 1 : 1	13	6	6	0	3	5
Within family selection	1 : 1 : 1	11	3	4	1	2	4
Shape emphasis, no growth change							
Family selection	1 : 3 : 3	1	3	4	-4	-1	1
Family/within family selection	1 : 3 : 3	1	7	8	-6	0	2
Within family selection	1 : 3 : 3	2	4	5	-3	1	2

Table 10.19. Distribution of final product in each size grade (% of total) when using different selection indices. Selections are made under a strategy of family and within family selection

Selection index	Length gain (%)	Percentage in each product grade [a]					
		Undersize <50 mm	Bistro 50-60	Buffet 60-70	Standard 70-85	Large 85-100	Jumbo >100
Base case	0	3	14	33	42	7	1
Growth only	13	0	3	13	43	33	7
Growth emphasis, no shape change	7	1	6	20	48	22	2
Equal emphasis growth & shape	0	3	14	33	42	7	1
Shape emphasis, no growth change	-4	5	22	38	32	3	0
Shape only	-8	10	32	39	19	1	0

a) Product grades are defined by length, and length classes for each grade are shown in mm. The 'base case' scenario assumes a mean of 70 mm and for other selection scenarios the mean was adjusted by the predicted length gain (e.g. the growth only scenario assumes a mean of 70 + 13 = 83 mm). All scenarios assume a coefficient of variation of 15%.

CHAPTER 11. Molecular Genetic Research

11.1. Introduction

We used molecular genetic techniques in this Project at multiple times and for various reasons. We wished to monitor genetic diversity, especially in the mass selection lines, to see what effect our procedures had on the level of diversity. We also wanted to genotype the parents of the family lines, for use in cases of disputed parentage. Additionally, we wanted to develop genotyping of young larval animals, for potential use in testing ploidy of individuals that might die before settlement. Allozyme techniques had earlier been deployed to examine Australian populations of Pacific oysters by English *et al.* (2000), and microsatellite loci had been used in earlier studies of some of our families (McGoldrick *et al.,* 2000).

11.2. Development of Additional Microsatellite Loci

McGoldrick *et al.*(2000) described the analysis of 21 di-nucleotide repeat microsatellite DNA loci in family 97-2 and four such loci in families 97-1 and 97-6. Null (non-amplifying) alleles were common in the Australian families, at an overall frequency of about 22%. This complicates interpretation of data, and we would prefer to use microsatellite loci without null alleles. We therefore spent some time developing and testing further microsatellite loci.

An enriched Pacific oyster microsatellite library (B. Evans unpublished data, March 2001, CSIRO Marine Research) was made to screen for tetranucleotide-repeat loci. Primers were developed for twelve candidate tetra loci, and the two best performing loci selected: *cmrCg204* and *cmrCg207*. These two loci were used with *ucdCg02*, *ucdCg22, cmrCg143*, *cmrCg61*, *ucdCg06* and *ucdCg08* (McGoldrick, 1997; McGoldrick *et al.*, 2000; Ward and Thompson, 2001) in multiplex reactions. These loci formed the basis of our microsatellite 'suite'. We were still keen however to increase the number of tri and tetra-nucleotide repeat loci.

At the beginning of 2004, eight new tri and tetra-repeat microsatellite loci (from Li *et al.,* 2003) were tested in family 97-2: *ucdCg-109* (tri), *ucdCg-126* (tetra), *ucdCg-167* (tetra), *ucdCg-192* (tri), *ucdCg-195* (tri), *ucdCg-198* (tri), *ucdCg-200* (tri) & *ucdCg-202* (tetra) in a single family 2B. Six of these eight show promise, but as yet we do not have information on whether null alleles are present in these loci.

11.3. Molecular Analysis in this Project

The suite of eight loci was then used in several studies. In the first study, we were interested to see if levels and patterns of genetic variation in the cultured lines were different to the Japanese/Tasmanian endemic and naturalised oyster collections (see section 11.4). However, more importantly, we were also interested to see what effect our culture activities had on the level of genetic diversity in the cultured lines. As part of this study, we also used allozyme loci developed by English *et al.* (2000).

Secondly, we felt that it was important to be able to genotype young larval animals; for potential use in testing ploidy of individuals that might die before settlement and

for early family identification. Again, only the suite of microsatellite loci were used for genotyping (see section 11.5)

Thirdly, we wanted to genotype the parents of family lines from F3, F4, and F5 for use in cases of disputed parentage. These animals were genotyped at the same microsatellite loci as those used in the above study (see section 11.6). We did not use allozyme loci for this as they were not considered variable enough to provide good discrimination power for parentage analyses.

11.4. Assessment of Genetic Diversity and Effective Population Size in Mass Selection Lines

11.4.1 Background

Virtually all commercial Pacific oyster spat in Tasmania and South Australia are hatchery-produced. The Australian Pacific oyster industry is thus well-poised to benefit from a genetic improvement program and, following a demonstration that genetic variation (for allozymes) was abundant in Tasmanian stocks (English *et al.* 2000), such a program was initiated in 1996/97 (Ward *et al*. 2000).

Selection programs elsewhere have been successful in improving oyster yields, particularly via faster growth rates (Newkirk and Haley, 1983; Paynter and Dimichele, 1990; Toro and Newkirk, 1990; Toro *et al*. 1995; Nell *et al*. 1999; Langdon *et al*. 2003). While these studies have demonstrated improvements from baseline, unselected control populations, loss of genetic diversity, reduced population sizes and deleterious effects of inbreeding remain a major concern (Hedgecock and Sly, 1990; Gaffney *et al.* 1992; Saavedra and Guerra, 1996; Launey *et al.* 2001).

Oysters are highly fecund animals and traditionally, only a few animals per generation used as contributing parents. Coupled with this, the census number of individuals (N) used per generation is likely to be much smaller than the actual numbers of oysters contributing their genes to the next generation (effective population size, N_e) (Lannan, 1980; Hedgecock and Sly, 1990; Gaffney *et al*., 1992). In addition, any selection procedure, be it family selection or mass selection, necessarily reduces the level of genetic diversity in the breeding pool compared with an outbred wild population (Hedgecock and Sly, 1990; Li *et al*., 2004 and references within).

We were particularly concerned that the mass selection procedures deployed in the Project may have led to the loss of high levels of variation in the later generations. To address the industry concerns, we used eleven allozyme loci and eight microsatellite loci to characterise the levels of genetic diversity in four cultured collections - the parents used to produce the first mass generation line, and then a single line from each of the M1, M3, and M4 generations.

We were also interested to see if levels of genetic variation in the cultured lines were different to naturalised (feral) Australian collections at Bridport (Tasmania) and Port Stephens (New South Wales, NSW) and two endemic Japanese collections (Sendai and Hiroshima, sources of the bulk of the original imports from Japan).

In addition, we were interested to see if genetic variation was impacted after successive generations of mass selection; perhaps due to reduced effective population sizes.

We observed genetic changes as allele frequency shifts and reductions and fluctuations in heterozygosity levels. We compared the census and effective population sizes of our cultured mass selection lines, the latter using sex ratio correction, and temporal variances in allele frequencies; a method particularly suited for use in aquaculture broodstocks (Pollack, 1983; Waples, 1989; Hedgecock and Sly, 1990).

11.4.2 Materials and Methods

Sample collections for Pacific oysters

We used the original allozyme data from the endemic and feral collections (English *et al.* 2000) as part of this genetic diversity study; as the original oysters from these populations were no longer available, it was not possible to undertake new allozyme screenings (Table 11.1). We re-sampled the population data from 50 individuals per collection. New allozyme data were obtained from individuals from several successive mass selected lines (Table 11.1). Varying census numbers of individuals had been used to develop these lines, depending on availability of putative parents.

For the microsatellite markers, we used genomic DNA from a random sample of oysters from the Japanese, Tasmanian and NSW collections (previously extracted, English *et al.* 2000). Genomic DNA was newly extracted from the same cultured individuals that had been just typed at the allozyme loci.

Allozyme loci

Based on previous allozyme studies in Pacific oysters (English *et al.*, 2000), and a pilot study undertaken on a random selection of individuals from the P1 collection, 11 allozyme loci were screened on Helena Titan III cellulose acetate (CA) plates (Table 11.2). Approximately 150 mg of adductor muscle was homogenized in three drops of distilled water. Homogenates were centrifuged at 13 000 rpm for five minutes at room temperature. The supernatant was then removed and centrifuged for a second time at 13 000 rpm for three minutes. We used the top layer for electrophoresis.

Electrophoretic running conditions and staining procedures were as described in Appleyard and Mather (2000), English *et al*. (2000) and Richardson *et al*. (1986) (Table 11.2).

Enzyme nomenclature, abbreviations, and allele designations of allozyme systems conformed to the recommendations of Shaklee *et al*. (1990). On each gel, an oyster previously genotyped was used as a standard. In each system, we designated alleles according to mobility and direction from the most common (100) allele. We estimated allelic frequencies from the presumptive genotypes assuming co-dominance of alleles.

DNA and microsatellite loci

We extracted total genomic DNA from approximately 50-100 mg of tissue from individual oysters using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle and Doyle, 1987; modified as in Appleyard, 1998). Genomic DNA pellets were resuspended in 150 μL of deionised water and stored at 4^0C. We used 1/10 dilutions of genomic DNA for working applications.

Two tetra-nucleotide repeat loci (obtained from an enriched Pacific oyster microsatellite library, B. Evans unpublished data, March 2001) and six di-nucleotide repeat loci (from a previous oyster study, GenBank submissions – McGoldrick, 1997; McGoldrick *et al*. 2000; Ward and Thompson, 2001) (Table 11.3) were used as part of our microsatellite suite. Oligonucleotides were synthesised by GeneWorks (Adelaide, South Australia) with one of the primer pairs being 5' end-labeled with a fluorescent tag - FAM, TET or HEX.

Four loci (*cmrCg204*, *cmrCg207*, *ucdCg02*, *ucdCg22*) were optimised for use in one multiplex reaction and the remaining loci (*cmrCg143*, *cmrCg61*, *ucdCg06*, *ucdCg08*) were optimised for use in another. PCR amplifications were performed in a PE-Applied Biosystems 9600 thermocycler in a total volume of 25 μL as per Appleyard *et al*. (2001) with between 0.14-0.18 μM for forward and reverse primers (varies according to primer). After an initial denaturing cycle of 93^0C × 10 minutes, samples were subjected to 93^0C × 30 seconds, 50^0C × 1 minute and 72^0C × 2 minutes for 35 cycles with a final extension step of 72^0C × 10 minutes.

Amplified products were used undiluted and mixed with formamide loading dye containing ABI Prism GeneScan 500 Tamra internal lane size standards (PE Applied Biosystems) and blue dextran loading dye. PCR products were analysed on an ABI Prism 377 DNA sequencer (PE Applied Biosystems) following Appleyard *et al*. (2001).

Statistical analysis

Genetic diversity in single collections

Allozyme and microsatellite data were analysed separately. The program FSTAT v. 2.9.3 (Goudet, 2001) calculated variation statistics (e.g., allele frequencies, mean number of alleles, F_{IS} values). F_{IS} is concerned with inbreeding in individuals relative to the subpopulation that the animal belongs (ranges from 0-1, with 1 representing complete inbreeding). While the mean number of alleles per locus is often used to measure genetic variation in populations, we also calculated allelic richness (R_s) using a rarefaction approach for R_S in FSTAT (i.e., standardisation of allelic richness to the smallest sample size).

Observed and Hardy-Weinberg expected heterozygosities (H_o and H_e respectively), and fits to Hardy-Weinberg equilibria (HWE), were calculated in ARLEQUIN v. 2.000 (Schneider *et al.* 2000). Conformation to HWE broadly tests for the random distribution of genotypes and expected allele frequencies from a sample of individuals (deviations from HWE may result from a number of forces including genetic drift). Chi-square analyses (CHIRXC; Zaykin and Pudovkin, 1993) were used to test for

differences in the number of alleles among the collections, and nonparametric Wilcoxon sign rank tests compared differences between mean H_o and H_e in each collection (Sokal and Rohlf, 1995). Linkage disequilibrium within collections was estimated using GENEPOP v. 3.2 (Raymond and Rousset, 2000). Microchecker v. 2.2.0 (van Oosterhout *et al*. 2003) assessed the probability of null alleles at each of the eight microsatellite loci.

Single locus exact tests in GENEPOP examined allele frequency differences among collections. We also used multi-locus exact tests in ARLEQUIN to test for collection differentiation across loci by testing the hypothesis of random distribution of individuals between pairs of collections. Pair-wise F_{ST} tests in ARLEQUIN also tested genetic differentiation among the eight collections across all loci. $F_{ST,}$ an inbreeding coefficient, is an estimate of the proportion of genetic variation attributed to population differentiation. Euclidean genetic distances among the collections were calculated in hierarchical AMOVAs (ARLEQUIN) resulting in Φ_{ST} estimations (Excoffier *et al*. 1992).

Significance levels in GENEPOP were determined after 500 batches of 5 000 iterations; in ARLEQUIN, significance was determined after 100 000 chain lengths. In all cases with multiple tests, we adjusted significance levels using a sequential Bonferroni procedure (Rice, 1989).

Temporal changes in genetic diversity of cultured collections and N_e

Changes in allele number, allelic richness, and mean heterozygosity over generations were assessed to see if culture activities affected levels of oyster genetic diversity.

The effective breeding size of each generation, N_e, was estimated from the numbers of males and females used to produce that generation (i.e., $N_e = 4N_mN_f/(N_m+N_f)$. Across multiple generations, it was estimated from the harmonic mean of the N_e values of the contributing generations.

We also estimated N_e from changes in allozyme and microsatellite diversity between generations, and these estimates were termed $N_{e,a}$ and $N_{e,m}$ respectively. The rationale is essentially that the larger the genetic fluctuations among generations, the smaller the effective breeding sizes.

Three different temporal methods were used in the program NeEstimator vers. 1.3 (Peel *et al*. 2004) to estimate population sizes (see Waples, 1989; Wang, 2001; Berthier *et al.* 2002). These methods required two temporally spaced samples (enabling temporal variance in allele frequency calculations).

Estimates of $N_{e.a}$ and $N_{e.m}$ assumed that the allele frequencies in the P1 collection were representative of the allele frequencies in the greater P1 generation and that this collection could be considered the founder generation at time $t = 0$. Allele frequencies in the M1 collection were estimated at time $t = 1$, in the M3 collection at time $t = 3$ and in the final M4 collection at $t = 4$. Unfortunately, samples were not available from the M2 generation.

11.4.3 Results

Allozyme diversity in oyster collections

Allele frequencies at the 11 allozyme loci were determined (see Appendix 7.1). Total numbers of alleles per locus ranged from 1 to 5. Mean numbers of alleles per locus across the eight collections ranged from 2.00 (*IDHP-2**) to 4.75 (*PEPS-1**), and H_o ranged from 0.061 (*IDHP-2**) to 0.688 (*PGM-1**) (Table 11.4). Individual locus F_{IS} values were generally modest, but were high for *IDHP-2** and *PGM-2** suggesting the possible presence of null alleles at these loci.

Average numbers of alleles per locus for the nine allozyme loci scored in all populations ranged from 3.18 in Port Stephens to 3.89 in M1 (Table 11.5). There were very few instances of private alleles across the eight collections. Chi-square tests demonstrated the total number of alleles per locus was not significantly different among the endemic ($\chi^2 = 1.279$, $P = 0.992$), naturalised ($\chi^2 = 3.114$, $P = 1.000$) or the cultured collections ($\chi^2 = 4.206$, $P = 0.999$). Overall, there was also no significant difference among the eight collections ($\chi^2 = 37.794$, $P = 0.999$). Likewise, there was no significant difference in the average number of alleles between the endemic (3.33 ± 0.28), naturalised (3.55 ± 0.000) and cultured oyster collections (3.64 ± 0.15) (P = 0.992).

Observed heterozygosity levels across the nine common loci were high. The highest levels were in the first two culture oyster collections, P1 and M1 (0.418 and 0.486 respectively), and the lowest (0.266) was seen in Port Stephens (Table 11.5).

We tested genotype proportions per locus and per collection for goodness-of-fit to HWE. After sequential Bonferroni correction for 79 tests, 17 of the tests demonstrated significant deviations from expectations. All were in the cultured oyster lines and were mainly due to a deficiency of heterozygotes. The cultured collections consequently had mean F_{IS} values which were about double those of the endemic/naturalised collections (Table 11.5). Wilcoxon sign rank tests demonstrated significant (although not large) differences between the overall observed and expected heterozygosity levels in each of the oyster collections ($P < 0.050$). The differences in these estimates were most pronounced in the M4 collection ($H_o = 0.381$ and H_e = 0.481).

Linkage disequilibrium tests demonstrated no significant loci association (data not shown) in any collection; therefore, it was assumed that the 11 loci were independent genetic markers.

The extent of allozyme differentiation among the collections was considerable. At eight loci, overall genic differentiation among the collections was significant (after Bonferroni correction) (all $P < 0.001$) (data not shown). There was however no significant differentiation among the collections at *GPI-1**, *PGM-1** (cultured collections) and *PGM-2** (cultured collections) ($P = 0.028$, $P = 0.113$, $P = 0.157$ respectively). Pair-wise exact tests were undertaken to find the source of this differentiation (Fisher's method, data not shown). Generally, the two endemic collections (Sendai and Hiroshima) and Port Stephens were not different to each other

but were different to all other collections. No significant pair-wise F_{ST} comparisons between the four cultured oyster collections (after correction) were detected (Table 11.6). Similarly, pair-wise F_{ST} values between the endemic/naturalised collections were also not significant (Table 11.6). However, we observed significant F_{ST} values between the endemic/naturalised collections and the cultured collections.

An AMOVA across the eight collections demonstrated a small and non-significant Φ_{ST} value (0.004, $P = 0.081$). When a hierarchical AMOVA analysis was undertaken on the endemic and naturalised collections versus the cultured oyster collections, 98.1% of the variance was observed within the collections ($\Phi_{ST} = 0.019$, $P = 0.078$). Variance among collections within the two groups was essentially zero ($\Phi_{SC} = -0.018$, $P = 1.000$). The endemic and naturalised collections were highly similar (within collection variance = 100.5%) as were the cultured oyster collections (within collection variance = 102.9%).

Microsatellite diversity in oyster collections

Allele frequencies at the 8 microsatellite loci were determined (see Appendix 7.2). The total numbers of alleles per locus ranged from 4 to 26. Mean numbers of alleles per locus per collection ranged from 5.13 (*cmrCg204*) to 20.38 (*ucdCg02*) (Table 11.4), although samples size across the collections were variable due to the non-amplification of some individuals. Average allelic richness per locus ranged from 2.61 (*cmrCg204*) to 7.34 (*ucdCg02*) (Table 11.4). Individual locus F_{IS} values were high for all loci except *cmrCg204* suggesting the probable presence of null alleles at most loci (see later).

Average numbers of alleles per locus for the eight loci ranged from 15.88 in Sendai to 8.62 in M4 (Table 11.4). Average allelic richness declined from highs of 6.56 and 6.50 in the endemic and naturalised collections of Sendai and Bridport respectively to lows of 4.77 and 4.15 in the cultured collections of M3 and M4. There were several instances of private alleles across the eight collections but no clear pattern.

Chi-square tests demonstrated that the number of alleles per locus was not significantly different between the endemic ($\chi^2 = 4.750$, $P = 0.699$), naturalised ($\chi^2 = 2.815$, $P = 0.908$) or cultured collections ($\chi^2 = 18.618$, $P = 0.617$). Likewise there was no significant differences between the endemic and naturalised collections ($\chi^2 = 10.186$, $P = 0.977$), cultured and endemic ($\chi^2 = 28.648$, $P = 0.778$) and cultured and naturalised ($\chi^2 = 27.400$, $P = 0.823$). There was also no significant difference among all collections ($\chi^2 = 34.905$, $P = 0.938$) with respect to number of alleles per locus.

The mean number of alleles per collection (across all loci) ranged from 8.62 (M4) to 15.88 (Sendai) (Table 11.5). With such hypervariable loci and differing sample sizes, allelic richness per locus was considered more appropriate for comparing different collections. This, on a per locus basis, ranged from 2.06 (*cmrCg204*, Bridport) to 8.53 (*cmrCg207*, Hiroshima). Average allelic richness (across all microsatellite loci, Table 11.5) declined from highs in the endemic/naturalised collections to lows in the cultured collections (with M4 showing the lowest R_S values).

Mean observed heterozygosities per microsatellite locus per collection were similar to the allozyme estimates, but the expected heterozygosities were substantially higher.

Observed variability levels across all loci were generally high throughout the collections and as expected, were higher than that observed at the allozyme loci (Table 11.5). The highest level of average observed heterozygosity was present in Sendai (0.444) and M4 (0.445) while the lowest observed heterozygosity (0.310) was seen in the M1 collection (Table 11.5).

Unlike the allozyme data, there were very large and significant differences between the overall average observed and expected heterozygosity levels across all collections ($P < 0.050$). Differences in observed and expected values are probably caused by the presence of null alleles at most of the loci (in particular at the dinucleotide repeat loci).
The majority of microsatellite loci did not conform to HWE expectations with the majority showing heterozygote deficiencies. Analyses from Microchecker showed probable evidence of null alleles (general excess of homozygotes for most alleles) at all loci except *cmrCg204*.

Of the 224 pair-wise comparisons for linkage disequilibrium, only *ucdCg08* and *ucdCg06* in the M4 collection were shown to be significantly linked (data not shown). However, as this relationship was only evident in the one collection, it was assumed that all loci represented independent genetic markers.

Allelic differentiation at the eight microsatellite loci was extensive, with exact tests demonstrating significant differences in allele frequencies among collections at each locus ($P < 0.001$). Fisher's exact tests across the eight loci indicated significant P values for all pair-wise collection comparisons (data not shown). However, exact tests on a per locus revealed Sendai, Hiroshima, Port Stephens and Bridport were not significantly different to each other at five loci, while there were significant differences at all eight loci among the four cultured collections (and between these collections and the other four collections).

While we observed significant allele frequency differences among all collections, the extent of this differentiation was relatively low but specific (Table 11.6). In most cases, the only significant F_{ST} comparisons were between the cultured collections and the endemic/naturalised collections (in particular M3 & M4 and all other collections) and among the four cultured oyster collections (Table 11.6). Pair-wise F_{ST} values between the endemic/naturalised collections were not significant.

AMOVA across the eight collections demonstrated a significant ($P < 0.001$) Φ_{ST} value of 0.028. When a hierarchical AMOVA analysis was undertaken on the endemic and naturalised collections versus the cultured oyster collections, 96.6% of the variance was observed within collections ($\Phi_{ST} = 0.034$, $P = 0.000$)**,** 1.4% of the variance was attributed to differences among the two groups and 2.0% attributed to variance among collections within the two groups.

Temporal changes and effective population sizes in cultured oysters

We were not able to calculate allelic richness in the allozyme data (as at least one locus in one collection had no genotyped individuals), but the average number of allozyme alleles and H_o remained relatively constant at about 3.60 and 0.395 respectively. However, signed rank tests demonstrated a significant difference ($P =$

0.050) in average microsatellite R_S values, with a gradual decrease over time from 5.76 in P1 to 4.15 in M4. (average numbers of alleles/locus also decreased from 14.38 to 8.62). As Table 11.5 indicates, while allelic richness and numbers of alleles per loci decreased steadily across the four cultured generations, average observed heterozygosity was not negatively affected (0.375 in P1 to 0.445 in M4, average 0.375). Average H_e was relatively constant for the allozymes, at about 0.450, but fell slightly for the microsatellites, from 0.793 in P1 to 0.697 in M4.

We then used various temporal methods (Table 11.7) to estimate a quantitative value of N_e for the cultured collections. These parameters were termed $N_{e,a}$ and $N_{e,m}$ for allozyme and microsatellite data respectively. $N_{e,a}$ estimates were generally larger than $N_{e,m}$ and their confidence intervals were usually much larger. Across time, each of the temporal analyses estimated a lower contributing N_e to the final cultured collection (Table 11.7).

Numbers of male and female broodstock used to produce each generation, together with total numbers (N_{census}), effective breeding numbers (N_e) and mean $N_{e,a}$ and $N_{e,m}$ (across all analysis types) are given in Table 11.8. Both sets of data showed the same trend; a progressive decrease in effective population sizes from P1 to M4. From hatchery records, 70 wild parents of unknown sex ratio (considered the P1) were used to produce the M1. While we did not sample the M2 (and do not have records on exactly how many parents from the M1 generation were used to produce the M2), records indicate that 33 females and 14 males from the M2 generation were used to produce M3. Following this, 6 females and 58 males from M3 were used as parents for the M4 generation. These putative census sizes were much higher than the estimated N_e values from all collections.

11.4.4 Discussion

Genetic diversity

Generally, all oyster collections were very similar in terms of allozyme diversity, with about 3.5 alleles per locus and average observed heterozygosity of around 0.350. Allozyme loci showed no significant allele loss from the original Japanese collections to the mass selected lines, and indeed we observed a slightly increased average number of alleles in the M4 collection (as compared to Sendai and Hiroshima). The average numbers of alleles in the cultured collections were also not different - levels fluctuated slightly across the generations with no clear pattern. This is probably due to one allele at each allozyme locus being at high frequency. English *et al*. (2000) also showed that farmed (not part of the selected lines) and feral Pacific oyster populations in Tasmania had similar allozyme diversity to feral and endemic populations. Smith *et al*. (1986) too showed no allozyme diversity loss between accidentally introduced Pacific oysters stocks in New Zealand and an endemic Japanese population.

There were no significant pair-wise F_{ST} values among the endemic and naturalised collections, or among the cultured collections, but there were some small and sometimes significant differences between the cultured and the introduced/endemic populations.

The microsatellite loci told a different story. Numbers of alleles were higher than for the allozymes and were highest in the endemic and naturalised collections. However, in most collections, the majority of loci did not show good agreement with HWE; there were significant differences between average observed and expected heterozygosity levels. The relative lack of observed heterozygotes probably reflects the known occurrence of non-amplifying or null alleles at many Pacific oyster loci (Magoulas *et al*. 1998; McGoldrick *et al*. 2000). In principle, other causes of such deviations could be gel-scoring errors, sampling effects, and inbreeding. However, these are likely to be minor contributors: the putatively outbred Japanese and naturalised collections also showed great differences in H_o and H_e.

We observed no significant pair-wise F_{ST} values among the endemic and naturalised collections, but some were present among the cultured collections and between some of the cultured and the endemic/naturalised collections.

Overall, the successive generations of mass selection led to a drop in diversity for the microsatellite but not allozyme loci. Average numbers of microsatellite alleles per locus fell from about 14 in P1 to about 9 in M4, with allelic richness also declining from about 6 in P1 to about 4 in M4. We attribute this to the loss of rare alleles and alleles of low frequency, and sampling effects (sampling only a small amount of the possible variation at the hypervariable microsatellite loci, small overall sample sizes per collection, and probably most importantly, bottleneck losses due to small numbers of parents in culture populations). We also looked at allelic richness in the cultured collections as it is considered more sensitive to the effects of severe bottlenecks than is heterozygosity (Luikart *et al.* 1998; Koljonen *et al.* 2002; Leberg, 2002; Säisä *et al.* 2003). Average allelic richness also declined across the generations. Heterozygosity values remained relatively constant for both allozymes and microsatellites. This is likely because rare alleles contribute little to the estimation of heterozygosity (Allendorf, 1986; Piry *et al*. 1999; Launey *et al*. 2001).

As indicated above, the decrease in the numbers of microsatellite alleles in the mass selection lines most likely reflects the rarity of many of the alleles: rare alleles will be lost before common alleles. Launey *et al.* (2001) observed a reduction in the number of alleles in three hatchery populations of *Ostrea edulis* as compared to their natural progenitors. Li *et al.* (2004) observed a loss of microsatellite alleles in hatchery produced *Haliotis discus hannai* strains as compared to wild populations, but they also reported lower expected heterozygosities. Evans *et al.* (2004) observed a loss of alleles per microsatellite locus in F1 progeny of *H. rubra* and *H. midae* but with no associated loss of heterozygosity. Losses of genetic diversity have also been commonly recorded in cultured finfish (e.g., *Salmo salar*, Verspoor 1988; Reilly *et al*. 1999; *Cyprinus carpio*, Aliah *et al*. 2000; *S. trutta*, Was and Wenne, 2002; *Paralichthys olivaceus*, Sekino *et al*. 2002; *Oncorhynchus mykiss*, Ward *et al*. 2003).

Clearly, microsatellite allele numbers have been affected to a greater extent than allozyme allele numbers. The Tasmanian stocks are descendants of millions of spat deliberately imported from Japan about 50 years ago, and thus far appear not to have gone through large enough bottlenecks to eradicate any of the common allozyme alleles. In other oyster studies, losses of allozyme variability in cultured populations have been reported, in some cases leading to monomorphism of the normally

polymorphic *GPI** and *AAT** loci (Gosling, 1982; Hedgecock and Sly, 1990, respectively).

However, we still consider that the oyster stocks retain sufficient heterozygosity for this loss not to be a major concern. As Beaumont and Hoare (2003) and others advise, it is not just the number of alleles at a locus that is important but also their relative frequency. Equal frequency alleles contribute strongly to heterozygosity; maintenance of heterozygosity ensures the deleterious effects of inbreeding do not accumulate. Therefore in our collections, if more common alleles start to be lost (e.g., allozyme alleles numbers decline), then that would become a concern and it might then be prudent to introduce new diversity into the mass selection lines. We recommend continual monitoring of all mass selected lines to ensure that genetic diversity is not seriously eroded.

Effective population sizes of the cultured oysters

We were also interested in estimating the effective population sizes of our breeding lines. In the mass selection collections, N_e was always less than N_{census}, sometimes markedly so when the sex ratio was highly skewed (as in the M3 broodstock).

Estimates of $N_{e,a}$ and $N_{e,m}$ were also derived from temporal fluctuations in allele frequencies between generations. Temporal estimates showed N_e reductions across the cultured generations. Both allozyme and microsatellite data reflected the same trends although confidence intervals were larger at the allozyme loci. Koljonen *et al.* (2002) suggested that the increased number of alleles observed at microsatellite loci provide better precision (hence smaller CIs) for N_e estimation.

Perhaps the more critical issue is the lack of concordance between N_e (N_{census} corrected for sex ratio) and genetically estimated N_e. The genetically derived estimates of $N_{e,a}$ and $N_{e,m}$ were always smaller than N_e. Since our sex ratio estimates take account of differing numbers of male and females used, the difference between the two types of estimates most likely reflects the high variance in family size commonly attributed to oysters (e.g., Hedgecock and Sly, 1990; Li and Hedgecock, 1998; Boudry *et al*. 2002). Family size variance results from: strip spawning of the oysters (there is no certainty around the actual parents that contributed as animals may not be fully ripe and able to fertilize successfully); varying family performance (sperm-egg interactions); fluctuating family sizes (due to poor survival of some families, gamete quality); differential viability among genotypes and most critically, uneven sex ratios (as in our study) (see also Hedgecock and Sly, 1990; Hedgecock *et al.* 1992; Boudry *et al*. 2002; Beaumont and Hoare, 2003; Shrimpton and Heath, 2003).

Low N_e/N ratios are not uncommon (see Frankham, 1995) and may be characteristic for species such as oysters that are highly fecund, have high juvenile mortality and slow growing families which may be lost from the grow-out system (Beaumont and Hoare, 2003). Reduced ratios have been observed in several oyster species previously (Hedgecock and Sly, 1990; Gaffney *et al*. 1992; Saavedra and Guerra, 1996; Launey *et al*. 2001).

In the breeding program, the various estimates of N_e declined as the breeding program progressed. This was largely due to a highly skewed sex ratio in the parents of the M4

generation. N_e, is reduced by unequal sex ratios and under these circumstances, the rate of inbreeding then largely depends on the numbers of the less numerous sex (Falconer and McKay, 1996). In the current study, we had very uneven sex ratios (particularly in the M4 generation, due to lack of availability of female parental oysters). We should also remember that sampling effects influence the genotypes that are analysed. As we only analysed a random sample of 50 individuals per generation, sampling likely provides a source of error in our calculations.

N_e can be increased in future generations by increasing broodstock numbers and by undertaking several 'mini' spawnings between equal numbers of males and females and then combining the embryos to produce a 'mass' generation (as suggested by Gaffney *et al.* 1992; Beaumont and Hoare, 2003). Alternatively, equal numbers of males and females directly used in the mass selections helps to increase N_e, assuming that adequate numbers of both sexes are available. If resources permit, the optimal method in a mass selection program is to carry out multiple single pair matings and then pool the progeny. We recommend these suggestions be considered in any future mass selection generations.

11.5. Larval Genotyping

We used the same eight microsatellite loci (as used in 11.4.2) to assess suitability of the markers for genotyping very young individuals. Our experiments also aimed to test the Mendelian inheritance of the alleles and the application of the eight loci for parentage assignment. Individual oyster larvae (spawned January 6, 2002, and sampled at 9 and 16 days of age) and tissue from their corresponding parents from two F4 family lines were obtained. We extracted DNA from the parental individuals as outlined in section 11.4.2.

For the offspring analysis, we collected larvae from each family (between 5-10 individuals) into ethanol and stored at them at -20^{0}C until DNA extraction. Using a Pasteur pipette, five larvae from each of the two families were transferred from ethanol to a microscope slide and isolated under a stereo-microscope. The larvae were left for several minutes under the microscope lamp to allow the ethanol to evaporate, the larvae were rinsed and agitated in distilled water, and then each individual larva was transferred to a 0.2 ml PCR tube with 4 μl of water. The PCR tubes were kept at -20^{0}C overnight, enabling ice crystals to form and break the larval cells, thereby exposing DNA for subsequent PCR reactions (see Evans *et al.*, 1998). From the 4 μl of water (containing individual oyster larvae cells), 2 μl was used for the first multiplex reaction and the remainder used as a template for the second multiplex reaction. Each larva and each parent was then genotyped at the eight microsatellite loci.

From this small-scale study, it appeared that the majority of microsatellite alleles were inherited according to Mendelian segregation ratios (deviations from Mendelian expectations have been recorded in other oyster studies, e.g. McGoldrick *et al.,* 2000) and genotyping of oysters at very early stages was possible. The loci therefore have wider applications for future parentage assignments, for monitoring family line 'purity', and possibly for checking ploidy status at an early stage from known crosses.

11.6. Screening of Parents from Family Lines

It is extremely difficult to monitor reproductive success and assign parentage in an aquaculture situation unless strictly controlled crossings are undertaken and families are maintained separately (or are easily identifiable by family tags). Genetic data from multilocus genotypes can play an important role in resolving parentage assignment and assessment.

The suite of microsatellite loci outlined in 11.4.2 was therefore used to genotype most of the parents that contributed to families from the F3, F4 and F5 generations (Appendix 7.3). PCR conditions, analysis on the DNA sequencer, and genotyping of individuals was as in 11.4.2. These parental genotypes are available for future reference.

The ability to determine successfully parentage or assign progeny back to a set of parents depends on a number of conditions: the number of putative or contributing parents, whether either one, two or none of the parents for each offspring are known, the genetic composition of the parents and the variability (i.e. the numbers of alleles/locus) of the genetic markers employed (Avise, 1994; Ferguson and Danzmann, 1998; Marshall *et al.*, 1998; Bernatchez and Duchesne, 2000).

Keeping this in mind, if the familial identity of an individual is questioned, the oyster should be genotyped at the eight microsatellite loci. We would then compare the multilocus genotypes from the oyster to the parental genotypes of the family to which it is thought the individual belongs. If genotypes of the parents that were thought to have contributed to the family are not available, this can be ‘back-tracked’ through the list of all known families to determine the possible genotypes for the family in question.

Table 11.1. Sample sizes of cultured mass selection lines, endemic and naturalised Pacific oyster collections used in allozyme and microsatellite analyses in the current study

Collection	Source	Source type	Sample size (allozymes, microsatellites)*
Sendai	Japan, 11/95	Endemic	50, 36
Hiroshima	Japan, 11/95	Endemic	50, 36
Bridport	Tasmania, 03/95	Naturalised	50, 36
Port Stephens	NSW, 06/95	Naturalised	50, 36
P1 Mass Sel^{n}	25/02/97	Cultured	50, 50
M1 Mass Sel^{n}	250/2/98	Cultured	50, 50
M3 Mass Sel^{n}	06/12/00	Cultured	50, 50
M4 Mass Sel^{n}	06/01/02	Cultured	50, 50

*individuals randomly sampled from collections

Table 11.2 Allozyme systems, allozyme number and number of putative loci screened in endemic, naturalised and cultured Pacific oyster collections (allozymes screened in adductor muscle supernatants)

Enzyme/protein name	Locus[a]	E.C. number[b]	Buffer	Structure
Aspartate aminotransferase (AAT)	*AAT-2**	2.6.1.1	TG	Dimer
Adenylate kinase (AK)	*AK**	2.7.4.3	TC	Dimer
Glyeraldehyde-3-phosphate dehydrogenase (GAPDH)	*GAPDH-2**	1.2.1.12	TC	Tetramer
Isocitrate dehydrogenase (NADP+) (IDHP)	*IDHP-1**	1.1.1.42	TC	Dimer
	*IDHP-2**			Dimer
Peptidase (PEP)	*PEPS-1**	3.4.11/13?	TG	Monomer
	*PEPS-2**			Dimer
Phosphogluconate dehydrogenase (PGDH)	*PGDH**	1.1.1.44	TC	Dimer
Phosphoglucose isomerase (GPI)	*GPI-1**	5.3.1.9	TC	Dimer
Phosphoglucomutase (PGM)	*PGM-1**	5.4.2.2	TC	Monomer
	*PGM-2**			

[a]analysis in cultured collections based on *PGDH**, *GAPDH-2**, *GPI-1**, *IDHP-1**, *IDHP-2**, *AK**, *PGM-1**, *PGM-2**, *AAT-2**, *PEPS-1**, *PEPS-2**: *PGM-1** &*PGM-2** not screened in endemic/naturalised collections

[b] E.C. number refers to the Enzyme Commission number assigned to each enzyme by the International Union of Biochemistry

Table 11.3 Microsatellite motif, primer sequences, number of alleles and allele size of Pacific oyster microsatellite loci. Motif listed is that obtained from the original oyster sequence used to generate primers

Locus	Motif	Primer sequences	GenBank submission	No. of alleles	Allele size range (bp)
cmrCg143[b]	$(TG)_8$ $(G)_{16}$	5'-CTTGCCATATTGCCATGTGT-3' 5'-CTTTTACATGGAATTGTCACAGG-3'	AF204061	18	137-177
ucdCg08[a]	$(GA)_{32}$	5'-CTTCTCACTTCACACACTCATCC-3' 5'-TTTAAACTTGTGTAAAGCATCTGGC-3'	AF051175	38	247-341
ucdCg06[a]	$(GA)_{12}$	5'-AAGCAACTATCAGTTTTTGGTAGC-3' 5'-AATGAGCTGACAGTTCATAGGC-3'	AF051172	20	141-193
cmrCg61[b]	$(CA)_6TA$ CATGTA $(TACA)_4$	5'-GATTGGTTGAAAAAATCACACG-3' 5'-TAACAGCAGCGCTACCATGC-3'	AF204062	20	193-233
ucdCg02[a]		5'-TTGCAGGAAGCAAGAGATGA-3' 5'-CTTGTTAACTGCCGGTGAGG-3'	-----	40	149-227
ucdCg22[a]		5'-CCCCAACTCAAACAGACGTT-3' 5'-TAGTCAGACGTTCCTAACTCTTCG-3'	-----	34	219-287
cmrCg204[b]	(GACA)	5'-ATTCCTCAAACGCATAAAAAAGTGT-3' 5'-TCCACCGAACAAAATAGAGCAATA-3'	-----	8	159-219
cmrCg207[b]	(CTAT)	5'-CATTGACGCAGTTTATATGACG-3' 5'-NNNNACGATAGAGTTATCTCTCCTA-3'	-----	34	253-397

[a]developed at the University of California, Davis (McGoldrick, 1997, McGoldrick *et al.*, 2000)
[b]developed at CSIRO Marine Research (McGoldrick *et al.*, 2000; Ward and Thompson, 2001; Evans 2001 unpublished data)

Table 11.4 Locus variability for Pacific oyster collections. Mean sample size per locus (n), number of alleles ($n_{alleles/collections}$), heterozygosity observed (H_o), heterozygosity expected under equilibrium conditions (H_e) (Nei, 1978), allelic richness (R_S, only estimated for microsatellite loci as at least one locus in one sample had no genotyped individuals) & F_{IS} = inbreeding coefficient (Weir and Cockerham (1984)).

	Average			Average heterozygosity		
Marker	n	$n_{alleles/collections}$	R_S	H_o	H_e	F_{IS}
Allozyme loci[a]						
*AAT-2**	49.5 ± 0.3	3.00 ± 0.00	-----	0.436 ± 0.059	0.434 ± 0.050	-0.100 ± 0.035
*AK**	49.4 ± 0.3	4.13 ± 0.30	-----	0.339 ± 0.030	0.410 ± 0.039	0.094 ± 0.053
*GAPDH-2**	43.9 ± 5.3	2.88 ± 0.35	-----	0.429 ± 0.018	0.532 ± 0.026	0.163 ± 0.052
*GPI-1**	49.6 ± 0.2	4.73 ± 0.18	-----	0.191 ± 0.031	0.200 ± 0.029	0.015 ± 0.034
*IDHP-1**	48.6 ± 0.6	3.63 ± 0.18	-----	0.434 ± 0.039	0.494 ± 0.018	0.248 ± 0.055
*IDHP-2**	48.9 ± 0.4	2.00 ± 0.19	-----	0.061 ± 0.018	0.175 ± 0.051	0.378 ± 0.145
*PEPS-1**	49.5 ± 0.2	4.75 ±0.16	-----	0.479 ± 0.053	0.513 ± 0.053	0.040 ± 0.046
*PEPS-2**	47.9 ± 0.6	3.88 ± 0.35	-----	0.571 ± 0.019	0.630 ± 0.014	0.080 ± 0.030
*PGDH**	37.0 ± 6.3	3.00 ± 0.44	-----	0.266 ± 0.048	0.345 ± 0.061	0.256 ± 0.069
*PGM-1**	49.8 ± 0.3	4.00 ± 0.00	-----	0.688 ± 0.011	0.665 ± 0.009	-0.046 ± 0.026
*PGM-2**	49.5 ± 0.5	3.25 ± 0.25	-----	0.470 ± 0.064	0.533 ± 0.026	0.264 ± 0.125
Microsatellite loci						
ucdCg22	31.4 ± 4.0	14.63 ± 1.76	6.46 ± 0.44	0.326 ± 0.055	0.892 ± 0.023	0.623 ± 0.074
ucdCg08	21.9 ± 4.5	9.62 ± 1.27	5.71 ± 0.38	0.176 ± 0.071	0.894 ± 0.028	0.795 ± 0.084
ucdCg06	39.1 ± 2.4	12.88 ± 0.81	6.37 ± 0.38	0.502 ± 0.040	0.880 ± 0.028	0.429 ± 0.037
ucdCg02	37.1 ± 4.1	20.38 ± 1.72	7.34 ± 0.44	0.505 ± 0.076	0.923 ± 0.021	0.451 ± 0.081
cmrCg143	38.4 ± 3.2	10.50 ± 0.63	5.61 ± 0.24	0.343 ± 0.039	0.856 ± 0.018	0.592 ± 0.052
cmrCg61	40.9 ± 2.6	9.50 ± 0.82	4.14 ± 0.26	0.296 ± 0.041	0.654 ± 0.036	0.553 ± 0.051
cmrCg204	42.1 ± 2.5	5.13 ± 0.35	2.61 ± 0.12	0.453 ± 0.052	0.419 ± 0.041	-0.089 ± 0.044
cmrCg207	32.3 ± 4.0	15.13 ± 2.63	6.33 ± 0.72	0.357 ± 0.066	0.867 ± 0.045	0.594 ± 0.068

[a]NB: the two allozyme loci *PGM-1** and *PGM-2** were typed only in the cultured collections.

Table 11.5 Collection variability for Pacific oysters. Mean sample size per locus (n), number of alleles ($n_{alleles}$), heterozygosity observed (H_o), heterozygosity expected under equilibrium conditions (H_e) (Nei, 1978), allelic richness (R_S) & F_{IS} = inbreeding coefficient (Weir and Cockerham (1984)).

	Average			Average heterozygosity		
Collection	n	$n_{alleles}$		H_o	H_e	F_{IS}
Nine allozyme loci						
Sendai	48.3 ± 0.5	3.44 ± 0.38		0.369 ± 0.060	0.402 ± 0.065	0.027 ± 0.037
Hiroshima	47.8 ± 0.9	3.22 ± 0.43		0.312 ± 0.063	0.343 ± 0.064	0.078 ± 0.049
Bridport	49.7 ± 0.2	3.56 ± 0.38		0.374 ± 0.058	0.410 ± 0.063	0.053 ± 0.039
Port Stephens	45.1 ± 4.1	3.18 ± 0.46		0.266 ± 0.006	0.297 ± 0.060	0.044 ± 0.033
P1	47.8 ± 0.5	3.67 ± 0.29		0.418 ± 0.075	0.466 ± 0.061	0.179 ± 0.112
M1	48.8 ± 0.6	3.89 ± 0.35		0.486 ± 0.064	0.494 ± 0.050	0.211 ± 0.089
M3	44.4 ± 4.9	3.44 ± 0.44		0.331 ± 0.078	0.398 ± 0.070	0.117 ± 0.055
M4	45.2 ± 4.8	3.56 ± 0.34		0.346 ± 0.059	0.449 ± 0.062	0.200 ± 0.101
Eleven allozyme loci						
P1	47.9 ± 0.4	3.63 ± 0.24		0.403 ± 0.074	0.492 ± 0.053	0.202 ± 0.100
M1	49.0 ± 0.5	3.91 ± 0.29		0.415 ± 0.058	0.514 ± 0.043	0.190 ± 0.078
M3	49.5 ± 4.1	3.45 ± 0.37		0.382 ± 0.064	0.426 ± 0.061	0.104 ± 0.046
M4	46.1 ± 3.9	3.55 ± 0.28		0.381 ± 0.061	0.481 ± 0.055	0.160 ± 0.086
Eight microsatellite loci			R_S			
Sendai	32.9 ± 2.1	15.88 ± 2.61	6.56 ± 0.64	0.444 ± 0.073	0.855 ± 0.065	0.443 ± 0.104
Hiroshima	25.3 ± 3.9	12.50 ± 2.06	5.91 ± 0.70	0.315 ± 0.074	0.844 ± 0.067	0.561 ± 0.135
Bridport	28.8 ± 2.3	15.00 ± 2.24	6.50 ± 0.75	0.360 ± 0.071	0.834 ± 0.089	0.525 ± 0.091
Port Stephens	25.8 ± 3.0	11.75 ± 1.37	5.95 ± 0.56	0.339 ± 0.050	0.844 ± 0.052	0.554 ± 0.106
P1	45.0 ± 2.1	14.38 ± 2.20	5.76 ± 0.66	0.375 ± 0.053	0.793 ± 0.078	0.471 ± 0.097
M1	35.8 ± 4.5	10.12 ± 2.06	4.96 ± 0.61	0.310 ± 0.068	0.758 ± 0.068	0.562 ± 0.107
M3	42.9 ± 2.0	9.50 ± 1.55	4.77 ± 0.46	0.370 ± 0.078	0.760 ± 0.044	0.488 ± 0.122
M4	47.4 ± 1.2	8.62 ± 0.98	4.15 ± 0.29	0.445 ± 0.060	0.697 ± 0.054	0.342 ± 0.100

Table 11.6 Pair-wise F_{ST} values for allozyme and microsatellite data between endemic, naturalised and cultured oyster collections. F_{ST} values below diagonal (allozymes) and above diagonal (microsatellites) with probabilities estimated after 100 000 randomisations of the data. Significant values (after sequential Bonferroni correction, per parameter estimate) are shown in bold. Allozyme comparisons among the cultured collections based on 11 loci, all other comparisons on 9 loci.

Collection	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
Sendai	-----	-0.061	-0.005	-0.039	**0.026**	-0.033	**0.056**	**0.089**
Hiroshima	-0.005	-----	-0.013	0.017	-0.028	0.026	0.011	0.004
Bridport	0.004	0.016	-----	0.006	0.012	0.016	**0.063**	**0.080**
Port Stephens	-0.030	-0.018	-0.001	-----	-0.005	**0.042**	**0.044**	**0.050**
P1	0.019	**0.034**	**0.025**	0.020	-----	-0.023	**0.060**	**0.093**
M1	**0.051**	**0.058**	**0.056**	**0.037**	0.015	-----	0.012	**0.022**
M3	-0.033	-0.008	-0.014	0.019	-0.020	-0.025	-----	**0.073**
M4	**0.027**	0.004	**0.045**	-0.073	0.003	-0.044	-0.124	-----

Table 11.7 Temporal estimates of effective population sizes of mass selected Pacific oysters (95% CI given in parenthesis)

Collection comparison	Gen. diff.	Allozyme TM3*	Allozyme MLNE**	Allozyme Temporal***	Microsatellite TM3*	Microsatellite MLNE**	Microsatellite Temporal***
P1 – M1	1	32.9 (0.7 – 97.1)	56.8 (29.9 – 251.4)	18.4 (8.4 – 44.4)	7.0 (5.5 – 9.5)	22.8 (19.0 – 27.6)	10.7 (7.2 – 16.0)
M1 – M2	1	Na	Na	Na	Na	Na	Na
M2 – M3	1	Na	Na	Na	Na	Na	Na
M3 – M4	1	5.6 (3.9 – 10.3)	14.6 (11.3 – 20.0)	7.7 (3.6 – 16.8)	6.5 (5.0 – 7.0)	21.1 (18.0 – 25.4)	5.3 (3.7 – 7.3)
M1 – M3	2	21.1 (10.6 – 36.2)	25.4 (17.3 – 42.1)	39.6 (16.1 – 142.5)	14.5 (11.7 – 19.0)	28.3 (22.9 – 35.0)	14.5 (9.7 – 21.4)
M1 – M4	3	26.3 (15.7 – 38.5)	31.5 (21.5 – 48.2)	32.2 (16.2 – 66.0)	14.5 (12.0 – 19.1)	28.4 (22.3 – 31.3)	14.0 (9.8 – 19.5)
P1 – M4	4	35.7 (21.7 – 52.7)	34.8 (24.0 – 52.9)	33.3 (16.2 – 62.8)	17.1 (14.4 – 20.6)	27.5 (23.5 – 32.5)	21.4 (15.7 – 28.6)

Na = not available

*Berthier *et al.* (2002) Bayesian likelihood estimation from TM3, external method provided in NeEstimator (Peel *et al.*, 2004), based on 5000 updates

**Wang (2001) pseudo-likelihood estimation from MLNE, external method provided in NeEstimator (Peel *et al.*, 2004)

***Moments based temporal estimation based on Waples (1989)

Table 11.8 Cultured oyster collections. N_f and N_m are numbers of female and male broodstock used, N_{census} the total number of broodstock, N_e the estimated effective numbers of broodstock corrected for sex ratio ($4N_mN_f/N_m+N_f$), $N_{e.a}$ the effective population size based on temporal variance in allozyme frequencies, $N_{e.m}$ the effective population size based on temporal variance in microsatellite frequencies. $N_{e.a}$ and $N_{e.m}$ are mean values of the three estimates from Table 5. Note: M2 progeny not genotyped.

Collection comparison	N_f	N_m	N_{census}	N_e	$N_{e.a}$	$N_{e.m}$
P1 – M1	<70	<70	70	≤70	36.0	13.5
M1 – M2	20	27	47	46.0	Na	Na
M2 – M3	33	14	47	39.3	Na	Na
M3 – M4	6	58	64	21.8	9.3	11.0
P1 - M4	<70, 20, 33, 6	<70, 27, 14, 58	≤70, 47, 47, 64	37.3	34.6	22.0
M1 – M3	20, 33	27, 14	47, 47	42.6	28.7	19.1
M1 – M4	20, 33, 6	27, 14, 58	47, 47, 64	32.2	30.0	19.0

Na = not available

CHAPTER 12. Commercialisation, Management and Communications

12.1 The Development of Australian Seafood Industries Pty Ltd

The work of this FRDC project (FRDC 2000/206), the previous FRDC project (97/321), and the CRC Aquaculture work on Pacific oyster genetics, was all directed to producing a more efficient and productive Pacific oyster industry. It was always anticipated that genetic improvement would lead to better-performing lines of oysters that would need to be accepted and deployed by the industry. Various meetings were held between the interested parties – the oyster industry, CSIRO Marine Research, Tasmanian Aquaculture and Fisheries Institute (TAFI), FRDC and the CRC Aquaculture – to plot a course forward. Perhaps the pivotal meeting was one at TAFI on July 26, 1999, when a working party was established to investigate the possibility a Joint Venture company. Members of this working party were: Patrick Hone (FRDC), Peter Montagu (CRC Aquaculture), Barry Ryan (TORC) and Mike Whillas (SAOGA). The working group met in Adelaide on September 28, 1999, to define its terms of reference and general goals. Subsequent meetings between the interested parties included those at CSIRO Marine Research (April 2000) and Wrest Point (Hobart) (May 2000 and October 2000).

A joint venture company, Australian Seafood Industries Pty Ltd (ASI), was established following a meeting of key Tasmanian and South Australian oyster industry representatives on November 16, 2000. The Board of the company comprised Bob Cox as Secretary, two directors (one from TORC, Barry Ryan, and one from SAORC, Gary Zippel) and two alternate directors (one from TORC, Vicky Wadley, who left soon afterwards and was replaced by Martin John, and one from SAOGA, Michael Whillas).

ASI circulated a draft business plan in February 2001. This stated that ASI had been established by the South Australian and Tasmanian oyster industries to: commercialise seafood research, drive industry focused shellfish research, develop the shellfish industry, pursue industry and government funds and, promote the growth of industry IP. It was recognised that this provides a broad scope of activity, but that in the first instance the company will concentrate on commercialising the outcomes of genetic research on the Pacific oyster conducted under the aegis of the CRC Aquaculture and FRDC. Essentially it was to provide a mechanism of technology transfer to the oyster industry and to ensure the ongoing nature of the selective breeding program on the termination of FRDC 2000/206. It was suggested that growers pay a premium for improved strains which would cover ongoing ASI costs.

ASI held its inaugural meeting on April 2 & 3, 2001, in Smithton. This was attended by the ASI Board, further industry members, and representatives from FRDC, CSIRO and TAFI. It was agreed that a collaborative agreement be established between ASI, FRDC, CSIRO, and TAFI.

A draft collaborative agreement was circulated by Tim Mangan of CSIRO Marine Research on July 2, 2001. Revisions of this draft were the subject of meetings

between ASI, FRDC, CSIRO and TAFI on July 20 and November 7, 2001. The agreed Management and License Agreements between ASI, FRDC, CSIRO, and UTAS were fully executed on December 20, 2001. An Equity Agreement between FRDC, CSIRO and UTAS was also signed at about the same time.

These Agreements, *inter alia*, established a Management Committee for FRDC2000/206. This met for the first time on April 11, 2002.

An August 2002 report from Barry Ryan, then chair of ASI, stated: ASI have identified the broad elements of its strategy, which will be developed as a combined Business Plan/Commercialisation Strategy. The intention is to employ an expert consultant to develop the strategy, with a completion target date of 30 November 2002. An application was submitted for Comet[4] assistance to fund the consultancy. This was successful, and COMET awarded $35k to ASI. In November 2002, ASI commissioned Mr Martin Rees of KPMG to draft a Commercialisation Strategy, which was provided to ASI in March 2003. Subsequently, in February 2004, ASI produced its Commercialisation Strategy, which drew heavily on the KPMG report.

A major milestone for ASI was the appointment of Scott Parkinson as General Manager, in August 2003.

ASI produced its first newsletter in June 2003, and its second in March 2004. Both were widely distributed to industry.

12.2. Management Committee Meetings

Membership of this committee was John Wilson (FRDC), Colin Buxton (TAFI), Peter Rothlisberg (CSIRO Marine Research), Barry Ryan (ASI) and Gary Zippel (ASI). The PI (Bob Ward) on the Project and/or Peter Thompson (CSIRO Marine Research) always attended to report on Project progress. Other participants in the Project were invited to attend as required. These included Andrew Swan (CSIRO Livestock Industries), Scott Parkinson (ASI) and Tim Mangan (CSIRO Marine Research).

This Committee, at its first meeting on February 4, 2002, established a Project Team. The second meeting was at Wrest Point on September 9, 2002. The third meeting was a teleconference on April 16, 2003. This welcomed Nick Elliot as CSIRO Marine Research's representative as Peter Rothlisberg had stepped down. The fourth meeting was at Port Lincoln on July 21, 2003, with two participants on the telephone. The fifth and sixth meetings were teleconferences on November 4, 2003, and March 22, 2004, respectively. These meetings provided formal contacts between ASI, CSIRO, TAFI, FRDC, and the Project. Both the progress of the Project and the progress of ASI were monitored. Meetings were chaired by FRDC.

12.3. Project Team Meetings

The Project Team consisted of Bob Ward (PI), Peter Thompson (CSIRO Marine Research), Di Murdoch (TORC & Tasman Isle Seafoods) and Gary Zippel (SAORC

[4] COMET (Commercialising Emerging Technologies) is an AusIndustry Commonwealth Government Program.

& Zippel Enterprises). Other people attended as required, including Andrew Swan, Barry Ryan and Scott Parkinson. The role of the Project Team was to assist with the day-to-day running of the project and its ongoing development, and to ensure good cooperation between the industry and the scientists.

The first meeting of the Project Team was April 11, 2002. The second Project Team meeting was on June 5/6 2002. This was a two day meeting at CSIRO Marine Research, the first one and a half days of which were devoted to the breeding program and included several invitees. Present for the breeding program component of the meeting were: Sharon Appleyard (CSIRO Marine Research), Graeme Cameron (Camerons of Tasmania), Nick Elliott (CSIRO Marine Research), Greg Kent (Aquaculture, University of Tasmania), Greg Kirby (Biology, Flinders University), Xiaoxu Li (Aquatic Sciences, SARDI), Di Murdoch (Project Team, ASI); Laurie Piper (CSIRO Livestock Industries), Richard Pugh (Shellfish Culture), Barry Ryan (ASI), Andrew Swan (CSIRO Livestock Industries), Peter Thompson (Project Team, CSIRO Marine Research), Bob Ward (Project Team, CSIRO Marine Research), Gary Zippel (Project Team, ASI). The major conclusions were: (1) All present agreed that combined selection (within and between families) should continue to be the basis for the breeding program, with relatively minor resources being allocated to continuing the mass selection and inbreeding lines, and (2) All present agreed that the experiment to estimate genetic parameters (i.e. the selection index experiment) needed to be repeated.

The third meeting was on December 20, 2002, and the fourth meeting on September 2, 2003.

There were many other meetings between Project Team members and ASI which were less formal and have not been documented here.

12.4. Other Interactions between the Project and Industry

June 8 2002 was the annual meeting of Tasmanian Shellfish Producers, held that year at St Helens. Barry Ryan and Di Murdoch explained the rationale behind establishing ASI and its relationship to FRDC2000/206, and Bob Ward described the selection program and results thus far. Ten representatives from each of nine F2 families and six F3 families were on display, identified by a new ‘public’ code (not the ‘private’ code used in reports). Some of the farmers nominated their best and worst families from those on display (Table 12.1). This was not intended as a realistic assessment of all the families, but was an exercise to raise farmers’ interests and knowledge of the Project. Interestingly, opinions were divided among the ‘best’ families presented, but were far more unanimous concerning the ‘worst’ families.

July 17 2002 saw a presentation by Mat Willis on the Project to a South Australian oysters growers Open Day.

October 11 2002 saw a presentation by Bob Ward on the selection index work to a TORC Annual Growers Forum at Hobart.

June 2003 saw publication of the inaugural ASI newsletter, which included an article and data on the Project supplied by Peter Thompson.

March 2004 saw publication of the second ASI newsletter. This comprised the Abstracts of talks given by Sharon Appleyard and Bob Ward at the conference Genetics in Aquaculture VIII (see below), together with a short article on the breeding program prepared by Sharon Appleyard.

12.5. Publications / Media Reports

January 17, 2002. A media release on the joint agreement to commercialise the selective breeding research was distributed by CSIRO and TAFI under the inspired header "Here Comes the Stud Oyster" (see Appendix 8). This received excellent media coverage. TV coverage included the ABC in Hobart and in Perth and Southern Cross in Hobart. Numerous radio stations gave the story including ABC Hobart, Adelaide, Sydney, WA, Northern Territory, Canberra, Radio National, and Radio 2EU (the ABC *Country Hour* in several states ran the story). Newspaper coverage included the Age, the Sydney Morning Herald, the Mercury (Hobart) and the Adelaide Sunday Mail. Bob Ward did most of the interviews, supported by Gary Zippel and Diana Murdoch.

September 21, 2002. Peter Thompson gave a presentation on the Project to Aquafest, Hobart.

May 21, 2003. Bob Ward presented an invited talk on the Project to World Aquaculture 2003, in Salvador, Brazil.

August 27, 2003. Sharon Appleyard gave a talk on the molecular genetics aspects of the Project to the School of Aquaculture, University of Tasmania, Launceston.

November 10 and November 12, 2003. Sharon Appleyard and Bob Ward , respectively, presented talks on the Project to Genetics in Aquaculture VIII, in Puerto Varas, Chile.

March 3, 2004. Peter Thompson presented an invited talk on the Project to World Aquaculture 2004, in Hawaii, USA.

July 13, 2004. Bob Ward presented an invited talk on the Project to the World Congress of Malacology, Perth.

September 29, 2004. Bob Ward presented an invited talk on the Project to Australasian Aquaculture, Sydney.

There are two publication thus far from this Project (others are in preparation):

Thompson, Peter and Ward, Bob. (2005). Genetic improvement of Pacific oysters in Australia. Global Aquaculture Advocate 8 (1), 46-49.

Appleyard, S.A. and Ward, R.D. 2005. Genetic diversity and effective population size in mass selection lines of Pacific oysters (*Crassostrea gigas*). *Aquaculture*, in press

Publications derived from earlier related projects include:

Ward, R.D., English, L.J., McGoldrick, D.J., Maguire, G.B., Nell, J.A. and Thompson, P.A. 2000. Genetic improvement of the Pacific oyster, *Crassostrea gigas*, in Australia. *Aquaculture Research*, 31, 35-44.

English, L.J., Maguire, G.B. and Ward, R.D. 2000. Genetic variation of wild and hatchery populations of the Pacific oyster, *Crassostrea gigas* (Thunberg), in Australia. *Aquaculture*, 187, 283-298.

McGoldrick, D.J., Hedgecock, D., English, L.J., Baoprasertkul, P. and Ward, R.D. 2000. The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. *Journal of Shellfish Research*, 19, 779-788.

English, L.J., Nell, J.A., Maguire, G.B. and Ward, R.D. 2001. Allozyme variation in three generations of selection for whole weight in the Sydney rock oyster (*Saccostrea glomerata*). *Aquaculture*, 193, 213-225.

Ward, R.D. 2003. Selective improvement of Pacific oysters (*Crassostrea gigas*) in Australia. In, *Proceedings of the Joint Australia-Taiwan Aquaculture, Fisheries Resources and Management Forum III*, *2001*, pp. 71-74. Australian Academy of Technological Sciences and Engineering, Canberra, Australia. 148pp.

Table 12.1. F2 and F3 Families presented to Tasmanian Shellfish Producers meeting, with nominations of 'best' and 'worst' families.

F2 Family number		No. of growers classifying as:		F3 Family number		No. of growers classifying as:	
Public	Private	Best	Worst	Public	Private	Best	Worst
2E	5			3B	51		
2L	13	1		3C	52		1
2M	14	4		3H	57	4	
2N	15			3J	59	4	
2O	16N			M3D	M3-2b	2	
2V	23	5		CTL	CONTROL		8
2AA	28						
2AL	41		9				
2AN	43						

Note: F2 family 41 has a high degree of frill (excess shell) possibly as a consequence of selecting broodstock from an area of low average salinity.

CHAPTER 13. Benefits and Adoption

The benefits of this Project are clear – the development of more productive Pacific oyster strains and the means of supplying these strains to the industry. These strains are faster growing than the unimproved strains the industry has hitherto been using. They are also more genetically uniform and therefore more morphologically uniform than conventionally-produced spat. Proper efficient adoption of these faster-growing oysters is likely to involve some change in the management practise of farmers – without this, some of the potential benefits may not be achieved. A faster, more efficient turn-over of oysters on farms means that farms will be able to grow a larger crop in a given area of lease. The additional cost of genetically improved spat will be low, and farmers who adopt this technology will achieve greater profits. Furthermore, as the areas of water released by state governments for inter-tidal leases become increasingly scarce, inter-tidal oyster growers who wish to expand production will either have to use these improved strains or will have to develop off-shore sub-tidal farm facilities.

The adoption by the industry of these improved strains has been good. A non-profit Tasmanian/South Australian company has been formed to facilitate this process – Australian Seafood Industries Pty Ltd (ASI). This company has not only championed the improved, 'thoroughbred', oysters, but has also helped to bring the Tasmanian and South Australian Pacific oyster industries together for their mutual benefit.

For the long-term sustainable improvement of the Pacific oyster industry in Australia, ASI needs to be viable and needs to be successful. It needs to achieve sufficient income to maintain the on-going genetic improvement program and to ensure the increasing adoption of thoroughbred oysters by the industry. The first few years of ASI may be financially difficult until industry uptake of the improved strains reaches around 50%. At that point ASI should be secure. Currently, two of the three main hatcheries in Tasmania are producing thoroughbred spat as a significant component of their production.

Bob Cox (ASI Secretary), in ASI newsletter volume 1 issue 2 (March 2004) estimates cost savings from the use of thoroughbred oysters as:

1. $24,000 per million spat for increased uniformity of growth enabled handling operations to be halved,
2. $32,000 per million spat for a 20% reduction in death rate, and
3. $12,000 per million spat for stock reaching market size at 21 months rather than 27 months.

These represent real dollar benefits to the farmer in return for paying an extra $2,000 to $4,000 per million thoroughbred spat (the former is the current extra cost, the latter is the proposed extra cost later on as improvement continues).

On a total industry-wide production of about 5,000 t per annum (approximate figures for South Australia and Tasmania totalled in 2002/03, see ABARE 2004), equating to about 70 m oysters (at 70 g per oyster), this represents cost savings across the industry of about $5 m per annum. The total production value for South Australia and Tasmania was about $27 m in 2002/03 (ABARE 2004).

CHAPTER 14. Further Development

Ongoing genetic improvement of the lines through selective breeding will be undertaken by ASI. In January 2004 ASI through their General Manager, Scott Parkinson, spawned the F6 generation. The breeding plans for this were produced by Dr Andrew Swan (CSIRO Livestock Industries) of the FRDC 2000/206 project team. While a long term breeding plan, incorporating hatchery advice, has been delivered to ASI (see Appendix 4), this will need to be continually re-visited as new data become available from the breeding program. ASI will always require quantitative genetics advice to maintain genetic gains for the program and to minimise inbreeding rates.

The breeding program is expected to evolve over the years. Traits that at the inception of the project were not deemed a top priority have become more important to the industry. Growth rate of the improved oyster families is now deemed sufficient and this characteristic is being supplanted by shape and uniformity as key traits, with preference also given to lines with the low mortality rates.

The breeding program will also benefit from a selection index approach, as outlined in Chapter 11. However, the objectives of the oyster breeding program need to be clearly identified in terms of increasing enterprise profit. Traits that influence profitability need to be identified through an economic evaluation of the market values of products and production costs. This will define the economic weights of traits, which define the dollar value of a unit change in a trait. Breeding objectives and breeding traits have been identified as part of this study, but this has not been done from an economic basis. Consequently, the appropriate weighting of traits that maximises profit remains unclear.

It also became apparent during this project that more work on defining and measuring condition index was required. Condition refers to the volume of the shell that is filled with meat, and is a measure of quality. It is a complex trait, and it is most probably affected by many factors such as spawning time/frequency and recovery after spawning. Data from this study suggests condition is under reasonably strong genetic control and that it is adversely correlated with other economically important traits (growth rate and shape). However, we are not confident about the measure used in this study (it was taken from the literature). Furthermore, condition was measured at only one point in time and on only one site so any results from this study needed to be treated with caution. Much more work is needed to understand this trait, and to decide how best to use it in a selective breeding program.

Attention also has to be paid to other ways of improving the genomes of Pacific oysters to the benefit of oyster growers. Genetic engineering solutions (transgenesis) are unlikely to find favour with either the industry or with the consumer in the short to medium term. However, plant breeders have had considerable success with F1 hybrid production. Here, chosen inbred lines of plants are crossed with the commercial product being the F1 hybrid. The F1 hybrids that are successful display heterosis (higher values than either of the parental inbred lines) and are also genetically highly uniform and therefore perform very uniformly. Another important aspect of F1 hybrids is that they make poor parents themselves: their progeny will be genetically and morphologically highly variable. A proposal to inbreed and then outcross chosen lines of Pacific oysters was developed and submitted to FRDC in November 2003

(Hybrid Vigor and Inbreeding Depression in Pacific Oysters). Unfortunately it was not approved. We have already produced several inbred lines of oysters in the present project which could have been used in that project had it been approved. A related project that aims to very rapidly inbreed oysters to homozygosity – double haploidy – has recently been funded by FRDC (Development of Techniques for Production of Homozygous Pacific Oysters, FRDC 2002/204, Principal Investigator Dr Xiaoxu Li, SARDI) but at the time of writing its achievements are uncertain. A similar project has still more recently been initiated at the University of Tasmania.

More work needs to be done on mortality issues. There was evidence (not presented here as it was carried out by ASI) for mortality differences among selected lines. While none of the selection lines has a proven high level of mortality, ASI stocks need to have low mortality. More data needs to be collected on mortality levels in selected and in unselected lines. This data needs to be reliable and not confounded by stock losses resulting from environmental causes.

Disease susceptibility in Pacific oysters may also become a more important trait. Currently Pacific oysters in Australia suffer little from known diseases: the occasional instances of high mortality in juveniles (which have been recorded especially from South Australia) have an unknown aetiology. In contrast, Sydney rock oysters (*Saccostrea glomerata*) in New South Wales suffer severely from two major diseases – QX and Winter disease. There is evidence that suppression of the phenoloxidase cascade leaves Sydney rock oysters more susceptible to QX (Peters and Raftos, 2003) and that oysters bred for QX disease resistance have novel forms of the phenoloxidase gene that seem to protect them from infection (Newton *et al.* 2004). There is preliminary evidence (Raftos, Butt and Bezemer, unpublished) that phenoloxidase levels vary among the family lines of Pacific oysters that were tested from our breeding program. This needs to be followed up. It will be interesting to see if PO levels are related to growth rate or mortality differences among families, including inbred and outbred families.

In the medium to long term, gene mapping for commercially valuable traits will prove useful. This will enable chosen traits to be more readily selected and combined to make still more valuable oysters. Selection processes will be speeded up. Some limited gene mapping was carried out as part of the CRC Aquaculture work (see Ward and Thompson 2001), and is also underway in California (D. Hedgecock, pers.com). Any larger scale implementation of this approach in Australia might need to wait on the successful commercial uptake of the current procedure by industry.

In the long-term, genetic engineering of Pacific oysters, should it prove acceptable to consumers, will facilitate rapid advances. Not only will oysters be able to be produced at reduced cost, but the problem of unwanted feral populations of Pacific oysters will also be able to be tackled. CSIRO Marine Research has already done much work on producing reversibly sterile oysters – i.e. oysters that are only fertile if provided with a supplement in the hatchery. This approach should be able to prevent farm animals reproducing on the farms. This will contain the spread of feral populations and might be able to reduce feral populations or even eliminate them altogether.

CHAPTER 15. Planned Outcomes

The Planned Outcomes described in the submitted project proposal were stated thus:

"The major outcome will be a more efficient and productive industry using genetically improved lines of Pacific oysters.

We expect to produce for the use of industry:

(i) lines of oysters with reduced time to harvest resulting in increased economies in production costs. An 8% improvement in growth rate in the first selected generation should result in a significant reduction in costs (perhaps of the order of ~$1m per year), and increasing growth rates over future selected generations will yield proportionately greater benefits.

(ii) lines of oysters free of the deleterious trait 'curl-back' and with enhanced growth and other desirable traits.

(iii) a breeding plan providing for a sustainable system of genetic improvement and incorporating both non-additive and major gene effects (Note: it is anticipated that this will be produced by the Joint Venture Company should it be established).

We also expect to provide a commercialisation strategy, with the industry taking the majority of the responsibility for developing this strategy."

The major Planned Outcome has, to a significant extent, already been met. A joint venture company – Australian Seafood Industries (ASI) – was established by the Tasmanian and South Australian Pacific oyster industries to oversee technology transfer from the Project to the industry, and to ensure that the genetic improvement program continues. The Pacific oyster industry in Tasmania and South Australia is using genetically improved lines in their production. These lines are being marketed by ASI as "thoroughbred oysters" and are being sold as spat from hatcheries with a small additional royalty. About 17 m thoroughbred oyster spat were produced by Shellfish Culture and Camerons in the 2003/04 season – this represents perhaps 15-20% of the total market. ASI are anticipating a gradually increasing market share for thoroughbred oyster spat for the next four or five years.

The growth rates of selected families are substantially better than unselected controls, for some families as much as double (section 7.3). Lines of oysters free of the deleterious trait "curl-back" have been produced, although this trait was present in some fast-growing F1 families (e.g. RB003), a few F2 families (e.g. 34), a small number of individuals in some F3 families, and was not evident in the early F4s**).** A long-term sustainable breeding plan has been provided (see Appendix 4). ASI has produced a Commercialisation Strategy (February 2004); however that is commercial-in-confidence in nature.

CHAPTER 16. Conclusions

This Project had six original 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

These objectives have been achieved, although more work is needed on economic modelling for developing a selection index. We have produced five generations of mass selection and family lines, the latter being cross-bred to incorporate desirable traits from different families. The commercial trials, whilst producing some valuable data, generally were less useful than expected for the Project. However, they did have the important benefit of bringing the program to the attention of a wider span of farmers than would otherwise have known of it. A long-term sustainable breeding plan has been produced. A new joint venture company, Australian Seafood Industries Pty Ltd, has been established by the Pacific oyster industries of Tasmania and South Australia, and has produced a commercialisation strategy. Both the long-term breeding plan and the commercialisation strategy are commercial-in-confidence documents.

The increase in growth rate of the mass selection lines appeared to be, on average, rather similar to the family selection lines. However, the family lines can be subjected to greater control, for example of inbreeding, and are a better long-term proposition for sustainable improvement. The fourth generation selected product grew, on average, about 1.6x faster than the unselected control in that generation. This needs to be confirmed with the larger number of lines produced in the fifth generation, but it is certainly true that selection has effected sizable growth rate gains.

There is some genotype x environment interaction, that is, the ranks of families do vary a little among farms. This was significant for the F1, F2 and F4 generation but not the F3 generation. One question that should be answered is whether or not all farms show similar interfarm pairwise correlations in family performance, or do one or more farms show significantly different rank orders from other farms?

This can be addressed, for any one cohort, by taking one farm and comparing the pairwise farm correlations including that farm with the pairwise correlations not including that farm. Given that there were five farms, this gives a sample size of four correlations for the former and six for the latter. These two sets of correlations can be compared with a two-tailed t-test and a probability value estimated. The process is repeated for the next cohort and that probability estimated. The probabilities can then be combined across the four cohorts (using the method detailed in Sokal and Rohlf, 1995) to arrive at a final probability value for that farm. The process is then repeated for the next farm.

Results (Table 13.1) show that two (Coles Bay and Pittwater) of the five farms show significant deviations at the 5% level. However, following Bonferroni adjustment for five comparisons, only one farm remained significant. This was Coles Bay. For this farm, when each cohort was taken separately, the mean pairwise correlation including Coles Bay was always lower than the mean pairwise correlation excluding that farm, sometimes significantly so (P values of 0.003, 0.063, 0.012 and 0.098 for cohorts A to D respectively). Thus, while the overall correlations of pairwise comparisons with Coles Bay were still high (0.713), they were nevertheless significantly lower than comparisons not including Coles Bay. So, it seems that the environment of Coles Bay – presumably its subtidal nature – does influence rank orders compared with the other, intertidal, environments examined. However, this effect is quite small, and a family that performs well at one of the intertidal farms will generally be expected to perform almost as well at Coles Bay.

A good oyster shell shape has been maintained in the course of the program. There is evidence that at a pre-market stage the condition of the faster growing oysters may be a little less than that of slower growing oysters, but very limited additional evidence suggests this effect might have diminished or even vanished at market size. This needs further study. There is also evidence that the selected lines give a more uniform product than unselected lines. Increasing uniformity will be a trait that ASI work towards.

Towards the end of the project, differential mortality was raised as a potential issue. The project has little data that address that point. Losses of stock on farms can be due not only to genetic factors but also to environmental factors. The latter include losses due to stresses arising from improper handling, temperature, salinity, food and/or predation and losses due to equipment failure. There was some evidence of differential mortality arising from genetic causes among selected lines (an ASI sponsored analysis of data[5]). However, these differences were generally small. The very large mortality events observed in some South Australian farms included both selected and unselected stock. More work needs to be undertaken on the mortality issue.

In general, and given the difficulties faced when attempting to do experimental work under oyster hatchery and farm conditions (including failures of some experimental hatchery runs and farm stock losses resulting from tag losses and from losses of baskets and whole racks in storms), we were very pleased with the outputs and outcomes of this project. The industry, through our work and through the formation of ASI, now has the basis for a continually improving and increasingly efficient Pacific oyster industry.

[5] Dr Steve Candy 2003. Familial analysis of ASI SA oyster mortality and weight data. The analysis used 21 F3 and M3 families from Coffin Bay using survivorship and weight data from September 2002. Some families showed mortalities significantly higher than the mean, some lower than the mean. Subsequently a crude estimate of heritability for mortality was given as a high 0.68 (the error term for this is unspecified but large)

Table 13.1. Cohorts A-D. Mean family final weight correlations (with standard deviations) across farm pairs for each farm, averaged across cohorts. Correlations come from the final weight checks. See text for further explanation.

Farm	Pairwise comparisons	Mean+SD	*P*
Pittwater	including Pittwater	0.818±0.126	0.023
	excluding Pittwater	0.781±0.077	
Coles Bay	including Coles Bay	0.713±0.094	<0.001
	excluding Coles Bay	0.851±0.067	
Smithton	including Smithton	0.822±0.077	0.624
	excluding Smithton	0.778±0.096	
Coffin Bay	including Coffin Bay	0.785±0.079	0.842
	excluding Coffin Bay	0.803±0.080	
Smoky Bay	including Smoky Bay	0.841±0.061	0.350
	excluding Smoky Bay	0.765±0.093	

REFERENCES

ABARE (2004). *Australian Fisheries Statistics 2003*. Australian Bureau of Agriculture and Resource Economics, GPO Box 1563, Canberra 2601. ABARE & FRDC.

Aliah, R.S., Sato, S. and Taniguchi, N. (2000). An evaluation of genetic variability of Nishikigoi, *Cyprinus carpio*, stock from Niigata Prefecture based on microsatellite DNA markers. *Suisanzoshoku* 48, 25-31.

Allen, S.K. (1998). Commercial applications of bivalve genetics: not a solo effort. *World Aquaculture* March, 38-43.

Allendorf, F.W. (1986). Genetic drift and the loss of alleles versus heterozygosity. *Zoological Biology* 5, 181-190.

Appleyard, S.A. (1998). *Application of Genetic Markers to Fijian Tilapia Stock Improvement*. Ph.D Thesis, Queensland University of Technology, Australia, 219pp.

Appleyard, S.A., Grewe, P.M., Innes, B.H. and Ward, R.D. (2001). Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean, inferred from microsatellite loci. *Marine Biology* 139, 383-393.

Appleyard, S.A. and Mather, P.B. (2000). Investigation into the mode of inheritance of allozyme and random amplified polymorphic DNA markers in tilapia *Oreochromis mossambicus* (Peters). *Aquaculture Research* 31, 435-445.

Avise, J.C. (1994). *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York, USA, 511pp.

Baghurst, B.C. and Mitchell, J.G. (2002). Sex-specific growth and condition of the Pacific oyster (*Crassostrea gigas* Thunberg). *Aquaculture Research* 33, 1253-1263.

Bartley, D., Bagley, M., Gall, G., and Bentley, B. (1992). Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conservation Biology* 6, 365-375.

Beaumont, A.R. and Hoare, K. (2003). *Biotechnology and Genetics in Fisheries and Aquaculture*. Blackwell Publishing, United Kingdom, 170pp.

Bentsen, H.B. and Olesen, I. (2002). Designing aquaculture mass selection programs to avoid high inbreeding rates. *Aquaculture* 204, 349-359.

Berg, P. and Henryon, M. (1998). A comparison of mating designs for inference on genetic parameters in fish. In *Proceedings of the Sixth World Congress on Genetics Applied to Livestock Production*, 27, pp. 115-118.

Bernatchez, L. and Duchesne, P. (2000). Individual-based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles? *Canadian Journal of Fisheries and Aquatic Sciences* 57, 1-12.

Berthier, P., Beaumont, M.A., Cornuet, J.M. and Luikart, G. (2002). Likelihood-based estimate of the effective population size using temporal changes in allele frequency: a genealogical approach. *Genetics* 160, 741-751.

Boudry, P., Collet, B., Cornette, F., Hervouet, V. and Bonhomme, F. (2002). High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. *Aquaculture* 204, 283-296.

Boudry, P., Degremont, L. and Samain, J.F. (2004). French program studies selective breeding in oyster spat. *Global Aquaculture Advocate* October, 78-79.

Bourdon, R.M. (2000). *Understanding Animal Breeding, 2nd Edition*. Prentice-Hall Inc, USA. 537pp.

Brake, J., Evans, F. and Langdon, C. (2003). Is beauty in the eye of the beholder? Development of a simple method to describe desirable shell shape for the Pacific oyster industry. *Journal of Shellfish Research* 22, 767-771.

Brookfield, J.F.Y. (1996). A simple new method for estimating null allele frequencies from heterozygote deficiency. *Molecular Ecology* 5, 453-455.

Campton, D.E. (1987). Natural hybridisation and introgression in fishes: methods of detection and genetic interpretations. In: Population *Genetics and Fisheries Management* (eds Ryman, N. Utter, F.) Washington Sea Grant Program, University of Washington Press, Seattle, pp 161-192.

Cornuet, J.M. and Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144, 2001-2014.

Cotterill, P.P. and Dean, C.A. (1990). Successful tree breeding with index selection. CSIRO Australia. 80 pp.

Crosby, M.P. and Gale, L.D. (1990). A review and evaluation of bivalve condition index methodologies with a suggested standard method. *Journal of Shellfish Research* 9, 233-237.

Crozier, W.J. (1914). The growth of the shell in the lamellibranch *Dosinia discus* (Reeve). *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere* 38, 577-584.

Davis, C.V. (2000). Estimation of narrow-sense heritability for larval and juvenile growth traits in selected and unselected sub-lines of eastern oysters, *Crassostrea virginica*. *Journal of Shellfish Research* 19, 613.

Davis, G.P and Hetzel, D.J.S. (2000). Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquaculture Research* 31, 3-10.

Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19, 11-15.

Dupont-Nivet, M., Vandeputte, M. and Chevassus, B. (2002). Optimisation of factorial mating designs for inference on heritability in fish species. *Aquaculture* 204, 361-370.

English, L.J., Maguire, G.B. and Ward, R.D. (2000). Genetic variation of wild and hatchery populations of the Pacific oyster, *Crassostrea gigas* (Thunberg), in Australia. *Aquaculture* 187, 283-298.

Evans, B., White, R.W.G. and Ward, R.D. (1998). Genetic identification of asteroid larvae from Tasmania, Australia by PCR-RFLP. *Molecular Ecology* 7, 1077-1082.

Evans, B., Bartlett, J., Sweijd, N., Cook, P. and Elliott, N.G. (2004). Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). *Aquaculture* 233, 109-127.

Excoffier, L., Smouse, P. and Quattro, J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131, 479-491.

Falconer, D.S. and Mackay, T.F.C. (1996). Introduction to Quantitative Genetics, 4th edition. Longman, Essex, England.

Ferguson, M.M. and Danzmann, R.G. (1998). Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting. *Canadian Journal of Fisheries and Aquatic Science* 55, 1533-1563.

Frankham, R. (1995). Effective population size, adult population size ratios in wildlife – a review. *Genetical Research* 66, 95-107.

Gaffney, P.M., Davis, C.V. and Hawes, R.O. (1992). Assessment of drift and selection in hatchery populations of oysters (*Crassostrea virginica*). *Aquaculture* 105, 1-20.

Gaffney, P.M. and Scott, T.M. (1984). Genetic heterozygosity and production traits in natural and hatchery populations of bivalves. *Aquaculture* 42, 289-302.

Gall, G.A.E. and Bakar, Y. (2002). Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day body weight in tilapia. *Aquaculture* 212, 93-113.

Galtsoff, P.S. (1964). The American oyster, *Crassostrea virginica* Gmelin. *Fishery Bulletin of the USA, Fish and Wildlife Service* 64, 1-480 (pp 20, 29-30).

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. and Thompson, R. (2002). *ASReml User Guide release 1.0.* VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Gitterle, T., Rye, M., Salte, R., Cock, J., Johansen, H., Lozano, C., Suarez, J.A. and Gjerde, B. (2005). Genetic (co)variation in harvest body weight and survival in *Penaeus* (*Litopenaeus*) *vannamei* under standard commercial conditions. *Aquaculture* 243, 83-92.

Gjedrem, T. (1983). Genetic variation in quantitative traits and selective breeding in fish and shellfish. *Aquaculture* 33, 51-72.

Gosling, E.M. (1982). Genetic variability in hatchery-produced Pacific oysters (*Crassostrea gigas* Thunberg). *Aquaculture* 26, 273-287.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Guo, X., Hedgecock, D., Hershberger, W.K., Cooper, K. and Allen, S.K. Jr. (1998). Genetic determinants of protandric sex in the Pacific oyster, *Crassostrea gigas* Thunberg. *Evolution* 52, 394-402.

Haley, L.E. (1977). Sex determination in the American oyster. *Journal of Heredity* 68, 114-116.

Haley, L.E. and Newkirk, G.F. (1982). The genetics of growth of *Crassostrea virginica* and *Ostrea edulis*. *Malacologia* 22, 399-401.

Handley, S.J. (2002). Optimizing intertidal Pacific oyster (Thunberg) culture, Houhora Harbour, northern New Zealand. *Aquaculture Research* 33, 1019-1030.

Hauser, L., Adcock, G.J., Smith, P.J., Bernal Ramírez, J.H. and Carvalho, G.R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Science* 99, 11742-11747.

Hedgecock, D., Chow, V. and Waples, R.S. (1992). Effective population numbers of shellfish broodstock estimated from temporal variance in allelic frequencies. *Aquaculture* 108, 215-232.

Hedgecock, D., Cooper, K. and Hershberger, W. (1991). Genetic and environmental components of variance in harvest body size among pedigreed Pacific oysters *Crassostrea gigas* from controlled crosses. *Journal of Shellfish Research* 10, 516.

Hedgecock, D., Cooper, K., Hershberger, W. and Guo, X. (1993). Body size at harvest, sex ratio and mantle colour of pedigreed Pacific oysters *Crassostrea gigas* from controlled crosses. *Aquaculture* 111, 299.

Hedgecock, D., Langdon, C., Bouin, M and Allen, S.K. (1997). Genetic improvement of cultured Pacific oysters by selection. 1996 Coastal Marine Experiment Station Annual Report, Agricultural Experiment Station, Oregon State University, 40pp.

Hedgecock, D. and Sly, F. (1990). Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 88, 21-38.

Henderson, C.R. (1973). Sire evaluation and genetic trends. In, *Proceedings of the Animal Breeding and Genetics Symposium in Honor of Dr Jay L Lush*., Champaign, IL. American Society of Animal Science, pp.10-41.

Higgins, E. (1938). Progress in biological inquiries, 1937. *Bulletin of the US Bureau of Fisheries, Admin. Report* no. 30, 1-70.

Hill, W.G. (1981). Estimation of effective population size from data on linkage disequilibrium. *Genetical Research* 38, 209-216.

Honkoop, P.J.C. and Bayne, B.L. (2002). Stocking density and growth of the Pacific oyster (*Crassostrea gigas*) and the Sydney rock oyster (*Saccostrea glomerata*) in Port Stephens, Australia. *Aquaculture* 213, 171-186.

Jarayabhand, P. and Thavornyutikarn, M. (1995). Realised heritability estimates on growth rate of oyster, *Saccostrea cucullata* Born, 1778. *Aquaculture* 138, 111-118.

Kinghorn, B., van der Werf, J. and Ryan, M. (1999). *Animal Breeding, Use of New Technologies*. The Postgraduate Foundation in Veterinarian Science of the University of Sydney, Australia. 329pp.

Koljonen, M-L., Tähtinen,J., Säisä, M. and Joskiniemi, J. (2002). Maintenance of genetic diversity of Atlantic salmon (*Salmo salar*) by captive breeding programmes and the geographic distribution of microsatellite variation. *Aquaculture* 212, 69-92.

Langdon, C., Evans, F., Jacobson, D. and Blouin, M. (2003). Yields of culture Pacific oysters C*rassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture* 220, 227-244.

Lannan, J.E. (1972). Estimating heritability and predicting response to selection for the Pacific oyster. *Proceedings of the National Shellfish Association* 62, 62-66.

Lannan, J.E. (1980). Broodstock management of *Crassostrea gigas* IV. Inbreeding and larval survival. *Aquaculture* 21, 353-356.

Launey, S., Barre, M., Gerard, A. and Naciri-Graven, Y. (2001). Population bottleneck and effective size *in Bonamia ostrea*-resistant populations of *Ostrea edulis* as inferred by microsatellite markers. *Genetical Research* 78, 259-270.

Lawrence, D.R. and Scott, G.I. (1982). The determination and use of condition index of oysters. *Estuaries* 5, 23-27.

Leberg, P.L. (2002). Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology* 11, 2445-2449.

Li, G., Hubert, S., Bucklin, K., Ribes, V. and Hedgecock, D. (2003). Characterisation of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. *Molecular Ecology Notes* 3, 228-232

Li, Q., Park, C., Endo, T. and Kijima, A. (2004). Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). *Aquaculture* 235, 207-222.

Losee, E. (1979). Relationship between larval and spat growth rates in the oyster (*Crassostrea virginica*). *Aquaculture* 16, 123-126.

Luikart, G., Allendorf, F.W., Cornuet, J.M. and Sherwin, W.B. (1997). Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity* 89, 238-247.

Luikart, G., Sherwin, W.B., Steele, B.M. and Allendorf, F.W. (1998). Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology* 7, 963-974.

Magoulas, A.N, Gjetvaj, B., Terzoglou, V. and Zouros, E. (1998). Three polymorphic microsatellites in Japanese oyster *Crassostrea gigas* (Thunberg). *Animal Genetics* 29, 69-70.

Mallet, A.L. and Haley, L.E. (1984). General and specific combining abilities of larval and juvenile growth and viability estimated from natural oyster populations. *Marine Biology* 81, 53-59.

Marshall, T.C., Slate, J., Kruuk, L.E.B. and Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7, 639-655.

McGoldrick, D.J. (1997). An Experimental Investigation of the Genetic Basis of Heterosis in the Pacific Oyster *Crassostrea gigas* (Thunberg). PhD. Thesis, University of California, Davis, USA.

McGoldrick, D.J., Hedgecock, D., English, L.J., Baoprasertkul, P. and Ward, R.D. (2000). The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. *Journal of Shellfish Research* 19, 779-788.

Meuwissen, T.H.E. (1997). Maximising the response of selection with a predefined rate of inbreeding. *Journal of Animal Science*. 75**,** 934-940.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583-590.

Nell, J.A., Sheridan, A.K. and Smith, I.R. (1996). Progress in a Sydney rock oyster, *Saccostrea commercialis* (Iredale and Roughley), breeding program. *Aquaculture* 144, 295-302.

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

Newkirk, G.F. (1980). Review of the genetics and the potential for selective breeding of commercially important bivalves. *Aquaculture* 19, 209-228.

Newkirk, G.F. and Haley, L.E. (1982). Progress in selection for growth rate in the European oyster *Ostrea edulis*. *Marine Ecology Progress Series* 10, 77-79.

Newkirk, G.F. and Haley, L.E. (1983). Selection for growth rate in the European oyster, *Ostrea edulis*: Response of second generation groups. *Aquaculture* 33, 149-155.

Newkirk, G.F., Haley, L.E., Waugh, D.L. and Doyle, R. (1977). Genetics of larvae and spat growth rate in oysters (*Crassostrea virginica*). *Marine Biology* 41, 49-52.

Newton, K., Peters, R. and Raftos, D.A. (2004). Phenoloxidase and QX disease resistance in Sydney rock oysters (*Saccostrea glomerata*). *Developmental and Comparative Immunology* 28, 565-569.

Norris, A.T. and Cunningham, E.P. (2004). Estimates of phenotypic and genetic parameters for flesh colour traits in farmed Atlantic salmon based on multiple trait animal model. *Livestock Production Science* 89, 209-222.

Paynter, K.T. and Dimichele, L. (1990). Growth of tray-cultured oysters (*Crassostrea virginica* Gmelin) in Chesapeake Bay. *Aquaculture* 87, 289-297.

Peel, D., Ovenden, J.R. and Peel, S.L. (2004). *NeEstimator: software for estimating effective population size.* Version 1.2. Molecular Fisheries Laboratory, Queensland Department of Primary Industries, Australia.

Peters, R. and Raftos, D.A. (2003). The role of phenoloxidase suppression in QX-disease outbreaks among Sydney rock oysters (*Saccostrea glomerata*). Aquaculture 223, 29-39.

Piry, S., Luikart, G. and Cornuet, J.M. (1999). Bottleneck: A program for detecting recent effective population size reductions from allele data frequencies. *Journal of Heredity* 90, 502-503.

Pollak, E. (1983). A new method for estimating the effective population size from allele frequency changes. *Genetics* 104, 531-548.

Pudovkin, A.I., Zaykin, D.V. and Hedgecock, D. (1996). On the potential for estimating the effective number of breeders from heterozygote excess in progeny. *Genetics* 144, 383-387.

Quinton, C.D., McMillan, I. and Blebe, B.D. (2005). Development of an Atlantic salmon (*Salmo salar*) genetic improvement program: Genetic parameters of harvest

body weight and carcass quality traits estimated with animal models. *Aquaculture* 247, 211-217.

R Development Core Team (2004). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org.

Raymond, M. and Rousset, F. 2000. Updated version of GENEPOP (v. 3.2) as described in: Raymond, M. and Rousset, F. (1995). GENEPOP (v. 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.

Reilly, A., Elliott, N.G., Grewe, P.M., Clabby, C., Powell, P. and Ward, R.D. (1999). Genetic differentiation between Tasmanian cultured Atlantic salmon (*Salmo salar* L.) and their ancestral Canadian population: comparison of microsatellite DNA and allozyme and mitochondrial DNA variation. *Aquaculture* 173, 459-469.

Rice, W.R. (1989). Analysing tables of statistical tests. *Evolution* 43: 223-225.

Richardson, B.J., Baverstock, P.R. and Adams, M. (1986). *Allozyme Electrophoresis: a Handbook for Animal Systematics and Population Studies*. Academic Press, Australia, 410pp.

Saavedra, C. and Guerra, A. (1996). Allozyme heterozygosity, founder effect and fitness traits in a cultivated population of the European oyster, *Ostrea edulis*. *Aquaculture* 139,203-22.

Säisä, M., Koljonen, M-L. and Tähtinen, J. (2003). Genetic changes in Atlantic salmon stocks since historical times and the effective population size of a long-term captive breeding programme. *Conservation Genetics* 4, 613-627.

Schneider, S., Roessli, D. and Excofier, L. (2000). Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

Sekino, M., Hara, M. and Taniguchi, N. (2002). Genetic diversity within and between hatchery strains of Japanese flounder *Paralichthys olivaceus* assessed by means of microsatellite and mitochondrial DNA sequence analysis. *Aquaculture* 213, 101-122.

Shaklee, J.B., Allendorf, F.W., Morizot, D.C. and Whitt, G.S. (1990). Gene nomenclature for protein coding loci in fish. *Transactions of the American Fisheries Society* 119, 2-15.

Sheridan, A.K. (1988). Agreement between estimated and realised genetic parameters. *Animal Breeders Abstracts* 56, 877-889.

Sheridan, A.K. (1997). Genetic improvement of oyster production – a critique. Aquaculture 153: 165-179

Shrimpton, J.M. and Heath, D.D. (2003). Census vs. effective population size in chinook salmon: large- and small-scale environmental perturbation effects. *Molecular Ecology* 12, 2571-2583.

Smith, P.J., Ozaki, H. and Fujio, Y. (1986). No evidence for reduced genetic variation in the accidentally introduced oyster *Crassostrea gigas* in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 20, 569-574.

Sokal, R.R. and Rohlf, F.J. (1995). *Biometry, The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, USA, 887pp.

Tave, D. (1993). *Genetics for Fish Hatchery Managers*, 2nd Edition. Van Nostrand Reinhold, USA. 350pp.

Thompson, P.A. (2005). *Selective Breeding of Pacific Oysters*. FRDC Final Report, Project 97/321. FRDC, Canberra, Australia.

Toro, J.E., Aguila, P., Vergara, A.M. and Newkirk, G.F. (1994). Realised heritability estimates for growth from data on tagged Chilean native oysters (*Ostrea chilensis*). *World Aquaculture* 25, 29-30.

Toro, J.E. and Newkirk, G.F. (1990). Divergent selection for growth rate in the European oyster (*Ostrea edulis* L.): Response to selection and estimates of genetic parameters. *Marine Ecology Progress Series* 62, 219-228.

Toro, J.E. and Newkirk, G.F. (1991). Response to artificial selection and realised heritability estimate for shell height in the Chilean oyster *Ostrea chilensis*. *Aquatic Living Resources* 4, 101-108.

Toro, J.E., Sanhueza, M.A., Winter, J.E., Aguila, P. and Vergara, A.M. (1995). Selection response and heritability estimates for growth in the Chilean oyster *Ostrea chilensis* (Philippi, 1845). *Journal of Shellfish Research* 14, 87-92.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P.F. (2003). Microchecker version 2.2.0. The University of Hull, Cottingham Road, Hull HU67RX.

Verspoor, E. (1988). Reduced genetic variability in first-generation hatchery populations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* 45, 1686-1690.

Wada, K.T. (1986). Genetic selection for shell traits in the Japanese pearl oyster *Pinctada fucata martensi*. *Aquaculture* 57, 171-176.

Wang, J. (2001). A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetical Research* 78, 243-257.

Waples, R.S. (1989). A generalised approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121, 379-391.

Ward, R.D. and Thompson, P.A. (2001). Genetic Studies and Selective Breeding of Table Oysters. Final Report to CRC Aquaculture. 90pp.

Ward, R.D., English, L.J., McGoldrick, D.J., Maguire, G.B., Nell, J.A. and Thompson, P.A. (2000). Genetic improvement of the Pacific oyster, *Crassostrea gigas*, in Australia. *Aquaculture Research*, 31, 35-44.

Ward, R.D., Jorstad, K.E. and Maguire, G. (2003). Microsatellite diversity in rainbow trout (*Oncorhynchus mykiss*) introduced to Western Australia. *Aquaculture* 219, 169-179

Weir, B.S. and Cockerham, C.C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution* 38, 1358-1370.

Willis, M.B. (2001). *Dalton's Introduction to Practical Animal Breeding*, *4th Edition*. Blackwell Science Ltd, UK. 159pp.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics* 15, 323-354.

Zaykin, D.V. and Pudovkin, A.I. (1993). Two programs to estimate significance of chi-square values using pseudo-probability tests. *Journal of Heredity* 84, 152.

Appendix 1. Intellectual Property

The intellectual data arising from this research are:

- Performance data from selected and control lines
- Performance data from commercial trial lines
- Pedigree information and crossing tables
- Genotype and allele frequency data
- The proposed breeding framework
- Copyright in this Report

There also exist Management and License Agreements between Australian Seafood Industries Pty Ltd, the Commonwealth Scientific & Industrial Research Organisation, University of Tasmania and the Fisheries Research & Development Corporation.

Appendix 2. Staff

Staff engaged on the Project:

Principal Investigator	
Dr Robert Ward	CSIRO Marine and Atmospheric Research
Coinvestigator	
Dr Peter Thompson	CSIRO Marine and Atmospheric Research
Research Assistants	
Mr Mathew Willis	TAFI, University of Tasmania
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Dr Peter Kube	CSIRO Marine and Atmospheric Research
Mr Mathew Willis	University of Tasmania
Ms Bronwyn Innes	CSIRO Marine and Atmospheric Research

Appendix 3. Mean Individual Weight and Condition Index for F1 Families.

See Table 3.5 for more information

	Pittwater						Coles Bay					
	Weight			Condition index			Weight			Condition index		
	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
RB000	20	15.545	4.54	20	36.15	5.68	19	23.268	9.29	19	46.04	8.13
RB002	20	18.090	6.31	20	39.11	6.70	20	28.350	12.98	20	43.64	8.95
RB003	20	26.820	9.68	20	30.43	3.33	18	35.483	12.50	18	42.37	13.88
RB004	20	25.250	10.08	20	38.96	7.47	20	33.020	18.49	20	51.75	13.07
RB006	20	25.015	5.90	20	34.09	7.35	20	32.310	11.67	20	46.43	8.36
RB011	20	19.800	4.78	20	33.68	5.39	20	35.540	15.30	20	41.35	9.76
RB012	20	18.600	5.97	20	37.54	5.34	20	33.805	11.89	20	45.37	10.43
RB015	20	22.380	5.92	20	34.09	3.82	20	35.165	15.38	20	44.43	12.65
RB022	20	18.819	7.80	20	36.19	4.41	18	33.444	13.58	18	43.89	8.56
RB029	20	14.330	5.05	20	40.66	7.18	20	25.500	9.09	20	54.47	12.67
RB032	20	16.037	6.45	20	36.38	5.53	20	19.015	8.27	20	50.17	10.97
RB042	20	21.275	7.86	20	35.17	7.77	20	40.920	14.19	20	39.56	10.87
RB044	20	18.890	7.55	20	37.44	8.53	20	31.565	10.02	20	39.18	7.42
RB048	20	21.190	8.87	20	35.24	5.31	20	22.385	8.16	20	42.64	12.16
WA213	20	18.025	6.28	20	31.67	5.39	20	29.720	11.51	20	44.74	9.72
WA214	20	19.850	8.28	20	38.42	6.03	20	26.875	9.88	20	46.63	8.64
WA219	20	23.340	5.59	20	33.18	5.89	19	32.021	14.11	19	44.54	8.18
WA225	10	17.030	5.54	10	37.72	4.68	20	28.545	7.34	20	48.66	17.65
WA234	20	21.125	5.94	20	36.87	4.50	20	28.890	12.81	20	38.37	9.69
WA921	20	18.090	7.63	20	37.53	7.79	20	29.040	8.15	20	46.87	15.41
WA925	20	24.275	7.13	20	35.18	4.70	18	38.817	14.68	18	44.29	10.46
WA928	10	23.310	3.69	10	33.96	4.42	20	29.810	10.08	20	39.29	12.93
WA930	20	23.910	7.72	20	38.19	5.17	20	32.755	7.12	20	41.76	11.44
WA979	20	18.180	5.77	20	39.20	5.78	20	18.355	8.49	20	54.51	13.84
WA98	20	17.690	7.69	20	35.86	7.51	15	18.907	11.06	15	49.04	9.72
WA981												
WA987	20	18.115	7.91	20	35.99	5.71	20	26.225	10.39	20	41.81	9.82
WA992	20	20.430	5.86	20	39.52	4.69	20	10.680	5.78	20	50.25	14.11
WA993												
WA996	20	9.715	3.82	20	43.54	7.13	20	28.210	10.97	20	43.18	16.01
YA002	20	18.025	5.56	20	33.88	6.70	20	31.365	7.33	20	44.68	10.25
YA006	20	16.715	5.99	20	38.62	7.95	15	24.793	10.06	15	41.05	18.34
YA011	20	15.345	6.26	20	38.35	12.71	20	26.260	10.70	20	51.05	16.73
YA013	20	13.140	4.12	20	35.16	4.18	20	29.680	10.18	20	44.16	13.82
YA018	20	23.450	4.56	20	35.95	4.30	10	18.140	4.65	10	50.75	4.47
YB008	20	17.940	6.58	20	32.99	7.76	19	21.379	12.53	19	54.09	11.11
YB020	20	17.290	5.63	20	36.91	6.46						

Appendix 3 – continued

	Smithton						Coffin Bay					
	Weight			Condition index			Weight			Condition index		
	n	mean	sd	n	mean	sd	n	mean	sd	n	mean	sd
RB000	39	27.694	5.82	19	53.83	6.71	19	39.289	15.39	14	48.08	9.58
RB002	40	28.495	7.90	20	54.52	8.69	10	26.650	14.49	10	42.73	7.32
RB003	35	43.606	11.54				14	41.184	11.30	9	36.36	6.44
RB004	39	31.414	12.20	20	56.33	12.73	19	50.163	14.43	14	54.46	15.85
RB006	40	35.080	8.24	20	48.63	4.20	15	40.419	15.12	15	58.58	21.04
RB011	40	28.565	7.60	20	50.22	8.22	20	47.383	18.78	10	45.10	9.52
RB012	40	36.045	8.90	30	46.20	7.24	19	40.863	12.85	14	45.91	10.31
RB015	40	37.504	8.60	30	46.18	15.77	15	47.507	21.80	10	43.69	10.25
RB022	40	31.383	9.34	10	48.01	4.65	8	44.750	10.96	8	42.50	5.12
RB029	40	26.383	9.25	20	54.50	10.58	17	36.459	14.26	13	50.20	10.08
RB032	40	23.808	6.94	40	55.53	10.01	20	32.436	15.01	20	58.26	15.23
RB042	40	37.340	10.31				19	54.558	17.21	11	35.38	17.66
RB044	40	32.760	8.40	40	49.10	7.60	20	51.620	18.51	15	40.58	6.58
RB048	40	28.095	8.74	20	51.77	8.34	15	26.213	5.47	15	51.69	18.68
WA213	40	28.474	7.82	20	49.24	4.43	19	44.767	13.84	15	51.03	19.87
WA214	39	32.044	8.61	10	50.46	3.51	15	38.260	13.17	13	57.30	10.07
WA219	40	36.508	12.44	20	44.12	8.39	5	34.960	7.18			
WA225	40	32.195	7.21	20	53.96	9.03	14	42.793	17.02	9	70.91	14.35
WA234	35	40.097	9.44	25	48.05	6.38	20	38.936	14.19	5	55.69	10.97
WA921	40	32.526	10.07	30	58.51	7.88	15	43.485	14.26	10	80.27	19.25
WA925	40	37.398	11.38	30	44.41	6.95						
WA928	40	35.183	9.58	10	48.15	8.31	5	33.680	13.83	2	41.61	1.68
WA930	40	34.644	10.04	30	54.72	9.29	20	46.625	20.20	10	66.97	8.69
WA979	40	25.732	6.49	20	50.97	5.13	17	33.459	18.78	8	50.25	6.23
WA980	40	31.173	9.32	40	49.83	10.06	13	41.874	15.87	8	56.24	14.82
WA981	40	35.602	8.75	10	54.16	4.88						
WA987	40	30.978	12.49	20	45.08	6.19						
WA992	40	31.573	9.38	20	57.70	11.48	10	13.230	6.54	5	86.59	10.56
WA993	25	36.870	5.61									
WA996	40	19.873	7.07	10	44.40	3.99	15	31.541	9.55	9	70.88	11.56
YA002	40	28.385	7.72	20	41.89	8.17	13	51.179	16.67	9	46.76	7.04
YA006	40	28.785	6.72	30	57.61	8.78	17	50.818	14.44	5	117.06	21.77
YA011	35	24.254	5.08	20	50.49	9.97	12	49.013	11.36	8	58.50	12.28
YA013	40	24.225	7.22	30	55.73	9.13	11	47.111	9.06	5	58.29	10.64
YA018	40	39.158	8.72	20	47.53	5.78	19	52.944	13.01	9	67.47	9.73
YB008	39	26.961	6.31	19	53.70	8.08	17	48.768	9.03			
YB020	40	25.603	6.21	30	58.74	8.60	20	44.202	11.24	5	52.60	8.37

Appendix 3 – continued

	Overall					
	Weight			Condition index		
	n	mean	sd	n	mean	sd
RB000	97	26.45	9.92	72	46.03	7.36
RB002	90	25.40	4.94	70	45.00	6.64
RB003	87	36.77	7.46	47	36.38	5.97
RB004	98	34.96	10.67	74	50.38	7.84
RB006	95	33.21	6.41	75	46.93	10.06
RB011	100	32.82	11.65	70	42.59	6.96
RB012	99	32.33	9.61	84	43.75	4.16
RB015	95	35.64	10.33	80	42.10	5.44
RB022	86	32.10	10.63	56	42.65	4.90
RB029	97	25.67	9.05	73	49.96	6.52
RB032	100	22.82	7.16	100	50.09	9.73
RB042	99	38.52	13.68	51	36.70	2.48
RB044	100	33.71	13.49	95	41.58	5.18
RB048	95	24.47	3.23	75	45.34	7.98
WA213	99	30.25	11.01	75	44.17	8.75
WA214	94	29.26	7.81	63	48.20	7.87
WA219	84	31.71	5.88	59	40.61	6.44
WA225	84	30.14	10.63	59	52.81	13.83
WA234	95	32.26	8.97	70	44.75	8.82
WA921	95	30.79	10.46	80	55.80	18.44
WA925	78	33.50	8.02	68	41.29	5.30
WA928	75	30.50	5.30	42	40.75	5.88
WA930	100	34.48	9.35	80	50.41	13.13
WA979	97	23.93	7.26	68	48.73	6.62
WA980	88	27.41	11.40	83	47.74	8.55
WA981	40	35.60		10	54.16	
WA987	80	25.11	6.50	60	40.96	4.60
WA992	90	18.98	9.36	65	58.52	20.15
WA993	25	36.87				
WA996	95	22.33	9.74	59	50.50	13.60
YA002	93	32.24	13.86	69	41.80	5.64
YA006	92	30.28	14.58	70	63.58	36.64
YA011	87	28.72	14.34	68	49.59	8.34
YA013	91	28.54	14.17	75	48.33	10.72
YA018	89	33.42	15.78	59	50.43	13.02
YB008	95	28.76	13.85	58	46.93	12.07
YB020	80	29.03	13.78	55	49.42	11.26

Appendix 4. Breeding Plans for Australian Seafood Industries Pacific oysters.

This appendix has been removed for commercial-in-confidence reasons.

Appendix 5. Example of Protocol Request supplied by Shellfish Culture along with Improved Stock in a Commercial Trial

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Date

Farm address

Dear X,

Thank you for purchasing some of this seasons improved oyster lines from Shellfish Culture Ltd. The particular line you have purchased is Family Line 28, aka "non curlback line", and it has been produced as part of the on going Oyster Genetics Program. This program was formerly conducted under the Cooperative Research Centre (Aquaculture) and is now managed by Australian Seafood Industries Pty Ltd (ASI) in cooperation with CSIRO, UTAS and FRDC. ASI is an industry based company registered by the Tasmanian Oyster Research Council, the South Australian Oyster Research Council and the South Australian Oyster Growers Association to commercialise the improved stocks.

On (date) you were supplied with (number specified) FL-28 oysters(number specified) PD mix 1 and (number specified) PD mix 2 oysters. The latter two groups are the standard SCL product and can be called the control lines. The control and 'improved' lines have been treated identically throughout production however, Shellfish Culture Ltd will not be held responsible for the way in which the improved seed performs on a farm. There is no premium charged on the improved line seed.

It is a condition of the project that SCL report to the Project how many improved and control seed are sold and to whom, including the size of the product and the date of supply. Furthermore, the hatchery must organise for the grower, at the time of sale, to put aside 50 seed oysters from each line for measuring. These oysters are to be placed in suitable mesh bags for separate on-growing for future assessment by researchers. However, in this particular case, where the size of the stock is small, an on growing period in trays would seem more appropriate until the stock is large enough to be placed in mesh bags. Please discuss this with me if you have any questions.

I have included below some correspondence from ASI that outlines the grow-out protocols for commercial trials of the improved oysters. This will make clear what is expected of you during the grow-out period. The information that you provide contributes to the on going improvement of Pacific oyster lines for the whole industry and your contribution is greatly appreciated.

Yours Sincerely,

Richard Pugh, General Manager, Shellfish Culture Ltd

Appendix 5 continued – the attachment to the Shellfish Culture letter

In the grow-out phase

The growers with the representative mesh bags of 50 oysters from each line must keep these in baskets, bags, or trays (depending on the culture system) separate from any other oysters. They are not to be graded or mixed with other oysters.

Once the oysters are big enough they should be removed from the mesh bag and put into one basket (bag or tray). When the grower is ready to sell the bulk of his experimental oysters to market he should contact the Project. Where possible we will arrange to inspect the 50 oysters from each line. In most cases these 50 oysters will be turned over to the grower after suitable measurements have been made.

Ideally all growers will track their lines of improved and commercial oysters as two separate batches. If the grower has a highly developed management system, then information such as how long the batches take to reach market size (i.e. when an where they sold and at what size) would be extremely useful to the Project. Growers collecting this sort of information are requested to provide it to the Project.

The Project reserves the right to purchase, at normal commercial rates, from any grower or hatchery any oyster produced as part of this experimental program. Growers must not knowingly supply the improved oysters to any hatchery for use as broodstock.

Appendix 6. Considerations for estimating heritabilities in Pacific oysters.

(inserted as a JPEG file)

Evaluating a design for estimation of genetic parameters in Pacific oysters

Andrew Swan
CSIRO Livestock Industries, Armidale

14th August 2002

1 Introduction

Knowledge of parameters such as heritabilities and genetic correlations is critical in the process of setting goals for animal breeding programs and in designing selection methods. An experiment designed to generate the information necessary to obtain precise estimates of these parameters is most definitely required for the oyster breeding program. This simulation study compares variations on one experimental design possible within the resources of the project, in terms of the expected precision of parameter estimates.

2 Basic design

The "partly factorial" design described by Berg and Henryon (1998) has been used previously in this project. This design estimates genetic parameters using information from both half and full sib matings. Each sire is mated to two dams, and each dam to two sires, as shown in Table 1 where sires 1 to 10 are mated to dams *a* to *j*, with cells filled with "x"s representing matings.

Table 1: Partly factorial design of Berg and Henryon (1998) with sires 1 to 10 and dams *a* to *j*

	1	2	3	4	5	6	7	8	9	10
a	x	x								
b		x	x							
c			x	x						
d				x	x					
e					x	x				
f						x	x			
g							x	x		
h								x	x	
i									x	x
j	x									x

1

Table 2: Number of progeny for each simulation combination

	Progeny per mating				
Matings	25	50	75	100	200
16	400	800	1200	1600	3200
24	600	1200	1800	2400	4800
32	800	1600	2400	3200	6400
40	1000	2000	3000	4000	8000
48	1200	2400	3600	4800	9600
56	1400	2800	4200	5600	11200
64	1600	3200	4800	6400	12800
72	1800	3600	5400	7200	14400

3 Simulation procedures

3.1 Mating structures

The partly factorial design was tested with varying numbers of mating pairs (16, 24, 32, 40, 48, 56, 64 and 72), and progeny measured within each mating pair (25, 50, 75 ,100 and 200). The number of progeny generated for each of these combinations is shown in Table 2. The simulations which follow assume that measurements are made on all progeny. Clearly, the experimental effort needed to gather these data is substantial.

An alternative structure was also modeled in which the mating pairs were divided between two groups, to represent different spawning times. Simulations of this structure were performed with no genetic links between the two groups, and compared with the basic structure described above. The purpose of these simulations was to examine the loss in precision of parameter estimates from dividing the matings across different spawning times.

Finally, the two group structure was simulated with varying degrees of genetic linkage, by using full sib males as parents across groups. For example, with three "link" matings in each group, three full sib families are chosen from a hypothetical base population, and two males selected from each, with the first mated in group 1 and the second mated in group 2. The purpose of these simulations was to see whether the expected loss in precision of parameter estimates from dividing the matings could be regained through the use of genetic links.

3.2 Simulation of records

Single and two trait models were used, with records for each trait simulated according to the following model:

$$y_{ij} = sg_i + a_{ij} + e_{ij}$$

where sg_i is the effect of the i^{th} spawning group, a_{ij} is the breeding value of the j^{th} animal in the i^{th} spawning group, and e_{ij} is the residual term, representing to unexplained environmental variation.

Spawning group effects for each trait were sampled from a normal distribution with mean, 0, and variance, $0.2\sigma_p^2$, where σ_p^2 is the phenotypic variance of the trait.

In the single trait model, the breeding value and residual effects were simulated assuming variances of 0.2 and 0.8 respectively, implying a heritability of 0.2. In the two trait model, the breeding values and residual effects were simulated assuming genetic (G) and residual (R) covariance matrices of:

$$G = \begin{bmatrix} 0.3 & 0.15 \\ 0.15 & 0.3 \end{bmatrix}, R = \begin{bmatrix} 0.7 & 0.25 \\ 0.25 & 0.7 \end{bmatrix}$$

implying heritability of 0.3 for both traits, a genetic correlation of 0.5, and a phenotypic correlation of 0.4.

Breeding values for progeny were simulated as:

$$a = 0.5\,(a_s + a_d) + seg$$

where a_s and a_d are the breeding values of the sire and dam respectively, and seg is the term due to Mendelian segregation, with variance equal to one half of the genetic variance.

3.3 Replication and data analysis

1000 replicates of each mating structure were generated, and data from each replicate were analysed using the ASREML software package (Gilmour *et al*, 1999), fitting the model shown in equation 3.2. Parameter estimates and their standard errors were collated. The average standard error of parameter estimates across replicates was used as the criterion for assessing precision.

4 Results and discussion

4.1 Single trait analyses

Figure1 shows the mean standard error of heritability estimates when all matings are carried out in 1 spawning group (Ngroup: 1) or in 2 spawning groups (Ngroup: 2). The results are according to expectation. Overall, the mean standard errors range between 0.03 and 0.1. Increasing the number of mating pairs increases precision (ie. lower standard error). This is because to estimate genetic variance, we require a reasonable sample of the population from which the parents are derived. Genetic variation is regenerated in the progeny through segregation, and as a consequence, increasing the number of progeny measured per mating also increases precision. However, this effect is not as large as the effect of increase the number of mating pairs. Therefore, for a given resource it is better to increase the number of base parents than the number of progeny per mating.

Dividing the mating groups between two spawnings reduces precision, but not by a large amount. Under the assumptions of these simulations, it is clearly better to have 60 matings across two spawnings than 40 matings in one spawning.

4.2 Two trait analyses

Figure 2 shows the mean standard error of genetic correlation estimates. This is the only parameter discussed from the two trait analyses because it is the hardest to estimate. That is, for a given data set, the genetic correlation will be the parameter with the highest standard error. This is demonstrated in Figure 2 with the standard errors ranging between 0.1 and 0.3. Apart from this difference, the results show the same trends as those for the single trait analyses in Figure 1.

4.3 The effect of linking the two spawning groups

These simulations showed no trend at all. I will have to check the programming and re-do them. I would have expected the inclusion of link matings to improve the precision up to a point.

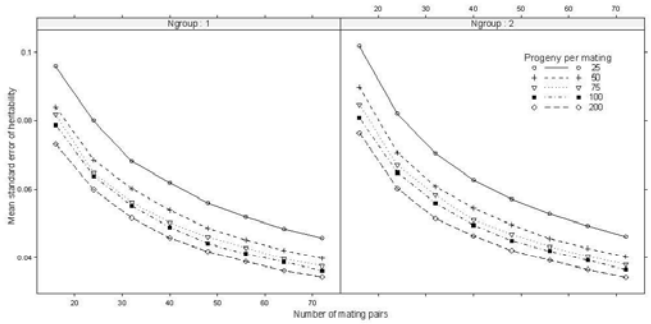

Figure 1: Mean standard error of estimated heritability for a partly factorial design with varying numbers of mating pairs and number of progeny per mating pair. Ngroup = 1 and 2 refers to the number of spawning groups.

4

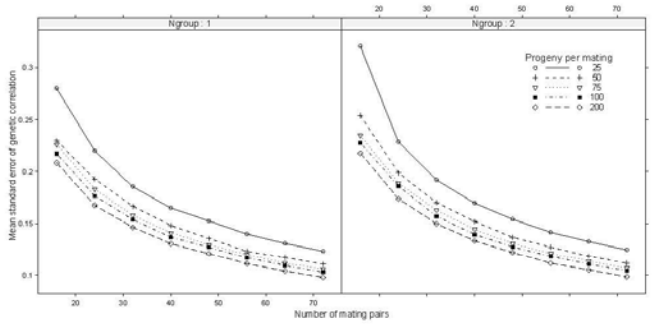

Figure 2: Mean standard error of estimated genetic correlation for a partly factorial design with varying numbers of mating pairs and number of progeny per mating pair. Ngroup = 1 and 2 refers to the number of spawning groups.

5

4.4 Other considerations

Simulation assumptions: Any simulation study is limited by the assumptions made. In this study, the following may affect the conclusions made:

- A simple model of inheritance was assumed (the infinitesimal model in which traits are assumed to be controlled by a large number of genes acting additively with small effect). This model is easy to work with and has been shown to be quite robust in many situations.
- The results for two spawning groups may be influenced by the size of the difference between groups (in this case 20% of the phenotypic variance).
- Possible confounding of genetic effects with spawning tank effects was not modeled. However, Berg and Henryon (1998) did consider these effects.

Linking spawning groups: While the simulations with genetic links between spawning groups are to date inconclusive, intuitively, links are desirable. It would be sensible to have at least 10% of the matings in each group linked through the use of full sibs (either male or female).

Making use of the families from the 2000 spawning: There are a limited number of families remaining from the 2000 spawnings of the heritability experiment. While these families have not been considered specifically in these simulations, they should be measured, and individuals from a proportion of the mating groups used to link the two spawning groups from the forthcoming trial.

Replication within and between sites: Previous attempts at the heritability experiment have replicated the mating groups both within and between sites. Each of these constitutes an experimental unit in its own right. It is sufficient to concentrate a measurement program on just one replicate (this is what happens in similar experiments in other animal species), although of course highly desirable to measure a number of replicates. Given past experience, the within and between site replicates should be constructed simply as an insurance policy. A comprehensive measurement program should be undertaken in one replicate, with the effort in the remaining replicates at a much lower scale, either with no measurement at all, or measurements on groups of animals.

Traits measured at different ages: Measuring traits at different ages poses a problem due to the fact that it is impractical to maintain individual animal identity across time at the scale required. It will not be possible to model the genetic and environmental covariances in a group of animals between traits measured at different times without maintaining animal identity. To partly overcome this, different samples of animals from each mating should be used for measurements taken at different times. This strategy will enable estimation of genetic but not environmental covariances. This is not a major drawback.

The magnitude of the task: The number of animals which it will be necessary to measure is substantial, with between two and three thousand animals required. This will take significant effort: our group recently recorded 12 trait measurements on 1600 individual fresh water crayfish. Three people worked full time on this task for ten days. A realistic estimate of the costs involved in a measurement program for oysters should be given a high priority.

5 Conclusion

An investment in research to estimate genetic parameters is vitally important for the success of the ASI oyster breeding program. A partly factorial design with 30 matings in each of 2 spawnings, and 25-50 progeny measured per mating will provide parameter estimates of reasonable precision. The inclusion of data from the 2000 spawning of this experiment will also be worthwhile.

In future, it will also be possible to add data from the breeding program itself to refine parameter estimates.

6 References

Berg, P and Henryon, M (1998) A comparison of mating designs for inference on genetic parameters in fish. *Proc. 6th World Congr. Genet. Appl. Livest. Prod.* 27: 115 - 119.

Gilmour, A.R., Cullis, B.R., Welham, S.J. and Thompson, R. (1999) ASREML Reference Manual. NSW Agriculture Biometric Bulletin No 3. NSW Agriculture, Locked Bag 21, Orange, NSW, 2800, Australia, 210pp.

Appendix 7. Molecular Genetic Information

Appendix 7.1. Allele frequencies at 11 allozyme loci in eight populations of Pacific oyster (number of individuals sampled given as *n*)

Locus	Allele	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
*AAT-2**	800	0.250	0.184	0.340	0.170	0.316	0.220	0.140	0.040
	100	0.656	0.734	0.590	0.780	0.541	0.680	0.750	0.950
	-700	0.094	0.092	0.070	0.050	0.143	0.100	0.110	0.010
	n	*48*	*49*	*50*	*50*	*49*	*50*	*50*	*50*
*AK**	120	0.041	-	-	-	0.010	0.020	-	-
	110	0.112	0.092	0.204	0.040	0.219	0.120	0.100	0.020
	100	0.745	0.837	0.694	0.860	0.635	0.680	0.740	0.860
	85	0.061	0.071	0.082	0.060	0.125	0.160	0.130	0.120
	75	0.041	-	0.020	0.040	0.010	0.020	0.030	-
	n	*49*	*49*	*49*	*50*	*48*	*50*	*50*	*50*
*GAPDH-2**	120	-	-	-	-	0.041	0.204	0.156	-
	100	0.643	0.602	0.730	0.590	0.520	0.531	0.615	0.643
	80	0.357	0.388	0.270	0.410	0.398	0.214	0.208	0.357
	60	-	0.010	-	-	0.041	0.051	0.021	-
	n	*49*	*49*	*50*	*50*	*49*	*49*	*48*	*7*
*IDHP-1**	115	-	-	-	-	0.022	0.051	-	-
	110	0.061	0.094	0.010	0.061	0.078	0.194	0.071	0.090
	100	0.582	0.646	0.670	0.622	0.656	0.704	0.653	0.800
	85	0.357	0.260	0.310	0.316	0.244	0.051	0.245	0.070
	80	-	-	0.010	-	-	-	0.031	0.040
	n	*49*	*48*	*50*	*49*	*45*	*49*	*49*	*50*
*IDHP-2**	110	0.051	-	0.010	0.032	0.104	0.214	0.100	0.180
	100	0.949	1.000	0.990	0.968	0.896	0.786	0.900	0.780
	95	-	-	-	-	-	-	-	0.040
	n	*49*	*48*	*50*	*47*	*48*	*49*	*50*	*50*
*PEPS-1**	125	0.020	0.020	-	0.020	0.031	0.030	0.030	0.020
	115	0.112	0.082	0.080	0.082	0.337	0.320	0.190	0.280
	100	0.796	0.806	0.760	0.765	0.541	0.430	0.600	0.460
	90	0.061	0.082	0.110	0.092	0.092	0.160	0.130	0.120
	85	0.010	0.010	0.050	0.041	-	0.060	0.060	0.050
	n	*49*	*49*	*50*	*49*	*49*	*50*	*50*	*50*
*PEPS-2**	115	0.023	0.010	0.041	0.031	-	-	-	-
	105	0.409	0.333	0.337	0.235	0.468	0.448	0.427	0.390
	100	0.455	0.531	0.357	0.551	0.404	0.417	0.490	0.450
	95	0.114	0.104	0.224	0.173	0.128	0.135	0.083	0.160
	90	-	0.021	0.041	0.010	-	-	-	-
	n	*44*	*48*	*49*	*49*	*47*	*48*	*48*	*50*
*PGDH**	165	0.041	-	0.020	-	0.022	0.011	-	0.040
	130	0.173	0.146	0.173	-	0.152	0.182	-	0.220
	100	0.786	0.854	0.806	-	0.793	0.739	1.000	0.590
	80	-	-	-	-	0.033	0.068	-	0.150
	n	*49*	*41*	*49*	-	*46*	*44*	*5*	*50*
*GPI-1**	175	-	-	0.010	0.030	-	-	-	-
	135	0.010	0.010	0.060	0.010	0.031	0.040	0.030	0.050
	110	0.020	0.020	-	-	-	0.030	0.020	0.060
	100	0.949	0.939	0.840	0.920	0.929	0.900	0.880	0.810
	90	-	-	0.020	0.020	0.020	0.010	0.020	0.050
	85	0.020	0.031	0.070	0.020	0.020	0.020	0.050	0.030
	n	*49*	*49*	*50*	*50*	*49*	*50*	*50*	*50*
*PGM-1**	150	-	-	-	-	0.153	0.050	0.080	0.140

Locus	Allele	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
	130	-	-	-	-	0.306	0.250	0.230	0.240
	100	-	-	-	-	0.439	0.500	0.520	0.520
	80	-	-	-	-	0.102	0.200	0.170	0.100
	n	-	-	-	-	*49*	*50*	*50*	*50*
*PGM-2**	125	-	-	-	-	-	0.020	-	-
	105	-	-	-	-	0.208	0.300	0.220	0.320
	100	-	-	-	-	0.656	0.610	0.710	0.570
	90	-	-	-	-	0.135	0.070	0.070	0.110
	n	-	-	-	-	*48*	*50*	*50*	*50*

Appendix 7.2 Allele frequencies at eight microsatellite loci in eight populations of Pacific oyster (number of individuals sampled given as *n*)

Locus	Allele	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
cmrCg143	137	-	-	0.019	-	-	-	0.011	0.031
	141	-	0.031	0.019	-	-	-	-	-
	143	0.188	0.375	0.074	0.086	0.021	0.125	0.032	0.041
	145	0.156	0.125	0.204	0.103	0.266	0.193	0.277	0.204
	147	0.094	0.109	0.204	0.190	0.043	0.045	-	-
	149	0.156	0.109	0.056	0.207	0.309	0.227	0.085	0.031
	151	0.141	0.094	0.074	0.155	0.021	-	1.191	0.367
	153	0.078	-	0.111	0.052	0.032	-	-	0.010
	155	0.016	-	0.074	0.017	0.160	0.182	0.074	-
	157	0.063	0.016	0.037	0.103	0.053	0.068	0.021	-
	159	0.078	0.016	0.056	0.034	-	0.114	-	-
	161	-	0.063	0.037	-	0.032	0.023	0.138	0.041
	163	-	-	-	-	-	0.023	0.170	0.245
	165	-	0.016	-	-	-	-	-	0.031
	167	0.031	0.016	-	-	-	-	-	-
	169	-	-	0.019	0.034	-	-	-	-
	171	-	-	0.019	-	0.064	-	-	-
	177	-	0.031	-	0.017	-	-	-	-
	n	*32*	*32*	*27*	*29*	*47*	*44*	*47*	*49*
ucdCg08	247	-	-	0.029	-	0.016	-	-	-
	249	-	-	-	-	0.109	0.111	-	-
	257	-	0.200	-	-	-	-	-	-
	259	-	0.00	0.059	-	-	-	-	-
	261	-	-	-	0.167	-	-	-	-
	263	0.053	-	-	-	-	-	-	-
	265	-	-	0.059	0.056	-	-	-	-
	267	0.053	-	0.059	-	-	0.111	0.125	0.012
	269	0.026	-	0.059	-	0.031	-	-	-
	271	0.026	-	0.176	-	0.125	0.167	-	-
	273	0.053	-	-	-	-	-	-	-
	277	0.105	-	-	-	-	-	-	-
	279	0.159	-	0.059	0.167	-	-	-	-
	281	-	0.200	-	-	0.234	0.222	0.063	0.279
	283	-	-	0.059	0.278	0.031	-	-	-
	285	-	-	-	-	-	-	-	0.012
	287	-	-	-	0.056	-	-	-	-
	289	-	0.200	-	-	0.016	-	-	-
	291	0.053	0.200	-	-	-	-	-	-
	293	-	-	0.059	0.056	0.031	0.056	-	-
	295	0.053	-	-	-	-	-	0.344	0.023
	297	0.053	-	-	-	-	-	-	-
	299	-	-	0.059	-	-	-	-	-
	301	0.105	-	-	-	0.109	0.222	0.375	0.105
	303	-	-	-	-	-	0.056	0.094	-
	305	0.079	0.200	-	-	0.016	-	-	-
	307	-	-	0.059	-	-	0.056	-	0.221
	313	-	-	-	-	-	-	-	0.047
	315	-	-	-	-	-	-	-	0.023
	317	0.026	-	-	-	0.016	-	-	0.035
	319	-	-	-	0.111	-	-	-	-
	321	-	-	-	0.111	-	-	-	-
	327	-	-	-	-	-	-	-	0.012
	329	-	-	-	-	-	-	-	0.209
	331	-	-	-	-	-	-	-	0.012
	333	-	-	-	-	-	-	-	0.012
	341	0.158	-	0.265	-	0.266	-	-	-
	n	*19*	*5*	*17*	*9*	*32*	*18*	*32*	*43*
ucdCg06	141	-	-	-	0.052	-	-	-	-
	145	-	-	-	-	-	0.011	-	-
	155	-	-	0.044	0.017	0.053	0.045	0.021	-
	157	0.042	-	0.059	0.052	0.032	0.023	0.096	-

Locus	Allele	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
	159	0.236	0.250	0.132	0.155	0.106	0.148	-	0.048
	161	0.069	0.147	0.044	0.052	0.138	0.114	0.351	0.488
	163	0.042	0.088	0.088	0.069	0.074	0.011	0.043	0.012
	165	0.069	0.044	0.059	0.052	0.064	0.057	-	-
	167	0.125	0.132	0.162	0.052	0.106	0.148	0.149	0.083
	169	0.056	0.044	0.059	0.172	0.021	0.045	0.021	0.012
	171	0.125	0.074	0.088	0.086	0.160	0.091	0.074	-
	173	0.028	0.074	0.0044	0.052	-	-	-	-
	175	0.083	-	0.044	0.069	0.043	0.136	0.011	0.012
	177	0.056	0.015	0.074	-	0.021	0.034	-	-
	179	0.042	0.044	0.059	0.086	0.032	0.011	-	-
	181	-	-	0.044	-	0.064	0.091	0.128	0.179
	183	0.014	0.044	-	-	0.085	0.034	0.096	0.167
	185	-	0.029	-	-	-	-	-	-
	189	0.014	0.015	-	0.034	-	-	-	-
	193	-	-	-	-	-	-	0.011	-
	n	*36*	*34*	*34*	*29*	*47*	*44*	*47*	*42*
cmrCg61	193	0.028	-	-	0.016	-	-	0.012	-
	195	-	-	-	-	0.021	-	-	-
	197	0.042	0.042	0.014	-	0.053	0.020	0.012	-
	201	0.028	0.014	0.029	-	0.032	0.070	0.049	-
	203	0.028	-	-	-	-	-	-	-
	205	0.028	0.014	0.014	0.047	-	-	0.049	-
	207	-	-	-	0.016	0.011	0.020	-	0.060
	209	0.028	-	0.014	-	-	0.010	-	-
	211	-	-	-	0.063	0.117	-	0.012	-
	213	0.417	0.514	0.414	0.547	0.585	0.680	0.646	0.660
	215	0.069	0.222	0.229	0.172	0.021	0.180	0.085	0.130
	217	0.125	0.028	0.057	-	0.032	-	-	-
	219	0.028	-	0.100	0.031	0.064	0.010	-	0.050
	221	0.153	0.083	0.071	0.094	0.064	-	-	0.100
	223	-	0.028	-	0.016	-	-	0.098	-
	225	0.028	0.014	0.014	-	-	-	0.037	-
	227	-	0.042	-	-	-	-	-	-
	229	-	0.028	0.014	-	-	-	-	-
	231	-	-	0.029	-	-	-	-	-
	233	-	-	-	-	-	0.010	-	-
	n	*36*	*36*	*35*	*32*	*47*	*50*	*41*	*50*
ucdCg02	149	-	-	0.037	-	-	-	-	-
	151	0.015	-	0.056	-	-	-	-	-
	153	0.015	0.023	-	-	-	-	-	-
	155	-	0.023	-	0.038	0.020	-	-	-
	157	-	0.023	-	0.038	0.010	0.013	-	-
	159	-	-	-	-	0.030	-	-	-
	161	0.015	-	0.037	-	-	-	-	-
	163	0.015	0.023	-	-	-	-	-	-
	165	0.029	-	0.037	-	-	-	-	-
	167	0.05	0.023	0.056	-	0.010	0.013	-	-
	169	0.015	0.068	-	0.038	0.030	0.063	0.073	0.290
	171	0.074	0.045	0.074	0.019	0.040	0.075	0.052	-
	173	0.029	0.045	0.037	0.212	0.040	-	-	-
	175	0.044	0.091	0.037	0.077	-	0.038	0.094	-
	177	0.044	-	0.148	0.096	0.060	0.025	0.219	0.010
	179	0.044	-	0.074	0.077	0.170	0.025	0.073	0.020
	181	-	0.068	0.037	0.038	0.010	-	0.010	0.010
	183	0.029	0.023	0.037	0.038	0.030	-	-	-
	185	0.088	0.045	-	0.019	0.050	0.013	0.010	-
	187	0.074	-	0.037	0.038	0.040	-	-	-
	189	0.015	0.068	-	-	0.020	-	0.010	-
	191	0.059	0.114	0.037	0.038	0.050	0.038	-	0.010
	193	-	0.045	-	0.038	0.020	-	0.021	0.040
	195	0.029	0.045	0.037	0.096	0.020	0.025	0.010	0.350
	197	0.044	0.045	0.019	-	0.070	0.025	0.052	0.130
	199	0.074	0.045	-	-	-	0.200	0.083	0.090

Locus	Allele	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
	201	0.015	0.045	0.037	0.058	0.130	0.025	0.146	-
	203	0.103	0.023	0.074	-	0.020	-	0.021	-
	205	-	0.045	0.019	-	-	-	-	-
	207	0.029	-	-	0.038	-	-	-	-
	209	0.015	-	-	-	-	0.013	0.010	0.010
	211	-	-	0.019	-	0.030	0.200	0.063	-
	213	0.015	-	0.037	-	0.070	0.100	0.042	-
	215	0.059	-	-	-	0.010	0.013	0.010	-
	217	-	-	-	-	-	0.013	-	-
	219	-	-	-	-	-	0.013	-	-
	221	-	0.023	-	-	0.010	0.013	-	-
	223	-	-	0.019	-	-	0.038	-	-
	225	-	-	-	-	-	0.025	-	-
	227	-	-	-	-	0.010	-	-	-
	n	*34*	*22*	*27*	*26*	*50*	*40*	*48*	*50*
ucdCg22	219	-	-	-	-	-	-	-	0.010
	221	-	-	-	-	-	-	0.049	0.204
	223	0.029	-	0.065	0.53	-	-	-	-
	225	-	-	0.022	-	0.012	-	-	-
	227	0.059	-	0.022	-	0.024	0.022	-	0.020
	229	0.074	0.024	0.130	0.026	0.183	0.043	0.110	0.296
	231	0.029	-	-	-	-	0.022	0.085	0.133
	233	0.074	-	-	-	-	-	0.024	-
	235	-	0.024	-	-	-	-	-	0.010
	237	0.029	-	-	0.237	0.024	-	0.085	0.051
	239	0.206	0.143	0.130	0.342	0.049	0.152	-	-
	241	-	0.048	0.065	0.053	0.207	-	0.024	-
	243	0.015	0.048	0.043	-	0.024	0.022	-	-
	245	0.044	-	-	0.079	-	-	-	-
	247	-	-	0.022	0.053	0.110	0.196	-	-
	249	0.015	0.048	0.130	0.053	-	-	-	-
	251	0.015	0.048	0.043	-	0.049	-	-	-
	253	0.029	-	0.043	-	0.037	-	-	-
	255	-	0.024	-	0.053	0.024	-	-	-
	257	0.088	-	0.022	-	-	-	-	-
	259	0.029	0.048	0.022	-	-	-	-	-
	261	-	0.048	0.043	-	-	-	0.085	-
	263	0.044	0.190	0.022	-	0.061	0.239	0.366	0.276
	265	-	0.048	0.043	-	-	-	-	-
	267	-	0.048	-	-	0.024	-	0.024	-
	269	0.029	0.024	-	-	-	0.174	-	-
	271	0.015	0.048	0.065	-	0.085	0.065	0.085	-
	273	0.044	-	-	0.026	0.012	-	-	-
	275	0.044	0.048	-	0.026	-	-	-	-
	277	0.044	0.048	0.065	-	0.012	-	-	-
	279	-	0.048	-	-	0.012	-	0.012	-
	281	-	-	-	-	0.049	0.065	0.049	-
	285	0.029	-	-	-	-	-	-	-
	287	0.015	-	-	-	-	-	-	-
	n	*34*	*21*	*23*	*19*	*41*	*23*	*41*	*49*
cmrCg204	159	0.014	-	-	0.014	0.010	0.022	0.031	0.040
	163	0.069	0.043	0.028	0.111	0.020	0.033	0.073	-
	171	0.750	0.743	0.875	0.653	0.830	0.717	0.604	0.780
	175	0.056	0.014	0.028	0.014	0.010	0.022	-	0.010
	179	-	-	-	0.014	0.010	-	-	-
	183	0.111	0.186	0.069	0.181	0.120	0.207	0.292	0.060
	195	-	0.014	-	0.014	-	-	-	-
	219	-	-	-	-	-	-	-	0.110
	n	*36*	*35*	*36*	*36*	*50*	*46*	*48*	*50*
cmrCg207	253	0.014	-	0.016	0.045	-	-	-	-
	257	-	-	-	-	-	-	-	0.228
	261	0.028	0.029	0.032	0.023	0.065	-	-	-
	265	0.069	0.118	0.081	0.023	0.120	0.595	0.308	0.359

Locus	Allele	Sendai	Hiroshima	Bridport	Port Stephens	P1	M1	M3	M4
	269	0.069	0.118	0.113	0.182	0.065	0.048	0.038	0.054
	273	0.042	0.118	0.065	-	0.022	-	-	0.011
	277	0.028	0.088	0.016	-	0.076	0.167	0.051	0.022
	281	0.083	0.118	0.048	0.023	0.163	-	0.026	-
	285	0.125	0.176	0.065	0.068	0.109	0.190	0.372	-
	289	0.056	-	0.065	0.045	-	-	0.026	-
	293	0.028	-	0.048	0.045	-	-	0.141	0.011
	297	0.011	-	0.016	-	-	-	-	-
	301	0.056	-	0.016	-	0.033	-	-	-
	305	0.028	-	0.016	-	0.011	-	-	-
	309	0.014	-	-	-	0.043	-	-	-
	313	-	-	0.016	0.091	0.011	-	-	-
	317	0.014	0.029	0.032	0.041	-	-	-	-
	321	-	0.029	-	-	-	-	-	-
	325	0.028	0.029	-	-	0.043	-	-	-
	329	-	-	0.048	-	0.065	-	-	0.011
	333	0.014	0.059	-	0.045	0.011	-	-	0.022
	337	0.014	0.029	-	0.045	0.043	-	-	-
	341	0.028	-	0.032	0.023	-	-	0.038	0.022
	345	0.042	-	-	0.091	-	-	-	-
	348	0.014	-	0.065	0.045	0.033	-	-	-
	353	0.028	-	0.032	0.045	-	-	-	-
	357	0.056	-	-	-	0.022	-	-	0.261
	361	-	-	0.065	-	0.022	-	-	-
	365	-	-	-	-	0.022	-	-	-
	369	0.014	-	0.032	0.114	-	-	-	-
	373	-	-	0.016	-	0.022	-	-	-
	377	-	0.029	0.048	-	-	-	-	-
	385	-	-	0.016	-	-	-	-	-
	397	-	0.029	-	-	-	-	-	-
	n	*36*	*17*	*31*	*22*	*46*	*21*	*39*	*46*

Appendix 7.3.

Genotyped parents from family lines (as per tissue provided for analyses during project (from the hatchery), not all parents were sampled - particularly in from the F5 generation of families)

Putative parents	Generation of putative parents	Putative family (ies)[##]	Generation of resultant family
6F from family 21	F2	52	F3
10M* from family 13	F2	52	
4F from family 21	F2	54	F3
1M from family 5	F2	54	
16F from family 10	F2	55	F3
10M* from family 13	F2	55	
18F from family 10	F2	56	F3
3M from family 5	F2	56	
F from family 21	F2	57	F3
M* from family 31	F2	57	
F from family 10	F2	58	F3
M* from family 31	F2	58	
F from family 21	F2	59	F3
M from family 10	F2	59	
4F from family 19	F2	60	F3
M from family 13	F2	60	
1F from family 31	F2	61	F3
1M* from family 19	F2	61	
F from family 5	F2	62	F3
1M* from family 19	F2	62	
1F from family 4	F2	64	F3
M* from family 13	F2	64	
F from family 31	F2	65	F3
2M from family 4	F2	65	
F from family 21	F2	66	F3
6M from family 14	F2	66	
wa213F	F2	67	F3
10M from family 15	F2	67	
3F from family 23	F2	68	F3
M* from family 13	F2	68	
1F from family 10	F3	100	F4
1M from family 61	F3	100	
1F from family 23	F3	101	F4
3M from family 10	F3	101	

Putative parents	Generation of putative parents	Putative family (ies)[##]	Generation of resultant family
2F from family 23	F3	102	F4
1M from family 43	F3	102	
3F from family 23	F3	103	F4
5M from family 43	F3	103	
2F from family 43	F3	104	F4
1M[#] from family 59	F3	104	
M[#]	F3	104	
5F from family 5	F3	106	F4
4M from family 10	F3	106	
1F from family 43	F3	107r	F4
1M from family 52	F3	107r	
2F from family 17	F3	110	F4
3M from family 59	F3	110	
3F from family 57	F3	111	F4
1M from family 17	F3	111	
3F from family 20	F3	112	F4
6M from family 57	F3	112	
5F from family 15	F3	114	F4
2M from family 59	F3	114	
1F from family 15	F3	115	F4
1M from family 14	F3	115	
M[#]	F3	115	
1F from family 25	F3	116	F4
1M from family 62	F3	116	
1F from family 21	F3	117	F4
1M from family 13	F3	117	
3F from family 67	F3	118	F4
1M from family 59	F3	118	
2F from family 57	F3	120	F4
7M from family 50	F3	120	
5F from family 16	F2	171?, 16-1?, 183?	F5
4F from family 16	F2	171?, 16-1?, 183?	F5
1F from family 16	F2	171?, 16-1?, 183?	F5
16F from family 16	F2	171?, 16-1?, 183?	F5
7F from family 16	F2	171?, 16-1?, 183?	F5
9F from family 16	F2	171?, 16-1?, 183?	F5
3M from family 16	F2	16-1	F5
5F from family 64	F3	192	F5
GF from family 62	F3	174?, 177?, 159?	F5
DF from family 62	F3	174?, 177?, 159?	F5

Putative parents	Generation of putative parents	Putative family (ies)[##]	Generation of resultant family
2F from family 62	F3	174?, 177?, 159?	F5
9M from family 62	F3	168?, 178?	F5
1F from family 66	F3	176	F5
1M from family 66	F3	175?, 177?, 186?, 169?	F5
6M from family 66	F3	175?, 177?, 186?, 169?	F5
5M from family 66	F3	175?, 177?, 186?, 169?	F5
JF from family 58	F3	178?, 182?	F5
KF from family 58	F3	178?, 182?	F5
1M from family 106	F4	172?, 179?, 194?	F5
2M from family 106	F4	172?, 179?, 194?	F5
4M from family 107	F4	163?, 180?, 182?, 164?, 193?	F5
10M from family 107	F4	163?, 180?, 182?, 164?, 193?	F5
1M from family 107	F4	163?, 180?, 182?, 164?, 193?	F5
8M from family 107	F4	163?, 180?, 182?, 164?, 193?	F5
2F from family 21	F2	21-1	F5
1M from family 21	F2	21-1?, 190?	F5
2M from family 21	F2	21-1?, 190?	F5
11M from family 102	F4	184?, 181?	F5
1M from family 102	F4	184?, 181?	F5
1F from family 110	F4	161?, 187	F5
5M from family 110	F4	166?, 170?, 199?	F5
1M from family 117	F4	159?, 174?	F5
3M from family 117	F4	159?, 174?	F5
2F from family 59	F3	160?, 181?	F5
LF from family 59	F3	160?, 181?	F5
5M from family 59	F3	154?, 158?, 187?	F5
6M from family 59	F3	154?, 158?, 187?	F5
2F from family 52	F3	172?, 184?,	F5
BF from family 52	F5	172?, 184?,	F5
1M from family 52	F3	165	F5
6F from family 60	F3	152?, 191?	F5
9M from family 60	F3	167?, 176?	F5
EF from family 67	F3	150?, 154?, 155?, 163?, 170?,179?, 180?, 198?	F5
CF from family 67	F3	150?, 154?, 155?, 163?, 170?,179?, 180?, 198?	F5
1F from family 67	F3	150?, 154?, 155?, 163?, 170?,179?, 180?, 198?	F5
HF from family 50	F3	153?, 157?, 175?, 189?, 188?	F5
2F from family 50	F3	153?, 157?, 175?, 189?, 188?	F5
7F from family 50	F3	153?, 157?, 175?, 189?, 188?	F5
1M from family 50	F3	185	F5
3M from family 112	F5	156?	F5
7M from family 112	F4	156?	F5
4M from family 112	F4	156?	F5

Putative parents	Generation of putative parents	Putative family (ies)[##]	Generation of resultant family
2F from Camerons	NEW	165	F5
3F from Camerons	NEW	173	F5

[*]individual(s) used in multiple families/combinations
[#]uncertain of exact male used, genotyped tissues from multiple males
individual records on which male or female used in the particular cross not available, all families that the individual could have contributed are presented

Appendix 8. The "Here comes the stud oyster" Media Release

Media Release

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CSIRO Media Releases are also available on the Internet: http://www.csiro.au

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HERE COMES THE STUD OYSTER

The stud oyster, sire of a long line of large, luscious, seductively-proportioned and sensuously-hued seafood to tempt the Aussie palate will soon become a reality.

Lines of superior oysters that grow faster and look better will be developed as a result of a landmark agreement to commercialise a selective breeding program developed by Tasmanian scientists.

The deal promises to boost the quality and efficiency of Australia's $25 million-a-year Pacific oyster farming industry.

The new technology will be adopted via Australian Seafood Industries, a company recently established by the Tasmanian Oyster Research Council, the South Australian Oyster Research Council and the South Australian Oyster Growers' Association.

It will ensure both oyster growers and consumers benefit from higher-yielding, faster growing and more uniform Pacific oysters, with the shape and colour favoured by local and overseas markets.
Scientists from CSIRO Marine Research and the Tasmanian Aquaculture and Fisheries Research Institute (TAFI) at the University of Tasmania began selectively breeding Pacific oysters in 1995, with funding from the Fisheries Research and Development Corporation (FRDC) and the former Cooperative Research Centre for Aquaculture. They also have received considerable support from industry.

The agreement – between Australian Seafood Industries, CSIRO, TAFI and FRDC – ensures the continuation of this collaboration.

Director of TAFI, Professor Colin Buxton, says the commercialisation of this research emphasises the importance of scientists working hand in hand with industry.

'It will provide for superior lines of Pacific oysters, and their associated breeding records, to be made available to commercial oyster hatcheries.'

Chairman of the Tasmanian Oyster Research Council, and of ASI, Mr Barry Ryan, says the industry stands to gain enormously from securing rights to the selective breeding technology.

'A better Pacific oyster will improve the viability of the Australian industry, helping it to compete more effectively with New Zealand products in domestic markets and to expand further into export markets,' he says.

Chief of CSIRO Marine Research, Dr Nan Bray, says the oyster-breeding program is using conventional genetic selection techniques similar to those used for centuries to improve the quality and productivity of domestic livestock.

'We're only just beginning to apply these selection techniques to aquaculture species and are hoping to make the same kind of gains as more established enterprises such as cattle and sheep,' Dr Bray says.

'Based on increased growth rates achieved so far, it looks as though we will be able to reduce the growing period by at least 25%.

Selection for maturity and uniformity of size will contribute to a further reduction in production costs due to reduced handling. Other benefits will include the development of Pacific oyster strains suited to particular farm locations, and the eradication of deleterious traits such as shell deformities.

'Tasmania and South Australia are among the few places in the world where Pacific oysters are farmed in a closed system,' Dr Bray says.

'In most other parts of the world, stocks are recruited from the wild, so they cannot be improved through genetic selection. This puts us ahead of the game.'

Research background

The selective breeding program takes advantage of the abundant genetic variation that exists in Tasmania's wild and farmed Pacific oyster stocks.

It began in 1996–97 with a few fast-growing oysters and six family lines and now involves more than 100 families, each containing some 6000 oysters. Some families are now in their fourth generation.

In close collaboration with industry, all families have been spawned at commercial hatcheries at Bicheno on Tasmania's eastern coast.

After spawning they are brought to TAFI in Hobart and grown for 12–15 weeks to almost 2 mm in diameter before being transferred a nursery site in northern Tasmania. They are then grown out at five farm sites, three in Tasmania and two in South Australia.

In related research, molecular markers have been developed so that Pacific oyster families can be identified through DNA analysis. A preliminary genetic map of the chromosomes locating major Pacific oyster genes for growth has also been developed.

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