

**Abalone Aquaculture Subprogram:  
Selective Breeding of Farmed Abalone to  
Enhance Growth Rates (II)**

**FRDC Final Report (Project No. 2001/254)**

**Xiaoxu Li**

**November 2008**

**SARDI Publication No: F2008/000813-1  
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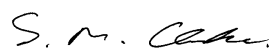
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## 1. TABLE OF CONTENTS

<b>2. NON-TECHNICAL SUMMARY:</b> .....	<b>4</b>
<b>3. ACKNOWLEDGEMENTS</b> .....	<b>11</b>
<b>4. BACKGROUND</b> .....	<b>12</b>
<b>5. NEED</b> .....	<b>15</b>
<b>6. OBJECTIVES</b> .....	<b>16</b>
<b>7. ABALONE FAMILY ESTABLISHMENT AND MAINTENANCE</b> .....	<b>17</b>
7.1. Family establishment and maintenance.....	17
7.2. Strength and weakness analyses of farm-based abalone selective breeding program.....	21
<b>8. PRELIMINARY GENETIC PARAMETER ANALYSES</b> .....	<b>28</b>
8.1. Introduction.....	28
8.2. Materials and methods .....	28
8.2.1. Experimental design.....	28
8.2.2. Statistical analyses .....	31
8.3. Results and discussion.....	33
8.3.1. Blacklip abalone .....	33
8.3.2. Greenlip abalone.....	43
8.4. Concluding remarks.....	46
<b>9. DEVELOPMENT OF BREEDING OBJECTIVES AND SELECTION INDICES AND SENSITIVITY ANALYSES ON SELECTED PARAMETERS</b> .....	<b>48</b>
9.1. Introduction.....	48
9.2. Material and methods.....	49
9.2.1. Assumed abalone aquaculture production system in Australia .....	49
9.2.2. Breeding objective .....	50
9.2.3. The selection index.....	54
9.2.4. Phenotypic and genetic parameters .....	55
9.2.5. Calculations of matrixes and selection responses.....	56
9.2.5. Calculation of economic benefits.....	58
9.2.6. Sensitivity analysis.....	62
9.2.7. Chance of success.....	63
9.3. Results and discussion.....	64
9.3.1. Genetic gains or cost savings.....	64
9.3.2. Sensitivity to biological parameters.....	68
9.3.3. Sensitivity to economic parameters .....	73
9.3.4. Sensitivity to operational factors .....	77
9.3.5. Sensitivity to nucleus population sizes .....	79
9.3.6. Chance of success.....	83
9.4. Concluding remarks.....	84
<b>10. BENEFITS</b> .....	<b>86</b>
<b>11. FURTHER DEVELOPMENT</b> .....	<b>87</b>
<b>12. PLANNED OUTCOMES</b> .....	<b>89</b>
<b>13. CONCLUSION</b> .....	<b>91</b>

**14. REFERENCES..... 92**

**APPENDIX 1: INTELLECTUAL PROPERTY ..... 98**

**APPENDIX 2: PROJECT STAFF ..... 99**

**APPENDIX 3: ABALONE TENTACLE SAMPLING..... 100**

## **2. NON-TECHNICAL SUMMARY:**

### **OUTCOMES ACHIEVED TO DATE**

As the result of this project the Australian abalone aquaculture industry's confidence to pursue a genetic improvement program has been further strengthened through: 1. more on-farm technical staff having been trained (most of them are currently involved in selective breeding projects on different farms); 2. demonstrating industry's capability to establish the targeted 100 families in the desired time period (1 month) through a collaborative approach among 3 farms; and 3. developing methodologies to establish breeding objectives for different production scenarios, and assess investments in genetic improvement programs.

The preliminary genetic analysis suggests genetic improvement in both body and processing traits can be achieved through selective breeding. The investment appraisals of the abalone genetic improvement program using the breeding objectives developed in this study show favourable economic benefit and benefit/cost ratio over the 15 year period evaluated. For example, if the progeny of selected parents are farmed at the production level of 300 ton per annum, the anticipated economic benefit and benefit/cost ratio over this period are AU\$ 12.7 and 10.4 million, and 19.2 and 15.9 for fixed farming period and fixed harvest weight production scenarios, respectively. Greater economic benefit and benefit/cost ratios can be expected when higher heritability values are applied, and/or more selected progeny are farmed.

The analyses also show that due to a significant negative correlation between harvest weight or growth rate and survival, the program would result in a reduction in abalone survival from 80% to about 70% after 5 generations (about 15 years), which may concern breeders because lower survival would also increase the chance of cross infection between animals in the highly intensive abalone farming system. However, this result needs to be treated cautiously because the correlation analysis was undertaken on a very small population size and tag losses were considered a random effect across families and replicate tanks. If a similar or higher magnitude of negative correlation is confirmed in the subsequent studies, a carefully designed selection

strategy or strategies would be needed to address this issue, and require greater attention in the program.

The methods used in the breeding objective and selection index development, and the sensitivity analyses to selected parameters, can also assist in identifying the research areas worthy of greater attention and in making decisions to obtain optimal return from investment. For example, investment in reducing the generation interval from 3 to 2 years would produce more than double the economic benefit than that reducing the extra number of broodstock selected for each parent required to breed the next generation from 2 to 0.25 individuals.

These methods can also be applied to breeding programs for other aquaculture species.

In addition, strengths and weaknesses of the farmed-based abalone selective breeding program have also been identified, and recommendations for further improvement have been provided.

The abalone aquaculture industry has developed significantly in Australia since the first attempts to farm in the early 1980s. Two species, the blacklip *Haliotis rubra* (Leach) and the greenlip *Haliotis laevigata* (Donovan) and their hybrids are currently farmed in Tasmania and Victoria, while only greenlip are farmed in South Australia and Western Australia. In comparison with some abalone species farmed in other countries, these species grow slowly and usually take about 4 years to grow to the market size of 110mm. As has happened with world prices for cultured salmon products, with the increase in world production of cultured abalone products, there is also likely to be a decrease in prices, mainly due to market competition. Therefore, the Australian industry needs to examine potential improvement technologies to ensure its continued viability and competitiveness. The exploitation of the, as yet, untapped genetic gains that are possible through well-designed genetic improvement programmes offers one of the logical and low-risk solutions (Elliott, 2000).

The application of selection techniques to shellfish has proven effective in recent years and will play a major role in the improvement of numerous quantitative traits such as growth rates and meat weight, especially for species with high market value and high production costs such as abalone.

In early 2000, an abalone selective breeding project (referred to as the first FRDC project hereafter), of 18 months duration, was established with funding support from the FRDC. The main objectives of the first FRDC project were to establish a national breeding protocol for abalone family establishments on participating farms, and demonstrate the feasibility of using farm facilities for the proposed selective breeding program (Li, 2004). A cooperative approach between farms for family production at larval stage was agreed at the start of the project, with all the families established in each state to be pooled on one farm after settlement (about 6 days post-fertilisation).

One of the outcomes of the first FRDC project was an interim breeding goal for the abalone selective breeding program, and this was established from interviews with participating farms, including:

1. An increase in growth rates (measured by shell size or whole weight);
2. An increase in meat weight at harvest; and
3. Improved survival to harvest.

At the same time, the following traits will also be monitored:

1. Difference in performance between male and female abalone in body traits;
2. Meat quality at harvest (assessment to be determined); and
3. Age at maturity, and sex ratios.

In November 2001 this project was approved by the FRDC to continue abalone selective breeding activities and further develop it into a comprehensive selective breeding program.

The 1<sup>st</sup> project objective was to establish new families, and this was achieved. In total, 235 families had been established from the summer of 2001/2002 to the summer of 2005/2006 (for details refer to Table 7.1. Number of families established). This number is much higher than the 200 families originally expected in the application. In



addition, 113 greenlip families were established in less than 1 month in the 2005/2006 summer among the three Victorian farms through the coordination provided by the project's principal investigator. This is the capacity level required in the proposed breeding program.

The main issues encountered during this period that affected the project were changes of research priorities in some farms, and frequent changes in on-farm project officers (for example, moved to a new farm, or left the industry). When new officers were recruited, one spawning season was normally required for them to be familiarised with the family establishment procedures. Measures to minimise these difficulties are suggested in section 7. ABALONE FAMILY ESTABLISHMENT AND MAINTENANCE.

The 2<sup>nd</sup> objective of the project was to continue to maintain established families; this was achieved until the outbreak of abalone viral ganglioneuritis on two of the three main farms participating in this project in Victoria, in early 2006. These farms were destocked according to regulatory requirements.

All the families established during this and the previous project were properly maintained on the participating farms, except that 18 greenlip families were discontinued due to either lack of tanks on the farm or too high levels of contamination after DNA pedigree analyses.

In addition, strengths and weaknesses of the farmed-based abalone selective breeding program have also been identified, and recommendations for further improvement have been provided.

The 3<sup>rd</sup> project objective was to upgrade the management system for easy maintenance, easy cross-reference, protection of the privacy of individual farms, and so on. This has been partially achieved. The project recording system was initially established with MS Excel in the first project. A method to link information between families and parent-progenies based on their family trees and/or farm, family and individual IDs was established. However, with the Excel system it is difficult to

establish a security system to protect an individual farm's privacy by allowing different access levels. A Microsoft Access-based system was considered and then abandoned, due to the fact that it was clear at that time that the system should also have the capacity to handle both electronic and manual data collection, and the potential to be extended to include data for new traits. Such an approach would require the services of a professional software developer and thus be too expensive.

Both objectives 4 and 5 are for the long-term development of the abalone selective breeding program, and will require regular updating and reviewing as new data and information become available.

The 4<sup>th</sup> objective, collection of data and determining genetic parameters for traits of economic importance and their correlations (if any), was completed.

The results from the preliminary parameter analyses for both abalone species in this study show that:

- There were preliminary indications of genetic variation in body traits (weight and length) in both species and in processing traits (meat weight, shell weight, rumbled meat weight and cooked meat weight) in blacklip abalone, suggesting that genetic improvement in these traits can be effectively achieved through selective breeding.
- Both spawning batch and replicate tanks had a strong influence on all the observed traits in both species, and the effect of sex was significant for harvest length and weight and processing traits in blacklip abalone (no gender data available in greenlip abalone), suggesting the necessity of including systematic fixed effects in the genetic evaluations in these species.
- The genetic correlations between body and processing traits (or among themselves) in blacklip abalone were highly favourable, and thus simultaneous improvement can be achieved by selection on any of these traits.
- The phenotypic correlation between body weight at harvest and overall survival in blacklip abalone were moderate, but negative.
- The measurements at the tagging stage are not good indicators for an animal's overall performance, thus selection at this stage is unlikely to deliver good genetic gain.

- Males and females performed differently in most traits measured, and have to be ranked and selected separately.
- In blacklip abalone, the ratio between male and female individuals is very close to 1:1.
- In order to estimate genetic parameters with a reliable level of accuracy, there is a strong need to increase the number of families, and the best fit of statistical models can be finalized when more data from different spawning years, and different farms, and states are accumulated in the future.

The 5<sup>th</sup> objective was to develop, respectively, the selection index for both greenlip and blacklip abalone with the data available. These indices will be used to select individuals for commercial production and production of the next generation. This objective was achieved. The methods for development of the breeding objective have been established and applied for both abalone production scenarios, that is, “fixed farming period” and “fixed harvest weight”. However, the definition of the breeding objective needs to be refined as a continuous progression, as production and marketing systems stabilise, new knowledge becomes available, and as more traits are considered in the future.

Sensitivity analyses on selected parameters were also conducted in this study. The results suggest:

- The chance of success of an abalone genetic improvement program is high, although failure due to unforeseen natural disasters can occur.
- Heritability levels can have a very strong impact, and can be increased through improved management practice in the selective breeding nucleus.
- A reduction in the generation interval from 3 to 2 years would have an extremely strong effect on economic benefits (*EB*) (>50%), which is much higher than that which could be achieved through a reduction in the extra number of broodstock selected for each parent required to breed the next generation from 2 to 0.25 individuals (<20%).

- The cost of increased feed intake should be considered in the breeding objective, to avoid over estimations of *EB* and benefit cost ratio (*BCR*) of the program.
- *EB* is not sensitive to changes in either initial investment and annual recurrent costs, whereas the discount rate can have very strong impact on *EB* and *BCR*.
- Both *EB* and *BCR* are very sensitive to abalone prices in the fixed farming period production scenario, but not sensitive in the fixed harvest weight production scenario.
- The earlier the first returns are achieved after the initiation of the breeding program, the greater the *EB* and *BCR* will be because it can have a very strong effect.
- The industry adoption levels could have the greatest impact on both *EB* and *BCR*. When the industry adoption levels increase from 100 to 500 ton the *EB* increase by about 400%, suggesting that the abalone genetic improvement program should start with the species with the higher expected industry adoption level in the future.
- The methods used in this study in the sensitivity analyses could assist in evaluation of investment and breeding strategies.
- The methods can also assist in assessment of R&D options to maximise returns from the limited available funding.

Finally, the methods developed in this project for definition of breeding objectives and appraisal of investment in genetic improvement programs can also be applied to breeding programs in other aquaculture species.

### **3. ACKNOWLEDGEMENTS**

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## 4. BACKGROUND

Abalone are one of the most important cultured aquatic species in Australia. Production has increased substantially during the last few years, from less than 100 ton in 2001 to about 500 ton in 2006, valued at approximately AU\$25 million. In Australia, two abalone species, blacklip and greenlip, and their hybrid, are cultured in southern temperate waters with various levels of management. Most commercial operations use land-based raceway systems in the grow-out phase. Off-shore cage systems are also used by a few farms in South Australia, and are under development in Victoria, Tasmania and Western Australia.

In Australia, abalone aquaculture began in the late 1980s, and the majority of farmed stock are produced directly from wild broodstock, although broodstock selected from commercially-farmed stocks have been used in some instances. The industry has not yet benefited from a structured selective breeding program.

Genetically based breeding programs have made an enormous contribution to increases in agricultural yield during the last century. Estimates suggest that at least 30% of the increase in the rate and efficiency of land-based protein production since 1900 is the direct result of genetic improvement (USDA, 1988). This same improvement in production is possible with any species of aquatic animals and plants, provided the life cycle can be controlled.

The application of selection techniques to farmed aquatic animal species has proven effective over the last 50 years. Since 1960, many improvements have been made in this area, and numerous quantitative traits such as growth rate and weight have been manipulated. The most successful example has been selection for improved growth rate and other desirable characteristics in Atlantic salmon (*Salmo salar*) in Norway over the past 20 years (Gjedrem, 1998). The effective application of genetic selection principles in bivalve molluscs such as Pacific oysters (*Crassostrea gigas*) has also been successfully practised in recent years (Kenneth, 1997; Ward et al., 2005) and is receiving increasing attention.

Abalone are slow-growing gastropod molluscs. In this study, more than four years were required for blacklip abalone produced in the 2000/2001 season to grow to an average weight of 110g. Consequently, improved growth rates would result in considerable cost savings for the abalone aquaculture industry. These could be achieved by selection for faster growing strains, and by other genetic improvement methods such as chromosome manipulation. These methods have proven to be effective methods in some cultured species.

High fecundity and phenotypic variance typify many marine organisms (Gjedrem, 1983). Providing that the phenotypic variance includes a substantial genetic component, the combination of these factors allows rapid genetic improvement via high levels of selection intensity. This is because selection intensity is dependent upon (1) the degree by which individuals of families deviate from the population mean, and (2) the proportion of individuals that can be selected from individual families. A high variance for a trait potentially allows for greater intensity of selection, because there are more individuals further from the mean value. High fecundity allows high levels of selection intensity because a smaller proportion of individuals are needed to prevent inbreeding effects.

This project is the extension of the first FRDC abalone selective breeding project which finished in November 2001. The main aims of the first project were to demonstrate the feasibility of establishing abalone families on participating farms, and to build up a working team which could manage the on-farm activities required for a genetic R&D program.

The new project continued the development of a structured genetic improvement program that has a high degree of industry involvement and ownership, and strengthens this through a substantial increase in abalone families and the development of breeding objective(s) and selection strategy(ies). These will provide a significant entry into the development of a comprehensive selective breeding program and the development of an economically viable and sustainable abalone breeding company for the future development of the industry.

The research component of this project was supervised by the Fisheries Research and Development Corporation's Abalone Aquaculture Subprogram. The critical milestones of the project were: the establishment of 200 additional abalone families, and the establishment of desired number of families (100) within the targeted time period (less than 1 month), the development of breeding objective and selection indices, and the appraisal of investment into a genetic improvement program.



## **5. NEED**

A major problem facing abalone farmers in temperate Australia is the high operating costs associated with holding animals for 4 years until they reach market size. In other shellfish, selective breeding has substantially, and in some cases radically improved a number of traits (particularly growth rate and disease resistance). It is expected that an appropriately designed selective breeding program could produce abalone with growth rates enhanced by up to 30% over 3 generations of selection. This could shorten the production cycle by over a year, and thus substantially reduce farm operating costs.

The first FRDC abalone selective breeding project was funded for 18 months and finished in November 2001. The project can be regarded as a step-up towards establishing selectively bred stock. In that project, a protocol manual was produced, technical officers trained, and families established in South Australia and Victoria. A business model was developed for the future commercialisation of stocks. The industry and subprogram were confident that the selected model for establishing families on-farm, where the onus was on industry to maintain families, was a successful one.

With the continuing enthusiasm for abalone aquaculture both on-shore and off-shore across southern Australia, as well as developing in northern Australia, significant growth of the industry can be expected. Within the next decade it is possible that abalone aquaculture production will exceed the wild fishery in tonnage and value.

## **6. OBJECTIVES**

The project objectives were to:

1. Establish new families.
2. Continue to maintain established families.
3. Upgrade the management system for easy maintenance, easy cross-reference, protection of the privacy of individual farms, and so on.
4. Collect data and determine genetic parameters for traits of economic importance and their correlations (if any), and
5. Develop, respectively, the breeding objective and selection indices for both greenlip and blacklip abalone with the data available. (The indices will be used for selecting improved broodstock for commercial production and production of the next generation.)

## **7. ABALONE FAMILY ESTABLISHMENT AND MAINTENANCE**

### **7.1. Family establishment and maintenance**

Establishment of the required number of families is critical to the long-term development of abalone selective breeding business in terms of 1) achieving the expected return from investment; 2) minimising potential negative effects from inbreeding, and 3) maximising genetic diversity in the founding population established.

Families were established and maintained on each of the participating commercial farms according to the protocol developed in the first FRDC project. These families were produced by full-sib design and included only wild broodstock collected within each state until the 2004/2005 summer. In the 2005/2006 summer both local wild stocks and farm stocks from interstate were included in the family establishment in Victoria, resulting in 113 families (including 90 half-sib families). In total 265 families have been established (Table 7.1), including 210 greenlip families, 53 blacklip families, and 2 selfing families (resulting from the two hermaphroditic parents used). The number of families produced from 2001/2002 to 2005/2006 was 235 (excluding 6 discontinued families), which is higher than the 200 families expected in the application. Most importantly, the target to establish 100 families within a one month period was achieved in Victoria.

During this project period, the following general procedures were used for family establishment and maintenance. Gametes were obtained by the standard spawning method used in commercial hatcheries. Similar numbers of individuals were maintained in each family during fertilisation and settlement, and reduced by random culling to equal numbers per family within three months post-fertilisation. Animals were held separately prior to being tagged. During this period, environmental conditions were maintained as uniformly as possible in order to reduce environmental effects on trait variation between families. At approximately one year post-fertilisation, 420 randomly selected individuals per family were tagged and divided

into three groups (140 individuals per group). The tagged animals from one group per family were then pooled in one grow-out tank, resulting in three replicates per generation. At tagging each individual's length was measured. Follow-up measurements were conducted at approximately one year intervals and included both length and weight. The animal's gender status was assessed visually at each measurement, and recorded when known. At the final measurement extra information on shell weight, meat weight, and meat weight after each processing required for canned product, were collected from subsamples.

Prior to tagging, information on broodstock collection, spawning, fertilisation and hatch-out, larval rearing and settlement was also collected.

The other critical measure is to determine whether there was any contamination in the established families prior to statistical analyses using DNA markers. These would require:

1. a procedure to collect tissue samples from live abalone without significant impact on their survival;
2. a practical procedure for transportation of tissue samples from remote abalone farms to the Victorian Institute of Animal Sciences (VIAS) without negative effects on the quality of the DNA extracted from these tissues and
2. a set of DNA markers suitable for pedigree analyses.

In November 2003 tentacle samples from 20 abalone were collected at SARDI and couriered to VIAS in about 24 hours in an esky filled with ice. DNA samples were successfully extracted from these tentacle samples. However, 4 animals (20%) died after tentacle sampling, which was higher than expected. High sampling mortality did not occur when tentacle sampling was conducted in April 2004, suggesting that the unexpected abnormal sampling mortality might have resulted from high temperature in November 2003. A protocol "Sampling Abalone tentacles for pedigree checks" was then developed (for details refer to Appendix 3).

In this study tentacles from 168 blacklip individuals (6 families, 26 individuals per family and their parents) have been analysed using 6 DNA markers. Although no contamination was found in any of the progenies analysed, the DNA results from the

tissues of one parent did not match up with the loci of the progenies in that family. Mislabelling the tissues collected from other broodstock used at the same spawning time was considered as the reason.

For greenlip abalone, tentacle samples from 250 individuals in 12 families were collected in 2004. These animals had lost their tags and were re-tagged after tissue sampling. It was anticipated that the pedigree results could reassign the re-tagged individuals to their respective families and they could be used in the subsequent data analyses and selections. Nine microsatellite markers were applied. The DNA results were then run through the parental assignment program Probmax. The outcomes showed that of the 250 individuals tested, 147 individuals (41.2%) were not from the original family crosses, indicating a high level of contamination in these families. In June 2005 a second set of tentacle samples were collected from the individuals with their original tags in these families to determine when the contamination had occurred. Analysis using Probmax (also checked manually) showed that out of the 34 individuals tested, a total of 18 individuals (52.9%) were unable to be identified to their original family crosses. This indicates that the contamination occurred before they were originally tagged. These results and methods that could minimise contamination were discussed with the farms participating in the selective breeding project and reported at the Abalone Aquaculture Subprogram annual meeting in 2005 at McLaren Vale, SA.

**Table 7.1. Number of abalone families established**

State	Farm	Species	Year					
			2000/2001	2001/2002	2002/2003	2003/2004	2004/2005	2005/2006
WA	Great Southern Marine Hatcheries (GSMH)	Greenlip		<b>3</b>	<b>3</b>	<b>5</b>		
SA	SAM Abalone	Greenlip	<b>12</b>			<b>3</b>		
	Kangaroo Island Abalone (KIAB)	Greenlip	5 (5 of the 12 families at SAM Abalone were split and sent to KIAB for environmental effects trial in 2002)		<b>4</b>			
	SA Seafoods	Greenlip				<b>2</b>		
VIC	Southern Ocean Mariculture (SOM)	Greenlip		<b>15</b>	<b>10</b>	<b>5</b>	<b>13</b>	<b>35</b>
		Blacklip	<b>14</b> (4 of which came from OWS as 5 day old larvae)	<b>3</b>	<b>6</b>	<b>5</b>	<b>11</b>	
		Others			<b>1</b>		<b>1</b>	
	Ocean Wave Seafood	Greenlip		<b>1</b>				
		Blacklip	4 (moved back from SOM for environmental trial)	<b>2</b>				
	Great Southern Waters	Greenlip				<b>6</b>	<b>13</b>	<b>40</b>
		Blacklip				<b>2</b>	<b>2</b>	
Costal Seafarms	Greenlip						<b>38</b>	
<b>Total</b>			<b>26</b>	<b>24</b>	<b>24</b>	<b>28</b>	<b>50</b>	<b>113</b>
<b>Cumulative Total</b>			<b>26</b>	<b>50</b>	<b>71</b>	<b>102</b>	<b>152</b>	<b>265</b>

## **7.2. Strength and weakness analyses of farm-based abalone selective breeding program**

In the summer of 2005/2006 the collaborative approach for abalone family establishment among three Victorian farms (Southern Ocean Mariculture, Coastal Seafarms and Great Southern Waters) was very successful, and thus could be considered as a model for other states. However, there is room for further improvement and some potential risks still exist. Assessments of these issues would help participating farms preparing for these challenges and newcomers or investors to address these issues at the start of their selective breeding activities. These include: resource allocation, biosecurity, selection strategies, and selective breeding business. For easy reference the assessments and suggested improvement are grouped in the following 7 areas: 1. abalone family establishment; 2. grow-out; 3. on-farm technical staff; 4. selection strategies; 5. biosecurity; 6. technical improvement and 7. selective breeding business and are provided in Table 7.2.

**Table 7.2. Strengths and weaknesses of the farm-based abalone selective breeding program and recommended improvements**

<b>Subjects</b>	<b>Strengths</b>	<b>Weaknesses</b>	<b>Recommendations for further improvement</b>
1. Family establishment	Sharing existing commercial production facilities for spawning, larval rearing, settlement and nurseries.	The existing facilities are normally larger than the requirements for family establishment, which only needs many small replicated units.	<ul style="list-style-type: none"> <li>• Establish required number of replicated units to maintain families separately; or</li> <li>• Develop cost effective DNA parentage assignment technique(s) so that progenies can pooled during their early developmental stages.</li> <li>• Need cost/benefit analyses prior to the implementation of a selected recommendation.</li> <li>• Involve R&amp;D agency to enhance the capacity for family establishment.</li> </ul>
	Previous knowledge on the potential performances of some local broodstock (including both farmed and wild).	Lack of detailed information on the origin of some farmed stock, which could result in increased inbreeding by mating of relatives.	<ul style="list-style-type: none"> <li>• Broaden the genetic diversity using broodstock from farms in different regions and/or wild broodstock from different localities.</li> <li>• Avoid inbreeding through DNA pedigree analyses or through limiting the use of broodstock with no background information.</li> </ul>
	Sharing the existing broodstock conditioning system.	The broodstock conditioning systems are highly variable between farms. They are also not designed for selective breeding purposes, for example, to achieve pair mating.	<ul style="list-style-type: none"> <li>• Involve R&amp;D agencies.</li> <li>• Develop techniques to: 1. enhance gonad development; 2. predict spawning potential; 3. synchronise the spawning of selected broodstock so that desired mating can be realised.</li> <li>• Further refine cryopreservation techniques.</li> </ul>



<b>Subjects</b>	<b>Strengths</b>	<b>Weaknesses</b>	<b>Recommendations for further improvement</b>
	Family establishments can be partially combined with commercial production.	Competing resources (labour and facilities) with commercial production.	<ul style="list-style-type: none"> <li>• Develop capabilities for off-season family establishment, including broodstock condition system, water temperature control system, off-season settlement system, etc.</li> </ul>
		Difference in time schedule for commercial productions between participating farms.	<ul style="list-style-type: none"> <li>• Develop family establishment schedule that is suitable for all participating farms.</li> <li>• Avoid commercial production period as much as possible.</li> <li>• Improve the coordination among participating farms.</li> </ul>
		Potential contamination from commercial productions.	<ul style="list-style-type: none"> <li>• Separate the resources (facility, equipment and staff) required by the commercial production and family establishment, or</li> <li>• Conduct these two functions separately.</li> <li>• Use DNA pedigree analysis to confirm if contamination occurred prior to genetic parameter analyses and selection.</li> </ul>
		Farm R&D priority changes.	<ul style="list-style-type: none"> <li>• Clarify the nature of selective breeding business and potential short-term and long-term gains.</li> <li>• Secure clear long-term commitment from participating farms.</li> </ul>

<b>Subjects</b>	<b>Strengths</b>	<b>Weaknesses</b>	<b>Recommendations for further improvement</b>
2. Grow-out	Animals' breeding values are evaluated under the environmental conditions where their progenies will be farmed.	Only the environmental conditions of the participating farms are assessed. If genetic and environmental interaction is high, the progeny produced from selected stock on one farm(s) might not perform well on others.	<ul style="list-style-type: none"> <li>• Encourage farms having different farming environments/systems to participate in the selective breeding program, or at least as a test centre.</li> <li>• Investigate the level of genetic and environmental interactions as a matter of emergency.</li> </ul>
	Investment saving by using existing grow-out tanks.		
	Saving maintenance costs by sharing supply with commercial production.	No specific backups to the nucleus breeding stocks.	<ul style="list-style-type: none"> <li>• Establish separate backups for key maintenance requirements such as water supply.</li> </ul>
3. On farm project technical staff	On-farm technical staff are trained and shared with other on-farm R&D activities.	On-farm technical staff are often overloaded.	<ul style="list-style-type: none"> <li>• Develop a clear annual time schedule for activities required for all farm-based R&amp;D projects.</li> <li>• Develop a clear contingency plan when activities from different projects are overlapped.</li> </ul>
		High turnover in technical staff, resulting in delay in required on-farm activities.	<ul style="list-style-type: none"> <li>• Develop a clear backup strategy on all participating farms.</li> <li>• Develop an emergency staff sharing mechanism between participating farms.</li> </ul>
		The data quality provided to the project is highly variable between farms and between staff.	<ul style="list-style-type: none"> <li>• Provide staff refreshment training prior to data collection.</li> <li>• Share a couple of core staff for data collection across the participating farms (these staff can be based at a research institution(s) for data maintenance as well).</li> </ul>

Subjects	Strengths	Weaknesses	Recommendations for further improvement
4. Selection strategies		Breeding objective and selection strategies might differ between farms using different systems, species, at different geological regions and/or for different markets. These might affect the genetic gains within the limited program resources.	<ul style="list-style-type: none"> <li>• Carefully design the selection strategies that could provide optimal return to industry, and at the same time compensate the specific requirement of individual farms.</li> <li>• Periodical review on these objectives and strategies.</li> </ul>
5. Biosecurity	Existing on-farm biosecurity measures are shared by the breeding program.	These measures are highly variable between farms and normally do not meet the standard required in the breeding program.	<ul style="list-style-type: none"> <li>• Establish the required standard and implement across all participating farms.</li> <li>• Ensure this standard being one of the preconditions for participating in the selective breeding program.</li> </ul>
		The breeding stocks are held with commercial stocks. If diseases occur on commercial stocks the nucleus stock could be exposed as well. Thus many generations' selection could be lost.	<ul style="list-style-type: none"> <li>• Establish measures to isolate nucleus stocks from commercial stocks. This is necessary although could increase the costs substantially.</li> </ul>
	All participating farms can back up each other if diseases do not occur on all the farms.		<ul style="list-style-type: none"> <li>• Ensure each participating farm has all families established within the selective breeding program, or at least within each state.</li> </ul>

<b>Subjects</b>	<b>Strengths</b>	<b>Weaknesses</b>	<b>Recommendations for further improvement</b>
		Many maintenance facilities/equipment are shared by commercial production.	<ul style="list-style-type: none"> <li>• Make sure farms use dedicated equipment for selective breeding stock maintenance.</li> </ul>
		If required by the government regulators, assessing all farms involving in stock transfer in the selective breeding program would be expensive.	<ul style="list-style-type: none"> <li>• Gradually centralise the breeding program on a few key farms (potentially one farm per state).</li> </ul>
6. Technical improvement	Data collection can be assisted by other on-farm staff.	Sometimes it was very difficult to coordinate the data collection among participating farms.	<ul style="list-style-type: none"> <li>• Use digital measurement equipment that can be connected to computer directly to improve the data collection efficiency and quality (computer linked digital calliper and balance have been used on some participating farms).</li> <li>• Avoid periods with strong commercial activities such as spawning, harvest, summer months, etc.</li> <li>• Establish an agreed data collection period each year among participating farms.</li> </ul>
		The optimal environmental conditions (such as temperature) cannot be achieved with current on-farm settings. This is needed to shorten the breeding cycle and thus increase genetic gains.	<ul style="list-style-type: none"> <li>• Upgrade the facilities specific for the selective breeding program on all participating farms if the breeding cycle can be shortened through this management change because very favourable effects have been showed in the sensitivity analysis on this parameter, or</li> <li>• Upgrade the facilities on an agreed farm per state to save the upgrading costs.</li> </ul>

Subjects	Strengths	Weaknesses	Recommendations for further improvement
		The techniques that could potentially improve the gains or efficiency of the breeding program need to be prioritised.	<ul style="list-style-type: none"> <li>• Conduct sensitivity analyses on each of the techniques identified and prioritise them accordingly.</li> <li>• Preliminary analysis suggests that a reduction in the reproductive cycle from 3 to 2 years could increase the economic benefit by more than 50% (however, caution needs to be taken to avoid selection of early maturity which could direct their energy to gametogenesis rather than to meat growth).</li> <li>• Develop techniques to achieve desired mating through either cryopreservation or synchronised spawning between male and female.</li> </ul>
7. Selective breeding business (Only key items are discussed)	Equal investment and equal share arrangements can be achieved by participating farms.	Mechanism for new farms/investors to join the program or existing farms to withdraw from the program needs to be developed.	<ul style="list-style-type: none"> <li>• Full project costs per family plus opportunity cost can be used as basis for new farms or investors to join the program.</li> </ul>
		The ownership of the selected stock and its commercialisation strategy need to be clarified.	<ul style="list-style-type: none"> <li>• Establish agreed method to sell improved progenies (individually or collectively).</li> </ul>
		Strategy to use selected individuals on other farm(s) for the genetic improvement program needs to be developed.	<ul style="list-style-type: none"> <li>• Using cryopreserved gametes would be the best option.</li> </ul>
	An agreement among participating farms has to be established prior to the initiation of the genetic improvement program or as soon as possible thereafter.		

## **8. PRELIMINARY GENETIC PARAMETER ANALYSES**

### **8.1. Introduction**

Table 7.1 shows that in total 259 families have been established and held on different participating farms, and only a small number of families per species per farm were produced in most spawning seasons. At the time when statistical analyses were conducted, only the data collected from the 14 blacklip families established in the 2000/2001 spawning season at the Southern Ocean Mariculture and the 15 greenlip families established in the 2001/2002 spawning season at the same farm were used. The data from other seasons (prior to the 2003/2004 spawning season) or from other farms either had limited family numbers per spawning season, or had been highly contaminated (more than 40% of animals were found to be the result of contamination as revealed by DNA pedigree analysis), thus were not included in the analyses. At the final data submission request in 2006, the 2004/2005 and 2005/2006 families had not been tagged, and were maintained in separate family nursery tanks. Therefore no information was available from these families for analyses.

### **8.2. Materials and methods**

#### **8.2.1. Experimental design**

##### *8.2.1.1. Blacklip abalone*

Fourteen full-sib blacklip abalone families were produced by parents originating from the following locations of the Victorian coast: Drain Bay, The Cutting, and Port Fairy River Wall (all in the Port Fairy area), Thunder Point (Warrnambool), and Port Phillip Bay.

Due to hatchery infrastructure limitations and the fact that spawning was not synchronized, only four families could be established per spawning run and in some runs only one family was produced. Family establishments had also to work around

commercial spawning, which added to the time lag between batches. The 14 families used in the present study were produced over a period of 12 weeks (between 3/12/2000 and 24/2/2001).

#### *Spawning, fertilisation and larval rearing*

The gametes were obtained by the standard spawning method used in commercial hatcheries (temperature increase (2 to 4°C) plus UV-treated seawater). Single pair crosses were used to establish each family. Similar numbers of individuals were maintained in each family during fertilization and larval rearing. The larvae from each family were raised together and kept separate from other families.

#### *Settlement and grow-out on plates*

A similar number of larvae for each family (80,000 larvae/family) were transferred to separate nursery tanks once the appearance of the third tubule on the cephalic tentacle formed on the larvae and 80% of the larvae displayed foot-testing behaviour across the families. At week 8 post-settlement a scraper was used to thin the plates to a juvenile density of approximately 120 per plate.

Ten days prior to larval settlement the nursery plates (held in baskets) were seeded with *Ulveella lens* in a large commercial tank. After five days with the tank remaining static, 5 µm filtered seawater at a 10% per hour exchange rate was introduced to the tank. The inflowing seawater allowed a wild biofilm to develop over the *U. lens* as an initial food source for newly settled juvenile abalone. When the larvae were ready to settle, the baskets from the large commercial tank were randomly divided and transferred into smaller nursery tanks designed for family establishment. Each smaller tank held 6 baskets (6 x 15 = 90 plates/tank).

#### *Intermediate grow-out*

At week 30 post settlement, each family was transferred and raised together in a tier of four raceways (2.5m x 0.5m) and kept separate from other families (4 x 2000 juveniles/raceway = 8000 juveniles/tier/family).

*Transfer of tagged juveniles and pooled families into replicated grow-out tanks*

Three replicated commercial slab tanks were used to house the families. At the age of about 13 months, when the smaller individuals across all the families were at least 20mm in shell length, 420 individuals were randomly tagged per family. The 420 individuals from each family were then randomly separated into 3 groups (140 individuals/group) and one group from each family were pooled into a slab tank and mixed with a commercial cohort to meet the stocking density requirements under the commercial management.

*General maintenance during grow-out stages in slab tanks and data collection*

After being transferred into the slab tanks the tagged abalone were maintained in the same way as the commercial stock, including feed types and feeding frequencies, water flow rates, and tank cleaning methods.

At the time when juveniles were tagged their length was measured to the nearest 0.1mm. Twelve months later all the tagged animals were measured to the nearest 0.1mm for shell length and 0.01g for whole body weight. At the same time the animals were sexed by visual observation of their gonad. However, the animals' gender could only be determined in half of the tagged animals. The measurements were repeated 15 months later. This time the animals' gender could be determined in almost all the individuals. The final data collection was conducted in April 2005, about 4 years and 2 months post-fertilization and about 10 months after the previous data collection. When the measurements of shell length and whole weight were completed 22 abalone per replicate per family were harvested and their meat and shell weights were weighted separately. Meats from 8 harvested individuals per replicate were then tagged by cotton thread with different knots and further processed according to the procedures used in the processing factory for production of canned abalone. The meat weights were measured again individually after rumbling and cooking.

*Survival estimation*

Survival was estimated from the time when animals were tagged in May 2002 to April 2005, when all tagged individuals within each replicate (tank) were counted and measured.



#### 8.2.1.2. *Greenlip abalone*

The parents used to produce the 15 full-sib greenlip abalone families originated from the following Victorian coast locations: Water Tower (Portland), Dutton Way (Portland), Armstrong Bay, Mallacoota, Point Lonsdale (Queenscliff).

The greenlip families were established in 4 spawning runs over a period of 5 weeks (18/12/2001 to 18/01/2002), with a minimum of 3 families being produced per run.

The procedures and methods used for greenlip abalone family establishment, the subsequent maintenance at different farming stages and data collection were the same as described for blacklip abalone in the previous section (8.2.1. Blacklip abalone) except that the greenlip abalone were measured on only two occasions. Length was only measured when they were tagged in March 2003 (15 months post-fertilisation) but length and weight were measured in August 2004. The gender of most greenlip abalone could not be visually identified during this period. The gender effect was, therefore, not analysed.

### 8.2.2. Statistical analyses

#### 8.2.2.1. *Blacklip abalone*

Blacklip data included records from 5895 progenies from 14 full-sib families across 7 spawning batches. Preliminary analyses using statistical software known as SAS were firstly carried out to detect systematic effects associated with body and processing traits. The effects of spawning batch and replicate tanks were highly significant ( $P < 0.05$  to 0.001) for all traits. The effect of sex was significant ( $P < 0.01$  to 0.001) for traits recorded in the later phase of growth. Linear regression analysis of age at respective measurements on the studied traits was statistically significant ( $P < 0.05$  to 0.001) except for shell weight.

Genetic parameters were estimated using a statistical software package for fitting linear mixed models using restricted maximum likelihood known as ASREML (Gilmour et al., 1999). For the random term, only the additive genetics of an individual animal was included in the model. Attempts to fit maternal and common environment effects as an additional random effect were unsuccessful because convergence was not reached, or parameters were out of space (limit).

The final statistical models used to analyse body and processing traits are summarized in Table 8.1. The  $R^2$  value ranged from 0.13 to 0.26 across traits. Trait heritability was obtained from univariate analysis. Phenotypic and genetic correlations were estimated from a series of bivariate models.

**Table 8.1. Statistical models used to analyse body and processing traits in blacklip abalone<sup>1</sup>**

Traits	Fixed effects <sup>2</sup>			Covariate <sup>2</sup>	Random effects	$R^2$
	Tank	Batch	Sex	Age	Additive Genetics	
L1	***	***	***	*	✓	0.19
L2	***	***	ns	***	✓	0.18
L3	***	***	ns	***	✓	0.18
L4	***	***	**	***	✓	0.19
W2	***	***	ns	***	✓	0.18
W3	***	***	ns	***	✓	0.16
W4	***	***	***	***	✓	0.19
SW	***	***	***	ns	✓	0.26
MW	ns	***	***	***	✓	0.13
MS	***	***	***	***	✓	0.21
RW	*	***	***	*	✓	0.20
CW	*	***	***	*	✓	0.19

<sup>1</sup> L1, L2, L3, L4, and W1, W2, W3 and W4 are length and weight at the first (424 days), second (779 days), third (1241 days) and fourth (1530 days) measurements, respectively. SW: shell weight; MW: meat weight; MS: meat/shell; RW: rumbled meat weight; CW: cooked meat weight.

<sup>2</sup> \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ , ns: non-significant.

### 8.2.2.2 *Greenlip abalone*

Greenlip data had records from 6286 individuals in 15 full-sib families across 4 spawning batches. In a similar manner to blacklip data, the general linear model (GLM) in SAS (SAS software Inc, 1997) was used to identify fixed effects. They included spawning batches and replicate tanks ( $P < 0.001$ ). Age at measurements showed a linear relationship ( $P < 0.001$ ) with body weight and length, and thus was included as a linear covariate for these traits. Besides the additive genetics of each individual animal, this dataset also allowed the maternal and common environmental effects ( $c^2$ ) to be fit as an additional random term in models (Table 8.2). A series of univariate and bivariate analyses were carried out to obtain genetic parameters for body weight and length (ASREML, Gilmour et al., 1999).

**Table 8.2. Statistical model used to analyse body traits in greenlip abalone**

Traits	Fixed effects		Covariate	Random effects			R <sup>2</sup>
	Tank	Batch		Model 1	Model 2	$c^2$	
			Age	Additive genetics	Additive genetics		
Length 1	***	***	***	✓	✓	✓	0.18
Length 2	***	***	***	✓	✓	✓	0.15
Weight 2	***	***	***	✓	✓	✓	0.15

$c^2$ : Maternal and common environmental effects

## 8.3. Results and discussion

### 8.3.1. Blacklip abalone

#### 8.3.1.1. Basic statistics

The actual number of records, means, standard deviations (SD), coefficients of variation ( $CV$ ) for body and processing traits in blacklip are given in Table 8.3. Body dimensions (length and weight) of blacklip increased linearly over growth periods from stocking in 2002 to harvesting in 2005. The average daily gain of blacklip

between stocking and harvest is only 0.086 g/d. Shell weight accounted for a large proportion of total live weight at harvest (28.2%). The percentage of meat is about 38.8% of the total live weight at harvest. Of particular interest is that there is a little loss in cooked meat weight relative to fresh meat weight (only 7.5%). Figure 8.1 shows the cooked meats from four families.

Besides the raw means, the coefficients of variation (*CV*) for measurements of body weights over different periods were high, consistent with those reported in other aquaculture species (Ponzoni et al., 2005). The *CV*'s for length measurements were the lowest, whereas those for processing traits were intermediate, but closer to those of weight measurements. Also note that the *CV*'s for both weights and lengths tended to decrease in later stages of growth.

**Table 8.3. Basic statistics for body and processing traits in blacklip abalone**

Category	Traits	Unit	N	Mean	SD	CV (%)	Min	Max
Length	L1	mm	5895	25.4	3.0	11.9	17.0	37.0
	L2	mm	5025	44.0	5.5	12.6	4.9	67.5
	L3	mm	4583	75.4	7.5	10.0	39.0	97.0
	L4	mm	3815	87.2	7.2	8.3	45.2	125.7
Weight	W2	g	5025	13.1	4.7	35.9	2.4	42.9
	W3	g	4583	69.2	19.1	27.6	9.0	143.0
	W4	g	3814	107.9	24.2	22.4	11.4	212.1
Processing	SW	g	892	30.4	6.3	20.6	9.6	56.0
	MW	g	893	41.9	10.6	25.4	9.1	86.3
	MS	ratio	892	1.4	0.2	17.0	0.8	2.3
	RW	g	330	36.3	7.2	19.9	21.0	59.2
	CW	g	329	31.5	6.7	21.3	17.2	56.2



Figure 8.1. Cooked meats from different blacklip abalone families established in the 2000/2001 summer.

#### 8.3.1.2. *Effects of spawning batch*

The least square means of body and processing traits in different spawning batches are presented in Tables 8.4a and b. Although the interval between spawning batches was approximately two weeks, their effects accounted for a large proportion of total variation in the model, ranging from 90 to 98% across traits. These results indicate that in breeding programs for species where synchronization of spawning (e.g. by induced breeding) is impossible or is hardly met, the effect of batch should be included in statistical models. The effect of spawning batch was also reported in Atlantic salmon (Bailey and Loudenslager, 1986) and in Nile tilapia (*Oreochromis niloticus*) (Eaknath et al., 1993).

It should also be noted that in this study, the broodstock used in different spawning runs (batches) were from different localities (or sources) and on average only two families were produced per spawning run. Therefore it is very difficult to separate the spawning date (batch) effects from the effects of different genetic make-ups of abalone from different localities. The batch effect might not be so important if batches

are from the same localities or their genetics are properly mixed at the establishment stage of the families. Further investigation on this topic would merit priority for the breeding program.

**Table 8.4a. Least square means of body traits in spawning batch for blacklip abalone**

Batch	L1	L2	L3	L4	W2	W3	W4
03/12/00	27.0±0.18	42.9±0.49	66.9±0.65	82.4±0.69	12.6±0.42	52.4±1.69	97.1±2.32
12/01/01	25.6±0.08	44.4±0.15	75.6±0.20	87.5±0.23	13.5±0.13	70.3±0.53	110.6±0.78
20/01/01	26.1±0.09	44.6±0.16	74.9±0.18	87.0±0.20	13.1±0.14	66.0±0.48	104.6±0.67
01/01/01	23.3±0.10	42.2±0.19	73.1±0.25	83.9±0.26	12.1±0.16	64.3±0.64	96.1±0.88
12/02/01	24.2±0.19	43.2±0.38	81.8±0.51	92.5±0.54	12.5±0.33	86.2±1.33	127.1±1.82
22/02/01	26.5±0.17	48.2±0.42	88.1±0.57	97.4±0.62	15.7±0.37	99.0±1.47	138.3±2.08
24/02/01	25.4±0.20	49.1±0.45	81.4±0.59	88.6±0.62	17.6±0.39	83.3±1.54	112.7±2.09

**Table 8.4b. Least square means of processing traits in spawning batch for blacklip abalone**

Batch	SW	MW	MS	RW	CW
03/12/00	29.0±1.27	33.7±2.21	1.1±0.05	33.2±2.61	29.3±2.40
12/01/01	31.9±0.42	43.1±0.72	1.3±0.02	35.9±0.85	31.4±0.78
20/01/01	28.7±0.35	38.5±0.60	1.4±0.01	34.8±0.63	30.0±0.63
01/01/01	27.3±0.47	39.0±0.82	1.4±0.02	33.1±0.96	28.7±0.88
12/02/01	33.7±0.97	50.3±1.67	1.5±0.04	38.8±1.72	33.3±1.61
22/02/01	37.7±1.01	52.0±1.76	1.4±0.04	45.9±2.03	39.2±1.87
24/02/01	29.4±1.04	43.9±1.81	1.5±0.04	35.5±2.01	30.2±1.85

### 8.3.1.3. Effects of replicate tanks

Tables 8.5a and b show least square means for body and processing traits in replicate tanks. Rearing of the animals in replicate tanks is expected to eliminate some environmental differences among families. Their effect was generally more pronounced for body traits than for processing characteristics (shell weight, meat weight, meat to shell ratio, rumbled meat weight and cooked meat weight).

**Table 8.5a. Least square means of body traits in replicate tanks for blacklip abalone**

Tank	L1	L2	L3	L4	W2	W3	W4
ST66	25.9±0.08	46.9±0.16	77.7±0.20	88.4±0.22	15.9±0.14	75.9±0.51	110.6±0.73
ST11	25.3±0.08	44.5±0.16	78.8±0.20	90.3±0.22	13.2±0.14	76.8±0.53	118.1±0.74
ST22	25.2±0.08	43.4±0.15	75.7±0.20	86.7±0.22	12.5±0.13	70.8±0.53	108.4±0.73

**Table 8.5b. Least square means of processing traits in replicate tanks for blacklip abalone**

Tank	SW	MW	MS	RW	CW
ST66	30.8±0.38	42.7±0.66	1.4±0.02	36.4±0.72	31.2±0.67
ST11	32.4±0.36	44.8±0.63	1.4±0.02	38.2±0.72	33.1±0.68
ST22	30.2±0.36	41.2±0.63	1.4±0.02	35.7±0.68	30.9±0.63

#### 8.3.1.4. Effects of gender

At the final (fourth) measurement the ratio between male and female individuals in this species was 1:1.02.

Unlike other farmed aquaculture species (such as tilapias and carps), sexual dimorphism in abalone occur for traits recorded in the later phases of growth, especially at harvest (Tables 8.6a and b). The body weight of males was on average 4.1% greater than that of females. Differences between sexes in processing characteristics were of much larger magnitude, for instance, 8.2 and 11.5% for cooked and fresh meat weights, respectively. The effect of sex was reported in several other species, for example in Nile tilapia by Ponzoni et al. (2005) and Nguyen et al. (2007).

**Table 8.6a. Least square means of body traits in female and male blacklip abalone**

Sex	L1	L2	L3	L4	W2	W3	W4
Female	25.4±0.07	44.9±0.13	77.6±0.17	88.2±0.19	13.9±0.12	74.6±0.15	110.1±0.63
Male	25.6±0.07	45.0±0.14	77.2±0.18	88.8±0.19	13.9±0.12	74.3±0.16	114.7±0.64

**Table 8.6b. Least square means of processing traits in female and male blacklip abalone**

Sex	SW	MW	MS	RW	CW
Female	30.2±0.33	40.6±0.58	1.4±0.01	35.4±0.64	30.5±0.59
Male	32.0±0.30	45.3±0.52	1.4±0.01	38.1±0.59	33.0±0.54

### 8.3.1.5. Heritability

The estimates of heritability for body and processing traits are from moderate to high (Table 8.7). However, the estimates are generally biased due to common environment and maternal effect components of non-additive genetic variance. They are thus of less significance in the context of selective breeding. A parameter of great importance which determines the magnitude of response to selection is the additive genetic variance since this is the component that determines how much of the observed superiority of the parents will be transmitted to the offspring. Processing traits generally had higher heritability than body traits, as also observed in other farmed aquaculture or livestock species (El-Ibiary and Joyce, 1978; Massey and Vogt, 1993).

Similar magnitudes of heritability for body traits were also found in most abalone species studied so far using data from different mating designs. For example, when estimating the heritability of growth-related traits at 12 months of age in the tropical abalone *Haliotis asinine*, Lucas et al. (2006) created a single cohort of 84 families in a full-factorial design and found that the heritability of shell length, shell width, and total weight were 0.48, 0.38, and 0.36, respectively. The study by Jonasson et al. (1999) using 100 full- and half-sib red abalone (*Haliotis rufescens*) families showed that the heritability for shell lengths was 0.34 when animals were 24 months old. They concluded that the growth rates in both red and tropic abalone could be doubled in four generations of selection (Jonasson et al., 1999; Lucas et al., 2006). The study by Hara and Kikuchi (1992) on mass selection of growth rates in *Haliotis discus* reported that by the third generation there was a 21% increase in daily growth rate with juveniles (20-30mm) and a 65% increase in maturing abalone (30-70mm).

Table 8.7 also shows that the heritability of body traits increase with increases in abalone age to 3.5 years, with heritability being 0.49 and 0.54 for shell length at



75mm and body weight at 79g, respectively. A similar trend was also reported by Jonasson et al. (1999) in red abalone with an age of 2 years (50mm in shell length). However, in this study these values reduce to 0.27 for shell length and 0.24 for body weight at the next sampling date. This phenomenon is not well understood because maternal and common environmental effects could not be included in the model and only a small number of full-sib families were used.

**Table 8.7. Heritability ( $h^2$ ) for body and processing traits in blacklip abalone**

Category	Traits	$h^2 \pm se$
Length	L1	$0.18 \pm 0.08$
	L2	$0.38 \pm 0.17$
	L3	$0.49 \pm 0.20$
	L4	$0.27 \pm 0.13$
Weight	W2	$0.40 \pm 0.18$
	W3	$0.54 \pm 0.21$
	W4	$0.24 \pm 0.12$
Processing characteristics	SW	$0.13 \pm 0.09$
	MW	$0.61 \pm 0.24$
	MS	$0.49 \pm 0.21$
	RW	$0.74 \pm 0.28$
	CW	$0.84 \pm 0.29$

#### 8.3.1.6. Correlations

Phenotypic and genetic correlations among body and processing traits are shown in Table 8.8. The genetic correlations among the body and processing traits were high and positive ( $\geq 0.65$ ), with the exception that the estimates between body length at stocking and other traits do not significantly differ from zero, due to high standard errors. Correlations between traits measured at some dates are not estimable due to convergence not being reached, or parameters being out of the upper limit ( $> 1.0$ ). Whenever convergence was obtained, the genetic correlations between body weight and length (except L1) are very high ( $\geq 0.77$ ). Regardless of the measurement periods, the genetic correlations of body weight and length with processing traits are very favourable, ranging from 0.65 to 0.92. The estimates of the genetic correlations in this

study indicate that both body and processing traits can be simultaneously improved if selection were to be carried out for any of these traits.

#### 8.3.1.7. *Survival*

From Table 8.3 and Figure 8.2 it can be seen that the average survival from tagging to the final measurement was  $64.1 \pm 8.9\%$  (SD), ranging from  $50.0 \pm 7.9\%$  (F14) to  $76.2 \pm 4.3\%$  (F11). The average survival rate is much lower than the survival rate ( $>80\%$ ) in commercial production. This is due to the fact that in this study the losses cannot be differentiated as tag losses or the death of animals. However, it would be reasonable to assume that tag losses were a random event across families and replicate tanks. A preliminary analysis on the correlation between family replicate survivals and family replicate final average weights (Figure 8.3) shows that these two traits are significantly negatively correlated ( $R^2 = -0.350$ ;  $P = 0.023$ ). This result needs to be treated cautiously because very small sample sizes have been used and other uncounted factors, such as differences in the care taken to dry abalone and glue tags between batches (families), might also have affected survival. A negative genetic correlation between survival and shell length was also found by Jonasson et al (1999) in red abalone (*H. rufescens*). Further investigation on this topic is important because it will influence the decision on selection criteria (details refer to 9. DEVELOPMENT OF BREEDING OBJECTIVES AND SELECTION INDICES AND SENSITIVITY ANALYSES ON SELECTED PARAMETERS).

Refstie (1990) also suggests that identification of dead fish, together with reasons for mortality, will give better selection criteria than percentage survival for each progeny group.

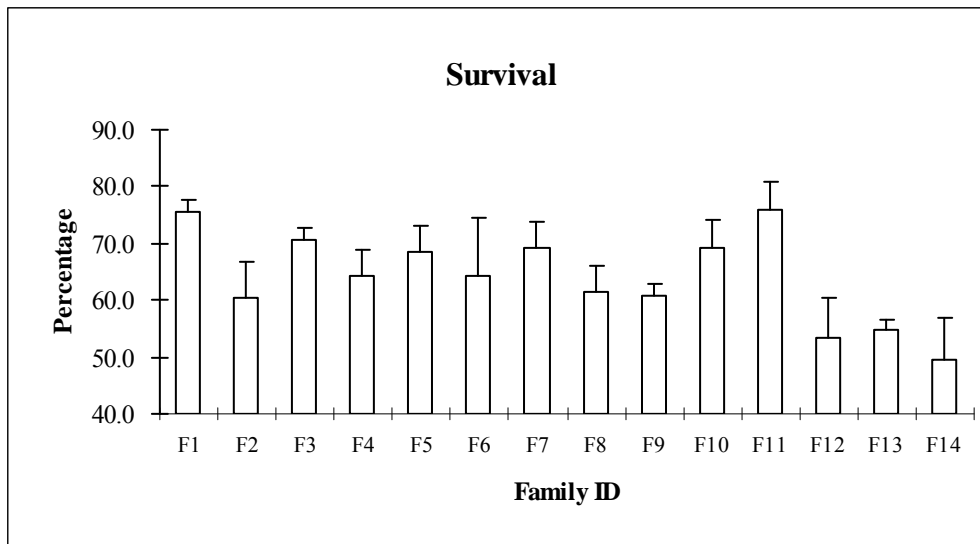


Figure 8.2. The survival rates in blacklip abalone families at the time of final data collection in April 2005 (tag losses were treated as death). Bars = SD.

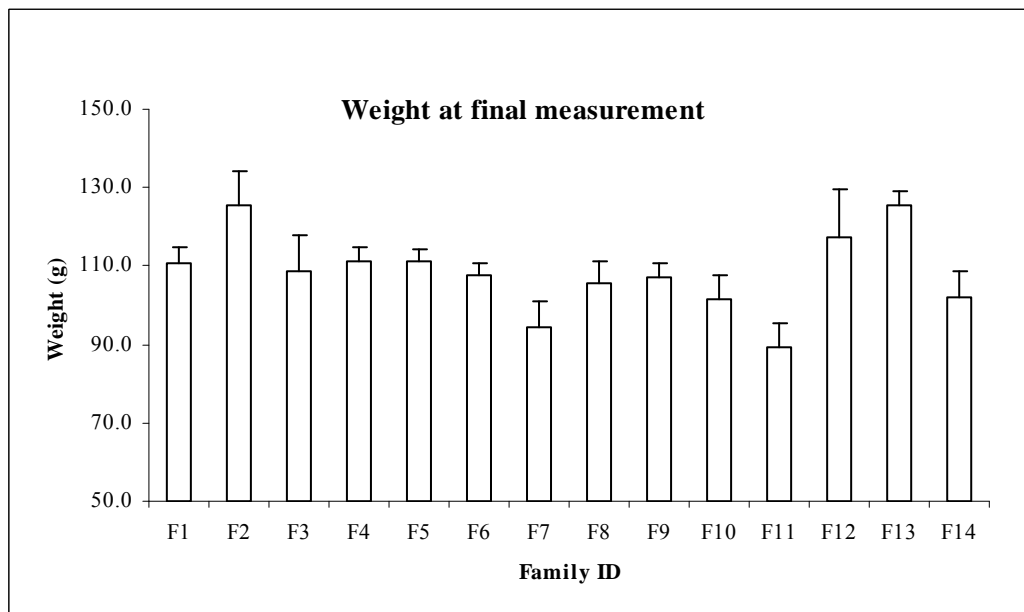


Figure 8.3. The harvest body weights of blacklip abalone families collected in April 2005. They are calculated using family replicate averages. Bars = SD.

**Table 8.8. Phenotypic (above) and genetic (below the diagonal) correlations among body and processing traits in blacklip abalone. Standard errors in parentheses**

Traits	L1	L2	L3	L4	W2	W3	W4	SW	MW	MS	RW	CW
L1		0.44 (0.06)	0.18 (0.07)	0.17 (0.05)	0.42 (0.06)	0.19 (0.07)	0.17 (0.05)	0.12 (0.05)	0.08 (0.07)	-0.002 (0.06)	-0.03 (0.07)	-0.03 (0.06)
L2	-0.21 (0.37)		0.59 (0.01)	ne	ne	0.67 (0.04)	0.53 (0.01)	0.43 (0.04)	0.47 (0.07)	0.22 (0.09)	0.45 (0.08)	0.45 (0.09)
L3	-0.33 (0.34)	0.87 (0.07)		ne	ne	ne	0.77 (0.02)	0.62 (0.03)	0.65 (0.05)	0.26 (0.10)	0.65 (0.06)	0.64 (0.07)
L4	-0.12 (0.38)	ne	ne		ne	0.93 (0.05)	ne	0.76 (0.02)	0.77 (0.03)	0.25 (0.07)	0.80 (0.03)	0.79 (0.03)
W2	-0.16 (0.37)	ne	ne	ne		ne	ne	0.44 (0.04)	ne	0.21 (0.09)	ne	ne
W3	-0.26 (0.36)	0.94 (0.05)	ne	0.77 (0.02)	ne		ne	0.63 (0.03)	ne	0.29 (0.10)	0.71 (0.05)	0.70 (0.06)
W4	-0.08 (0.09)	ne	0.90 (0.07)	ne	ne	ne		0.86 (0.01)	0.81 (0.02)	ne	ne	0.87 (0.02)
SW	-0.19 (0.41)	0.87 (0.13)	0.65 (0.27)	0.91 (0.10)	0.91 (0.10)	0.75 (0.21)	ne		0.68 (0.02)	0.03 (0.07)	0.68 (0.04)	0.66 (0.04)
MW	-0.10 (0.39)	0.91 (0.08)	0.91 (0.07)	0.89 (0.08)	ne	ne	0.91 (0.07)	0.84 (0.13)		0.60 (0.06)	ne	ne
MS	-0.03 (0.38)	0.81 (0.14)	0.86 (0.11)	0.73 (0.09)	0.86 (0.11)	0.89 (0.09)	ne	0.61 (0.29)	0.93 (0.06)		0.48 (0.09)	0.49 (0.09)
RW	-0.18 (0.30)	0.92 (0.07)	0.65 (0.06)	0.88 (0.10)	ne	0.93 (0.07)	ne	0.77 (0.19)	ne	0.82 (0.15)		ne
CW	-0.15 (0.32)	0.92 (0.08)	0.85 (0.12)	0.89 (0.09)	ne	0.92 (0.07)	0.94 (0.05)	0.78 (0.18)	ne	0.81 (0.15)	ne	

ne: non-estimable due to failure of convergence or parameters out of space (limit).

### 8.3.2. Greenlip abalone

#### 8.3.2.1. Basic statistics

As measurements of greenlip abalone were not consistent with those in blacklip, it is not possible to make a relative comparison of growth performance between the two species. However, both species show that the coefficient of variation (CV) is lower for body length than for body weight (Table 8.9).

**Table 8.9. Basic statistics for body measurements in greenlip abalone**

Category	Traits <sup>†</sup>	Unit	N	Mean	SD	CV (%)	Min	Max
Length	L1	mm	6286	21.8	2.4	11.2	13.0	34.0
	L2	mm	5345	65.6	7.5	11.4	27.0	89.0
Weight	W2	g	5345	37.3	12.0	32.0	5.2	90.8

<sup>†</sup> L1 measured at an average age of 412 days from spawning, and L2 and W2 at 930 days from spawning.

#### 8.3.2.2 Effect of spawning batch

For body weight and length at the second measurements, statistical differences are generally significant ( $P < 0.001$ ) between batches that are further apart (Table 8.10).

**Table 8.10. Least square means for body traits in spawning batches for greenlip abalone<sup>†</sup>**

Spawning batch	L1	L2	W2
18/12/01	22.9 ± 0.13 <sup>a</sup>	62.6 ± 0.41 <sup>a</sup>	37.3 ± 0.65 <sup>a</sup>
29/12/01	21.2 ± 0.06 <sup>b</sup>	64.3 ± 0.19 <sup>b</sup>	34.8 ± 0.30 <sup>b</sup>
07/01/02	21.9 ± 0.06 <sup>c</sup>	67.3 ± 0.20 <sup>c</sup>	38.5 ± 0.31 <sup>ac</sup>
18/01/02	21.3 ± 0.10 <sup>bd</sup>	67.6 ± 0.23 <sup>cd</sup>	38.8 ± 0.53 <sup>ad</sup>

<sup>†</sup> Means with different letters in a column differ significantly ( $P < 0.001$ ).

### 8.3.2.3. *Effect of replicate tank*

Differences among replicate tanks were statistically significant ( $P < 0.001$ ) for body weight and length measured at the second measurement (Table 8.11). Body length at the first measurement did not differ between tanks STA16 and STA20.

**Table 8.11. Least square means for body traits in replicate tanks for greenlip abalone<sup>†</sup>**

Replicate tank	L1	L2	W2
ST A16	21.8 ± 0.05 <sup>a</sup>	67.4 ± 0.16 <sup>a</sup>	40.3 ± 0.26 <sup>a</sup>
ST A20	21.7 ± 0.05 <sup>a</sup>	63.9 ± 0.16 <sup>b</sup>	34.8 ± 0.26 <sup>b</sup>
ST A21	22.0 ± 0.05 <sup>b</sup>	65.0 ± 0.17 <sup>c</sup>	36.9 ± 0.27 <sup>c</sup>

<sup>†</sup> Means with different letters in a column differ significantly ( $P < 0.001$ )

### 8.3.2.4. *Heritability*

The heritabilities, and maternal and common environmental effects estimated from two different statistical models, are given in Table 8.12. The results show that the model with only an additive genetic effect (model 1) overestimates the heritability for both body weight and length. The maternal and common environmental effects accounted for a large proportion of total variance, 11 to 20%. It is therefore concluded that in genetic evaluation systems where common environmental effects exist, they should be included in statistical models.

A study of genetic parameters in this species was also undertaken by Kube et al. (2007) using a small population of abalone established on a farm in Tasmania. Their population consisted of both full-sib and half-sib families of various sizes (number of individuals). In their study, genetic variation in both body length and weight were not detectable until 2.5 years old. Kube et al. (2007) suggested that the genetic variation appeared to be masked by maternal, larval, and settlement effects until this age. When the final measurement was taken at 3 years age in their study a low heritability (0.1)

was revealed for both traits. A similar magnitude of heritability was also found in this study for these traits based on a model that included maternal and common environmental effects. However, it should be noted that the final animal sizes used in these studies (73mm in length and 46g in weight in Kube's et al study, and 66mm in length and 37g in weight in this study) were much smaller than the targeted market size of 110g (approximately 90mm in length) aimed for by the Australian abalone aquaculture industry. Therefore, further study will be required to confirm these findings at this market size.

**Table 8.12. Heritability ( $h^2$ ), and maternal and common environmental effects ( $c^2$ ) for body traits in greenlip abalone**

Traits	Model 1	Model 2	
	$h^2$	$h^2$	$c^2$
Length 1	0.31 ± 0.12	0.08 ± 0.006	0.11 ± 0.06
Length 2	0.48 ± 0.16	0.07 ± 0.007	0.20 ± 0.08
Weight 2	0.39 ± 0.14	0.08 ± 0.007	0.16 ± 0.07

#### 8.3.2.5. Correlations

Table 8.13 shows phenotypic and genetic correlations between body weight and length. The genetic correlation between weight and length within the same measurement period was high (close to 1). The genetic correlation of length between the two measurement periods was only moderate (0.39). This means that the measurement at the tagging stage may not be good indicators for overall performance of the animal; similar results were also found by Kube et al. (2007) in the same species, and in blacklip abalone in this study. However, this hypothesis needs to be further investigated when additional data are accumulated.

**Table 8.13. Phenotypic (above) and genetic (below the diagonal) correlations between body traits in greenlip abalone**

Traits	Length 1	Length 2	Weight 2
Length 1		0.33 ± 0.04	0.36 ± 0.04
Length 2	0.39 ± 0.25		0.93 ± 0.09
Weight 2	0.39 ± 0.26	0.94 ± 0.04	

Correlations were estimated using Model 1. Convergence was not reached with Model 2.

#### 8.4 Concluding remarks

The results from the preliminary parameter analyses for both abalone species in this study show that:

- There were preliminary indications of genetic variation in body traits (weight and length) in both species and processing traits (meat weight, shell weight, rumbled meat weight and cooked meat weight) in blacklip abalone, suggesting that genetic improvement in these traits can be effectively achieved through selective breeding.
- Both spawning batch and replicate tanks had a strong influence on all the observed traits in both species, and the effect of sex was significant for harvest length and weight and processing traits in blacklip abalone (no gender data available for greenlip abalone), suggesting the necessity of including systematic fixed effects in the genetic evaluations of these species.
- The genetic correlations between body and processing traits (or among themselves) in blacklip abalone were highly favourable, so simultaneous improvement can be achieved by selection of any of these traits.
- The phenotypic correlation between body weight at harvest and overall survival in blacklip abalone were moderate, but negative.
- The measurements at the tagging stage are not good indicators for overall animal performance, and thus selection at this stage is unlikely to deliver good genetic gains.
- Males and females performed significantly differently in most traits measured and have to be ranked and selected separately.



- In blacklip abalone the ratio between male and female individuals is very close to 1:1.
- In order to estimate genetic parameters with a reliable level of accuracy, there is a strong need to increase the number of families and the best fit of statistical models can be finalized when more data from different spawning years, different farms and states are accumulated in the future.

## **9. DEVELOPMENT OF BREEDING OBJECTIVES AND SELECTION INDICES AND SENSITIVITY ANALYSES ON SELECTED PARAMETERS**

### **9.1. Introduction**

The Australian farm-based abalone selective breeding program started in the 2000/2001 spawning season through the first FRDC project (“selective breeding of farmed abalone in Australia to enhance growth rates”) and lasted for more than 7 years. It is expected that this implementation could have similar positive economic impact as those resulting from the selective breeding program in Atlantic salmon in Norway, and programs in many terrestrial animal and plant species in the world. To achieve this, however, a carefully designed breeding objective and its associated selection strategy or strategies will be required. This is crucial because it determines the direction of the abalone selective breeding program, as the breeding objective is about “where to go”. Without a well-defined breeding objective an industry or individual breeder has little chance of achieving genetic improvement (Ponzoni, 1988).

The traits that should be included in the abalone selective breeding program were identified in the first FRDC project. However, they need to be built into the breeding objective to guide program development. When a breeding objective is defined it also provides an opportunity to illustrate potential impacts to industry over a certain period (say 15 years). It can be judged solely with the achievable rates of genetic improvement, and can be predicted if sufficient information on parameters such as heritability, variance and covariance and the breeding population size are available. An alternative appraisal can be achieved by assessing its potential economic impact, which also involves market signals and costs required in the program, such as the initial capital investment, annual running expenditure for maintaining the selection stock, and data collection required by the breeding program. These appraisals would also provide valuable direction for future research activities and investment and selection strategies.

## **9.2. Material and methods**

### **9.2.1. Assumed abalone aquaculture production system in Australia**

Currently the abalone farmed in Australia are mainly sold in Asian markets as canned or frozen products, and a small amount is sold domestically. Prices of canned products are determined by the animal's meat weight, whereas frozen products are sold according to the animals' whole body weight. Most abalone farms in Australia are involved in all aspects of the abalone farming cycle: 1. production of larvae (including collection and/or maintenance of broodstock); 2. production of abalone juveniles and 3. growing juvenile abalone to market size using various grow-out systems. The live abalone are then sold to processors to produce canned or frozen products, or are frozen on-farm and sold directly.

In a selective breeding program the genetic improvements take place in a small population called the selection stock or nucleus. The genetic gain(s) achieved in the nucleus will then be disseminated either directly or through multiplications of improved animals to farmed stocks if the numbers of selected animals are not large enough to meet industry's demand on improved stock for commercial productions. Due to very high fecundity in abalone, they are well placed for the dissemination of genetic gains from the nucleus to farming stocks without the need of a tier for multiplication (Fig. 9.1). In this study it is assumed that the participating farms will invest the facilities required for the breeding program and annual recurrent costs, and run the program themselves. It is also assumed that the generation interval of selection stock is three years, which means every three years a new generation of genetically improved broodstock will be available for commercial production. The selected broodstock will be used for both establishment of next generation in the nucleus and the production of commercial stock without delay in dissemination of genetic gains to the industry. It is further assumed that only pure species (greenlip or blacklip abalone) will be farmed by the industry. Farms in Victoria and Tasmania also farm the hybrid of these two species and assume that the hybrid performs better than both pure species in terms of their growth rates, while performing better than blacklip abalone in slab

tanks, although these assumptions need to be tested with properly designed experiments.

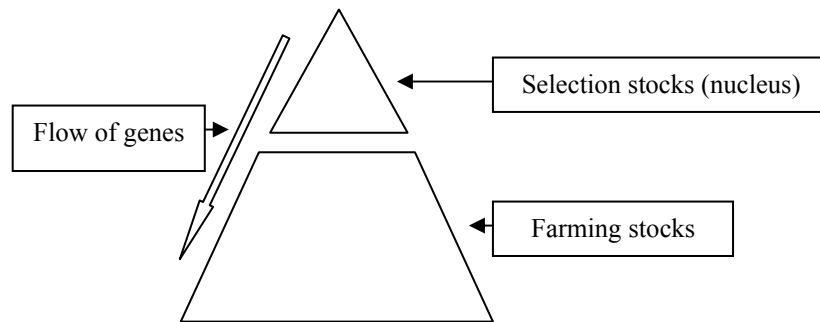


Fig 9.1. Diagram of gene flow from selection stocks to production stocks in abalone aquaculture industry.

Potentially there are two different kinds of production scenarios that can be applied to realise the benefits from farming with genetically improved progenies: 1. a fixed farming period production scenario (fixed farming period), where larger or heavier (and higher quality) abalone will be produced with each generation of genetic improvement; and 2. a fixed harvest weight production scenario (fixed harvest weight), where the period required to produce certain size (weight) abalone will decrease with the progress of selective breeding. Profit from the first scenario will be derived by producing heavier and potentially higher value products within the target farming period. Profit from the second scenario will, on the other hand, be mainly due to cost savings via a reduction in production period. Because of the long production cycle and generation interval (approximately 3 years) of the two species farmed in Australia, the first returns are expected to occur in year 6 after the start of the breeding program. The evaluation was conducted over a 15 year period to allow the sensitivity analysis on the delay in the year the first returns occur.

### 9.2.2. Breeding objective

In considering the development of any animal selection program, the first prerequisite is a meaningful definition of the breeding objective. According to Ponzoni and Newman (1989), the abalone breeding objective could be defined as the effective

combination of economically important traits in the production system. For a farm-based breeding program, the goal of abalone selection is to maximise the profit of the production system.

In the abalone production system described above, abalone are produced for consumption only. Two of the three biological traits identified in the first FRDC project are also included in this study. They are: 1. growth rates or whole weight at harvest; and 2. survival to harvest. The third trait, meat weight at harvest, is not included at this stage. The genetic parameter analyses show that this trait is highly correlated with whole body weight and will respond positively when selection on whole body weight (or growth rate) is applied. In addition, the abalone is currently sold by whole body weight at the farm gate, not by meat weight. A new trait, feed intake (calculated from feed conversion ratio), is included because it is expected that the increase in feed intake will accompany improvements in whole body weight or growth rates during selection, and would have a substantial impact on profit for industry. For example, if the average whole body weight at a fixed farming period (currently about 3 years) could be improved by 15g (about 13.6%) per generation through selection, the extra feed cost for this increase in weight could be AU\$ 0.074 per individual according to the current feed conversion ratio of 1:1.8 and the feed cost of AU\$ 2.75/kg if feed intake is not included in the objective. This consists of more than 12% of the profit from this 15g increase and would be higher if current farm gate price is less than AU\$ 42/kg or feed price is higher than AU\$2.75/kg.

The sources of income and expense in a commercial abalone farm can be expressed in the following profit equation:

$$\text{Profit } (P) = \text{Income} - \text{Expense}$$

The equation can be expanded as a function of biological traits and expressed respectively for a fixed farming period production scenario (A) and a fixed harvest weight production scenario (B). As used by Ponzoni et al. (2007) each equation is scaled up to a production unit of 1000 abalone.

A. For a fixed farming period abalone production scenario, the profit ( $P_p$ ) may be written as:

$$P_p = 1000 [(W)(S/100)(\text{price per unit weight of abalone}) - FI(\text{price per unit weight of feed})] - K$$

where  $W$  is the weight at harvest and equals 110g in this analysis,  $S$  is the survival from weaning to harvest and equals 80% in this analysis,  $FI$  is the total amount of feed consumed per abalone to harvest weight (at the feed consumption ratio of 1:1.8) and  $K$  represents constant costs. Constant costs are those that occur independently of the production level. These costs are ignored when obtaining the partial derivative of  $P$  with respect to each trait (Ponzoni, 1988).

The current farm gate price for whole wet weight abalone of 110g and the abalone feed price are provided in Table 9.1.

The economic value of each trait in the objective can be calculated as the partial derivative of  $P$  with respect to the trait in question, considering other traits as constants and evaluated at the mean values. The economic value (EV) of each trait can be computed as:

$$\begin{aligned} EV_W &= \partial P / \partial W \\ &= (1000)(0.80)(\text{AU\$}0.042) = \text{AU\$ } 33.60 \end{aligned}$$

$$\begin{aligned} EV_S &= \partial P / \partial S \\ &= (1000)(110\text{g})(1/100)(\text{AU\$}0.042) = \text{AU\$ } 46.20 \end{aligned}$$

$$\begin{aligned} EV_{FI} &= \partial P / \partial FI \\ &= - (1000) (\text{AU\$}0.00275) = - \text{AU\$ } 2.75 \end{aligned}$$

The breeding objective for a fixed farming period abalone production scenario can then be expressed as:

$$H_I = 33.60 BV_W + 46.20 BV_S - 2.75 BV_{FI}$$

where BV is the trait's breeding value.

B. For a fixed harvest weight abalone production system the profit ( $P_w$ ) equation may be written as:

$$\begin{aligned} P_w &= 1000 (S/100)[(\text{harvest weight})(\text{price per unit weight of abalone}) - (\text{harvest} \\ &\quad \text{weight}/G_r(\text{average yearly maintenance cost per abalone}) - FI_y(\text{price per unit} \\ &\quad \text{weight of feed})(\text{harvest weight})/G_r] - K \\ &= 1000 (S/100)\{(\text{harvest weight})(\text{price per unit weight of abalone}) - (\text{harvest} \\ &\quad \text{weight}/G_r[(\text{average annual maintenance cost per abalone}) + FI_y(\text{price per unit} \\ &\quad \text{weight of feed})]\} - K \end{aligned}$$

where  $S$  is the same as described for fixed farming period production scenario,  $G_r$  is the average growth rate (g/year) to harvest and equals 34.72 in this analysis,  $FI_y$  is the yearly average amount of feed consumed per abalone (at the feed consumption of 1.8):  $34.72 \times 1.8 = 62.50$ ;  $K$  is the same as that used for the fixed farming period equation.

The average annual variable maintenance cost per abalone is AU\$ 0.901 per year.

Similarly the economic value of each trait can be calculated as:

$$\begin{aligned} EV_{G_r} &= \partial P / \partial G_r \\ &= (1000)(0.80)(110)/[(34.72)(34.72)][0.901+(62.50)(0.00275)] \\ &= \text{AU\$}78.30 \end{aligned}$$

$$\begin{aligned} EV_S &= \partial P / \partial S \\ &= (1000)(1/100)\{(110)(0.042)-110/34.72[0.901+(62.50)(0.00275)]\} \\ &= \text{AU\$}12.22 \end{aligned}$$

$$EV_{FI_y} = \partial P / \partial FI_y$$

$$= - (1000)(0.80)\{(110)/(34.72)[(1)(0.00275)]\} = - \text{AU\$6.97}$$

The breeding objective for a fixed harvest weight abalone production scenario can be expressed as:

$$H_6 = 78.30 \text{BV}_{Gr} + 12.22 \text{BV}_S - 6.97 \text{BV}_{FI}$$

**Table 9.1. Parameters and their values**

Parameter	Abbreviation or symbol (units)	Value(s)
Discount rate	$d$ (fraction)	<b>0.05</b> , 0.075, 0.10
Discount factor	$r = 1/(1 + d)$	Calculated from $d$
Year when returns start	$y$ (years)	<b>6</b> , 7, 8
Number of years evaluated	$T_0$ (years)	15
Number of full-sib families	Family numbers	30, 50, <b>100</b> , 200, 300, 400
Individuals per family	Individuals/family	25, 50, <b>100</b> , 200, 300, 400, 500
Selection intensity in females	$i_F$	1.985383
Selection intensity in males	$i_M$	1.985383
Female generation interval	$gi_F$	3.0
Male generation interval	$gi_M$	3.0
Number of abalone sold/year	$Na$ (million)	0.9091, <b>2.7273</b> , 4.5455
Initial investment	$I$ (AU\$)	37475, <b>74950</b> , 112425
Annual recurrent cost	$Cu$ (AU\$)	42384, <b>84767</b> , 127151
Harvest whole body weight	$W$ (g)	110
Survival rate (grow-out)	$S$ (%)	80
Total cumulative feed intake	$FI$ (g)	198
Growth rate	$Gr$ (g/year)	34.72
Average feed intake per year	$FIy$ (g/year)	62.50
Farm gate abalone price	Price (AU\$/g)	0.032, <b>0.042</b> , 0.052
Feed cost	Cost (AU\$/g)	0.00275

### 9.2.3. The selection index

It was assumed that the breeding population considered as the initial and the base in this study consists of 100 full-sib families per generation with 100 progenies per family. For harvest weight or growth rate the data from individuals and their full-sibs



were used in the index. For survival the data are from their full-sibs only. Feed intake was included in the breeding objective. However, it was not treated as a selection criterion at this stage because it is difficult to quantify.

#### 9.2.4. Phenotypic and genetic parameters

Tables 9.2 and 9.3 show the phenotypic and genetic parameters used in this study for fixed farming period and fixed harvest weight abalone production scenarios respectively. Values are mainly from this study, Jonasson et al. (1999), Lucas et al. (2006) and Kube et al. (2007). For survival, family performance was used, which would be conservative because the within-family variation was not accounted for. There are no published data available for feed intake. The mean value is calculated by multiplying the mean harvest whole body weight or mean yearly increment in whole body weight with the feed conversion ratio. As used by Ponzoni et al. (2007), the phenotypic standard deviation of feed intake was calculated assuming a coefficient of variation of 0.3. A very high correlation value between feed intake and harvest whole body weight or growth rate is assumed. The correlation between survival rate and feed intake is considered at a slightly lower magnitude than between survival rate and harvest weight.

**Table 9.2. Phenotypic and genetic parameters for harvest whole body weight (*W*), survival rate (*S*) and feed intake (*FI*)**

	<i>W</i> (g)	<i>S</i> (%)	<i>FI</i> (g)
Mean	110.0	80.0	198.0
$h^2$	0.2	0.1	0.2
$\sigma_p$	24.2	8.9	59.4
<i>Phenotypic (above) and genetic (below) correlations</i>			
<i>W</i>		-0.3	0.85
<i>S</i>	-0.3		-0.2
<i>FI</i>	0.85	-0.2	

**Table 9.3. Phenotypic and genetic parameters for growth rate (*Gr*), survival rate (*S*) and annual feed intake (*FIy*)**

	<i>Gr</i> (g/year)	<i>S</i> (%)	<i>FIy</i> (g/year)
Mean	34.72	80.0	62.5
$h^2$	0.2	0.1	0.2
$\sigma_P$	7.9	8.9	18.8
<i>Phenotypic (above) and genetic (below) correlations</i>			
<i>W</i>		-0.3	0.85
<i>S</i>	-0.3		-0.2
<i>FI</i>	0.85	-0.2	

### 9.2.5. Calculations of matrixes and selection responses

According to the standard selection index theory (Hazel, 1943; Lin, 1978; Cameron, 1997) three matrixes were created. The ***P*** matrix is a square matrix of phenotypic variances and co-variances among the traits used as selection criteria. The ***G*** matrix comprises of genetic variances and co-variances between the selection criteria and breeding objective. The ***C*** matrix is square and comprises genetic variances and co-variances among the traits in the breeding objective. The ***a*** is a vector of economic values and calculated in 9.2.2. Breeding objective. The ***b*** is a vector of index weights, which is calculated according to the following equation:

$$\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\mathbf{a}$$

The index variance ( $\sigma^2_I$ ), the variance of the breeding objective ( $\sigma^2_H$ ) and the column vector of genetic gains corresponding to each trait ( $\Delta$ ) are calculated from the following three equations respectively.

$$\sigma^2_I = \mathbf{b}'\mathbf{P}\mathbf{b}$$

$$\sigma^2_H = \mathbf{a}'\mathbf{C}\mathbf{a}$$

$$\Delta = \mathbf{G}\mathbf{b}/\sigma_I$$

The index standard deviation ( $\sigma_I$ ) and the breeding objective deviation ( $\sigma_H$ ) are the square roots of index variance and breeding objective variance, respectively.

In this study there are 3 traits in the breeding objectives and 2 traits in the initial selection index. As used by Pitchford (2007) in his study on improving accuracy of selection of young bulls, the full-sib information on harvest body weight or growth rate is treated as an additional selection criterion correlated with the harvest body weight or growth rate. Therefore, the dimensions of  $\mathbf{P}$  and  $\mathbf{G}$  were 3x3 respectively. The  $\mathbf{a}$  and  $\mathbf{b}$  vectors were both 3.

Selection response is calculated as a truncation on the index value. To allow for losses and unsuccessful matings, the number of males and females is three times greater than that actually required. For example, when 100 full-sib families are produced only 100 broodstock of each sex will be required. To allow for the above mentioned uncertainties 300 broodstock of each sex are selected.

The following equation is used to calculate the annual genetic gain ( $gg/yr$ ) in economic units (AU\$):

$$gg/yr = (i_F \sigma_I + i_M \sigma_I) / (g_{i_F} + g_{i_M})$$

where  $i_F$  and  $i_M$  are the selection intensity for male and female respectively,  $g_{i_F}$  and  $g_{i_M}$  are the generation interval for male and female respectively, and  $\sigma_I$  is the standard deviation of the index.

The effective breeding number and inbreeding value are calculated according to Tave (1993):

$$N_e = (4N_m N_f) / (N_m + N_f)$$

$$F = 1/(2N_e) \\ = (N_m + N_f) / (8N_m N_f)$$

where  $N_e$  is the effective breeding number,  $N_f$  is the number of females that produce viable offspring and  $N_m$  is the number of males that produce viable offspring, and  $F$  is the average offspring inbreeding value.

All calculations were conducted in Microsoft® Excel (2003). The results were checked by comparing with published results, where available.

### **9.2.5. Calculation of economic benefits**

Economic benefits can be calculated from different perspectives, for example Ponzoni's et al (2007) calculation for Nile tilapia from a national perspective. We will calculate the economic benefits from the perspective of investing farms. They want to know what kind of return they should expect from their current level of investment in abalone selective breeding, and its chance of success. In addition, what increase in return can they expect if additional investment is available under the current cost structure for maintaining the selective breeding nucleus?

An industry adoption level of 300 ton is used as the base for the economic benefit calculation. This quantity was chosen because the production level among the three farms participating in the 2005/2006 abalone breeding activities had the capacity to produce at least 300 ton of abalone per year. It should be noted that many abalone farms in Australia cultivate both greenlip and blacklip abalone with variable production amounts.

The economic parameters and other values used to calculate the economic benefit from the selective breeding program are provided in Table 9.1. The initial investments for program setup and the annual recurrent costs for program maintenance are shown in Tables 9.4 and 9.5 respectively. Some data have been used within this project as references for budget calculations.

**Table 9.4. Initial program investment required on participating farms<sup>1</sup>**

Items	No of units	Unit price	Cost (AU\$)
Larval rearing tanks	39	250	9750
Settlement + nursery tanks	105	250	26250
Grow-out tanks*	9	4000	
Algae facilities*		2000	
Broodstock conditioning tanks*	9	500	
Biosecurity measures			10000
Quarantine tanks for newly imported broodstock	12	400	4800
UV units*	3	4500	
Spawning tanks	105	30	3150
Chiller for broodstock conditioning	3	5000	15000
Heater for broodstock conditioning	3	2000	6000
<b>Total</b>			<b>74950</b>

<sup>1</sup> The values provided are the requirements to establish 100 families with 100 individuals per family. It is also assumed that three farms will participate in the breeding program and that these families will be produced in three spawning runs.

\* These facilities will be required. However, it is assumed that the existing facilities on participating farms will be made available to the program.

**Table 9.5. Annual recurrent costs for the program, and return from selling excess nucleus stock after final data collection<sup>1</sup>**

Items	No of unit	Unit price	Cost (AU\$)
<b>Costs</b>			
Feed (AU\$/g)		0.00275	4303
Tagging material (AU\$/piece)		0.15	1000*
Labour for tagging (AU\$/individual)		0.96	6400*
Electricity prior to tagging (AU\$/family)	100	100	3333*
Electricity for grow-out stage			4500
Electricity for broodstock chillers	3		1600*
Electricity for broodstock heaters	3		800*
Technical staff (FTE)		48000	31200
Labour for final data collection			6400*
One standard data collection			5333*
Transportation of tagged abalone			167*
<b>Annual recurrent costs</b>			<b>84767</b>
<b>Return</b>			
Selling excess stock after final data collection			24640*
<b>Annual recurrent costs less selling return</b>			<b>60127</b>

<sup>1</sup> The values provided are the requirement to establish 100 families with 100 individuals per family. It is also assumed that three farms will participate in the breeding program and that these families will be produced in three spawning runs.

\* These values are calculated by dividing generation values with the average male and female broodstock generation intervals, which are 3 years currently.

The variable costs for running a 100 ton farm provided by Primary Industries and Resources SA (1998) are also used in this study, with some modifications (Table 9.6).

**Table 9.6. Annual major variable costs for a 100 ton farm**

Items	No of unit	Unit price	Cost (AU\$)
Labour	12	48000	576000
Materials			150000
Electricity (pumping, etc)			350000
Feed			550000
Marketing & Freight			500000
<b>Total</b>			<b>2126000</b>

In this study, economic benefits of the selective breeding program are calculated using the discounted cash flow technique. As stated by Hill (1971), this method can equate all expenditure and returns made in different years to a same base year and compute an aggregate profit to any year. Thus breeding programmes which lead to very different time patterns of returns can be compared in a simple way. This method has also been applied by Ponzoni et al. (2007) to assess genetic improvement programs in Nile tilapia.

According to Ponzoni et al. (2007), the undiscounted annual return ( $Ru$ ) from the abalone selective breeding program is calculated by multiplying the number of abalone sold per annum ( $Na$ ) with the genetic gain per year:

$$Ru = Na \text{ gg/yr}$$

The discounted return ( $Rd$ ) over  $To$  years with the first returns being expected at year  $y$  can be calculated as:

$$Rd = Ru [r^y + 2r^{y+1} + \dots + (To - y + 1)r^{To}]$$

$$Ru \{ (r^y - r^{To+1}) / (1-r)^2 - [(To - y + 1) r^{To+1}] / (1-r) \}$$

where  $r$  is the discounted factor.

The discounted cost ( $Cd$ ) over  $To$  years are calculated as the product between the annual undiscounted costs ( $Cu$ ) and the discounted factor:

$$Cd = Cu(r + r^2 + \dots + r^{To}) = Cu r (1 - r^{To}) / (1 - r)$$

The economic benefit ( $E_B$ ) over  $To$  years is then calculated as:

$$EB = Rd - Cd - I$$

where  $I$  is the initial investment.

The benefit/cost ratio ( $BCR$ ) over  $To$  years is conducted using the equation:

$$BCR = Rd / (Cd + I)$$

The parameter values used in the initial and the base situation are considered moderate and achievable. It is expected the  $EB$  and  $BCR$  would be better if improved scenarios are applied.

#### 9.2.6. Sensitivity analysis

Sensitivity analyses are conducted by examining the consequences of altering a number of parameters from the base values (one parameter a time) on  $EB$  and  $BCR$  in both models. For ease of discussion, these parameters are grouped into the following four categories: 1. biological (heritability values, inclusive of feed intake, generation interval, extra number of broodstock selected and one trait objective), 2. economic (annual costs, initial investment, discount rate, and abalone market price), 3. operational (year when first return occurs, and industry adoption level), and 4. population size in the nucleus. The last category is conducted mainly for the fixed farming period model, and by varying both family numbers per generation and individuals in each family to maintain the same level of inbreeding value as used in the base situation (0.25% per generation). This inbreeding value is half the inbreeding level (0.5%) considered by Gjedrem et al. (2005) as acceptable in a fish breeding program. The logic behind this is that with the further extension in the breeding population in the nucleus, what are the cumulative genetic gains (in AU\$) and the cumulative economic benefits and benefit cost ratios we could expect from the abalone breeding program? Answers to these will provide information to help the



abalone industry understand the potential impact from their selected investment strategies.

In this study the impact from sensitivity analyses are classified into 5 levels according to the percentage changes against the base situation:

< 5%	not sensitive
5% - 20%	moderate
21% - 35%	strong
36% - 50%	very strong
> 50%	extremely strong

These classifications are arbitrary and are used to compare the relative impact of magnitudes of different parameters or different levels of a parameter under the assessment only.

The *BCR* values are provided on top of the bars in figures.

### 9.2.7. Chance of success

One of the most important practical issues is to manage the effects of genetic drift and inbreeding because it will vary the selection responses predicted by the commonly-used formulae. A simple measure that provides a very useful guideline is the coefficient of variation of response (*CVR*), and this can be calculated using the equation of Nicholas (1989):

$$CVR = (gi_F + gi_M)^{0.5} / [Q(N_e T_o)^{0.5}]$$

where *gi* is the generation interval, *Q* is the average of the product of selection intensity (*i*) and accuracy of selection (*s*) for male and female, and *T<sub>o</sub>* is the number of years the breeding program is evaluated. Because *CVR* is the ratio of the standard deviation on the mean, re-arranging the equation can set confidence limits (*CL*) on the response to selection for both *EB* and *BCR* (Ponzoni et al., 2007):

$$CL = \text{mean response} \pm (v)(\text{standard deviation})$$

where  $v$  is the appropriate table value for the chosen confidence level (e.g. 1.96 for 95% confidence).

### **9.3. Results and discussion**

In this study a vertically integrated business structure is assumed, which controls the two tiers described in Figure 9.1, that is, the nucleus and the production sector. It is also assumed that the participating farms will collaborate under an equal contribution basis, that is, farms will each produce the same number of families in each spawning season. It should be noted that the findings in this study are also applicable to the whole abalone aquaculture industry.

Abalone farms can realise genetic improvements in two ways. For example, they can choose to produce heavier animals in a set period (3 years) or produce certain weight individuals (110g) in a reduced time period (less than 3 years). Therefore, they should receive extra benefits from selling either heavier animals being produced (a genetic gain) or animals being produced in a shorter period (which equates to a cost saving). Both strategies are evaluated in this study.

#### **9.3.1. Genetic gains or cost savings**

The annual genetic gain in each trait in the breeding objectives and the overall gain or cost saving for fixed farming period and fixed harvest weight production scenarios are shown in Tables 9.7 and 9.8, respectively. The parameters chosen for the initial and the base situation are considered reasonable. For example, abalone price is the current farm gate price for 110 g animals. The industry adoption level is the production capacity of the three Victorian farms participating in the program. The first returns are expected at year 6. For both production scenarios the *EB* turns from negative to positive in year 7, the second year after the expected first turns occur. By year 15 after the implementation of the program the *EBs* become higher than AU\$ 10 million (Figure 9.2) and *BCRs* are higher than 15 (Figure 9.2, Base) for both production scenarios. Higher *EBs* and *BCRs* can be expected if more farms will use the selected progeny for their commercial productions. Therefore the investment in the abalone

genetic improvement program would be highly beneficial if uptake across the whole industry occurs.

It is also demonstrated that the index (initial situation) including both survival and weight or growth rate is not as favourable as that including weight or growth rate only in terms of the improvements in each individual trait and genetic gains (Tables 8 and 9). This is due to the negative correlations between survival and other traits included in the breeding objective and selection criteria. As a consequence, the index using the individual and its full-sib data on weight or growth rate is considered as the Base(s) in the subsequent sensitivity analyses.

It should be noted that if selection is applied according to this Base strategy or strategies it could result in about a 10% reduction in survival after 5 generations of selection. This would be a serious concern to the breeding program and breeders because the increase in mortality also increases chance for cross contamination between abalone farmed in the intensive system, and might be more sensitive to other environmental changes. The correlation data used in this study was calculated from a very small sample size. If similar magnitudes of correlation between survival and other traits are confirmed later in both species, alternative selection strategies such as restriction on survival would be needed to address this issue. It should be noted that any alternative selection strategies to compensate for the loss in survival would result in a reduction in genetic improvement in other traits, if they are negatively correlated.

**Table 9.7. Annual genetic gain for each trait, standard deviations of breeding objective ( $\sigma_H$ ) and selection index<sup>a</sup> ( $\sigma_I$ ) and overall gain in economic units (Fixed farming period model)**

Breeding objective & indices	Harvest weight (g)	Survival rate (%)	Feed intake (g)	$\sigma_H$ (AU\$)	$\sigma_I$ (AU\$)	Gain in economic units (AU\$)
Initial (abalone price AU\$ 42/kg) $H_1 = 33.60 BV_W + 46.20 BV_S - 2.75 BV_{FI}$ $I_1 = 4.89 P_W - 18.56 Pf_S - 1.87 Pf_W^d$	3.6	-0.7	7.2	291.11	101.60	67.24
Base (weight only selection criteria) $H_1 = 33.60 BV_W + 46.20 BV_S - 2.75 BV_{FI}$ $I_2 = 0.94 P_W + 39.13 Pf_W$	6.6	-0.5	13.7	291.11	241.36	159.73
Weight only objective $H_2 = 33.60 BV_W$ $I_3 = 1.30 P_W + 54.18 Pb_W$	6.6	-	-	363.64	334.31	221.25
FI economic values set at 0.0 $H_3 = 33.60 BV_W + 46.20 BV_S$ $I_4 = 1.16 P_W + 48.38 Pf_W$	6.6	-0.5	13.7	347.52	298.54	197.51
Lower heritabilities <sup>b</sup> $H_1 = 33.60 BV_W + 46.20 BV_S - 2.75 BV_{FI}$ $I_5 = 0.73 P_W + 33.75 Pf_W$	4.3	-0.3	9.0	205.85	158.18	104.68
Higher heritabilities <sup>c</sup> $H_1 = 33.60 BV_W + 46.20 BV_S - 2.75 BV_{FI}$ $I_6 = 1.10 P_W + 41.19 Pb_W$	8.3	-0.6	17.3	356.64	304.06	201.23
Lower price AU\$ 32/kg $H_4 = 25.60 BV_W + 35.20 BV_S - 2.75 BV_{FI}$ $I_7 = 0.66 P_W + 27.61 Pf_W$	6.6	-0.5	13.7	209.60	170.30	112.71
Higher price AU\$ 52/kg $H_5 = 41.60 BV_W + 57.20 BV_S - 2.75 BV_{FI}$ $I_8 = 1.22 P_W + 50.65 Pf_W$	6.6	-0.5	13.7	356.73	302.50	199.89

<sup>a</sup> Index accuracy =  $\sigma_I/\sigma_H$

<sup>b</sup> Equal to 0.1, 0.05 and 0.1 for harvest weight or growth rate, survival and feed intake, respectively.

<sup>c</sup> Equal to 0.3, 0.1 and 0.3 for harvest weight or growth rate, survival and feed intake, respectively.

<sup>d</sup> Pf represents full-sib phenotypic information.

**Table 9.8. Annual genetic gain for each trait, standard deviation of the breeding objective ( $\sigma_H$ ) and of the index<sup>a</sup> ( $\sigma_I$ ) and overall saving in economic units (Fixed harvest weight model)**

Breeding objective & indices	Growth rate (g/year)	Survival rate (%)	Yearly feed intake (g)	$\sigma_H$ (AU\$)	$\sigma_I$ (AU\$)	Saving in economic units (AU\$)
Initial (abalone price AU\$ 42/kg) $H_6 = 78.30 BV_{Gr} + 12.22 BV_S - 6.97 BV_{YFI}$ $I_9 = 11.94 P_{Gr} - 14.67 Pf_S - 0.18 Pf_{Gr}$	1.1	-0.6	2.2	221.10	99.27	65.70
Base (growth rate only selection criteria) $H_6 = 78.30 BV_{Gr} + 12.22 BV_S - 6.97 BV_{YFI}$ $I_{10} = 2.37 P_{Gr} + 98.94 Pf_{Gr}$	2.2	-0.5	4.3	221.10	199.49	132.02
Growth rate only objective $H_7 = 78.30 BV_{Gr}$ $I_{11} = 3.02 P_{Gr} + 126.30 Pf_{Gr}$	2.2	-	-	276.98	254.64	168.52
FI economic values set at 0.0 $H_8 = 78.30 BV_{Gr} + 12.22 BV_S$ $I_{12} = 2.92 P_{Gr} + 121.95 Pf_{Gr}$	2.2	-0.5	4.3	268.67	245.16	162.24
Lower heritabilities <sup>b</sup> $H_6 = 78.30 BV_{Gr} + 12.22 BV_S - 6.97 BV_{YFI}$ $I_{13} = 1.87 P_{Gr} + 85.33 Pf_{Gr}$	1.4	-0.3	2.8	156.34	130.73	86.52
Higher heritabilities <sup>b</sup> $H_6 = 78.30 BV_{Gr} + 12.22 BV_S - 6.97 BV_{YFI}$ $I_{14} = 2.78 P_{Gr} + 104.14 Pf_{Gr}$	2.7	-0.6	5.5	270.79	251.31	166.31
Lower price AU\$ 32/kg $H_9 = 78.30 BV_{Gr} + 1.22 BV_S - 6.97 BV_{YFI}$ $I_{15} = 2.48 P_{Gr} + 103.18 Pf_{Gr}$	2.2	-0.5	4.3	228.33	208.03	137.67
Higher price AU\$ 52/kg $H_{10} = 78.30 BV_{Gr} + 23.22 BV_S - 6.97 BV_{YFI}$ $I_{16} = 2.27 P_{Gr} + 94.71 Pf_{Gr}$	2.2	-0.5	4.3	218.06	190.95	126.37

<sup>a</sup> Index accuracy =  $\sigma_I/\sigma_H$

<sup>b</sup> Equal to 0.1, 0.05 and 0.1 for harvest weight or growth rate, survival and annual feed intake, respectively.

<sup>c</sup> Equal to 0.3, 0.1 and 0.3 for harvest weight or growth rate, survival and annual feed intake, respectively.

<sup>d</sup> Pf represents full-sib phenotypic information.

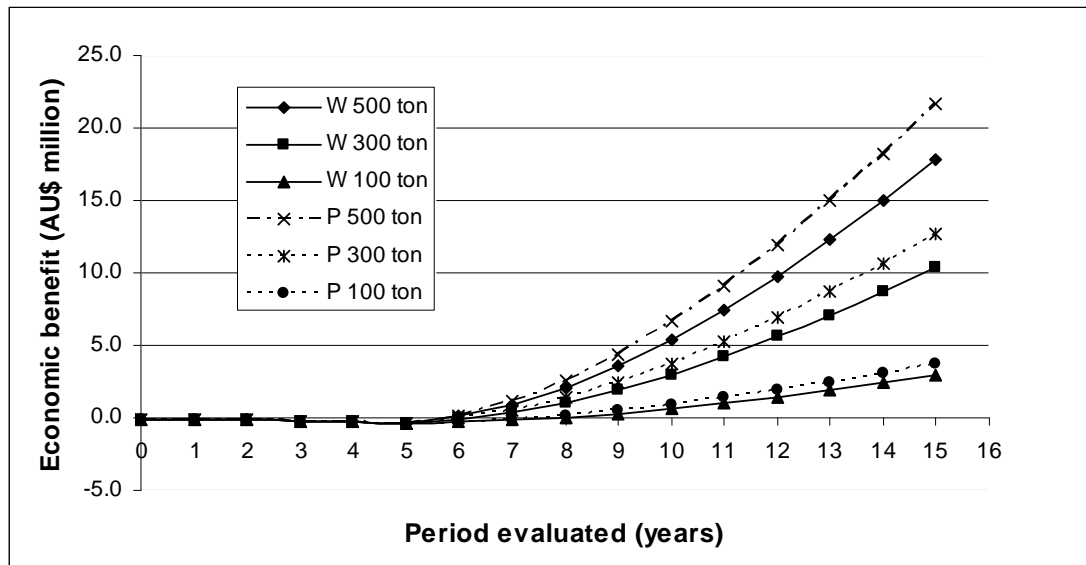


Figure 9.2. Accumulation of economic benefits under the three industry adoption levels evaluated with the fixed harvest weight model (W) and the fixed farming period model (P).

### 9.3.2. Sensitivity to biological parameters

In this study four biological parameters are assessed: they are the heritability, feed intake, generation interval, and extra number of broodstock selected. Both models show a similar magnitude of sensitivity to each parameter assessed. According to the effect standard described above, heritability shows strong or very strong impacts on *EB* (Table 9.9 and Figure 9.3). As expected, greater heritabilities result in higher *EB* and *BCR*. Heritabilities can be improved by reducing the environmental variance by managerial means (Ponzoni et al., 2007). However, even for the lower values that are used the *EB* and *BCR* are favourable, being AU\$ 6.6 million and AU\$ 8.1 million, and 10.4 and 12.6 for fixed harvest weight and fixed farming period production scenarios respectively. Setting economic values of food intake at 0 has a strong impact (more than 24%), whereas reducing the extra number of broodstock selected for production of next generation has only a moderate impact on *EB* (close to 20%) (Table 9.9, Figures 9.4 and 9.6). Shortening the generation interval from 3 years to 2 years causes an increase in *EB* by more 50% and in *BCR* at the similar magnitude (Table 9.9, Figure 9.5). Similar trends of responses in heritability and feed intake were also

reported by Ponzoni et al. (2007) for a Nile tilapia genetic improvement program with the fixed farming period model.

In the present study, the feed intake in the breeding objective for the fixed farming period model is handled in the same way as that handled by Ponzoni et al. (2007) to account for the increased production cost due to the assumed greater feed intake of faster growing abalone. As discussed by Ponzoni et al. (2007), setting the economic value of feed intake to zero is equivalent to assuming that faster growing abalone do not have a greater feed intake, or that the additional intake has no cost. It is clear that these assumptions are not correct. In brown trout (*Salmo trutta*), selection for growth rate was correlated with feed intake response (Mambrini et al., 2006). If the correlated response reported for fish species is confirmed in abalone, ignoring feed intake in the breeding objective would overestimate the benefit of a selective breeding program emphasizing harvest weight. In the present study, it could result in 24% overestimation. A greater over-estimation rate can be expected if a higher feed price is used.

With regard to generation interval and extra number of broodstock selected, reducing the generation interval from 3 years to 2 years and reducing the extra number of broodstock selected from 2 to 0.25 would be the maximal levels we could expect at this stage, and both would require substantial investments in R&D activities and facility upgrade. If we assume a similar investment and time period would be required to achieve each of the suggested levels, and we have to make a decision on which improvement to target first due to the limited available funding, results from this study suggest that reducing the generation interval would potentially provide more than double the economic benefit than if the investment is put on reducing the extra number of broodstock selected (53% vs 19%).

**Table 9.9. Economic benefit deviations (%) from the Base scenario in the parameters assessed in the sensitivity analyses**

Category	Parameter	Production system	Comparison	% deviated from Base
Biological	Heritability	FFP <sup>a</sup>	Lower vs Base	-36.3%
			Higher vs Base	27.4%
		FHW <sup>b</sup>	Lower vs Base	-36.8%
			Higher vs Base	27.7%
	EV <sub>FI</sub> = 0	FFP	EV <sub>FI</sub> = 0 vs Base	25.0%
		FHW	EV <sub>FI</sub> = 0 vs Base	24.4%
	Generation interval	FFP	2 yr vs 3 yr (Base)	52.6%
		FHW	2 yr vs 3 yr (Base)	53.1%
	Extra individuals selected for each parent required	FFP	0.25 vs 2 (Base)	18.8%
		FHW	0.25 vs 2 (Base)	18.9%
	One trait objective	FFP	Weight only objective vs Base	40.7%
		FHW	Growth rate only objective vs Base	29.5%
Economic	Annual cost (50% change)	FFP	Lower vs Base	3.5%
			Higher vs Base	-3.5%
		FHW	Lower vs Base	4.2%
			Higher vs Base	-4.2%
	Initial investment (50% change)	FFP	Lower vs Base	0.3%
		Higher vs Base	-0.3%	
		FHW	Lower vs Base	0.4%
			Higher vs Base	-0.4%
	Discount rate	FFP	7.5% vs 5% (Base)	-24.5%
			10% vs 5% (Base)	-42.5%
		FHW	7.5% vs 5% (Base)	-24.7%
			10% vs 5% (Base)	-42.7%
Abalone market price	FFP	\$32 vs \$42 (Base)	-31.6%	
		\$52 vs \$42 (Base)	31.6%	
	FHW	\$32 vs \$42 (Base)	3.9%	
		\$52 vs \$42 (Base)	-4.0%	
Operation efficiency	Years 1 <sup>st</sup> returns occur	FFP	7 yr vs 6 yr (Base)	-20.7%
			8 yr vs 6 yr (Base)	-38.8%
		FHW	7 yr vs 6 yr (Base)	-20.9%
			8 yr vs 6 yr (Base)	-39.3%
	Industry adoption level	FFP	100 tons vs 300 ton (Base)	-70.3%
			500 tons vs 300 ton (Base)	70.4%
	FHW	100 tons vs 300 ton (Base)	-71.2%	
		500 tons vs 300 ton (Base)	71.1%	
Nucleus population size	Base situations	FHW vs FFP	-19.3%	

<sup>a</sup> Fixed farming period production scenario.<sup>b</sup> Fixed harvest weight production scenario.



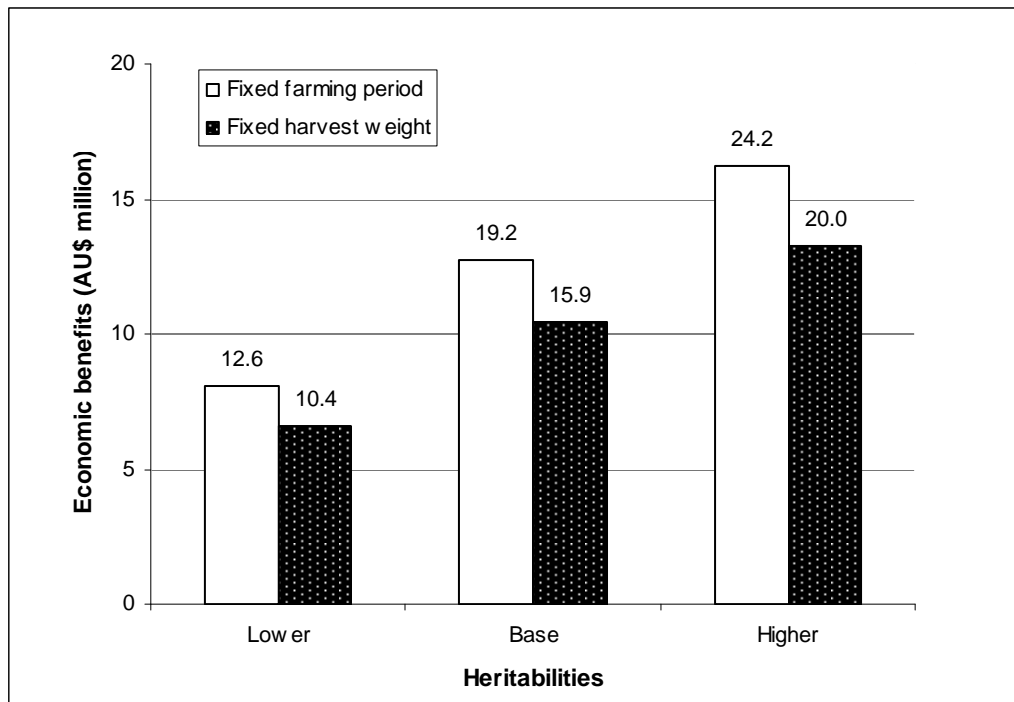


Figure 9.3. Sensitivity to heritability values. They are evaluated over 15 years of selective breeding. The value details are provided in Tables 9.2, 9.3, 9.7 and 9.8. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

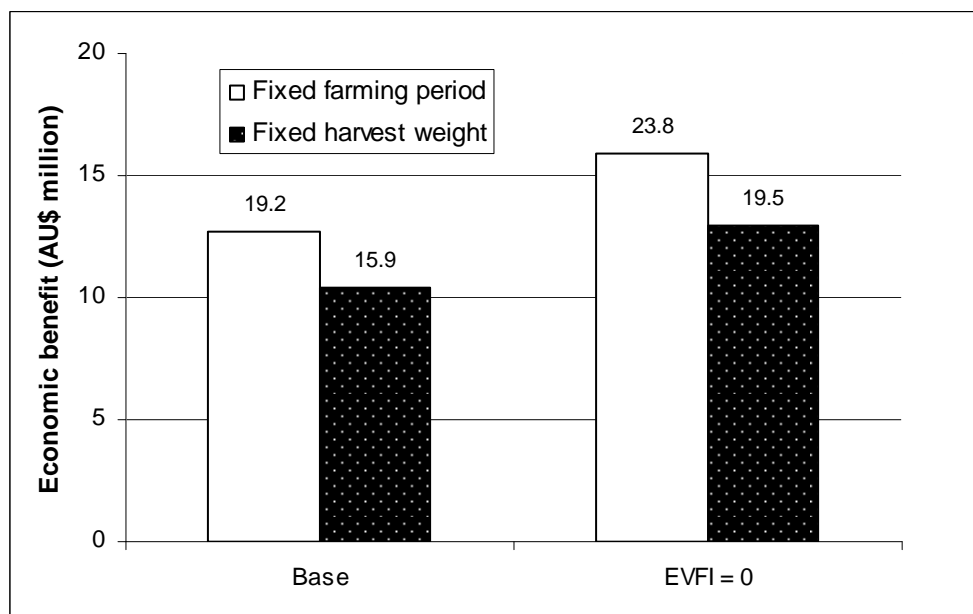


Figure 9.4. Differences in economic benefit between selections on the base indices and the indices with economic value for feed intake being set as 0. They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

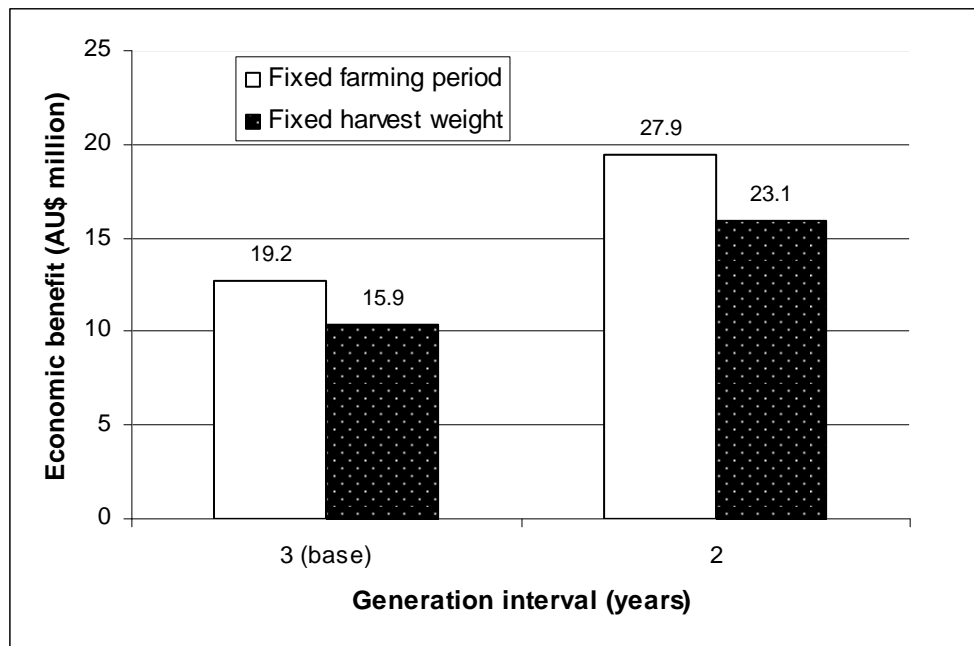


Figure 9.5. Sensitivity to generation interval (years). They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

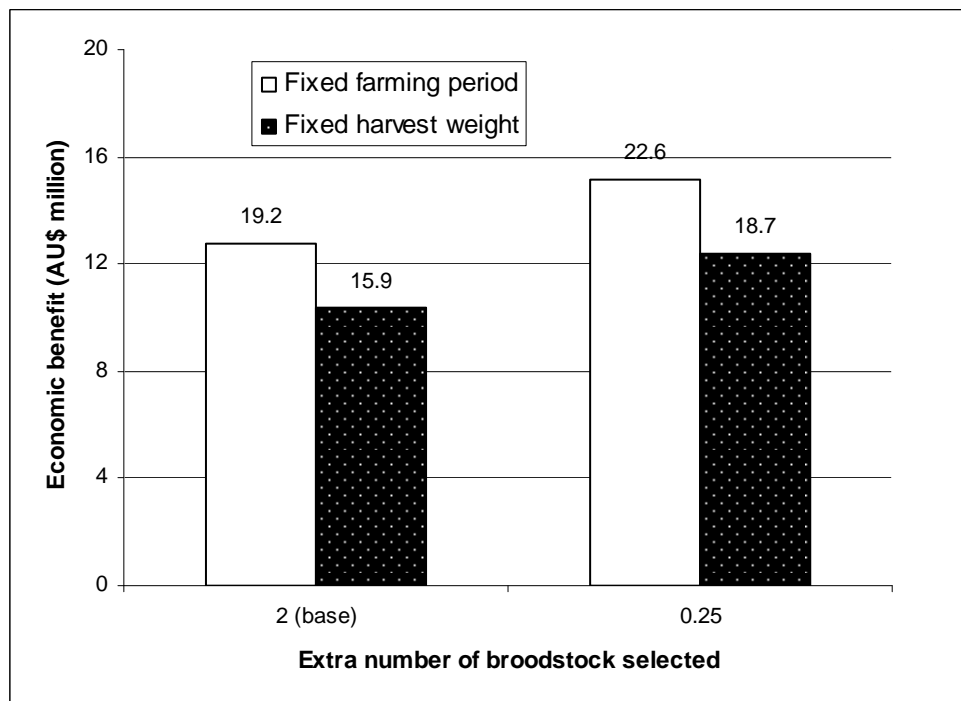


Figure 9.6. Sensitivity to the extra number of broodstock selected for each parent required to breed next generation. They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

This study also shows that if only harvest weight or growth rate is included in the breeding objective and the selection criteria, which is the case used by some farms for their own breeding projects, the economic benefit predicted could be overestimated by at least 30% (Table 9.9; Figure 9.7). This is partially due to the reasons discussed above for the feed intake and partially due to the negative correlation between these traits with survival.

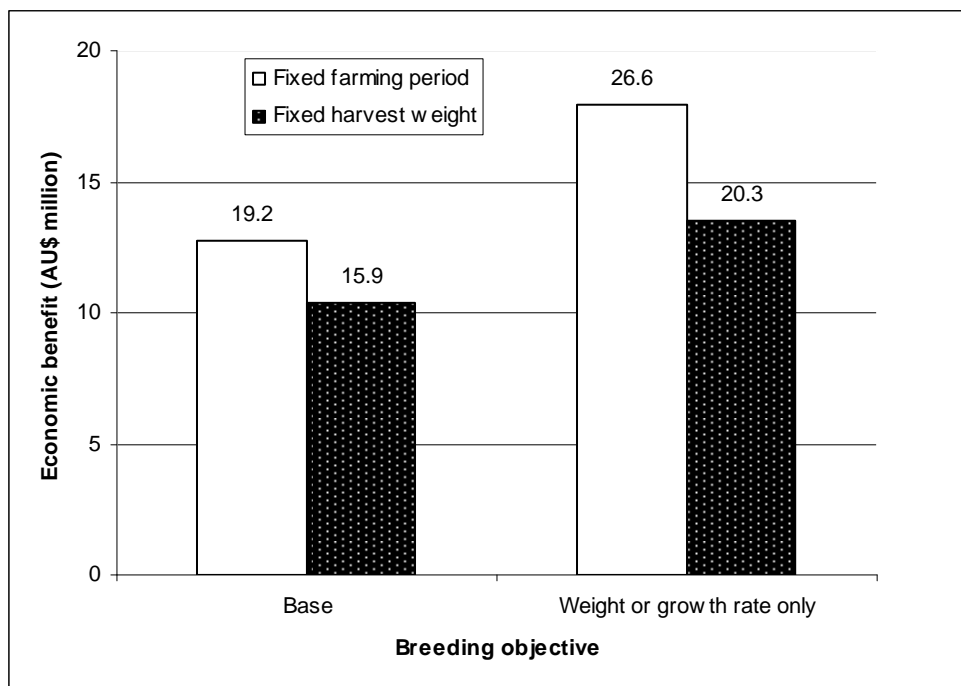


Figure 9.7. Comparisons in economic benefit and benefit/cost ratio between the base breeding objective and selection strategy used in this study and that including only harvest weight or growth rate in both breeding objective and selection criteria. They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

### 9.3.3. Sensitivity to economic parameters

Four economic parameters - annual recurrent costs, initial investment, discount rate and abalone market price - have been analysed in this study. Both models show a similar magnitude of sensitivity to annual recurrent cost, initial investment and

discount rate (Table 9.9; Figures 9.8, 9.9 and 9.10). The responses to abalone prices differ between these two models (Table 9.9; Figure 9.11).

*BEs* are not sensitive to either annual recurrent costs or initial investment when the values in both parameters are changed by 50% from one level to the next. *BCRs*, on the other hand, show much higher sensitivity to annual recurrent cost than to initial investment; it decreases by about 40% when the annual recurrent cost increases from one level to the next.

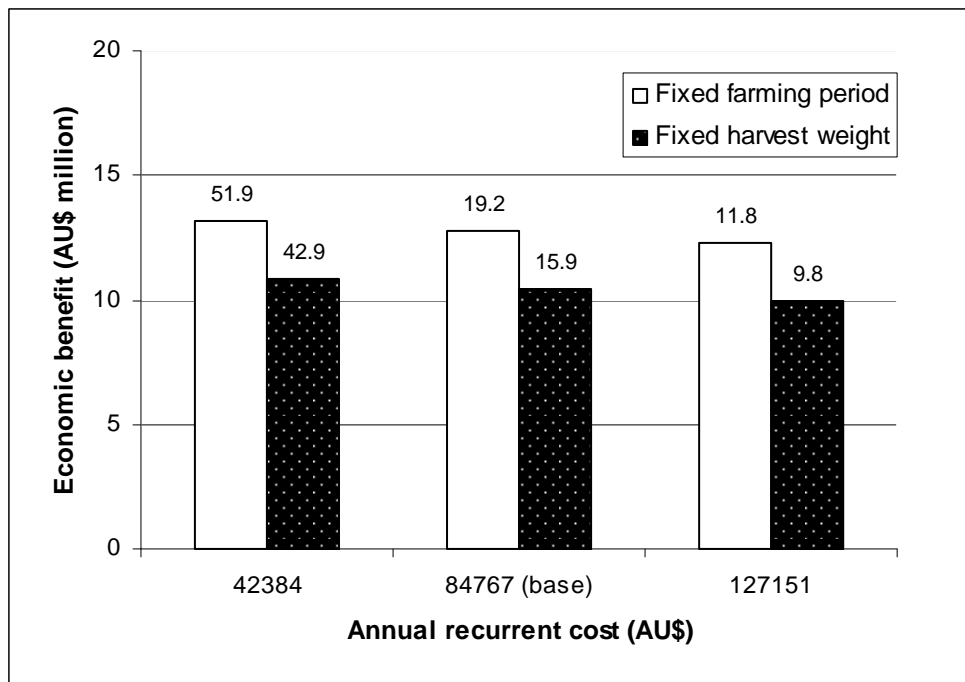


Figure 9.8. Sensitivity to annual recurrent costs (at 50% increment). They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

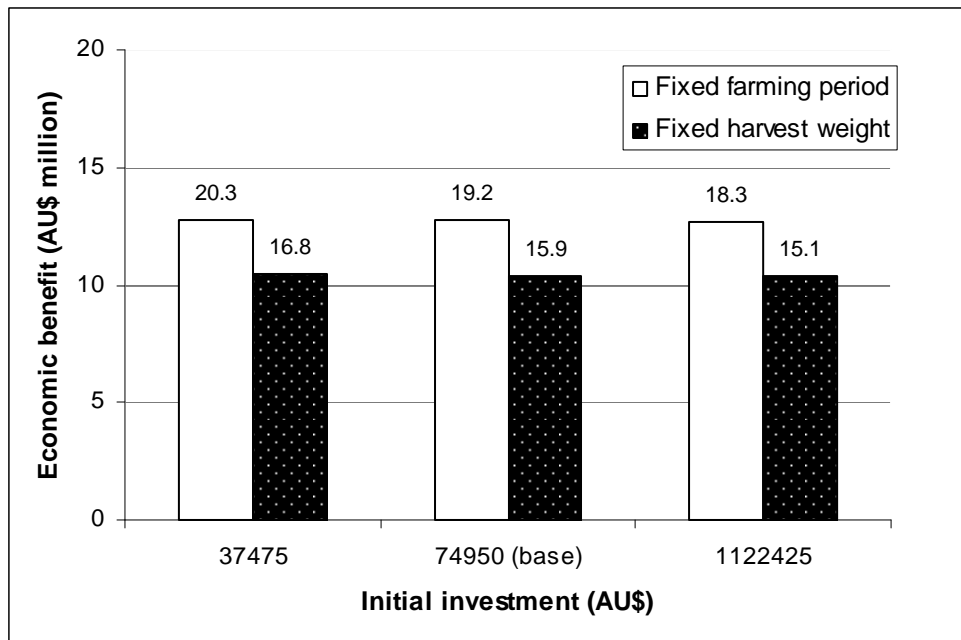


Figure 9.9. Sensitivity to initial investment levels (at 50% increment). They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

Increasing the discount rate from 5% to 10% reduces economic benefit and benefit/cost ratio at a similar magnitude (approximately 40%) (Table 9.9, Figure 9.10). The choice of a discount rate in a study like this is always an open question. In discussing this for animal breeding, Bird and Mitchell (1980) suggested that the discount rate should be in the order of 3% to 5%. However, selection of greater discount rates can be used as a way of accounting for risk. In this study, even at a high discount rate of 10%, a highly positive *EB* still results.

Both *EB* and *BCR* are not sensitive to abalone market prices in the fixed harvest weight model. In the fixed farming period model, abalone prices have a strong impact on these two measures, resulting in a more than 30% increment in *BE* when abalone price increase from AU\$32 to \$42 or from \$42 to \$52 per kilogram (Table 9.9; Figure 9.11). The differences in responding to abalone prices between these two models are due to the fact that the fixed harvest weight production scenario mainly receives the benefit from saving the operational costs by producing the targeted size animals within the reduced production period. The fixed farming period production scenario, on the other hand, receives their predicted economic benefit from the genetic

improvement by producing larger or heavier abalone in the predetermined production period.

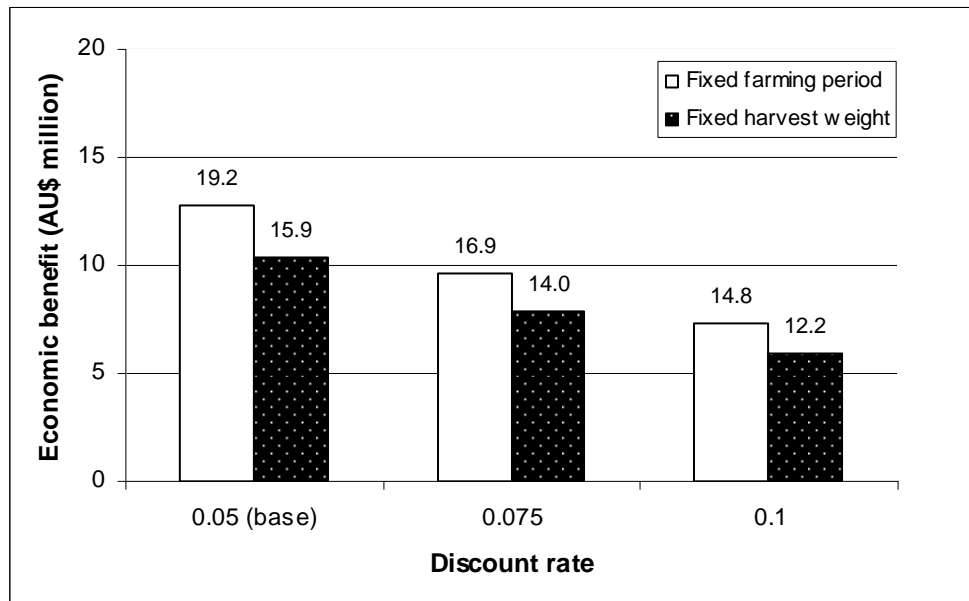


Figure 9.10. Sensitivity to discount rate. They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; filled bars: fixed harvest weight model. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

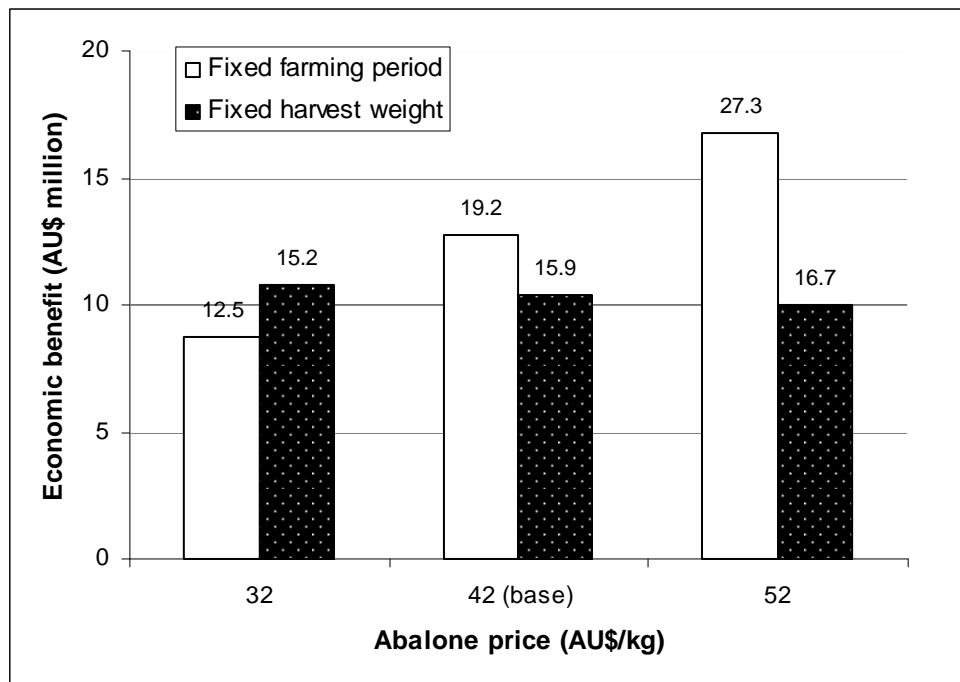


Figure 9.11. Effects of abalone prices on economic benefit and benefit/cost ratio; harvest weight model. They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

#### 9.3.4. Sensitivity to operational factors

The two parameters assessed in this study have strong to extremely strong impacts on *EB* in both models used (Table 9.9; Figures 9.11 and 9.12).

When a genetic improvement program is fully established, the distribution of genetic gains to the industry can be another critical factor influencing the economic benefit resulting from the program, especially for a species having a long generation interval such as abalone (3 years). The year when the first returns occur have strong effects (21%) on *EB* if it is delayed for one year to very strong (39%) if delayed for two years. Similar magnitudes of impact were also reported by Ponzoni et al (2007) in Nile tilapia. It should be noted that even with a delay in two years, *EB* and *BCR* are still highly favourable (Figure 9.12).

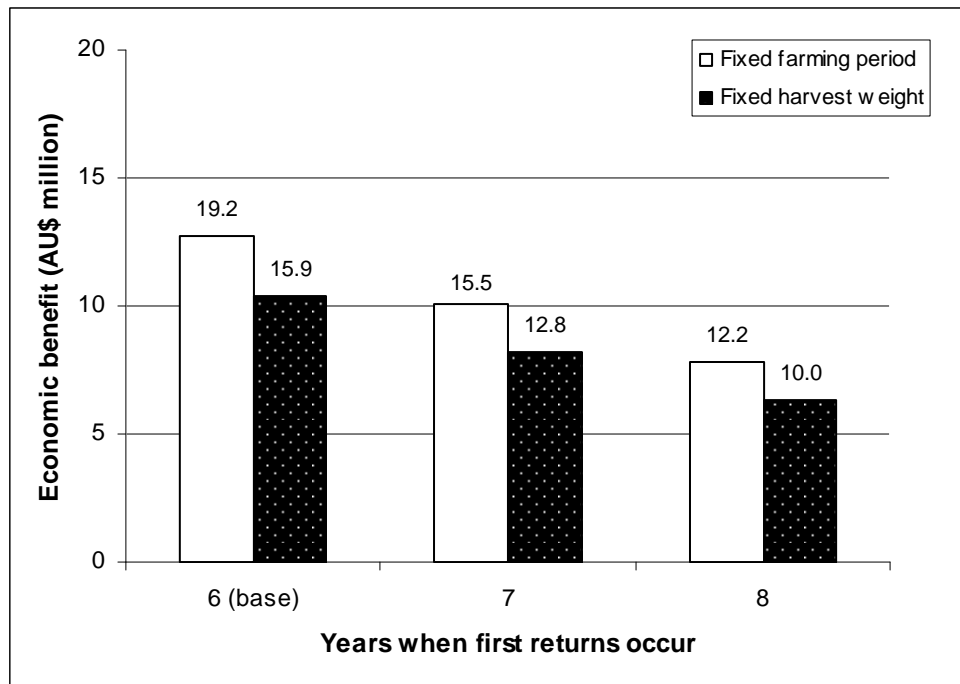


Figure 9.12. Sensitivity to the year when first returns occur. They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

The industry adoption levels are one of the business issues a breeding program needs to take into account. It will not affect the genetic gains at the individual abalone level. However, it can change the anticipated economic benefit and benefit/cost ratio dramatically. In the present case, when industry adoption level increases from 100 ton to 500 ton, both *EB* and *BCR* would increase by approximately 400% (Figure 9.13). At the 500 ton level, the program becomes highly favourable even over a 10 year period (Figure 9.2). For example, at 100 ton industry adoption level, the *EB* is AU\$ 3.78 million over a 15 year period, whereas at the 500 ton level the *EB* becomes AU\$ 6.61 million over 10 years (using the fixed farming period model). This result also suggests that the abalone genetic improvement program should start with the species with higher expected industry adoption level in the future, say ten years.



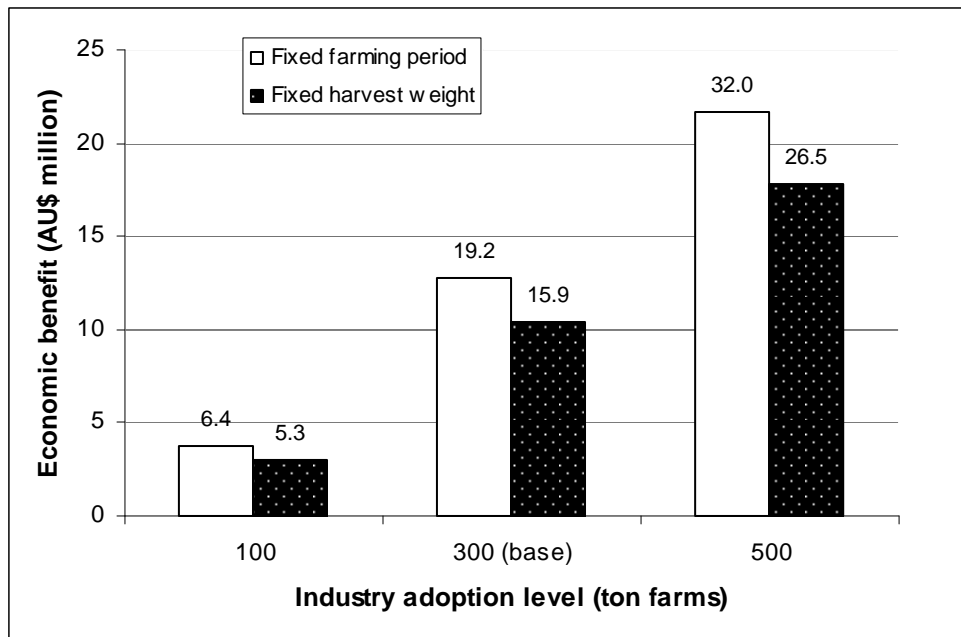


Figure 9.13. Sensitivity to industry adoption levels (ton farms). They are evaluated over 15 years of selective breeding. Open bars: fixed farming period model; Filled bars: fixed harvest weight model.

### 9.3.5. Sensitivity to nucleus population sizes

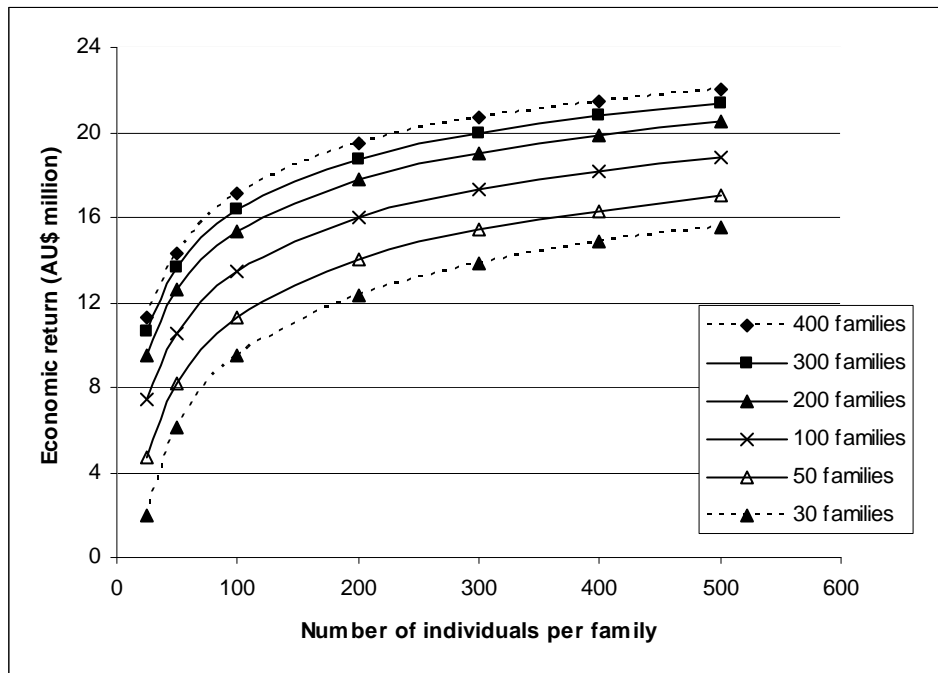
A genetic improvement program can and should be considered as a business. Therefore, we need to understand what are the expected gross return and profit under the different investment levels. In this study, the number of families and the number of individuals per family are treated as investment levels because they are linked to the costs for facility and maintenance requirements. The simulation is conducted in the same manner as for the base situation, but maintaining the inbreeding level at 0.25% per generation. As mentioned above, this level of inbreeding is half the acceptable value suggested for a fish breeding program (Gjedrem, 2005).

In this study the gross return is defined as the return that does not include any costs involved in the genetic improvement program. The gross return can also be converted to percentage genetic gain over the period evaluated and both would provide a similar trend. The gross return is used to compare the differences in the ways the breeding program is assessed in the following sections.

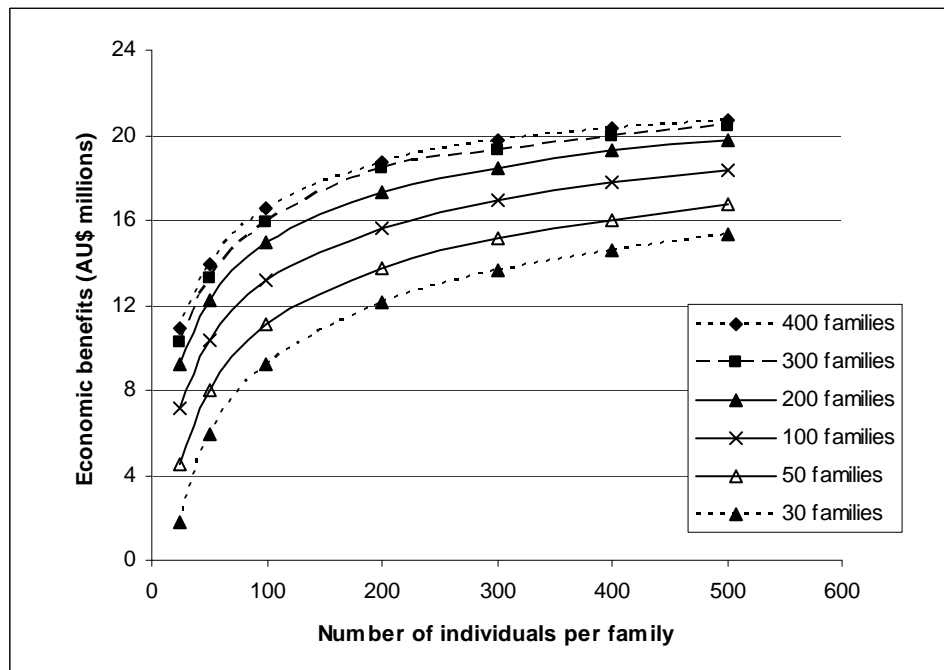
One would expect that at each number of individuals per family level, an increase in family numbers will result in an increase in the gross return. However, this increase is not linear, with the increase in the gross return from 300 to 400 families being much smaller than that from 30 to 100 families. A similar trend also occurs with an increase in individual numbers per family; an increment of abalone number by 100 after 300 individuals per family only makes a marginal improvement in gross return in all the family number scenarios (Figure 9.14A).

When the costs (initial investment and annual recurrent costs) required for the abalone genetic improvement program are included in the assessment, the economic benefit reduced in all nucleus population sizes, especially for the higher family number and higher individuals per family scenarios. Actually the *EBs* in 400 families with higher number of individuals per family are lower than those with same number of individuals per family in 300 and 200 families groups (Figure 9.14C). The *EBs* have improved in the analyses by halving the annual recurrent costs (Figure 9.14B). These results address the importance of proper cost estimation in anticipating the gains from the proposed investment strategy in the genetic improvement program. It should be noted that the analyses conducted here are for a 15 year period only (about 5 generations). Higher numbers of families and individuals per family would increase the capacity to maintain the genetic diversity in the nucleus for longer generations of selection. The potential benefit from maintaining genetic diversity has not been included in the present study.

A.



B.



C.

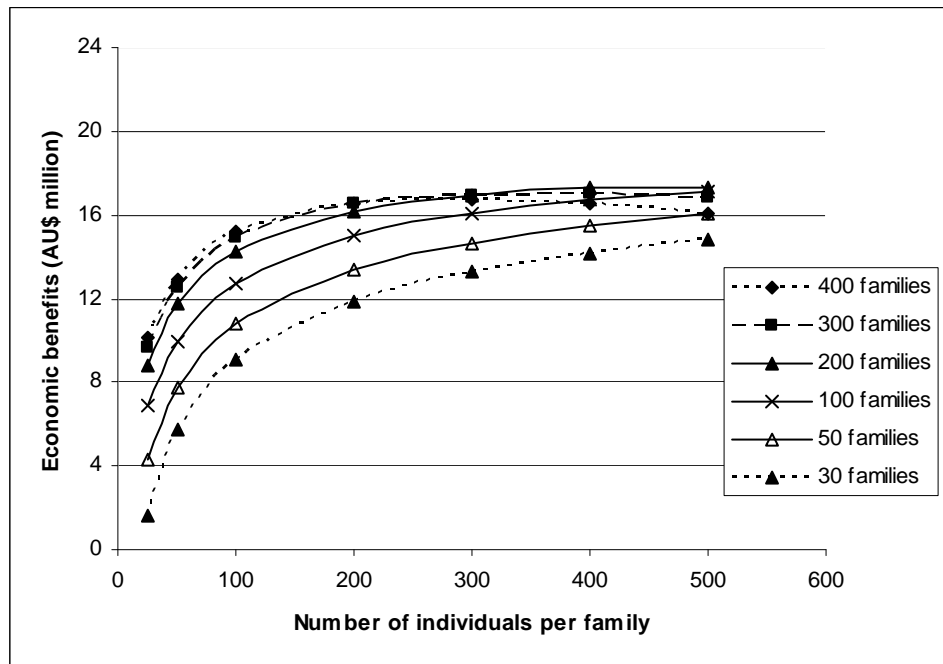


Figure 9.14. The expected gross return or economic benefit by varying both the number of families and the number of individuals per family by maintaining the inbreeding level at 0.25% (calculated using the fixed farming period model). A: costs are not included; B: 50% annual recurrent costs; C: 100% annual recurrent costs.

The benefits from investment in a genetic improvement program can also be assessed from a benefit/cost ratio point view. In fact, this might provide a tool to determine the most efficient investment strategy. For example, if maintaining 100 families per generation is decided by the program, and the facility requirement and funding are also allowed, maintaining 100 individuals per family would result in the optimal *BCR* under the costing structure used in this study. This will not be affected by the industry adoption level (Figure 15) and the evaluation period (data not shown). However, if the annual recurrent cost is halved, the maintenance of 200 individuals per family would provide a significantly higher *EB*, with the *BCR* being similar to maintaining 100 individuals per family (Figures 14C and 15). Again these results further emphasise the importance of correct cost estimation in decision making. Please also note that all analyses conducted in this study are based on the farm-based genetic improvement program and many costs are subsidised through sharing the facilities with commercial

productions. The sensitivity analysis results would change if a different costing structure were applied.

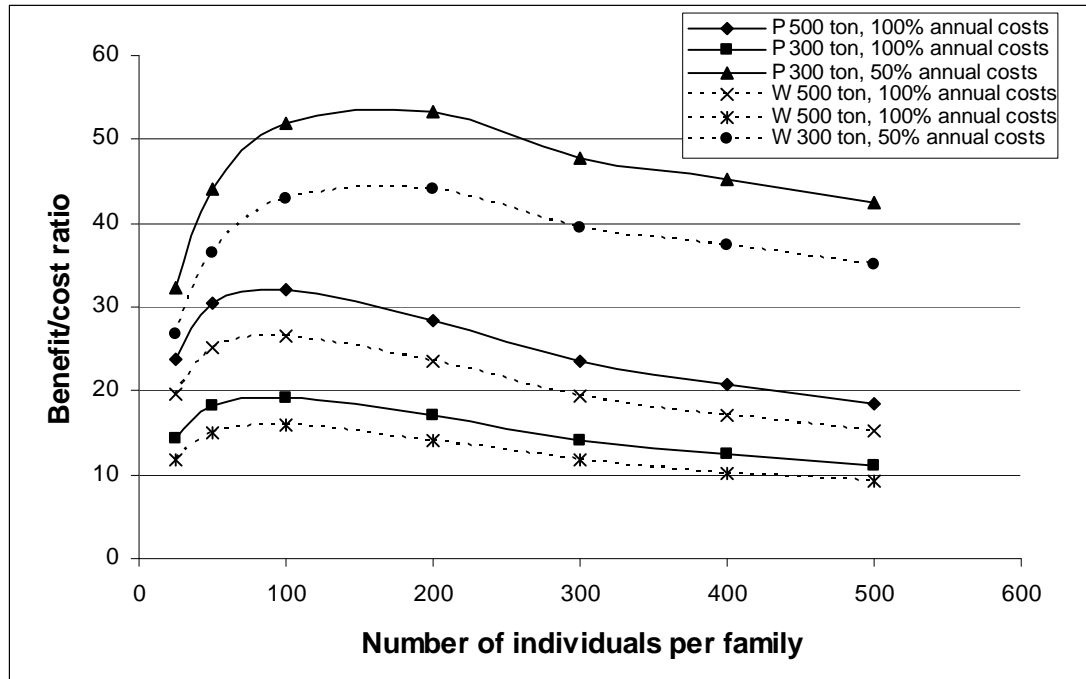


Figure 9.15. Benefit/cost ratio at different number of individuals per family in a 100 families per generation breeding nucleus. P: fixed farming period model; W: fixed harvest weight model.

### 9.3.6. Chance of success

According to Nicholas' equation (1989), the coefficient of variation of responses to selection corresponding to the design and time horizon evaluated in this study were 2.72% and 2.50% for fixed farming period and fixed harvest weight models, respectively. Table 9.10 shows the 95% probability limits for *EB* and *BCR* at different industry adoption levels. The way of assessing the chance of success of a genetic improvement program with Nicholas' equation is judged by the anticipated variability in response to selection. It was suggested by Nicholas (1989) that if the *CV* is less than 5% it would be quite confident of achieving the expected response results from the genetic improvement program. We could then conclude that the risk of failure due to technical reasons in the program evaluated here is very low. As shown in Table 9.10, even at the lowest industry adoption level both *EB* and *BCR* are still very

favourable. It should be noted that the industry adoption level itself does not affect *CV* values. It is the factor that determines the number of abalone upon which genetic improvement is expressed. Therefore it can change the *EB* and *BCR* substantially, which could in turn influence both industry and individual farms' investment priority decisions.

**Table 9.10. The *CV* and upper and lower limits at 95% probability for *EB* and *BCR* at the different levels of industry adoption evaluated with fixed farming period (FFP) or fixed harvest weight (FHW) models**

Industry adoption level (ton)	Limit for <i>BE</i> and <i>BCR</i>	<i>EB</i> (AU\$ million)		<i>BCR</i>		<i>CV</i>	
		FFP	FHW	FFP	FHW	FFP	FHW
100	Upper	4.02	3.18	6.75	5.56	2.72%	2.50%
	Lower	3.54	2.82	6.07	5.04		
300	Upper	13.46	10.95	20.25	16.67	2.72%	2.50%
	Lower	12.02	9.87	18.20	15.11		
500	Upper	22.89	18.72	33.75	27.78	2.72%	2.50%
	Lower	20.51	16.91	30.34	25.19		

#### 9.4. Concluding remarks

Breeding objectives have been established for both abalone production scenarios ("fixed farming period" and "fixed harvest weight") using the biological, economic and production costs information available so far or from best estimations. Results from the analyses conducted in this study suggest:

- The chance of success of an abalone genetic improvement program is high, although failure due to unforeseen natural disasters can occur.
- Heritability levels can have a very strong impact and can be increased through improved management practice in the nucleus.

- Reduction in generation interval from 3 to 2 years would have an extremely strong effect on *EB* (>50%), which is more than double that which could be achieved through a reduction in the extra number of broodstock selected for establishing next generation from 2 to 0.25 individuals (<20%).
- The cost of increased feed intake should be considered in the breeding objective to avoid overestimations of *EB* and *BCR* of the program.
- *EB* is not sensitive to changes in either initial investment or annual recurrent costs, whereas the discount rate can have very strong impact on *EB* and *BCR*.
- Both *EB* and *BCR* are very sensitive to abalone prices in the fixed farming period production scenario, but are not sensitive in the fixed harvest weight production scenario.
- The earlier the first returns are achieved after the initiation of the breeding program, the greater the *EB* and *BCR* will be because it can have a very strong effect.
- The industry adoption levels could have the greatest impact on both *EB* and *BCR*. When the industry adoption levels increase from 100 to 500 ton the *EB* increases by about 400%, suggesting that the abalone genetic improvement program should start with the species with the higher expected industry adoption level in the future.
- The methods used in this study in sensitivity analyses could assist in evaluation of investment and breeding strategies.
- The methods could also assist in the evaluation of R&D options to maximise returns from the limited available funding.

## 10. BENEFITS

Benefits from this project are mainly to the abalone aquaculture industry and can be described from the following four aspects:

### 1. Capability development for farm based abalone selective breeding programs

Firstly, more than 5 on-farm research officers have been trained through this and the previous FRDC projects. Most of them are currently actively involved in or managing the various selective breeding programs on different abalone farms. Secondly, the confidence to pursue farm-based genetic programs has been enhanced in the Australian abalone aquaculture industry by this project. Concerns about whether a proper genetic improvement program can be established on commercial farms have proved unwarranted because the targeted breeding strategy has been achieved through collaboration among three farms within a short period.

### 2. The identification of strengths and weaknesses of the farm based genetic improvement program and the provision of recommended improvements

This will help the existing abalone genetic program(s) to overcome identified weaknesses or to avoid these weaknesses in developing a new program on new farms or in new states.

### 3. The estimation of genetic parameters

This is important for the design and implementation of a selective breeding program. The existence of genetic components in all observed traits suggests that these traits can respond effectively to selection. In addition, the survival trait needs to be managed carefully because it might be negatively correlated to many production traits (only phenotypic correlation between body weight and survival has been analysed with very limited data) and we do not want to decrease the survival rate substantially through selection of other traits.



#### 4. The development of methods for defining breeding objectives and assessing abalone genetic improvement programs

These methods provide tools for developing breeding objective and selection criteria that could maximise the economic value of the abalone produced. They can also assist industry in decision-making on investment strategy and optimisation of returns from limited R&D funding. For example, sensitivity analyses show that the program should start with the abalone species with expected higher industry adoption level and the investment in R&D to reduce the abalone reproduction cycle would generate greater economic benefit than to improve the spawning efficiency.

In addition, the methods used for defining the breeding objective and appraisal of genetic improvement program can also be applied to breeding programs for other aquaculture species.

## **11. FURTHER DEVELOPMENT**

The breeding objective defined in this study includes harvest weight or growth rate, survival and feed intake. It should be noted that the reliability of phenotypic and genetic parameters is critical to the development of a breeding objective and selection strategy. The parameter information used in this study is from preliminary analyses on data collected from a very small population with only full-sibs. The required information is also limited for other abalone species in published papers. It is expected the data on body traits and survival will be improved when more data from families bred according to initial project design are available. In contrast, information on feed intake (feed conversion ratio) would require a separate experiment. Attention to genetic and environmental interactions is also required. The abalone families established so far are all held on the farms where they were produced. To ensure that the selected stock will perform as expected on other farms or environments, cross-environment assessments should be organized as early as possible. The inbreeding effect is not known in abalone species. The aimed inbreeding level of 0.25% per

generation is well within the recommended value (0.5% per generation) for fish breeding programs. However, management of the inbreeding level might be critical to an abalone breeding program because research in humans and livestock suggests that the fitness (fertility and health) of an individual is negatively correlated with its level of inbreeding (Miglior et al., 1995). The preliminary results from this study indicate that in blacklip abalone, survival is negatively correlated with harvest weight at a moderate level. Increases in inbreeding level could increase an abalone's sensitivity to other environmental changes substantially.

The inclusion of other traits, such as processing traits and resistance to specific diseases, may be worthy of consideration in the future. However, prior to their incorporation in the breeding objective, reliable phenotypic and genetic parameters and adequate economic importance assessments will be required.

Smith (1988) and Ponzoni (1992) suggest that the measurement of genetic gains should be an essential part of a genetic improvement program. This should provide the way to check if the selection program works, and demonstrate to industry and funding agencies that it is worthwhile for long term effort and investment. However, the changes in abalone's performance over time can be influenced by both genetic and environmental changes such as improvement in production system, nutrition, and so on. Therefore, it is important to separate genetic changes from environmental ones. This can be achieved by using frozen semen from males born in the first generation (Smith, 1988). A method developed for abalone sperm cryopreservation (Li, 2004) is encouraging, although further assessment on its application to farmed stock is needed.

At this early stage of the genetic improvement program, many techniques could potentially improve the economic benefit and/or genetic gain directly or indirectly, while the R&D funding and the funding to support the breeding nucleus are very limited. The methodologies developed or applied in this project provide the opportunities and capabilities to assess this from both individual farms and/or whole industry perspectives to not only maximise the economic value of the abalone produced, but also optimise the return on limited R&D resources. Some of these have been assessed in this study. These need to be extended to include other parameters or techniques, such as marker assisted selection and tagging methods. Results from the

simulation on marker assisted selection for abalone breeding programs are encouraging (Hayes et al., 2007).

The Microsoft Excel data sheet developed in the first FRDC project is an interim measure only, for data management. It was critical for the early stage of the program. It needs a major review when selection criteria have been decided. This is because many aspects of the performance recording service will be influenced by the nature of the information we decide to collect, and by the way in which it is to be combined. In addition, functions for automatically managing electronic data should also be considered in upgrading or developing this system.

Since Abalone Viral Ganglioneuritis broke out in Victoria in the 2005/2006 summer, biosecurity has become a major concern to the breeding program. Obviously upgrading the facility to meet the high biosecurity standards of all the project participating farms would be expensive. Furthermore, a cooperative approach will also be needed to disseminate the genetically improved stock to the whole abalone aquaculture industry in Australia. Otherwise the dissemination can be slow, due to limited market size, and the extra costs for obtaining stock transfer permission from governmental authorities. One of the options for tackling these potential problems would be gradually centralising the breeding nucleus on fewer farms, or even on one farm or a purpose built facility for the breeding program.

## **12. PLANNED OUTCOMES**

The planned outcomes include:

1. Capability development to produce 100 abalone families nationally per season within one month and the establishment of a total of 200 families (100 families per species) with about 250 individuals per family at the time for selection.

It was expected that this would be a reasonable breeding population size, and underpin the technical requirements for building up a long-term farm-based selective breeding program that could deliver substantial genetic gains and/or

economic benefit to the abalone aquaculture industry. This expectation was supported by the results from the genetic improvement program evaluation conducted in this study. The number of families produced from 2001/2002 to 2005/2006 was 235 (excluding 6 discontinued families), which is higher than the 200 families expected in the application. Most importantly, the goal to establish 100 families within one month was achieved in Victoria.

## 2. Genetic parameter estimations

As proposed, preliminary genetic parameter analyses have been conducted on both body traits (lengths and weights) and processing traits (meat weight, shell weight, meat/shell ratio, rumbled meat weight and cooked meat weight) in both species, and preliminary phenotypic correlation assessment between final body weight and survival in the blacklip abalone. The results have been used in the development of a breeding objective and selection index, and sensitivity analyses to selected parameters.

## 3. Development of an abalone selection index.

Methods for defining a breeding objective have been developed for both fixed farming period and fixed harvest weight abalone production scenarios, with three traits: harvest weight or growth rate, survival, and feed intake being included. Furthermore, they have been used to assess different selection strategies and sensitivities to biological, economic and operational parameters and population sizes in the breeding nucleus. The potential benefits of their application have been provided in the examples in the BENEFITS section.

## 4. Communication of the project outcomes to the abalone aquaculture industry.

The project results have been presented and updated at each of the FRDC Abalone Aquaculture Subprogram annual meetings from 2002 to 2005 (4 in total). Specific technical and business issues were communicated personally to the farms involved in the project and the Subprogram Leader.

In addition, the strength and weakness analyses of the farm-based genetic improvement program were also conducted, and recommendations were provided to overcome the weaknesses identified.

### **13. CONCLUSION**

The results from this FRDC project have shown that a properly designed and managed abalone breeding program could deliver substantial economic benefits to the abalone aquaculture industry in Australia. Firstly, the project has demonstrated that the target breeding nucleus population size can be produced within the desired time frame through collaboration between participating farms. Secondly, genetic parameter analyses show that genetic components exist for all observed traits. This means that these traits could respond to selection effectively. The genetic correlations between body and processing traits and among themselves are highly favourable, and thus simultaneous improvements can be achieved by selection of any of these traits.

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## **APPENDIX 1: INTELLECTUAL PROPERTY**

N/A

**APPENDIX 2: PROJECT STAFF**

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## **APPENDIX 3: ABALONE TENTACLE SAMPLING**

### **PROCEDURES FOR TENTACLE SAMPLE COLLECTIONS**

**Xiaoxu Li**

South Australian Research & Development Institute

**NOVEMBER 2005**

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## **Sampling Abalone tentacles for pedigree checks**

### **Materials**

- 20L shallow plastic tray
- 250 $\mu$ m sieve (optional)
- 10L bucket
- Scalpel with disposable blades
- Forceps
- Eppendorf tubes
- Permanent marker

### **Method**

1. Fill the 20L tray with seawater from the abalone holding tank.
2. Place the 250 $\mu$ m sieve into the 20L tray for tentacle collection (optional).
3. Remove the abalone from its holding tank and hold it inverted underwater inside the 20L tray until the tentacles extend.
4. Once the tentacles have extended slowly remove the abalone from the water keeping it inverted. The tentacle should fall over the shell and start to retract.
5. Before the tentacles retract use a clean scalpel to cut down onto the shell and sever one of the tentacles collecting as much of the tentacle as possible. The tentacle should be taken from the respiratory pore side. Care must also be taken to not to cut the membrane or the frill. Collect the severed tentacle or allow it to fall onto the sieve.
6. Three to four tentacles need to be collected per broodstock used or per progeny randomly selected.
7. Once enough tentacles have been collected place the abalone back in its holding tank. Then place the severed tentacles into a clean Eppendorf tube for analysis. If the tentacles are on the sieve, remove the sieve from the tray and use a pair of clean forceps to collect the tentacles. All sample tubes should be marked with a permanent marker for future recognition.
8. The samples need be placed immediately into a freezer.
9. Clean the utensils used by thoroughly rinsing in seawater prior to repeating the sample collection with a new abalone.

10. Fill in the log for each broodstock or progeny with the following data: date, shell length, total weight and location of the tentacle samples.
11. Frozen samples will be shipped overnight in a Styrofoam container containing ice packs to SARDI when the establishment of families is completed each spawning season.