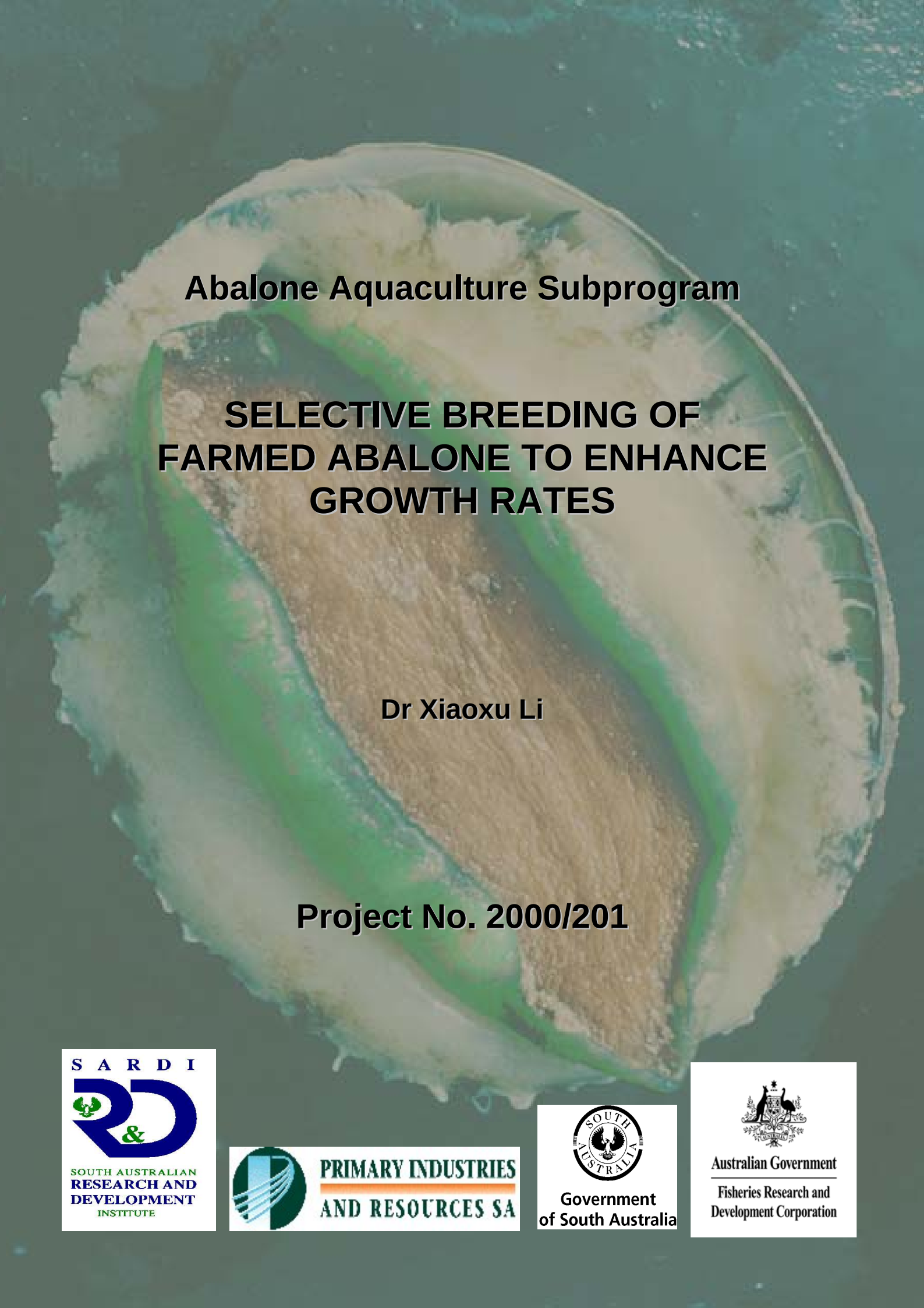

Abalone Aquaculture Subprogram

SELECTIVE BREEDING OF FARMED ABALONE TO ENHANCE GROWTH RATES

Dr Xiaoxu Li

Project No. 2000/201

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

PRIMARY INDUSTRIES AND RESOURCES SA

Government of South Australia

Australian Government
Fisheries Research and Development Corporation

2000/201 Abalone Aquaculture Subprogram: Selective breeding of farmed abalone to enhance growth rates

Dr Xiaoxu Li

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Fisheries Research and Development Corporation project final report
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TABLE OF CONTENTS

NON TECHNICAL SUMMARY

2000/201 Abalone Aquaculture Subprogram: Selective Breeding of Farmed Abalone to Enhance Growth Rates

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Objectives:

1. To develop a practical selective breeding protocol for commercially desirable traits in abalone.
2. To develop a genetic evaluation system.
3. To develop an R&D genetic business plan.
4. To establish and maintain a desired number of abalone families in each participating state.

OUTCOMES ACHIEVED

As a result of the protocols and plans developed during the course of this 18 month pilot project, the resources required for the development of a long-term industry-based breeding project have been built. Personnel responsible for the on-farm activities for the selective breeding project have been trained and construction and modification of the facilities required for the breeding project have been completed. The two step project commercialisation model has been accepted by the various shareholders and will structure the project's business activities and guide the breeding program into a business entity. In addition, 14 blacklip abalone families have been established in Victoria and 12 greenlip abalone families have been established in South Australia in accordance with the protocol. More families are expected to be produced in the coming abalone spawning season not only on the farms and in the states where the existing families are held but at other farms and in other states as well.

Non Technical Summary:

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 *H. 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 70-80mm. In China and Taiwan, for example, one of the main species cultured is the small abalone

H. diversicolor, which grows faster and reaches market size in less than ten months, although this species is not considered a premium product as are other species. China and Taiwan are not only the major producers of cultured abalone but also the major world markets for abalone. 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 improvement programmes 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 offer a logical and low-risk solution (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 long reproduction cycles such as abalone. This 18 month project has been treated as the starting point and pilot study for the development of a long term, structured breeding program. The main aims of the project were to demonstrate the feasibility of establishing abalone families on participating farms and building up a working team that could manage the on-farm activities required for a genetic R&D project.

The breeding objectives for the project were established from interviews with participating farms and the selection criteria able to be evaluated and include:
1. Increase in growth rates (measured by shell size);
2. Increase in meat weight at harvest and
3. Survival to harvest.

At the same time the following traits will also be monitored:
1. Difference in performance between male and female abalone;
2. Meat quality at harvest (assessment to be determined) and
3. Age at maturity and sex ratios.

The breeding protocol, genetic evaluation system and R&D genetic business plan objectives were addressed through the production of the following documents:
“General Protocol for Family Establishment of Farmed Abalone in Australia”,
“Project Recording and Managing Systems”,
“Project Arrangement”,
“Cost Benefit Analysis for a 15 Year Period” and
“Within Family Share Arrangements”.

These technical and business documents have set a basic guideline for the further development of the project. They are attached as appendixes to the main report.

In the 2000/2001 abalone spawning season, 14 blacklip families were produced in Victoria and 12 greenlip abalone families were established in South Australia. At the project’s Industry Training Workshop in Horsham in March 2001, procedures for family establishment described in the general

protocol were reviewed. Farms involved in the project were happy with the steps developed and were confident and ready to establish more families in the coming spawning seasons.

Given the success of the initial family establishments, it is reasonable to anticipate that at least 100 families could be established and maintained in each state in a few spawning seasons. To further develop the current project into a comprehensive selective breeding program, collaboration and long-term commitment from all parties is essential.

KEYWORDS: Abalone, selective breeding, aquaculture, genetics.

ACKNOWLEDGMENTS

Many people were involved in this study and made an essential contribution to this project. First of all we would like to thank Mr Mark Gervis and Mr Anton Krisnich of Southern Ocean Mariculture in Vic, Mr Colin Rohrlach and Mr Brent Smith of South Australian Mariculture in SA, Mr Peter Rankin of Ocean Wave Seafoods in Vic, Mr Steve Parsons of Great Southern Marine Hatcheries in WA, Mr Mike Wing of Tasmanian Tiger Abalone in Tas, Mr Tony Smith of Bay City Sea Farming in Vic, Mr Steve Rodis of Great Southern Waters in Vic and Mr Brett Stevens of Kilcunda Abalone Farm in Vic, who gave invaluable support to the project. The staff on the participating farms kindly provided help in the establishment of abalone families, their maintenance and data collection. We appreciate and thank Dr Tony Peacock of the CRC for Pest Animal Control for presenting the business models used by the livestock breeding programs in Australia at the project's business workshop in Melbourne in March 2001. Dr Raul Ponzoni of SARDI, Dr Chris Austin of Deakin University and Drs Burton, Subrahmanyam and Spackman of the Victorian Institute for Dryland Agriculture presented lectures on the principles and genetic basis of animal and plant breeding and the use of molecular markers in the breeding programs at the project genetic workshop in Horsham, Vic, in March 2001. Mr Anton Krsinich of Southern Ocean Mariculture, Ms Tania Kiley of South Australian Mariculture and Mr Julian Marcus of Ocean Wave Seafoods also presented talks on broodstock conditioning and abalone family establishment at the workshop. The Victorian Institute for Dryland Agriculture provided the opportunity for the project staff to visit their plant breeding research and selected seed storage facilities in Horsham, Vic. Dr Ann Fleming of the Abalone Aquaculture Subprogram provided help in managing the project. Dr Chris Austin and Mr Bob Collins of Deakin University provided help with organising the genetic workshop in Horsham. Dr Stephen Madigan and Ms Donna Cahill of SARDI helped organise the project's team meeting in Adelaide in July 2000. Prof. John Benzie of the University of New South Wales, Dr Patrick Hone of FRDC and Mr Steven Clarke of SARDI provided much helpful advice. We thank the managerial and academic staff at the Aquatic Sciences Centre of SARDI for their support and valuable discussion. We are also grateful to Dr Meegan Vandepeer and Steven Clarke of SARDI for editing this final report.

BACKGROUND

Genetically based breeding programs have made an enormous contribution to increases in agricultural yield during the last century. Estimates suggest that nearly all the 300 – 400% growth improvement in modern chicken strains was the result of selection (Havenstein *et al.,* 1994). This same improvement in production is possible with any species of aquatic animals and plants provided their life cycle can be controlled.

The application of selection techniques to farmed fish species has proven to be 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 is the selection for improved growth rate and other desirable characteristics in Atlantic salmon in Norway conducted over the past 20 years (Gjedrem, 2000). The effective application of genetic selection principles in shellfish such as Pacific oysters (*Crassostrea gigas*) has been successfully practiced in recent years (Kenneth, 1997; Ward *et al.,* 2000) and is receiving increasing attention.

Abalone are slow growing gastropods. Most commercial operations predict that it will take at least four years to grow abalone to a market size of 80-100mm. 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 in some cultured species (Gjedrem, 2000; Guo *et al.,* 1996).

High fecundity and phenotypic variance typify many marine organisms (Gjedrem, 1983). Providing the phenotypic variance includes a substantial genetic component, the combination of these factors allows for rapid genetic improvement via high levels of selection intensity. This is because selective intensity is dependent on (1) the degree by which individuals of families deviate from the population mean and (2) the proportion of individuals that can be selected for breeding 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.

Currently, there are established farms for two temperate abalone species, the blacklip abalone (*H. rubra*) and the greenlip abalone (*H. laevigata*) in South Australia, Victoria and Tasmania. Commercial production of greenlip abalone has just commenced in Western Australia. Research concentrating on improving production efficiency and reducing production time through growout system design and diet formulation has been successful and is being applied. The opportunity now exists to make use of genetic improvement technologies to further reduce the production time and advance the industry.

In addition, the establishment of abalone families and/or family lines not only opens the possibility to develop other genetic techniques such as DNA-based

tools and their applications that could be used to further improve the production and/or the quality of the products, but also provides the materials that can be used to test and confirm the predictions made in different techniques prior to their commercialisations.

The design and business management for this project was modelled on the Norwegian Atlantic salmon selective breeding program (Gjedrem, 1998) which has operated successfully over the past 20 years. Key differences are that in this project the establishment of families occurred on commercial farms and involved two abalone species. Furthermore, no artificial mixing of gene pools was possible between states for either species because of government regulatory translocation barriers. Therefore, if translocation issues could be resolved technically by using cryopreservation and sterile stocks, this project could utilize a gene pool larger than that which could be harnessed by any one state and/or hatchery. However, if the translocation issues can not be resolved in a short period, within state selection will be applied first.

This project delivered the first stages in the development of a structured genetic improvement program that involved a high degree of industry involvement and ownership. The program achieved this during its 18 month period through development of elite abalone family lines, performance recording and evaluation systems, and establishment of a R&D business case. These provided a significant entry to the development of a comprehensive selective 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 coordinated and managed with the Abalone Aquaculture Subprogram. The critical milestones of the project were the development of the abalone selective breeding protocol, the establishment of the desired number of families in each participating state, the agreement on intellectual property sharing arrangements and the establishment of an R&D business case.

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 improved a number of traits (particularly growth rates & disease resistance), however, no such program exists for abalone. An appropriately designed selective breeding program could produce abalone with growth rates enhanced by up to 30% over 3 generations of selection (6-8 years). This could shorten the production cycle by over a year, and thus substantially reduce farm operating costs.

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

OBJECTIVES

1. To develop a practical selective breeding protocol for commercially desirable traits in abalone.
2. To develop a genetic evaluation system.
3. To develop an R&D genetic business plan.
4. To establish and maintain a desired number of abalone families in each participating state.

METHODS

The project was one and half years in duration and composed of three subprojects, Subproject A: Development of a selective breeding protocol, Subproject B: On-farm technical assistance and Subproject C: Development of an R&D business plan.

SUBPROJECT A. Development of a selective breeding protocol

Related objectives:

1. To develop a practical selective breeding protocol for commercially desirable traits in farmed abalone.

Leader: Xiaoxu Li (SARDI)

Collaborators: Raul Ponzoni (SARDI), Chris Austin (Deakin University), Greg Maguire (WA Department of Fisheries) and Greg Kent (University of Tasmania)

Development of selective breeding protocol

From the project's onset the development of a selective breeding protocol was needed to ensure that it was structured appropriately and to allow for cohesion of the procedures on different farms and at different developmental stages.

At the start of the project an outline of the selective breeding protocol for farmed abalone in Australia was developed with respect to the selective breeding theory, the life cycle of the abalone and the spawning, hatchery and farming systems used by the abalone aquaculture industry. The outline was sent to the project team members that consisted of genetic experts, an abalone biologist and a nutritionist, and the abalone aquaculture industry for close consultation. To gain familiarity with the farming environments and the farming facilities used by the participating farms two rounds of on-farm visits were conducted by the state project coordinators, quantitative geneticist and the project's principal investigator. A draft protocol was then developed by inclusion of the feedback. The protocol was reviewed step by step at the project team meeting held in Adelaide in July 2000. State project coordinators, project scientific advisors and the representatives from the FRDC and the Abalone Aquaculture Subprogram attended the meeting. As specified in the project proposal the development of the general protocol was reported to the Abalone Aquaculture Subprogram Steering Committee in August 2000 in Dunedin, New Zealand, and presented and discussed at the following Abalone Aquaculture Subprogram annual meeting. After those discussions and consultations the general protocol was circulated to the participating farms for final comments. A manual entitled "General Protocol for Family Establishments in Farmed Abalone in Australia" was produced in October

2000 before the first round of abalone family establishments in the 2000/01 abalone-spawning season (Appendix 5).

Establishment of breeding objectives

At the same time a survey of biological traits that could be included in the breeding objectives was circulated to the farms participating in the project. The traits were:

- Growth rates;
- Survival to harvest;
- Meat/shell ratio at harvest;
- Disease resistance;
- Temperature tolerance;
- Food conversion efficiency;
- Size uniformity;
- Taste at harvest;
- Flesh colour;
- Shell shape

These traits were ranked by the industry and discussed at the project team meeting in July 2000 in Adelaide. A survey on the farming system(s) and facilities available for the project on the participating farms was also conducted.

Development of a recording and managing system

The project recording and managing systems (preliminary version) were developed according to the structures of the information and data that need to be collected during the course of abalone family or family line establishments in the general protocol developed for this project.

SUBPROJECT B. On-farm technical assistance

Related objectives:

4. To establish and maintain desired number of families in each participating state.
2. To develop a genetic evaluation system.

Leader: Xiaoxu Li (SARDI)

Collaborators: Raul Ponzoni (SARDI), Chris Austin (Deakin University), Greg Maguire (WA Department of Fisheries) and Greg Kent (University of Tasmania)

Establishment and maintenance of abalone families

The aim of this subproject was to ensure the success of the family establishments in all participating states through on-farm technical assistance, research-industry liaison, ensuring protocols were undertaken, ensuring animal husbandry issues were addressed and ensuring the desired data was collected.

At least one scientist from each state was responsible for industry liaison and ensuring that protocols were undertaken and animal husbandry issues addressed (Greg Maguire, WA; Raul Ponzoni and Xiaoxu Li, SA; Chris Austin, Vic and Greg Kent, Tas).

To facilitate the required activities on the participating farms for the family establishments, one half-time on-farm project technician was employed by South Australian Mariculture (SA) and Southern Ocean Mariculture (Vic) respectively. One part-time technician (20% FTE) was employed by Great Southern Marine Hatcheries (WA).

Families were established and maintained on commercial farms in each of the participating states according to the protocol developed by the protocol development team in Subproject A. This standard protocol was adjusted to meet the requirement of individual farms during the visits conducted by the project state coordinators and the project's principal investigator. The abalone families were established with an outbreeding design and included only wild broodstock initially. 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. Environmental conditions were maintained as uniformly as possible in order to reduce environmental effects on trait variation between families. Animals were held separately during the length of this project, and were expected to be tagged and mixed early in 2002 after the start of the new project (the extension of this project) in December 2001. Phenotypic measurements will be taken at the same time. The families required in each state were produced over four sequential hatchery runs occurring within one and a half months.

During the course of the project information on broodstock collection, spawning, fertilisation and hatch-out, larval rearing and settlement were collected when the families were established. The data collection on shell size at six month post-fertilisation had to be postponed due to the small sizes of the animals in most families. If these animals were weaned off high mortality was expected because small sized abalone are very vulnerable to handling. It is anticipated that the collection of this data will be conducted in early March 2002, thereby avoiding the clash with busy industry production in spring and summer and hot water temperatures in summer. Estimations of phenotypic and genetic parameters of economically important traits will be conducted soon after these data are available.

An Orientation Workshop for the on-farm technicians and the project state coordinators was organised in March 2001 in Horsham, Victoria. This workshop was initially suggested at the protocol development team meeting held in July 2000 in Adelaide. A proposal was then developed and submitted to the FRDC in October 2000 through the Abalone Aquaculture Subprogram and approved by the FRDC in January 2001. The purposes of the workshop were:

1) to get project members to work as a team and to understand what each desired;
2) provide participants with the background to this genetic project, and the problems likely to arise;
3) familiarise participants with the general procedures employed in breeding programs with terrestrial species;
4) describe the tagging and practical data collection procedures required to be used with abalone and
5) discuss any issues raised as a result of the last spawning season.

During the one and a half day workshop seminars were presented on: 1) Genetic basis of abalone improvement programs by Dr Raul Ponzoni of SARDI (project co-investigator); 2) The application of molecular genetic markers to aquaculture by Dr Chris Austin of Deakin University (project co-investigator); 3) Principles of plant breeding by Dr Wayne Burton of VIDA and 4) The use of molecular markers in plant breeding by Drs Subrahmanyam and Spackman of VIDA. Experiences from the previous season were presented by the farms participating in the abalone family establishments and technical issues relating to the present project and issues related to the further development of the project were discussed.

In addition, the workshop participants visited the facilities required for plant breeding research and seed storage (genetic diversity) at VIDA.

Development of genetic evaluation system

The method developed by Raul Ponzoni for calculation of the economic values of various traits in the breeding objectives was proposed in this project. It includes:

1. determination of related economic importance of various traits by close consultation with industry or through questionnaire;
2. specification of the production system;
3. identification sources of income and expense in abalone production;
4. determination of those traits that may be genetically modified, and
5. finally calculation of economic values.

SUBPROJECT C. Development of a R&D business plan

Related objectives:

3. To develop an R&D genetic business plan.

Leader: Anthony Francis (Technology Commercialisation Group Pty Ltd, SA)

Collaborators: Andrew Graham (Technology Commercialisation Group Pty Ltd, SA), Xiaoxu Li (SARDI) and Raul Ponzoni (SARDI)

An agreement on intellectual property share arrangements and the establishment of an R&D business case was needed from the project's commencement to ensure appropriate planning, resources and co-ordination and that ownership and development were understood by all parties.

This subproject was run in parallel to the R&D elements of the project (subprojects A and B) and was led by the Technology Commercialisation Group Pty Ltd.

Each research and industry participant was asked to respond to a survey to gauge views and commitment to a commercial model extending beyond the end of the current project. The ultimate aim was to determine what commercial business, if any, might best serve the future growth of the industry. Response to the survey was 100% with many different views expressed. The result of the feedback was presented and discussed at the project protocol development meeting in Adelaide in July 2000.

RESULTS

SUBPROJECT A. Development of a selective breeding protocol

Development of selective breeding protocol

A standard protocol for establishment of families or family lines ("General Protocol for Family Establishments in Farmed Abalone in Australia", Appendix 6) was developed as part of the first phase and then adjusted to the specific requirements of each participating farm. The protocol describes broodstock collection and procedures at the spawning, hatchery, nursery and long-term husbandry stages. The protocol also includes timing of measurements, procedures for standardising measurements, degree of accuracy required in obtaining the measurements, procedures for checking data and procedures for recording and storing the data.

Establishment of breeding objectives

The breeding objectives were decided by analysing the feedback on the biological traits ranked by the participating farms and the current capabilities of the project. They are:

Objectives:
1. Increase in growth rates (measured by shell sizes).
2. Increase in meat weight at harvest.
3. Survival to harvest.

At the same time the following traits will be monitored:

1. Differences in performance between male and female abalone.
2. Meat quality at harvest by taste.
3. Age at maturity and sex ratios.

Development of a recording and managing system

The project recording and managing systems (preliminary version) were developed in November 2000 using Microsoft Excel. The recording systems integrate data and information collected at different farming stages during the course of family or family line establishment, including the stages of broodstock collection, tissues storage, spawning, fertilisation and hatch-out, larval rearing, settlement and grow-out on plates, grow-out off plates, grading, maturation, physical data collections. Examples of some spreadsheets are attached in Appendix 5: Data recording spreadsheet samples.

SUBPROJECT B. On-farm technical assistance

Establishment and maintenance of abalone families

In Victoria, nursery facilities, with a capacity to hold 30 families, were constructed at Southern Ocean Mariculture before the commencement of the establishment of abalone families with funding support provided by the project. The re-allocation of the project budget was approved by the FRDC through the Abalone Aquaculture Subprogram. In the 2000/2001 abalone spawning season at least three coordinated spawnings were organised among the project's participating farms in order to produce families within a limited time frame. Ten blacklip abalone families were produced at Southern Ocean Mariculture and 4 families were produced at Ocean Wave Seafood. These 4 blacklip abalone families were reared at Ocean Wave Seafood for 5 days before being transferred to Southern Ocean Mariculture. The animals were then settled in the nursery system at Southern Ocean Mariculture and have been maintained at the same farm since.

In South Australia, separate larval rearing and nursery facilities required for simultaneous establishment of 12 to 15 families or family lines were constructed by South Australian Mariculture using their own funds. After a few initial trials, three consecutive spawnings were conducted to produce the required number of abalone families. Nineteen greenlip abalone families were initially produced by pair-mating the wild broodstock collected from the adjacent waters of Port Lincoln. The animals from those 19 families were weaned off the plates in May 2001. However, as some individuals in the families were too small to be plastic tagged they had to be kept separately. For 7 families the numbers of individuals weaned off were less than 50 and these were therefore culled. The total ongoing families in South Australia are therefore 12.

In Western Australia several spawnings were attempted and the resulting families were reared in separate tanks, however limited individuals survived beyond the larval stage. No ongoing families existed at the time the current project finished in November 2001 (although families have been successfully established since).

The Tasmanian farm that intended to participate in this project withdrew prior to any attempts to establish abalone families, due to management difficulties.

The abalone families established during the course of the project are summarised in Table 2.

Table 2:

Abalone Families Established in the 2000/01 Spawning Season

State	Farm	Species	00/01	***Total*** (Greenlip)	***Total*** (Blacklip)
SA	SA Mariculture	Greenlip	12	12	
VIC	Southern Ocean Mariculture	Blacklip	10 (+4)*		10 (+4)*
	Ocean Wave Seafood	Blacklip	4		4
Total			26	12	14

* Figures in the bracket indicate that these families are currently held on this farm, but spawned at another farm.

Development of genetic evaluation system

By the end of the project the only information available to the project were the management records from broodstock collection until the nursery stage for the ten blacklip families established and maintained at Southern Ocean Mariculture.

SUBPROJECT C. Development of a R&D business plan

A simple two-stage commercialisation model was developed, which is able to address the objectives of all the various stakeholders (researchers, industry, FRDC) including management of intellectual property, access to project outcomes, providing a public benefit, and the ability to perform future research. The initial stage, a simple cooperation between industry partners backed by the research providers, focuses only on managing access to the genetic improvements. From that basis a larger and more valuable business model can develop, giving the parties sufficient time to foster a management approach to both the cooperative and the competitive pressures that will exist. The development of the model in stages allows the parties enough time and information to make sensible decisions about whether the commercial opportunity warrants moving to the next stage. A third and final stage would be to propose a fully commercial self-sustaining operation. It is up to the participating parties to determine whether or when that stage might be reached.

This model was discussed at the project IP commercialisation meeting at the Melbourne airport in March 2001. Representatives from all farms intending to participate in the project attended the meeting. During this meeting Dr Tony Peacock of the CRC for Pest Animal Control presented the business models currently used by the livestock breeding programs in Australia. The working mechanisms of those models, the advantages and the disadvantages of those

working mechanisms if applied to our proposed IP commercialisation activities were discussed. The within family line share arrangement model developed by SARDI to resolve the technical issues in the initial two-step model was also discussed. After the meeting, the two-step business model was finalised by the TCG by integrating the comments and suggestions from the attendees at the meeting. The project's participating parties were happy with the incremental business development concept. The evolution of the model and the proposed elements of the stages business structure are described in the document "Intellectual Property Sharing Arrangement", which can be obtained from the project principal investigator or the Abalone Aquaculture Subprogram leader. In addition the cost/benefit analysis for a 15 year period was also developed by SARDI under the request of the farms participating in the project and is outlined in Appendix 3.

The outcomes of the "R&D business plan" investigation were reported by Mr Andrew Graham of TCG at the Eighth Abalone Aquaculture Subprogram Annual Workshop in Fremantle, WA in July 2001. The final document "Selective Breeding of Abalone – Project Arrangement" was then developed (Appendix 1).

DISCUSSION

SUBPROJECT A. Development of a selective breeding protocol

Development of selective breeding protocol

The standard protocol for establishment of families or family lines ("General Protocol for Family Establishments in Farmed Abalone in Australia", Appendix 6) developed in this project describes broodstock collection and procedures at the spawning, hatchery, nursery and long-term husbandry stages. The protocol also includes timing of measurements, procedures for standardising measurements, degree of accuracy required in obtaining the measurements, procedures for checking data and procedures for recording and storing the data.

Procedures described in the protocol were reviewed by the participating farms at the project's Industry Training Workshop in Horsham in March 2001 after the completion of the 1st family establishments in the 2000/2001 abalone spawning season. The farms involved in the project were happy with the general protocol and were confident and ready to establish more families in the following abalone spawning seasons.

Information from the established families will be collected for the estimation of phenotypic and genetic parameters for economically important traits. Phenotypic and genetic parameters are building blocks of a scientifically based genetic improvement program. The data will be collated, stored and analysed at SARDI.

The duration of this subproject was from May 2000 to September 2000. Over that period the primary focus of the subproject was to develop a manual for the establishment and maintenance of abalone families and/or family lines with the facilities currently existing on the participating farms. The protocol produced was lacking technical details regarding on-going selective breeding because at that time some critical information such as selection method(s) and the feasibility of conducting on-farm family establishment, etc required for its development were not clear. In addition, considering the length of this subproject, addressing technical issues relating to family establishment and maintenance were the only components proposed in the application.

However, a comprehensive selective breeding protocol should also include the following items:

1. Numbers of animals that should be selected as broodstock per generation for both the selective breeding project and commercial productions (if required);
2. Method(s) to avoid inbreeding;
3. Method(s) to manage improvements of different traits;

4. Age(s) and/or time(s) when selection can be applied for different traits;
5. Structure and interpretation of the results (breeding values of individuals, families and/or family lines in a specific environment and/or across different environments) produced by the genetic evaluation system used, and
6. Method(s) to use these breeding values for different breeding strategies.

It is expected that the inclusion of these items in the breeding protocol will need two stages: the preliminary stage and the advanced stage. Over the preliminary stage, which is the period when the families produced in the first few spawning seasons could be used to produce next generations, the breeding strategies will be decided according to the numbers of traits that can be included in the breeding, the numbers of families that are available to the project in each state and the numbers of families that can be produced and maintained in each state. It is expected that this stage will start from 2004/05 spawning season and last for about two to three years. A simple selection strategy(ies) (method) needs to be developed for this stage prior to the 2004/2005 spawning season if the extension of the project is approved because only a few families were produced in the 2000/01 abalone spawning season (12 blacklip families were established in Victoria and 14 greenlip families were produced in South Australia) and a similar number of families are expected to be produced in the 2001/2002 season. Over the advanced stage, which should start from the 2006/2007 abalone spawning season, a comprehensive breeding strategy will be required. It is expected that in late 2006 thirty to fifty families per species per state will be available to the project, most information required for genetic evaluation will have been collected and analysed (for details refer to "Development of a Genetic Evaluation System" section in this Chapter) and the capability to produce about 40 families within the required period in each state in one spawning season will have been established. The comprehensive breeding details should therefore be developed prior to the start of the advanced stage.

Establishment of breeding objectives

Determining the breeding objectives for the project was difficult as the relative immaturity of the abalone aquaculture industry in Australia means that market signals are unreliable. In addition, two species, greenlip abalone (*H. laevigata*) and blacklip abalone (*H. rubra*) and/or their hybrid will be worked with in different states. Therefore it is hard to detail accurately the major sources of income and expense in commercial operations. As a consequence, the breeding objectives were decided by analysing the feedback on the biological traits ranked by the participating farms and the current capabilities of the project.

These general breeding objectives will be viewed as a continuous process of refinement, progressing as the breeding, production and marketing systems stabilise, and new knowledge becomes available.

Development of a recording and managing system

The project recording and managing systems (preliminary version) were developed in November 2000. The original proposal aimed to utilise the software developed for abalone-farm management but it was found to be unsuitable. Consequently, the PI has developed a dedicated data management system.

The system was designed primarily for ease of use by the farmers. The main reasons for its development were:

1. Not all the farms intending to participate in the project at that time had the commercial abalone data management software suggested by the Subprogram.
2. The commercial software was designed to manage information at stock level, not at individual level, which was required in the general protocol produced by the project.
3. The commercial software was not designed to link the information across generations.
4. The project needed a standard hard copy of the data collection form for use by staff at different farm(s) and at different spawning times.
5. There were only a few days left from the time when the general protocol was finished in October 2000 to the proposed date for the first family establishment in late October 2000. The project did not have time to search for other options for data management.

SUBPROJECT B. On-farm technical assistance

Establishment and maintenance of abalone families

In Victoria the establishment of 30 abalone families was the target initially set by the 4 farms intending to participate in the project. They had planned for these families to be established in three consecutive spawnings before Christmas 2000. However, in late November some farms still had not finished the spawnings for their commercial productions. This was mainly because most broodstock collected from the wild were not in very good condition. Difficulties were experienced in getting enough eggs from those animals. More broodstock and spawnings were required to produce the numbers of animals targeted by these farms. Only 2 farms managed to produce abalone families, resulting in 14 blacklip families in Victoria in the 2000/2001 spawning season.

In South Australia, 19 greenlip abalone families were produced initially in three consecutive spawnings at South Australian Mariculture. It was late in December 2000 when the larvae from the third batch were transferred from larval rearing tanks to the settlement tanks. The plan to establish more families in January 2001 was abandoned because no mature broodstock could be found

from areas where the wild abalone normally spawn late. Therefore, the target to establish 30 families in 2000/2001 spawning season in SA could not be met.

The main difficulty experienced by the project in establishing abalone families in the 2000/2001 spawning season was that the condition of the gonads of the broodstock collected from the wild was unsynchronised. Therefore, fewer families could be produced each time than initially anticipated and more broodstock and extra spawnings were needed in order to produce the targeted number of families. In addition, it was very difficult to plan a time schedule for the family establishment in the later spawning season based on predictions of when the wild population would spawn naturally. To minimise these uncertainties the participating farms aim to use conditioned wild broodstock in the following rounds of family establishments if the broodstock conditioning units are available.

After the 2000/01 spawning seasons successful "preliminary" family establishment, it is reasonable to anticipate that the required numbers of abalone families for the proposed selective breeding purposes could be established and maintained in each state in a few spawning seasons.

For the last one and half years the project has received full support from the abalone industry, especially the participating farms, the Abalone Aquaculture Subprogram and the research providers. However, the success of the project will need long-term commitment from all parties involved.

To clarify the tasks of all parties participating in the project over the next few years a document entitled "Summary of Technical Requirements and Responsibilities for the Second Phase of the FRDC Project: Selective Breeding of Farmed Abalone to Enhance Growth Rates" has been developed. For details refer to the document, which can be obtained from the project's principal investigator or the Abalone Aquaculture Subprogram leader.

Development of a genetic evaluation system

To develop a selective breeding evaluation system the following information will be required: breeding objectives (traits), selection methods (family selections, individual selections, index selections), economic values of various traits in the breeding objective, genetic parameters (heritability of various traits in the objectives, correlations between these traits, interaction between environments and genetics, correlation between early and late performances in these traits) and phenotypic data of the traits included in the breeding objective. Obviously many experiments will be required to verify these factors.

By the end of the project the only information available to the project were the management records from broodstock collection until the nursery stage for the ten blacklip families established and maintained at Southern Ocean Mariculture. The proposed data collection on length and weight (October 2001) clashed with the commercial production on the farm and then high summer temperatures caused it to be postponed until early 2002.

The investigation on the economic value of various traits by using the two main farming systems (tank and pipe system and raceway system) was the only component proposed in the application. However, by the time when on-farm visits were conducted it was found that many of the participating farms were still in the construction stage (building up nursery and grow-out tanks) and the farms we intended to consult with were changing to the newly developed slab system. No farms had developed into the full production stage.

The development of a genetic evaluation system is an objective to build a sustainable selective breeding program. Its development in this project might need two stages: 1) use of the data collected from the families established at the family accumulation period and 2) use of the data collected from specifically designed crosses. This is mainly due to the unique set-up of the project:

1. The establishment and maintenance of abalone families were designed to use facilities currently existing on the participating farms, which are normally large, and were designed and constructed for commercial production only.
2. The on-farm project technical officers (selected by the participating farms from their existing staff) have limited knowledge on selective breeding and its requirements, and need to be trained with the development of the project.
3. Collaboration and coordination between farms in each state will be required in order to establish the required numbers of families within a short period.
4. Most participating farms are still in their developmental stages.

Experiences from the abalone family establishments in 2000/2001 spawning season showed that establishing five families per run per farm was the maximum capacity of the farms participating in the project at this stage. For example, the 12 families established in South Australia were produced in 3 consecutive spawnings. Obviously, in order to conduct the studies on heritability estimates, etc, a capability of establishing 30 or more families in a short period will be required in each state. This could be achieved by either involving more farms in the project, upgrading the facilities on the existing participating farms, or both, and is expected to be completed in two years (2003/2004 spawning season or 2004/2005 spawning season). The period prior to the completion of the scale up is considered by the project as the family accumulation stage. The data collected at this stage can be used in the analyses of correlation between early and late performances of the traits investigated, differences in the performances between male and female individuals, etc. Assumptions on some parameters will be needed if evaluations of individuals or families will be conducted at this stage. However, the evaluation can be postponed because it is not expected that a female abalone less than 3 years old could produce enough good quality eggs for producing the next generations in the project. When the participating states are advanced enough that the numbers of families needed for the proposed genetic analyses can be established within the required time period, the data collected will then be

used for the other proposed genetic analyses such as heritability estimates. It is anticipated that the family and individual breeding values, resulting from the analyses using the parameters produced in both stages, will be available in 2004 when the progeny from the 2000/2001 and 2001/2002 spawning seasons can be used for selective breeding of the next generation.

SUBPROJECT C. Development of a R&D business plan

The conclusion from analysing the feedback on the survey was that at the time there was not a commercial business case that could be built on the back of the current research. The stage of the technology was 'pre-application' i.e. not ready to form the basis of new products and services, and so to attempt to form any business on this basis would potentially only stunt future development of real products and services of commercial industry value.

What is of current commercial value is the selectively bred stock, provided it can form the starting point for an ongoing collaboration between the industry partners supported by the research groups. If this collaboration can be successful, then a platform is in place for future innovation from the research groups (post current project) to add real value to abalone aquaculture.

From this analysis a simple two-stage commercialisation model arises, which is able to address the objectives of all the various stakeholders (researchers, industry, FRDC) including management of intellectual property, access to project outcomes, providing a public benefit, and the ability to perform future research. The development of the model in stages also allows the parties enough time and information to make sensible decisions about whether the commercial opportunity warrants moving to the next stage. It is up to the participating parties to determine whether or when that stage might be reached.

BENEFITS

The abalone aquaculture industry is the direct beneficiary of this project. Because of the long reproduction cycle of the abalone currently farmed in Australia (about three years) the economic benefit of this project was not able to be determined at the project's completion. However, its economic value can be predicted by analysing the information available from published papers and the existing knowledge on the farming systems used in Australia and is described below.

Calculation of benefit to abalone industry

When additive genetic variation is present in a trait there will always be a response to selection if efficient selection methods are applied. In the literature there are several estimates of response to selection for increased growth rates in abalone. The following estimates should be mentioned (given as genetic gain in percentage per generation): Japanese abalone, 10~15% (Hara & Kikuchi, 1992) and red abalone, 20~25 % (Jonasson et al., 1999). An average figure of these estimates is 15~20 % genetic gain per generation for growth rate. This is a larger genetic gain than usually obtained in farm animals and similar to that in fish species. Fish and shellfish have larger genetic variation in growth rate and have higher fecundity than terrestrial animals; consequently, it is possible to apply a much higher selection intensity.

The benefits of genetic improvement in growth rate are reductions in both fixed costs and production costs, the latter due to lower energy requirement for maintenance. Often a correlated response can be observed in an improved feed conversion rate.

The Norwegian breeding program, which today supplies genetically improved eggs of Atlantic salmon and rainbow trout to more than 70 % of the farming industry, has a cost/benefit ratio of 1:15. Similar estimates are also found with breeding programs for farm animals. This ratio will depend largely on the total production output that benefits from the genetic improvement.

Initial analysis (Appendix 3) shows that the cost/benefit ratio over a 15 year period for the current project is about 1:15. This is according to 1) current contributions from the funding organisation and research providers, 2) costs for establishing and maintaining families required from participating farms, 3) benefit from a 10 % improvement in growth rates per generation of the selected broodstock, which was predicted from the data published for other abalone species, and 4) the current abalone market prices. This means that every dollar invested either as cash or in-kind should get A$15 return from the project (based on a production of 800 tonnes per year). This result is within the cost/benefit ranges for other existing genetic breeding projects with aquatic and terrestrial species.

Other beneficiaries

In addition to the abalone industry who are the direct beneficiaries of this project, secondary beneficiaries include the abalone feed manufacturers who will benefit through increased sales of manufactured diets due to increased production rates on farms. The decreased production costs due to enhanced growth rates will also mean that abalone aquaculture is more viable leading to expansion of the industry and an increase in job opportunities in rural communities.

FURTHER DEVELOPMENT

This project was seen as part of an ongoing research program established in abalone genetic breeding. The areas investigated in this project need to be addressed before the next stage can be commenced. In this project we have successfully demonstrated the feasibility of establishing abalone families on participating farms and have built up a working team that can manage the on-farm activities required for a genetic R&D project. To further develop the current project into a comprehensive selective breeding program, collaboration and long-term commitment from all parties are essential.

Experiences from the abalone family establishments in 2000/2001 spawning season showed that establishing five families per run per farm was the maximum capacity of the farms participating in the project at this stage and only 1 to 2 farms could actively produce families in each state. If this situation cannot be improved in the next 2 years it will reduce the capacity of the project in delivering the benefits to the industry or delay the delivery. These issues can be addressed by either more farms becoming actively involved in the family establishments or the facilities on the existing participating farms being upgraded or both.

PLANNED OUTCOMES

As a result of the protocols and plans developed during the course of this project the resources required for the development of a long term industry based breeding project have been built. Personnel who will manage the on-farm activities for the selective breeding project have been trained and construction and modification of the facilities required for the breeding project have been completed. The two step project commercialisation model has been accepted by the various shareholders and will structure the project's business activities and guide the breeding program into a business entity. In addition, 14 blacklip abalone families have been established in Victoria and 12 greenlip abalone families have been established in South Australia in accordance with the protocol with more families expected to be produced in the coming abalone spawning season.

CONCLUSION

The original objectives of the project have been met with several documents required for guiding the development of genetic breeding of abalone in Australia being developed. These include:

- "General Protocol for Family Establishment of Farmed Abalone in Australia",
- "Outline of Proposed Commercialisation Business Model",
- "Project Recording and Managing Systems",
- "Within Family/Line Share Arrangements",
- "Cost/Benefit Analysis over a 15 Year Period, Summary of Technical Requirements" and
- "Responsibilities for the Second Phase of the FRDC Project: Selective Breeding of Farmed Abalone to Enhance Growth Rates"

In addition both blacklip and greenlip abalone families have been successfully established on commercial farms in Australia by using or slightly modifying their current farming facilities. This project has also demonstrated that the on-farm activities required for an abalone R&D genetic breeding program can be managed by the industry technically and physically. With continued effort from all parties participating in the project enough numbers of abalone families will be able to be established and maintained for selective breeding purposes and the improved stocks and/or new technologies will be able to be commercialised.

However, issues relating to difficulties in establishing required numbers of families within a short period need to be addressed and could be resolved by either involving more farms in the family establishments or upgrading the facilities on the existing participating farms or both.

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APPENDIX 1: Intellectual Property

SELECTIVE BREEDING OF ABALONE
PROJECT ARRANGEMENTS – FOR CIRCULATION TO ALL PARTIES

PROJECT PARTIES

[Industry Parties]

(list here)

[Research Parties]

(list here)

BACKGROUND

- The parties are all supporting a Fisheries Research and Development Corporation ('FRDC') sponsored research project entitled *Selective Breeding of Abalone* (the 'project').
- Under the project the Industry Participants have agreed to make certain contributions (cash or in kind) including the provision of abalone brood stock selected from their own facilities.
- Under the project the research have agreed to contribute technical skills and research expertise and facilities to the delivery of the program.
- The parties wish by these cooperative activities to mutually enhance the breeding stock of the respective participating facilities and to otherwise improve the abalone aquaculture industry.
- The parties have agreed the following terms with respect to the expected outcomes of the project.

TERMS OF THE AGREEMENT

Project Commitments

1. Each party agrees to make the contributions of the kind, in the manner and according to the time frames set out in the proposal accepted by FRDC for the project.

2. Each party agrees to communicate to the project leader nominated by FRDC any issue or opportunity related to the project, in a timely manner, including the creation or emergence of any new valuable brood stock, blood line, innovation, technology or other creation or valuable outcome.

Confidentiality of a Parties Secret Information

3. Each party acknowledges that confidential information of a disclosing party is valuable and agrees:
   (a) to keep the confidential information confidential and secret;
   (b) not to disclose the confidential information to any person except in accordance with this agreement, unless with the disclosing party's prior written consent;
   (c) to use the confidential information only to the extent necessary for the purposes of this agreement and not for any other purpose;
   (d) to take proper and effective precautions to prevent persons from accessing any of the confidential information;
   (e) notify the disclosing party immediately it becomes aware of person using the confidential information other than for the purposes of and in accordance with this agreement.

4. The obligations of confidentiality under this agreement, do not extend to information that:
   (a) the receiving party can prove was rightly known to it prior to this agreement;
   (b) comes into the public domain after the date of this agreement, otherwise than as a result of a breach of this agreement; or
   (c) is required by law to be disclosed.

5. A party may disclose the confidential information to those of its employees or agents who have a need to know the confidential information for the purposes of this agreement, and who have been advised of, and are bound by, the obligations of this agreement.

6. All documents and other materials containing confidential information of a party will be returned to that party immediately upon written request.

7. Nothing in this agreement shall be construed as giving a party any rights by way of licence or otherwise, to the confidential information or any related intellectual property, possession or thing.

8. The obligation to keep secret and confidential the confidential information will survive the completion of this agreement.

Statements or Announcements

9. Neither party shall make, issue or permit or suffer to be made or issued, any public statement or announcement concerning any of the matters the subject of the project or this agreement unless such public statement or announcement is first approved as to the timing and content by the Program Leader appointed by FRDC or is required by law.

10. Any public statement or announcement concerning or touching upon the project will give due acknowledgement to the support provided by FRDC.

Information and Outcomes from the Project to be Generally Free

11. Subject to the confidentiality and other obligations of this agreement, a party shall be entitled to publish and otherwise communicate and use the outcomes of the project in their own research and operations, including commercial and non commercial use, so long as the use by that party does not infringe upon or damage confidential information or related intellectual property which, by virtue of this agreement or otherwise, rightly belongs to another party.

Ownership of Project Outcomes

12. The parties agree the following terms in relation to the outputs of the breeding program, being family lines and including all related information, genetic material and live breedstock (hereinafter referred to as the 'Family Lines'):
    a) The Family Lines will be owned jointly as tenants in common among the Industry Participants, in proportion to the number of family lines contributed by an industry party, and accepted into the breeding program by the project leader nominated by FRDC, in accordance with protocols determined for the project.
    b) An Industry Party who does not contribute family lines accepted into the breeding program in accordance with sub-clause (a) will not hold any ownership in the Family Lines.
    c) Notwithstanding sub-clause (b) each Industry Party to this agreement os hereby granted a free, perpetual licence and right to access to the, subject to:
        - that party continuing to meet it obligations pursuant to this agreement; and
        - payment by the party exercising the right of access, of the reasonable costs, including additional labour and out of pocket and transport expenses of accessing the project outputs, but excluding the costs of the breeding program itself.

13. The Industry Participants agree to form a cooperative arrangement to manage their joint ownership of the breeding program and contribution to further ongoing breeding programs, which may include arrangements for sub-contracting the breeding program, giving each Industry Participant access to the outcomes of the breeding program, the conduct of further R&D, and such other activities as the parties may agree.

14. The Industry Participants agree to develop the cooperative in good faith and in keeping with the project and will, pursuant to clause 13, develop a detailed plan for the collective.

15. The parties have agreed the following in relation to the other outcomes from the project:
    a) With the exception of items covered by clause 12, intellectual property developed under or arising out of the project will be owned by the Research Party under whose activities the intellectual property developed or arose.
    b) Intellectual property which pursuant to sub-clause (a) is owned by a Research Party shall remain subject to the FRDC's limited right to promote, publish or otherwise transfer the benefits of the project, subject to the need to protect any valuable intellectual property owned by any party.
    c) The parties acknowledge that the dissemination of knowledge is a key objective of the project and the parties agree to pursue in good faith opportunities for the dissemination or commercialisation of the intellectual property referred to in sub-clause 12 (a).

16. These arrangements are made subject to the terms of the FRDC's funding agreement and to the extent of any inconsistency the terms of the FRDC's funding agreement will prevail.

Industry Participants' Cooperation

17. The Industry Participants agree to work together to develop and implement a cooperative management process with respect to the brood stock, as well as other activities related to aquaculture of abalone, beneficial to each party.

18. The parties shall use their best endeavours to further their mutual interests and, so far as they are able to do within their limited resources, make available to the cooperative management process their expertise, resources and information.

19. A detailed plan for the cooperative management process will be agreed between the Industry Participants within 1 year of the signing of this agreement.

Terms and Relationships Between the Parties

20. This Agreement will continue in force subject to the termination by the parties upon six months written notice. If within 12 months from the date of execution no cooperative activities have been formalised, this Agreement shall be deemed to have expired and no party will have any claim against the other. In the event of termination, activities already in progress shall be carried out to an orderly conclusion.

21. The parties will not be or be deemed to be in any partnership or joint venture and no party will hold itself out as being in any way a partner or joint venturer of any other party and no party will pledge the credit or warrant the authority of any other party.

22. Each party will bear its own costs in relation to the negotiation and preparation of this agreement and, unless expressly provided by this agreement, the cost of performing any obligation under this agreement.

23. These terms are governed by the laws of South Australia and the parties submit themselves to the non-exclusive jurisdiction of the Courts of South Australia.

24. In the event of any dispute arising out of this agreement the matter shall be referred for alternative dispute resolution, or failing that arbitration, by a person nominated by the FRDC, and the parties agree to submit themselves to that process, as a precondition to any party pursuing its rights in any court of law. Nothing in this clause shall act to prevent a party petitioning the courts for any urgent interlocutory relief it may be entitled to, to preserve its rights or the value of its rights.

Dr Xiaoxu Li	**Dr Ann Fleming**
Project Principal Investigator	**FRDC Abalone Subprogram Leader**

APPENDIX 2: Project Staff

Principal Investigator:	**Dr Xiaoxu Li** (South Australian Research and Development Institute, SA)
Co-investigators:	**Dr Raul Ponzoni** (South Australian Research and Development Institute, SA)
	Mr Anthony Francis (Technology Commercialisation Group Pty Ltd, SA)
	Dr Chris Austin (Deakin University, Vic)
	Dr Greg Maguire (Department of Fisheries, WA)
	Mr Greg Kent (University of Tasmania, TAS)
	Mr Andrew Graham (Technology Commercialisation Group Pty Ltd, SA)
Independent Advisors:	**Dr John Benzie** (University of New South Wales, NSW)
	Dr Ann Fleming (FRDC Abalone Aquaculture Subprogram, Vic)
	Dr Patrick Hone (FRDC, ACT)
	Dr Nicholas Elliott (CSIRO, Tas)
	Dr Jon Havenhand (Flinders University, SA)
	Dr Kirk Hahn (Department of Fisheries, WA)
Technical Support:	**Mr Anton Krinich** (Southern Ocean Mariculture)
	Ms Tania Kiley (South Australian Mariculture)
	Ms Kylie Freeman (Fisheries WA)
	Ms Gaynor Jones (Great Southern Marine Hatcheries)
	Ms Hayley Eglinton (South Australian Mariculture)
Participating Farms:	Southern Ocean Mariculture
	South Australian Mariculture
	Great Southern Marine Hatcheries
	South Australian Abalone Development
	Ocean Wave Seafoods
	Great Southern Waters
	Bay City Sea Farming
	Kilcunda Abalone Farm

APPENDIX 3: Cost Benefit Analysis

What does the ten percent enhancement in growth rate mean?

Cost benefit analysis for abalone selective breeding project
(15 years)

Costs

To analyse the return on investment of the selective breeding project, we need to detail the costs specific to the project and the benefits produced. According to the protocol in this project, the project costs for farms are mainly from:

- labour for general maintenance;
- special care required at different development stages (tagging, measurements, data and tissue sample maintenance); and
- the cost of tags and other minor equipment.

Obviously it is difficult to quantify the cost for most of these items. To simplify the calculation, it is assumed that:

- all individuals tagged will grow for 6 years before being used for the next production and;
- of the 270 individuals per family tagged at ten months, 200 individuals will survive to year 4 and onward.

During the proposed 15 years of the breeding period, the first 6 years are classified as the family accumulating stage, in which the initial 100 families will be gradually established. Years 6 to 15 are classified as the constant stage, in which families of new generation(s) will replace the families from previous generations. The total number of families will remain at about 100.

According to current market price ($50.00/kg), a 6 year old abalone (about 100g in weight) should be worth $5.00. If an extra $1.50 per tagged animal is assumed to cover the extra costs from the activities specified above, the total value of the individuals held till year 6 would be:

100 (families) X 200 (individuals/family) X 6.50 ($, unit price) = 130,000 ($, value cumulated till year 6) ---------- equation 1

The total value of the abalone produced during 15 years would be:

130,000 (value till year 6) X 10 (10 years from year 6 to 15) = 1,300,000 ($) --- equation 2

The results in equations 1 & 2 are higher than their real values because equation 1 uses full prices for all individuals at year 6, which consist of animals from 2 to 6 years old. Actually, many individuals will be culled off when the data collected are analysed at year 4. Despite this, the values calculated from equations 1 & 2 are still considered reasonable because higher maintenance costs are expected at the early

stages of the project. This value (1.3 million dollars) is considered in this analysis as the total farm investment for one species. This method is called the unit price method.

The farm's investment could also be calculated by the direct method. In this method, it is expected that each year:

- the 20,000 tagged animals will be measured twice (determined by the general protocol),
- 30 new families will be established (according to current industry commitment) and;
- the animals will require general maintenance.

According to previous experience, 250 to 500 individuals per day could be tagged, measured and recorded by two full-time staff. Thus, less than 160 working days will be required for the two measurements per year (20,000 individuals ÷ 250 individuals per day X 2 measurements/year). If 10 families can be established by two full-time staff in 15 days, 45 days will be needed to produce 30 families. Because there are only 250 working days in a year, the other 45 days will be used by the two staff to conduct the general maintenance (feeding, cleaning, etc). Based on the existing analysis for running costs for abalone farming in Australia, a maximum of $20,000/year per farm should be required for electricity, feed, tags, etc to maintain animals produced for the project. If the current average on-farm staff salary ($35,000/annum – including oncosts) is used for the calculation, the total contribution for one species from a farm in the 15 year period should be:

(35,000 (annual salary) X 2 (full-time staff) + 20,000 (running costs)) X 15 (years) = 1,350,000 ($)

This value is similar to that calculated by the unit price method.

In this analysis the unit price method is recommended because it can be considered as a standard method for all farms, while the direct method might cause debate on the farming system used, the competence of staff, etc. The unit price method can also be used to separate the project costs from those for commercial production.

Other costs for the project are R&D components. These are currently contributed by the FRDC and research providers and amount to $200,000/annum. If this level remains constant for the next 15years, the total R&D costs should amount to:

200,000 X 15 = 3,000,000 ($)

contribution to each species should be $1,500,000.

Benefits

The expected benefits from the project are improved abalone broodstock. Enhanced production efficiency through increased growth rate was identified by the industry as its highest priority. According to the published results for other abalone species, it is reasonable to anticipate that a 10% enhancement in growth rate could be gained over one generation of selection for Australian farmed abalone species. If the first generation of improved stocks (10% enhancement in growth rate) are released for

commercial production at year 6, then a further 5% enhancement can be gained every three years by crossing broodstock from different or the same generations.

In 1999 the market price for 70 to 80mm abalone (in shell) was approximately $A 42.00 per kilogram. A farm that yields 100 tonnes/year of abalone of this size could generate sales revenue up to $A 4.2 million per annum. If the farm keeps producing 100 tonnes/year from year 6 (when the improved stocks start to be used for commercial production) to year 15 (the final year of the analysis), the following extra sales revenue could be generated by using the improved broodstock:

Years	**Enhancement in** growth rates	**Number of cohorts** reaching market size	**Extra sales value**
6 ~8	10 %	3	4,200,00 X 10% X 3 = 1,260,000
9 ~11	15 %	3	4,200,00 X 15% X 3 = 1,890,000
12 ~15	20 %	2.5	4,200,00 X 20% X 2.5 = 2,100,000

The total extra sales revenue generated from this period should be $A 5,250,000. For a farm that yields 200 tonnes per annum, this value will be doubled (5,250,000 X 2 = 10,500,000). Therefore, the cost benefit ratio for an abalone aquaculture industry that yields 100 tonnes per year should be:

1,300,000:5,250,000 ≈ 1:4

For the project (including R&D costs), this ratio should be:

(1,300,000 + 1,500,000):5,250,000 ≈ 1:2

The cost benefit ratios for other annual productions are listed in Table 1.

Table 1: Costs/Benefits for Different Production Levels (tons)

Production (tons)	Costs/benefits (including R&D)	Costs/benefits (industry)
100	1:2	1:4
200	1:4	1:8
300	1:6	1:12
400	1:8	1:16
500	1:10	1:20
600	1:11	1:24
700	1:13	1:28
800	1:15	1:32
900	1:17	1:36
1000	1:19	1:40

Depending on the number of animals being tagged per family and the fecundity of a 6 year old female, the current breeding program, when fully developed, could provide industry with the demand in both species for an annual production of 700 to 900 tonnes. 1000 tonne requests should be the upper limit. Therefore, the expected cost benefit ratio for this 15 year period should be 1:28 to 1:40 for the abalone aquaculture industry and 1:13 to 1:19 for the abalone selective breeding project.

The 6 year reproduction cycle used in this analysis is determined by the size of abalone that could produce enough eggs for commercial production. This reproduction cycle (6 years) may be shortened to 3 to 4 years, even 2 years. Therefore, it is possible to double, even triple those benefits in this 15 year period.

APPENDIX 4: Extension of Results

The following articles were produced during the length of the project and were presented at the FRDC Abalone Aquaculture Subprogram annual meetings.

Li X, 2000. Genetic improvement of farmed abalone in Australia. In: Proceedings of the Seventh Annual Abalone Aquaculture Workshop, Dunedin, New Zealand (edited by Fleming, A). Fisheries Research and Development Corporation, Canberra, Australia, pp 39-46.

Graham A, 2001. Development of an R&D business plan. In: Proceedings of the Eighth Annual Abalone Aquaculture Workshop, Perth WA (edited by Fleming, A). Fisheries Research and Development Corporation, Canberra, Australia, p 187.

Li X, 2001. Selective breeding of farmed abalone: Where should we go next? In: Proceedings of the Eighth Annual Abalone Aquaculture Workshop, Perth WA (edited by Fleming, A). Fisheries Research and Development Corporation, Canberra, Australia, pp 51-53.

Li X, 2003. General Protocol for Family Establishments in Farmed Abalone In Australia. South Australian Research and Development Institute.

APPENDIX 5: Data recording spreadsheet samples

FRDC -- Abalone Selective Breeding Project *(Broodstock Collection)*

Broodstock ID	State (1 digit)[1]	Farm (2 digits)[2]	Batch No. (3 digits)[3]

Collector		Date	
Location[4]			
Depth(s)			
Temperatures	Air		
	Seawater		
Numbers	Male		
	Female		
Transportation	Time out of seawater		
	Time packed		
	Packing method		
	Time released into conditioning or spawning tank(s)		
	Temperature in packing container after opening		
Comments on gonad Condition			
Note			

Note: [1] Victoria: 3; South Australia: 5; Western Australia: 6.

[2] SAM: 01; SOM: 02; GSMH: 03; GSW: 04; OWS:05; KAF: 06: BCA: 07.

[3] Batch No. is determined by collection date and collection locality, different combination of these two elements will have different batch numbers.

[4] Name of the collection location and the GPS coordinates if available.

FRDC -- Abalone Selective Breeding Project *(Larval Rearing)*

Year:

Family ID	Digit														
	Color code														
	Temporary*														
Larvae hatched	Numbers								Average			Date			
		Tank							Water Filter		Clean	Antibiotics			
	Date	ID	Type	Size	Larval No	Temp.	Salinity	Water flow	Type	Size	method	Type	Dosage	Note	
Maintenance															
Development	Date & time shell developed							Date & time > 80% larvae displaying foot testing							
Transportation	Packing method							Larval density & quantity							
	Date & time packed							Water temperature after packing							
	Date & time released in settlement tank							Water temperature before released into settlement tank							
	Sending Farm							Date							
	Receiving Farm							Date							

Note: * An identification given by the farm.

FRDC -- Abalone Selective Breeding Project (*Data Collection, Digits*)

Species: *Recorder:*

Individual ID						**Parental ID**		Fertilisation	Replicate	**Data (2)**		
State[1]	Farm[2]	Fn[3]	Sex[4]	Family	Individual	Male	Female	Date	No.	Date	Length	Weight

Note: [1] Victoria: 3; South Australia: 5; Western Australia: 6. [2] SAM: 01; SOM: 02; GSMH: 03.
[3] Generation. [4] Male: 1; Female: 2.

FRDC -- Abalone Selective Breeding Project *(Tissue Storage)*

Species:

Broodstock ID	State	Farm	Batch No.			
Animal's ID*						
Samples	Tissues	Location	Processor	Date	Sender	Date
		Location	Receiver	Date	Sender	Date
	Shell	Location	Processor	Date	Sender	Date
		Location	Receiver	Date	Sender	Date
Tissue Sub-samples	Type#	Location	Receiver	Date	Sender	Date
Animal characters	Soft body weight			Whole body weight		
	Shell length					
	Foot & mantle coloration		Black			
			Stripe			
			Grey			
			Others			
Label						

Note: * Animal's ID = Broodstock ID + animal number or Individual ID from second generation.

Alcohol preserved or deep frozen.

APPENDEX 6: General Protocol

"The key is man's power of accumulative selection: nature gives successive variation; man adds them up in certain directions useful to him." ---- Darwin

General Protocol for Family Establishments in Farmed Abalone in Australia

Version 1

Dr Xiaoxu Li

South Australian Research and Development Institute

Table of Contents

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How to Use This Protocol

1. Steps

The steps in the methods section are listed according to the circle on the chronicle of abalone farming and divided, for easy reference, by the stages commonly used by abalone farmers or in biological studies. The (C) and (G) at the beginning of each step stand for compulsory and guide respectively, indicating whether the step is compulsory or recommendatory in the establishment of abalone families.

2. Cross-reference

The four sections, Basic Materials and Equipment, Methods, Flow Charts and Figures are described in the same order of stages. The same stage names have been used in all sections. Therefore, four sections can be cross-referenced. For example, at the larval rearing stage, the required facilities are outlined in the Basic Materials and Equipment section and the methods for larval rearing and data collection are detailed in the Methods section and summarised in the Flow Charts and Figures sections. In the Flow Charts and Figures sections the implementation of the protocol on a commercial farm is illustrated.

3. Suggestions

Before commencement of establishing abalone families, read the Methods section carefully and familiarise yourself with the requirements of this protocol. Check and/or prepare materials and equipment in accordance with the Basic Materials and Equipment section. During the establishment of abalone families, use Flow Charts or Figures as a guideline (which ever is easier for you) and cross-check against the Methods and/or Basic Materials and Equipment sections when needed.

Introduction

This general protocol is the final product of the subproject: “Development of a selective breeding protocol”, part of the FRDC project: “Abalone Aquaculture Subprogram: Selective breeding of farmed abalone to enhance growth rates”. The protocol provides information to the Australian abalone farmers on the methods to successfully establish a desired number of families of *Haliotis rubra*, the blacklip abalone and *H. laevigata*, the greenlip abalone.

The main focus of the protocol has been on "how to do it" rather than "why do it". Each part is written in a recipe-like format and designed for direct practical use on farms. Each part therefore stresses the practical steps and is readily replicable. For further hatchery and farming techniques you are referred to the "Abalone Hatchery Manual for Australia" (Hone et al, 1997) and "The Culture of Abalone and Other Marine Gastropods" (Hahn, 1989).

The protocol consists of four sections: Basic Materials and Equipment, Methods, Flow Charts and Figures. The procedures and the basic requirement are outlined according to the chronicle of the abalone farming cycle and are regarded as the minimum needed for establishing 30 families in one production run. It is not intended to limit a farmer’s freedom to go beyond this number if desired, and will be modified according to the specific requirement of individual abalone farms participating in this project.

Basic Materials and Equipment

System Set Up

Settlement Tank Preparations

- water filtration unit
- settlement tanks (x 33) and tank fittings
- airpump (x 1) and airlines
- plates and plate buckets (at least enough to fill the settlement tanks)
- shade cloth
- microscope (compound)

Spawning System Set Up

- water filtration unit
- self-adhesive labels
- thermometer
- individual spawning tanks (x 90-100) and tank fittings
- UV unit (at least one)
- airpump (x 1)
- pipes and airlines

Broodstock

Collection

- esky
- catch bags
- spatula
- thermometer
- legal measuring device
- cloths to wrap abalone in
- dive gear and boat
- location of sites

Maintenance

- items in 'Spawning System Set Up'
- immersion heaters (only required if abalone do not immediately respond to the UV stimulation or if incoming water needs to be heated).
- chillers (only required if incoming water needs to be cooled).

- siphon hoses
- scrubbing brush

Breeding Protocol

Spawning

- items in 'Spawning System Set Up' and 'Maintenance'
- calculator (X1)
- timer (X1)
- measuring cylinders (1000mL X3; 100mL X5)
- microscope (compound)
- microscope slides
- haemocytometer
- Lugol's iodine solution
- pipettes, petri dishes

Fertilisation

- items in 'Spawning' section
- self-adhesive labels
- water filtration unit
- thermometer
- 10 litre white buckets (required for fertilisation, X35)
- disposable tubs (750mL X50)
- sieves (60 micron and 400 micron wet sieve system X35)

Broodstock Material Storage

- alcohol and benzocaine
- labels and marker pens
- 25ml glass bottles and their sealing lids (x 70)
- scissors (x 1)
- scalpels (x 2)
- tweezers (x 2)
- tissues or paper towel
- vernier callipers (x 2)
- balance (0.01g accuracy)
- -20 ^{o}C freezer
- paper towel
- aluminium foils

Hatch-out into Larvae (overflow system)

- water filtration unit
- self-adhesive labels
- thermometer
- pipettes
- microscope (compound)
- microscope slides or petri dishes
- larval hatch out tanks (x 35) and tank fittings

Larval Rearing (flow through system)

- water filtration unit
- thermometer
- siphon hoses
- pipettes, petri dishes
- microscope (compound)
- Lugol's iodine solution
- larval rearing tanks (x 33-35) and tank fittings
- airpump (x 1)

Settlement and Grow-out (on plates)

- items in 'Settlement Tank Preparations' section
- vernier callipers (x 2)

Grow-out (off plates)

- tagging equipment (tags, glue etc)
- tanks (pooling tagged individuals x 3-6) and tank fittings
- abalone diet
- vernier callipers (x 2)
- balance (0.01g accuracy)

Back-ups

Spawning and Fertilisation

- extra aquaria
- broodstock conditioning system and diet
- disposal tubs

Settlement tank preparation, Settlement and Grow-out Maintenance I (on plates)

- nutrition (aquasol)
- 20L carboys (x 8)
- *Navicula sp* store culture

In addition, the management sheet and the data collection sheet are required at all stages.

Methods

Before starting establishment of abalone families, clean, sterilise and dry all the equipment that will be used (step 4). Then mark or label them (steps 7 and 25). Pre-filter seawater to stop natural recruitment.

System Set Up

Settlement Tank Preparations (also see steps E & F in the back-ups)

1. (G) Set up the settlement tanks one to two weeks prior to the spawning if the microalgal layer on plates is to be established using the conventional method, because this method depends on the time of year, temperature and the nutrients in the water. If the algal culture method is to be used to establish the microalgal layer on plates, set up the settlement tanks 3 to 4 days prior to metamorphosis. The tanks are approximately 50cm deep, 150cm wide and 300~900cm long, and are marked with an ID number. Place two rows of baskets containing vertically stacked plates (30 x 60 cm) in each tank. Place, under the plates, at least two air lines along the complete length of the tank. Provide tanks with water filtered with sand filtration or cartridge filtration to 10 to 20 microns nominal.
2. (G) If the conventional algal film culture method is employed, examine the microalgal layer regularly under the microscope to ensure the size of the individual microalgae do not exceed 15 microns. Use shade cloth to slow algal growth if it has become overgrown.
3. (C) Fill the log with: tank's ID number, time tank was set up and the time the alga was added for the algal culture method.

Spawning System Set Up

4. (C) Clean, sterilise and air-dry all the equipment which will be used in handling broodstock, gametes and larvae.
5. (G) Set up the spawning system up one to two days prior to broodstock collection and set the tanks or aquaria in two groups, one for male broodstock and the other for female broodstock.
6. (G) Each tank or aquaria unit consists of a spawning tank, at least two inlet pipes (with a tap on each) and one outlet pipe. The outlet pipe directs the out flowing water into the drain. The two inlet pipes deliver, respectively, the UV treated filtered seawater and the filtered seawater to the spawning tank. The water supply is filtered to 10 microns nominal. If the temperature spawning method is to be used in conjunction with the UV method an extra inlet pipe will be required to deliver filtered ambient seawater while the two existing pipes carry temperature controlled water (one connects to the UV unit).
7. (C) Mark the female tanks using Arabic numerals and the male tanks using letters of the English alphabet.

> **Note**: *Avoid keeping tanks too close to each other, avoid cascading tanks and avoid using tanks larger than 15 litres.*

Broodstock

Collection

8. (G) Go to the locations (maximum of 3 locations) where the broodstock are collected for commercial farms.
9. (C) Collect as wide a range of broodstock as possible with the following characteristics:
   - Legal in size;
   - Good gonad condition;
   - Free from obvious epifauna (algae growing on the back is acceptable);
   - Free from *Perkinsus*, and
   - Free from mudworm.
10. (G) Keep the abalone in a catch bag by placing 2 individuals foot to foot until enough broodstock have been collected.
11. (C) Note the seawater temperature at the depth the abalone were collected.
12. (G) Wrap each pair of broodstock foot to foot with a wet cloth.
13. (G) Put the wrapped abalone into an esky(ies) and transport them to the hatchery as soon as possible.
14. (G) Keep the inside temperature of the esky similar (± 1^{o}C) to the temperature of seawater where the abalone were collected.
15. (C) Fill the log book in with the following information: date, batch number, location and depth where the broodstock were collected, seawater temperature, air temperature, who collected the animals, time out of seawater, transportation method(s), number collected for both sexes and comments on gonad condition.

> **Note***: Avoid selection on shell shape and age, avoid any physical damage to the broodstock and also avoid water in the bottom of the Esky during transportation.*

Maintenance

16. (G) Open the esky having reached the hatchery.
17. (C) Turn on the water from the UV unit (do not turn on the UV unit) to the spawning tanks and keep the temperature variation at less than 2^{o}C from the temperature of the water where the abalone were collected.
18. (C) Scrub the shells of the abalone to remove any algae growing on the surface. Rinse in seawater and place one animal per spawning tank (see spawning system set up section).
19. (C) Fill in the log (see monitoring section) with the following data: water temperature and the time abalone were placed in the tanks.

> **Note**: *Avoid mixing male and female tanks.*

Breeding Protocol

Spawning (also see steps A, B, C & D in the back-ups)

20. (G) Reduce light and noise levels to an absolute minimum and allow the abalone to acclimatise for a few hours.
21. (G) Switch on the UV unit manually or with a timer at the desired time (preferably at about 9am).
22. (C) When an abalone starts spawning, turn off the tank's UV water and then turn on the filtered water to the tank. For males, turn off the tank's UV water first, empty the tank and then fill the tank with filtered water. Repeat emptying and refilling every half-hour until sperm have been used. Keep tanks clean by immediately removing any faeces with a siphon tube. Once the desired numbers of families have been established turn off the UV unit.
23. (C) Check the vigour of the sperm and examine the shape and colour of the eggs under the microscope. Discard any eggs of poor colour or that are clumped together. High quality eggs are about 250 microns in diameter and are olive green in colour (for greenlip abalone), having a characteristic hollow ring of lipid.
24. (C) Fill in the log for each individual broodstock with the following information (see monitoring section): abalone's ID number, number of eggs spawned, water temperature, spawning method(s), treatment duration for UV light and/or other method such as temperature, time spawning started and time gametes were used for fertilisation.

> **Note**: *Do not use dilute fresh sperm and microwaved sperm to stimulate female spawning, and avoid any contamination between tanks.*

Fertilisation (also see steps A, B, C & D in the back-ups)

25. (C) Label the disposable tubs according to the letters of the alphabet on the male tanks, label the siphoning tubes according to the numbers on all spawning tanks and mark the fertilisation containers, stirring rods and sieves by using the numbers on the female spawning tanks.
26. (C) Collect the eggs of one tank (from one female) by using a 60~90μm sieve with a 400μm sieve stacked on top to remove the faecal matter. The tank and the two sieves must have the same label number. Then wash the eggs gently and thoroughly to remove detritus.
27. (C) Transfer the eggs into a fertilisation container marked with the same number as the spawning tank, half fill to 5 litres and count the eggs, repeat twice. For egg counting, agitate to distribute evenly, and take a 0.5 mL sample using a pipette. Dispense the 0.5 mL sample as individual droplets onto a clean petri dish. Count the number of eggs in each drop using a microscope. Sum the total number of eggs and calculate the total number per container. Then work out an average per mL.
28. (C) At the same time as step 26, collect sperm from one male with a disposable tub marked with the same letter as on the tank. Determine the sperm concentration and recheck the quality under a microscope. Fertilise eggs from one female with sperm from one male at a 15:1 sperm/egg ratio.

29. (C) Gently agitate the fertilisation container to ensure complete mixing of the egg and sperm. Reduce the contents of the container to a volume (litre) in which the desired numbers (1~1.5 million) of eggs are obtained. Re-top to 5 litres with filtered seawater. The container should then be left undisturbed for 15 minutes. Using a pipette, remove a sample of eggs and place them on a slide under a compound microscope. If adequate sperm has been added, each egg should have 5-10 sperm tails surrounding it.
30. (G) After the sperm has been in contact with the eggs for 15 minutes, commence rinsing the eggs using the wet sieve (60~90 micron) technique. This is done by gently pouring the contents of the container into the sieve and rinsing with 0.2 micron filtered seawater with a flow rate of 5 litres per minute. Continue rinsing for 5 minutes to wash away excess sperm.
31. (C) Pour the eggs from one fertilisation container into one hatch-out tank (see hatch-out into larvae with a flow through system section for tank set up details). Distribute them evenly and allow them to settle.
32. (C) Fill in the log for each family with the following data: comments on egg quality (colour, form/shape, clumped/uniform layer), number of eggs spawned, number of eggs used, sperm/egg ratio, time fertilised and time washed.

> **Note**: *Avoid cross contamination between spawning tanks and between fertilisation tanks. Avoid using eggs more than 2 hours old and sperm no longer moving vigorously.*

Broodstock Material Storage

33. (G) Prepare 500 mL 80 % alcohol from the purchased alcohol with distilled water. Fill the 25ml bottles with 20 mL of 80 % alcohol.
34. (C) Remove, individually, the broodstock from the spawning tank, clean the soft body with filtered seawater, dry with paper towels and measure the length with a vernier calliper and the weight using a balance.
35. (G) Put the animal in 40ppm benzocaine seawater solution until abalone is narcotised.
36. (G) Take abalone out of benzocaine solution, rinse with fresh seawater and cut the soft body off the shell with a spatula or a scalpel. Dry the soft body with paper towels and measure the weight on a balance.
37. (C) Cut respectively one square centimetre of tissue from the gills, gonad, gut and mantle (with tentacles) from each individual used for family establishments. Wash the tissues with filtered seawater and put them into a 25 mL bottle with 20 mL 80% alcohol, then seal the bottle.
38. (C) Cut pieces of mantle, visceral mass, gills and foot tissue from individual parental broodstock, double wrap them in aluminium foils with an animal's ID label in between. Then store in a -20^{o}C freezer. (These samples will be sent to SARDI later).
39. (C) Mark the bottle with the date of storage, alcohol percentage and the animal's ID number, and then put the bottles in a dark, safe and well-ventilated storage place.
40. (C) Clean the shell, mark the shell with the animal’s ID number and air dry the shell.

41. (C) Fill in the log for each individual with the following data: date, shell length, total weight, soft body weight and locations of tissue and shell samples (also foot colour and stripes for blacklip abalone).
42. (G) Clean all the utensils used for tissue sample preparations.
43. Repeat steps 33 to 42 until all the abalone used for family establishment have been processed.

> **Note***: Avoid any contamination. Wear latex gloves when handling benzocaine and its solution.*

Hatch-out into Larvae (overflow system)

44. (G) The hatch-out system consists of individual units (35 units for this project). Each unit is made up of a hatch-out tank with a weir on the side that directs the surface water to the outlet, and an inlet pipe that delivers filtered water to the hatch-out tank. The tank is about 45cm in depth with a flat bottom of about 0.5m^2.
45. (C) When eggs settle on the bottom, turn on the inlet water and adjust the flow to the speed that does not disturb the egg layer. The outlet water is then received by a larval rearing tank (see next section for set-up details), resulting in the trochophores on the water surface of the hatch-up tank being transferred into the larval rearing tank.
46. (G) Take a 0.5 mL egg sample from the bottom using a pipette 8 hours post-fertilisation. Dispense the sample on a clean petri dish. Count the number of divided eggs in 100 eggs. Repeat this three times in total for each family. Then average the fertilisation rates by dividing the sum of the divided egg numbers from the three repeats by 3.
47. (G) Once the eggs have all hatched and no larvae remain in the surface layer of the hatch-out tank (this will take from 24 hours at 18 ^oC up to 36 hours at 14 ^oC), disconnect the hatch-out unit from the system and turn on the direct inlet water to the larval rearing tank.
48. (C) Fill the log with the following data for each family: water temperature, fertilisation rate, time hatch-out started and time hatch-out tank was disconnected from the system.

> **Note***: Do not sieve at this stage of development as the larvae are vulnerable to damage. Avoid cross-contamination. Maximum temperature: 18^oC for blacklip abalone and 20^oC for greenlip abalone.*

Larval Rearing (flow through system)

49. (G) A large number of tanks are required for rearing the larvae from each family (35 tanks are needed for this project). Each tank should be about 200~250 litres in volume and have a hemispherical bottom and steep sides. Filtered air is supplied through the bottom and filtered water through a pipe at the top. A 60 micron banjo sieve is connected to the outlet pipe to stop larvae from escaping.
50. (C) When hatch-out is finished, count the larvae in each family (tank). Agitate to distribute evenly, and take a 2 mL sample using a pipette. Dispense the 2 mL sample as individual droplets onto a clean petri dish. Count the number of larvae in each drop using a microscope. Sum the total number of larvae and calculate the

total number per tank. Repeat this step three times in total, then average the total number per tank.

51. (C) When all families have hatched and the larvae have been counted, adjust the tanks so that the same number of larvae are in each. First turn the water off to the tank with the higher larval concentration. Agitate to distribute the larvae evenly and siphon the water from the tank centre to reduce the water in the tank to the level at which the desired number of larvae remain (15 larvae/mL for over flow system, 2 larvae/mL for static system), then turn the water back on. Repeat this for all the other tanks.
52. (G) Adjust the water flow to the speed that does not create larval blocks on the banjo sieve screens. Siphon the bottom of the tank every day to remove dead larvae and detritus. First turn the air off for 5 minutes, siphon the bottom, then turn the air back on.
53. (C) Monitor and count the larvae everyday under the microscope. When more than 80% of the larvae are displaying the foot testing behaviour, collect the larvae on a sieve. Place the larvae from one family in one settlement tank (see next section for detail). The time from hatch-out to settlement varies depending on temperature. It ranges from 4-5 days at 20^{o}C to 9-10 days at 14 ^{o}C.
54. (G) Cull off 2 families with low survival or randomly cull off 2 families if there is no significant difference in survival between families and extra settlement tanks are not available at the settlement and on-plates grow-out stages.
55. (C) Fill the log with the following data for each family: total larvae hatched, initial larval density (as determined), total larvae surviving after the larval rearing stage, water flow, temperatures each day, time shell developed and time more than 80% larvae displayed the foot testing behaviour (or date larvae reached the three tentacle stage).

> **Note***: Avoid cross contamination between tanks. Maximum temperature: 18^{o}C for blacklip abalone and 20^{o}C for greenlip abalone.*

Settlement and Grow-out Maintenance I (on plates) (also see step F in the back-ups)

56. (C) Turn off the water and attach a banjo sieve to the outlet prior to the addition of the larvae to the tank. Add the larvae from one family to one settlement tank or one line of settlement tanks at a rate that allows for 50% survival during settlement, 10% survival to day 150 and will result in 100 juveniles per plate at the end of the plate stage. Leave the water off with only very gentle aeration for the first 24 hours, then turn on the water.
57. (G) Remove the banjo sieve when less than 5% of the larvae are observed in the water column.
58. (G) Reverse the plates every fortnight or when needed. Rotate racks and clean tanks every month or when required.
59. (C) At two months post-settlement count juveniles from three plates/rack and repeat with five racks/family. Use a narrow scraper and scrape randomly and vertically to thin the on plate juvenile density to about 120/plate for all families.
60. (C) Fill in the log with the following information for each family: time larvae were added, time banjo sieve was removed, larval number or densities, comments

on the microalgal layer quality when larvae were added, water temperature, date on-plate juveniles were thinned and densities before and after thinning.

> **Note***: Avoid cascading tanks holding juveniles from different families. Ensure that all plate surfaces receive good aeration and light. Ensure that all tanks receive the same number of larvae for settlement.*

Grow-out Maintenance II (off plates)

61. (G) Set up or allocate desirable numbers of tanks from the tanks used for commercial production in an area on the farm where the environmental factors can be kept uniform across the tanks. Mark each tank with an ID number.
62. (G) Transfer the juvenile abalone from plates into the grow-out tanks at 180 days post-fertilisation or when required. Wash off the juvenile from plates in one family into one container, then count. Cull off those families in which the numbers of juveniles left are not large enough for further maintenance.
63. (C) Choose at random 270 animals from each family, dry with paper towels, tag individually with their family ID number (colour dots combination), and measure the length with a vernier calliper and the weight on a balance.
64. (C) Separate randomly the 270 tagged abalone from each family into three groups (90 individuals/group), put one group from all families in one pooling tank (three replicates in total). Add equal numbers of individuals, tagged with control population's ID number (50 individuals/family) and untagged, from all families to match the animal density requirement in each tank. These individuals will be used as control populations.
65. (G) Mix and distribute evenly the individuals from different families in the pooling tanks. If required, feed artificial powder for a few days to facilitate the weaning of the abalone from the diatom film to the artificial diet.
66. (G) Maintain animals with the methods currently used on farms. Clean the tanks before feeding or when required and feed the animals every other day with the amount currently used on farms.
67. (C) At 360 days post-fertilisation dry the tagged abalone with paper towels and re-tag the animals previously tagged with a family ID with an individual ID number and re-tag the tagged control population with blank tags of the same size used for tagging individuals, measure length with a vernier calliper and weight on a balance after tagging.
68. (C) Collect the length and weight data from all individuals tagged with IDs in each replicate at 6 monthly intervals from the time of re-tagging and onwards.
69. (C) Monitor the sex maturation of individuals tagged with IDs by checking the gonad development during the spawning seasons from two years old.
70. (G) At six month intervals or when required, grade and restock the abalone at the densities used for commercial farming at each stage respectively. For example, a density of 700 individuals/m^2 is currently used for 1 to 2 year old greenlip abalone. These can be done by randomly adding or culling off the control population in the pooling tanks (and by randomly culling off untagged individuals in the family tanks if the families are kept separately).
71. (C) When the average shell lengths reach the market size (90mm, subject to review) in more than half of the families, collect the data as described in step 67.

72. (G) Randomly select 30~50 tagged animals from each family and put them in 40ppm benzocained seawater solution until the abalone are narcotised.
73. (C) Take the abalone out of the benzocaine solution, rinse with fresh seawater and separate the meat from the shell and the other parts of the soft body with a knife, dry the meat with paper towels and weigh individually on a balance.
74. (C) Fill in the log with the following data for each family and individuals: date abalone were removed from the plates, colour tagging or re-tagging date, abalone's family colour IDs or individual abalone's ID numbers, weight, length and measurement dates (at six monthly intervals), tank's ID numbers, daily temperature, age and sex of tagged individuals at first maturity, times abalone were graded and individual's grade, feeding and cleaning frequencies, food type(s) and amount(s) at different stages, length, whole body weight and meat weight at harvest.

> **Note***: Avoid reusing the escaped individuals if their identities are in doubt. Avoid cascading tanks holding different families. Wear latex gloves when handling benzocaine and its solution.*

Back-ups

Spawning and Fertilisation

A. Keep separately the extra male and female broodstock in a few aquaria and set the temperature at 2^{o}C less than the water temperature where the broodstock were collected. If broodstock in the spawning tanks did not produce enough spawn to establish the desired number of families within 24 hours or most broodstock did not spawn within 4 hours after the first abalone started to spawn, replace the spawned broodstock with the abalone in the back-ups. Use new spawning tanks if tanks are available, otherwise clean and rinse the existing tanks thoroughly and register them with a new ID number before use.
B. When not enough ripe broodstock can be collected from the wild for the establishment of families or spawning wild broodstock is difficult, use pre-conditioned broodstock instead. If pre-conditioned broodstock are not available, condition collected wild broodstock and start the family establishments when these broodstock are ready.
C. When eggs are nearly 2 hours old and no or not enough males have spawned, individually take the unspawned males from the spawning tank, massage the gonad using your fingers and collect the gamete in a container marked the same number as the spawning tank. Return the massaged male back to its spawning tank when finished and fertilise the eggs according to the methods described in the fertilisation section. Wash your hands thoroughly with detergent and then repeat this with the other individual males until the eggs have all been fertilised.
D. If male(s) have spawned and no eggs are available for fertilisation, take the sperm from one abalone with a disposal tub marked with the same letter as the spawning tank and keep them in a refrigerator at 5^{o}C. These sperm have to be used within 24 hours.

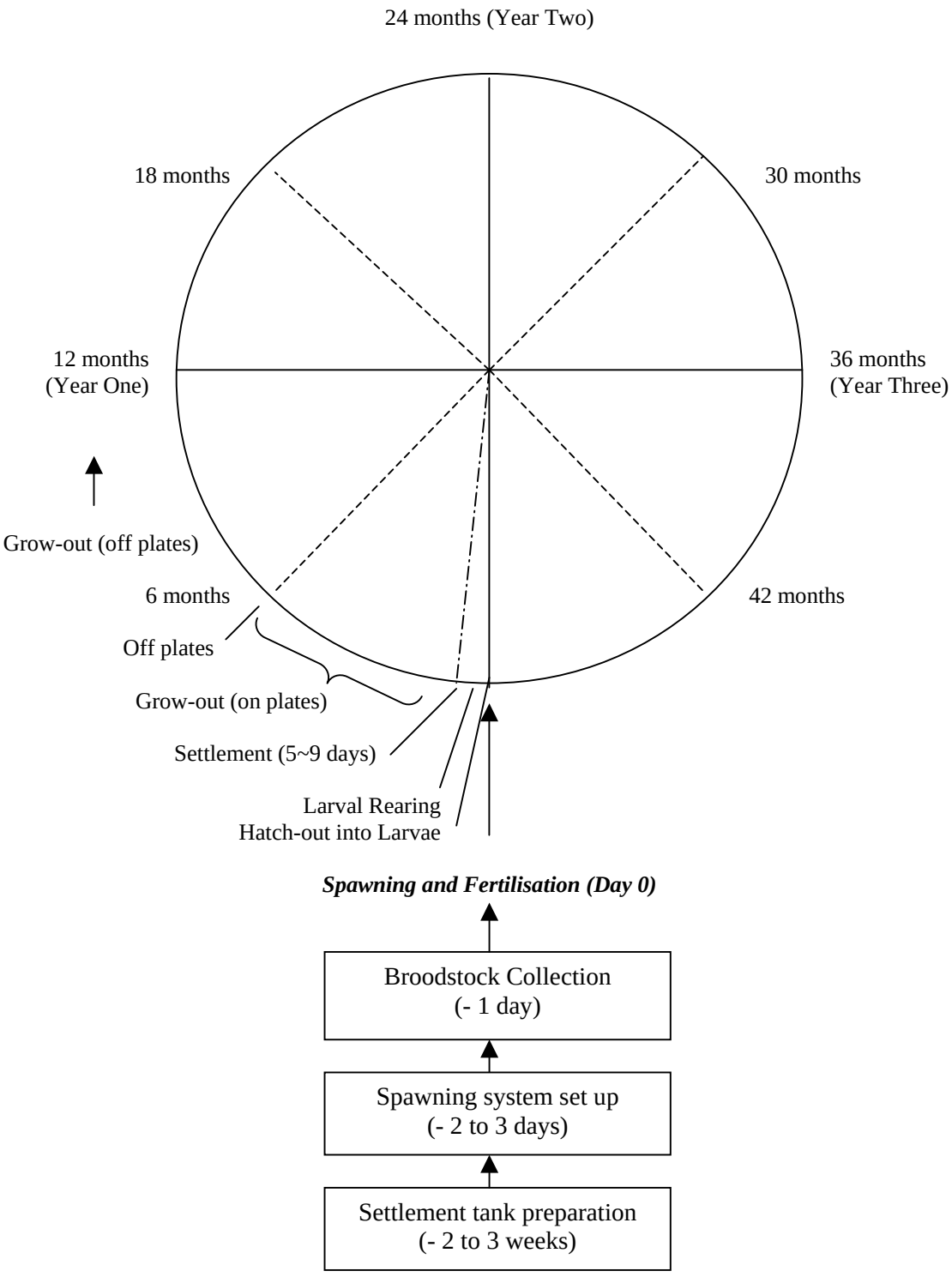
Settlement tank preparation, Settlement and Grow-out Maintenance I (on plates)

E. When diatom growth on plates is slow for the first two weeks add nutrients to promote growth. If Aquasol is used a dosage of 3 grams per 100 litres is recommended.

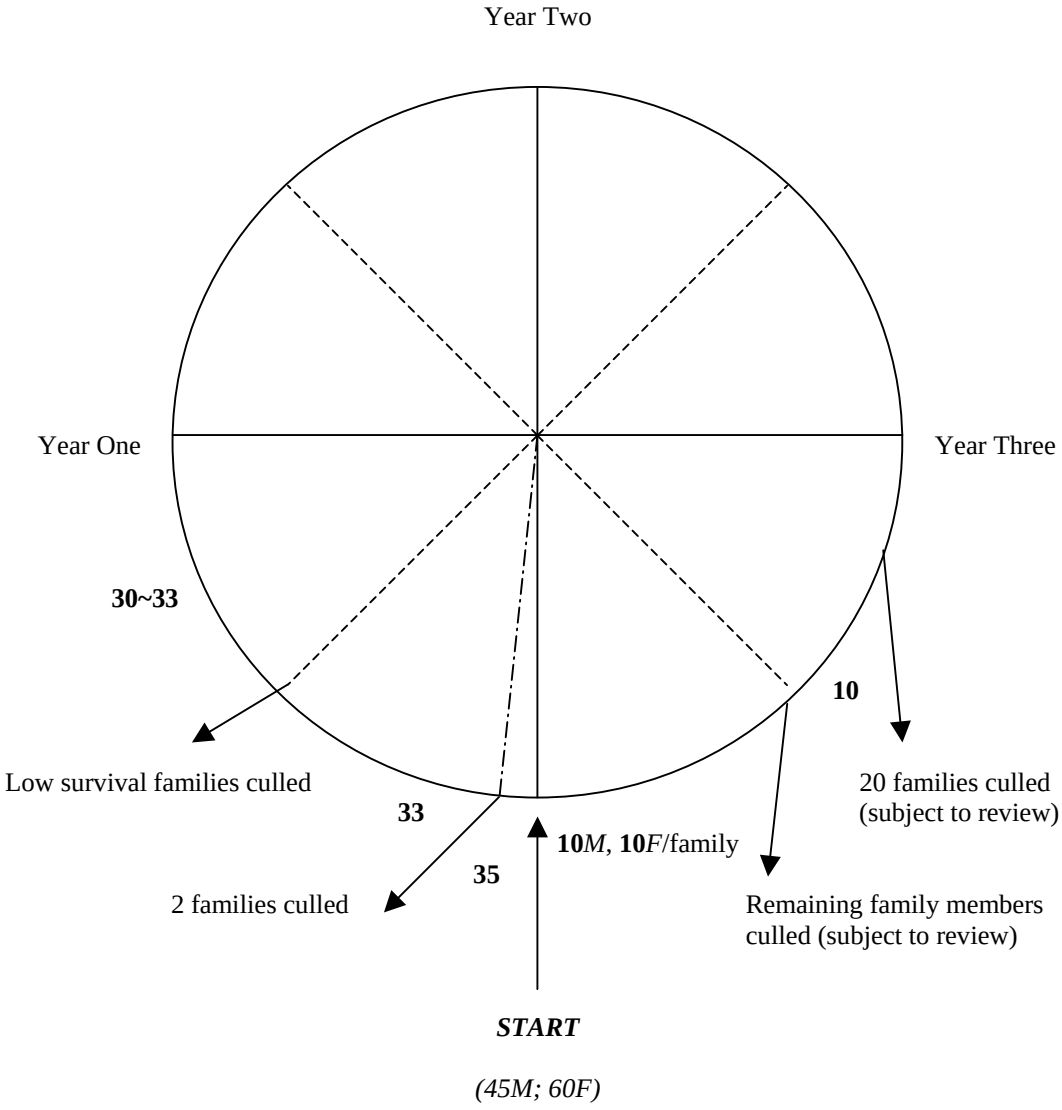
F. In case of a diatom shortage at the on-plates grow-out stage, start a *Navicula sp* culture in four 20L carboys at the time when preparing the settlement tanks, then start a second culture two to three weeks later. When the cell density of the second batch are high enough replace the first batch with the new culture. Keep replacements until the end of the on-plate grow-out stage. In this way at least one batch of high density algal culture (4 carboys) will be available if the diatoms on the plates are not enough for larval settlement or juvenile grow-out. When *Navicula sp* culture is required to promote the algal growth on the plates, turn off the water-flow to the tanks first, add an equal volume of algae to each of them and then turn the water-flow back on in 24 hours time.

Flow Charts

Flow-chart 1: Abalone Farming Stages

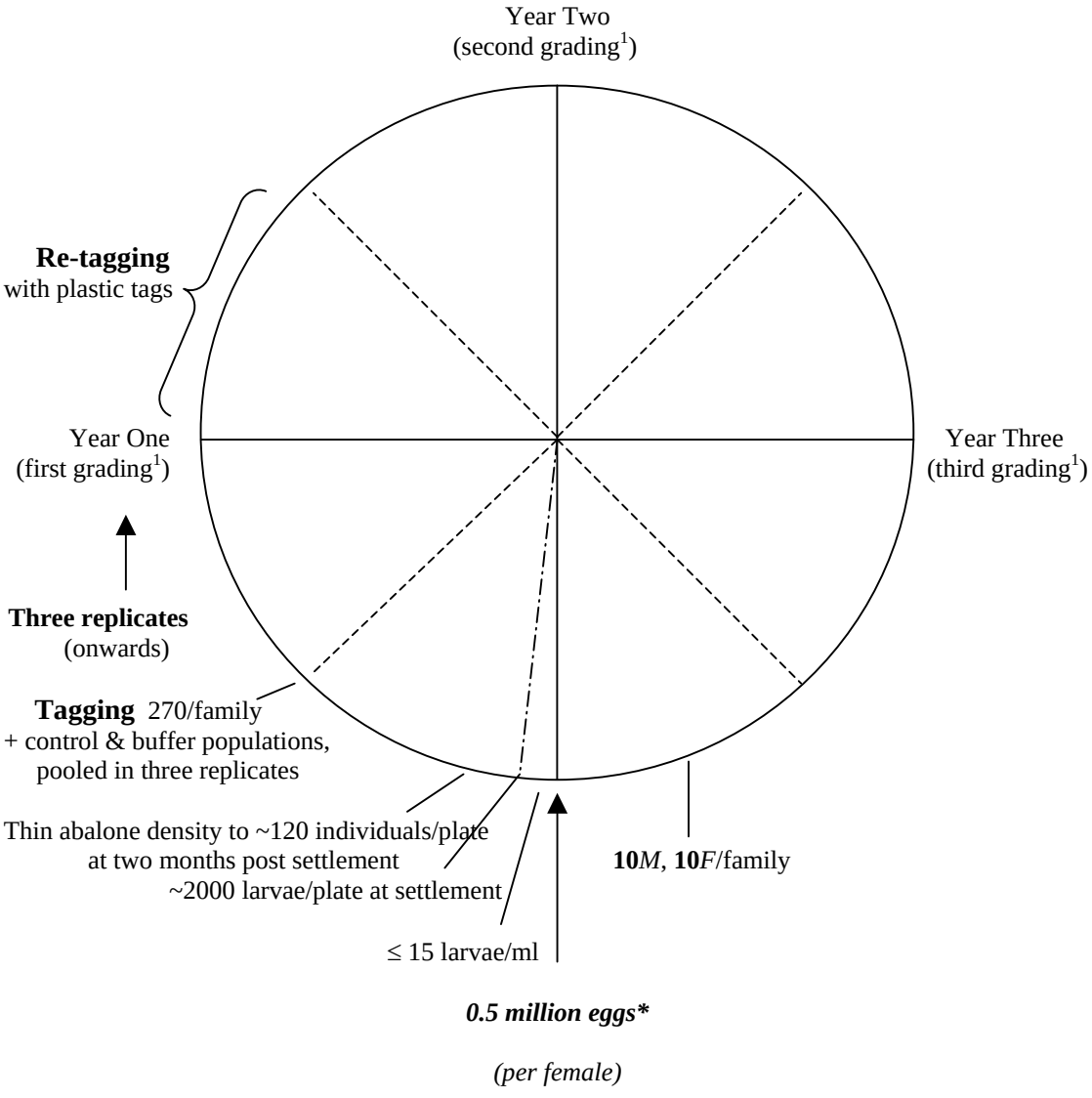


Flow-chart 2: Family Numbers at Different Developmental Stages*



* Note: The numbers are valid for 30 families only, they have to be modified if used for other family numbers.

Flow-chart 3: Individuals per Family at Different Developmental Stages

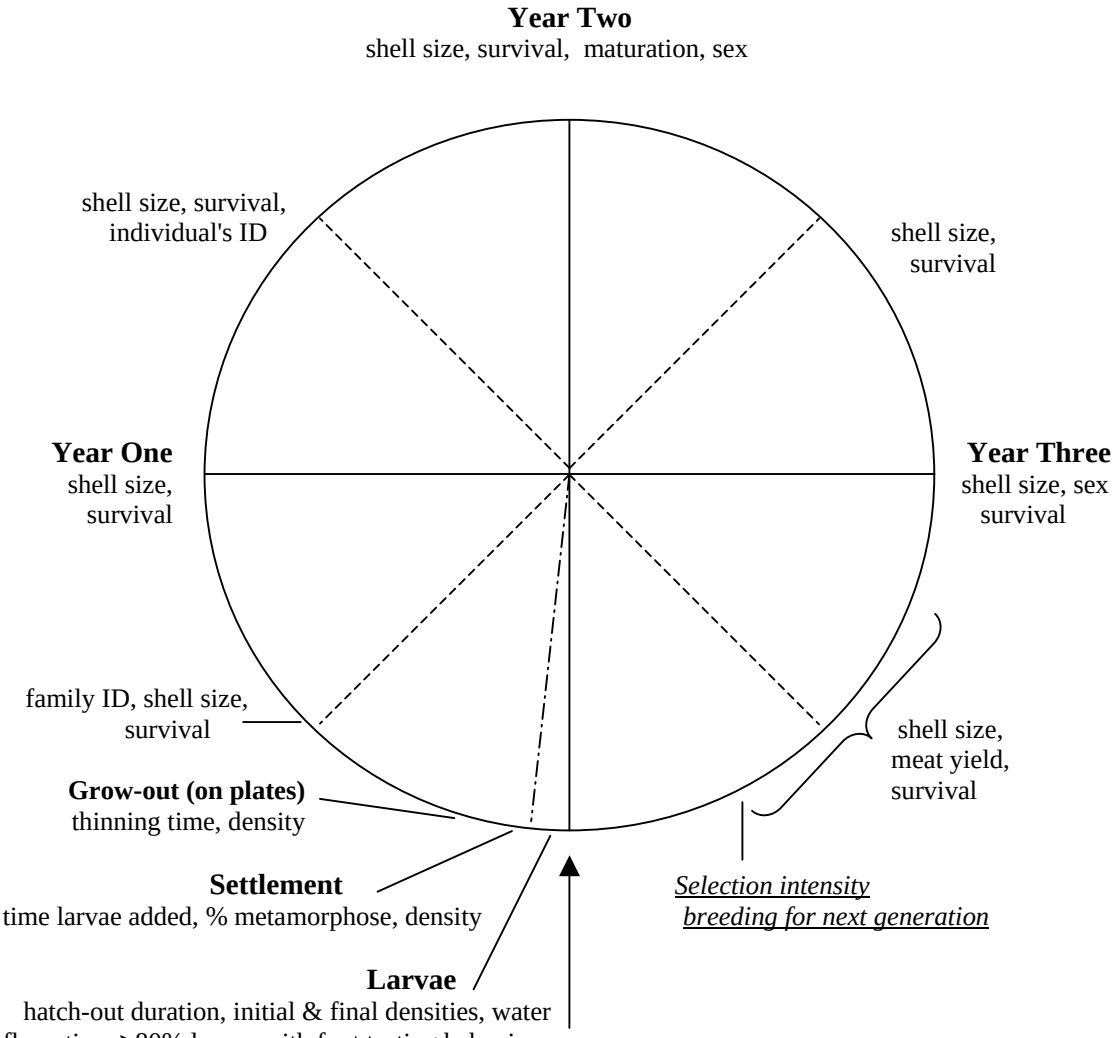


Note: * The number given here is the minimum egg requirement for the rearing systems which will be used in this project.

[1] The number of times needed for grading will depend on abalone species and the farming method.

Flow-chart 4: Data Collections at Different Stages

(Note: Male and female data will be collected separately)



In addition, temperature, salinity and amount of food used each time will also be recorded or monitored regularly.

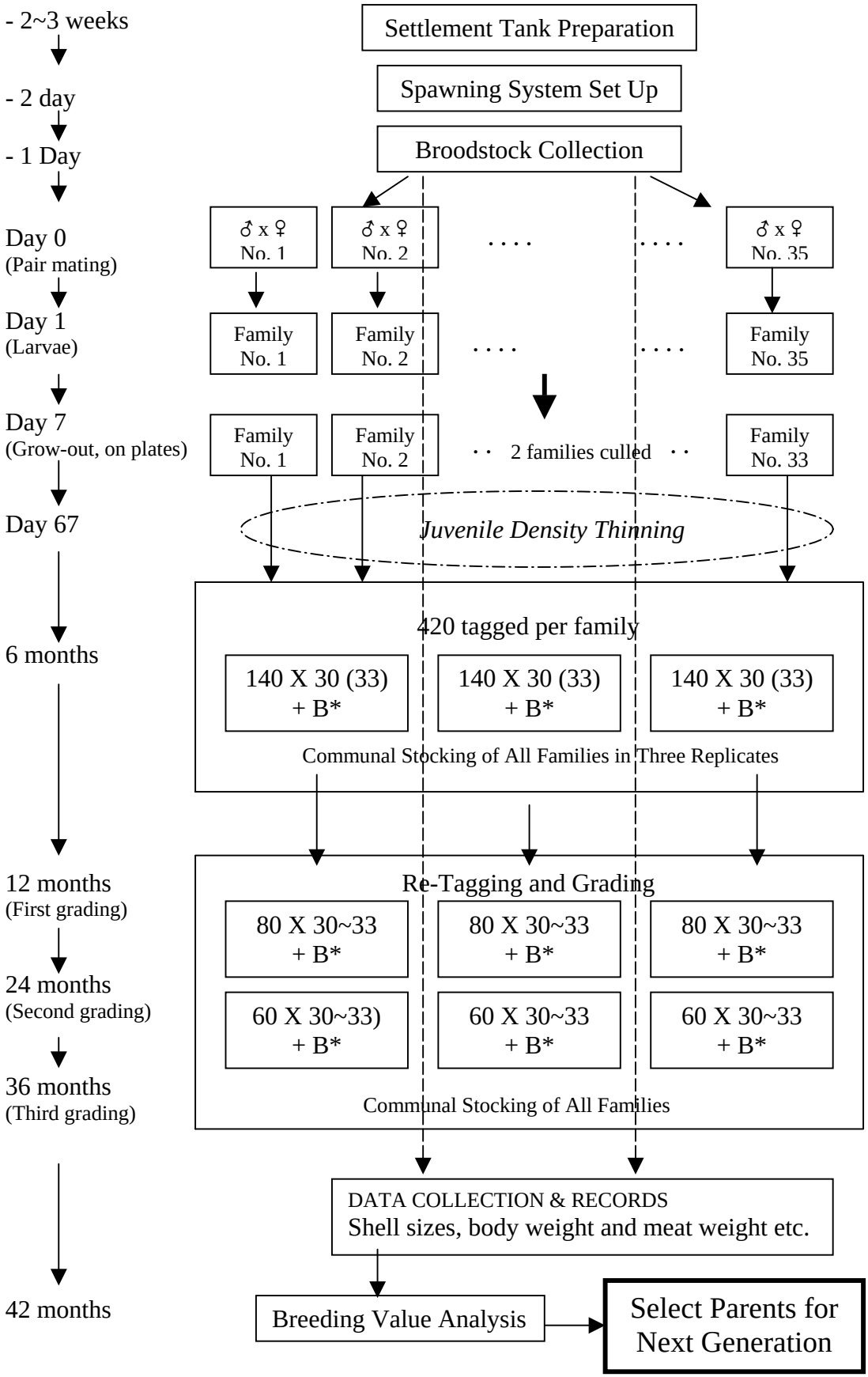
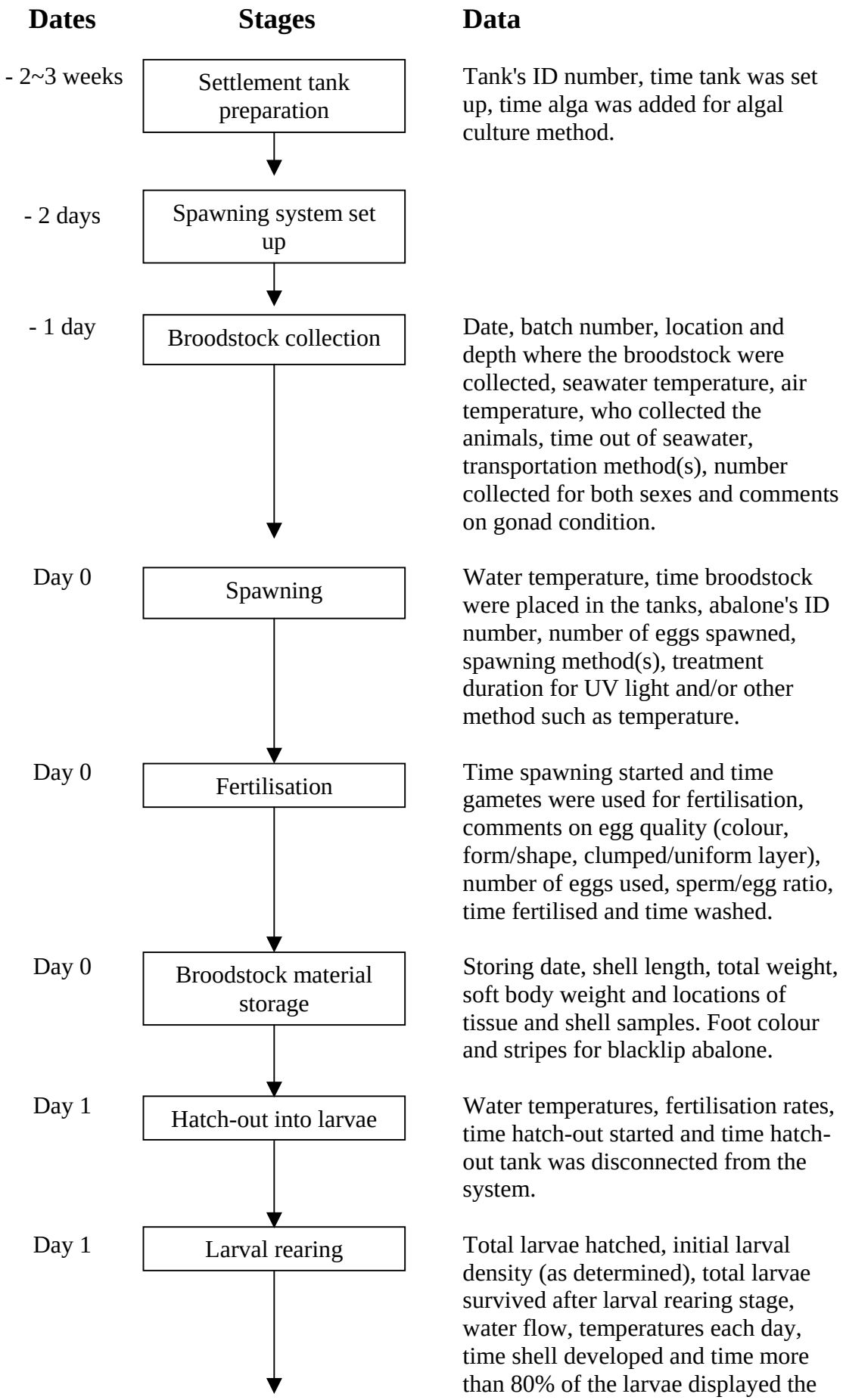
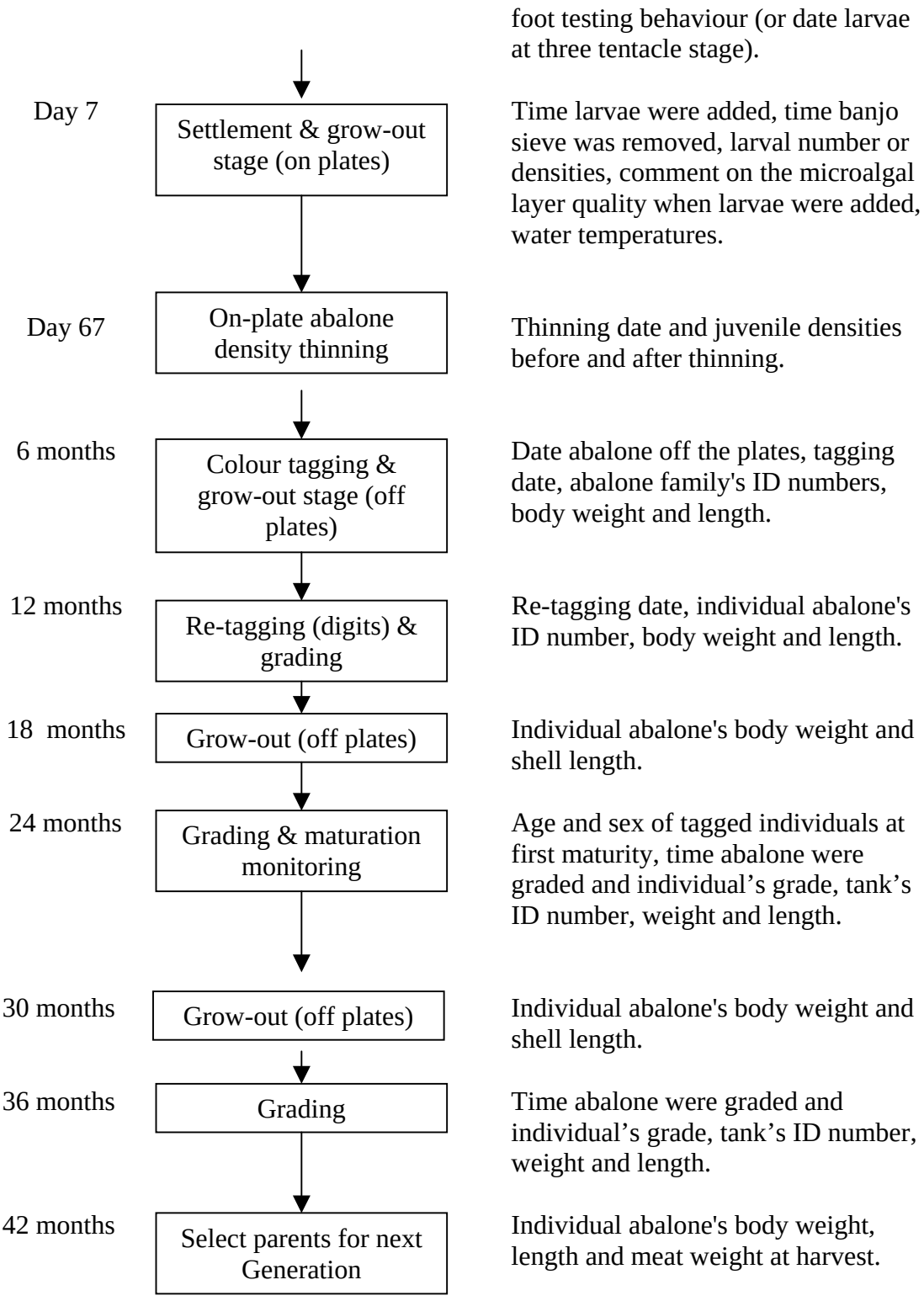


Figure 1: Example of Working Chart for One Round of Breeding. B*: buffer population


Dates
Stages
Data
- 2~3 weeks
Settlement tank preparation
Tank's ID number, time tank was set up, time alga was added for algal culture method.
- 2 days
Spawning system set up
- 1 day
Broodstock collection
Date, batch number, location and depth where the broodstock were collected, seawater temperature, air temperature, who collected the animals, time out of seawater, transportation method(s), number collected for both sexes and comments on gonad condition.
Day 0
Spawning
Water temperature, time broodstock were placed in the tanks, abalone's ID number, number of eggs spawned, spawning method(s), treatment duration for UV light and/or other method such as temperature.
Day 0
Fertilisation
Time spawning started and time gametes were used for fertilisation, comments on egg quality (colour, form/shape, clumped/uniform layer), number of eggs used, sperm/egg ratio, time fertilised and time washed.
Day 0
Broodstock material storage
Storing date, shell length, total weight, soft body weight and locations of tissue and shell samples. Foot colour and stripes for blacklip abalone.
Day 1
Hatch-out into larvae
Water temperatures, fertilisation rates, time hatch-out started and time hatch-out tank was disconnected from the system.
Day 1
Larval rearing
Total larvae hatched, initial larval density (as determined), total larvae survived after larval rearing stage, water flow, temperatures each day, time shell developed and time more than 80% of the larvae displayed the



In addition, temperature, salinity and amount of food used each time will also be recorded or monitored regularly.

Figure 2: Data Collection at Different Stages

Appendix:

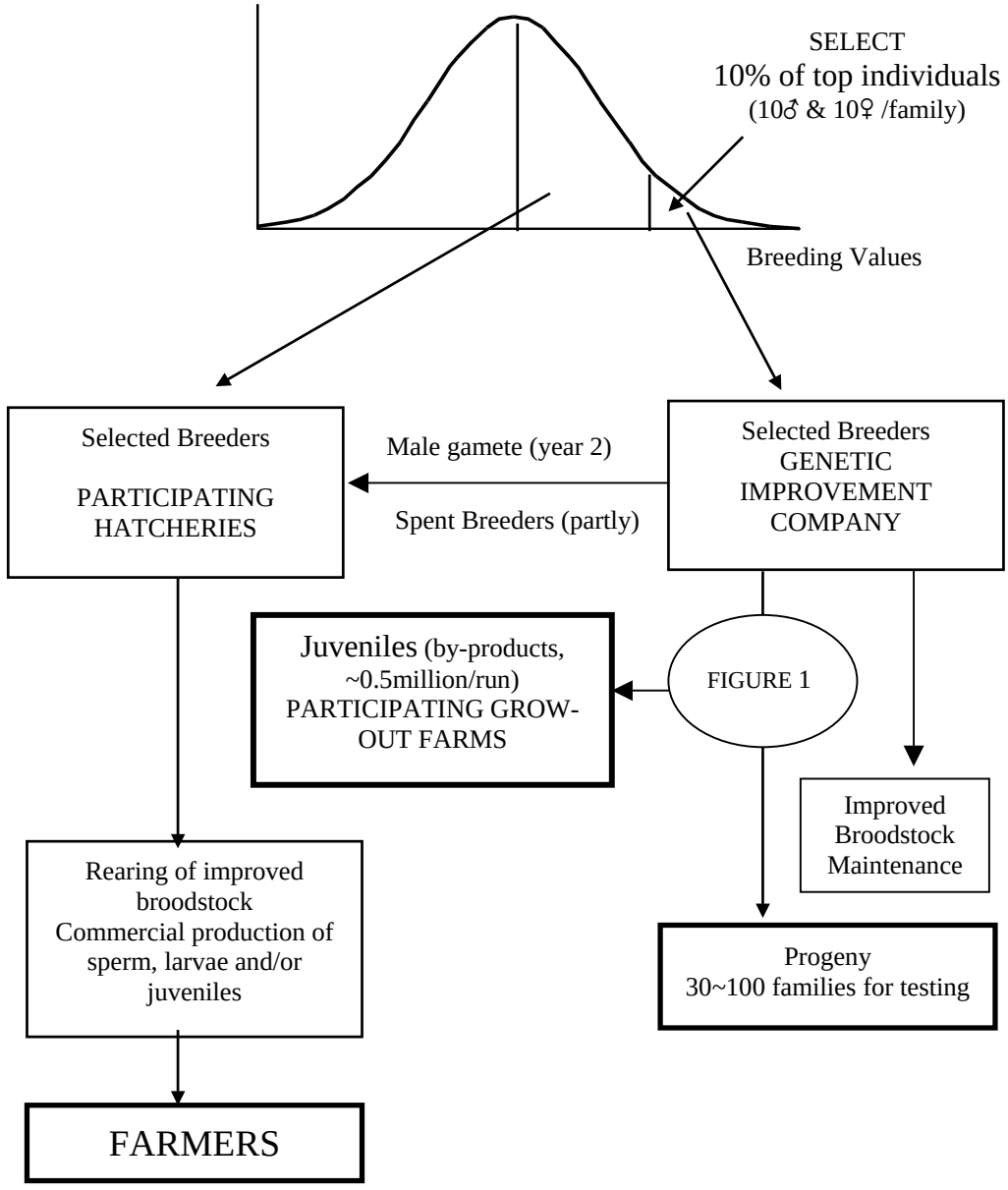


Figure 3. Tentative Schemes for the Selection of Breeders and Dissemination of Genetically Improved Abalone to Farms, in Each Round of Breeding

References:

Hahn, K.O., 1989. Handbook of Culture of Abalone and Other Marine Gastropods, CRC Press, Boca Raton, FL, USA.

Hone, P.W., Madigan, S.M. & Fleming, A.E., 1997. Abalone Hatchery Manual for Australia. South Australia Research and Development Institute, p. 34.