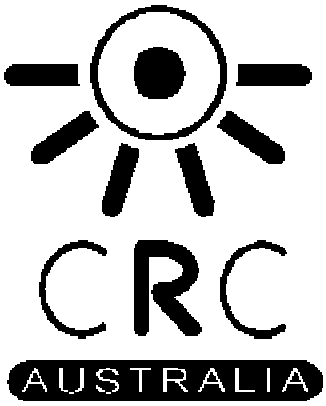
Development of a genetic management and improvement strategy for Australian cultured Barramundi

FINAL REPORT

Dr Nick Robinson & Dr Dean Jerry



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Development of a genetic management and improvement strategy for Australian cultured Barramundi

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1. Non-technical summary

The Australian Barramundi industry, through the Australian Barramundi Farmers Association (ABFA), have long appreciated the potential for improving the sustainability and profitability of production through the appropriate implementation of genetic management and improvement of the species. However, previous attempts to develop a coordinated national strategy for this species have not met with success due to a poor understanding of possible models by which a breeding program within the industry can be implemented, the resources required for implementation, R & D impediments and the potential benefits that will arise from a successful breeding program. The ABFA investment in the Seafood CRC is viewed by the association as a clear opportunity to catalyse efforts directed towards an industry wide approach to the sustainable and economically viable genetic management and improvement strategy for cultured barramundi in Australia. This project was carried out in order to address the following key constraints faced by the industry:

- lack of a broad, industry wide, understanding of the potential benefits (and risks) associated with genetic management and improvement
- lack of a clear understanding of the resources (including human, economic, infrastructure and genetic resources) required for the implementation of genetic management and improvement in this species
- gaps in knowledge required for the implementation of genetic improvement including key genetic parameters and economic weights of traits that could be improved.
- limited information on the genetic status of existing (highly valued) hatchery broodstock and thus their potential as founder stocks for genetic breeding programs
- lack of clear guidelines on appropriate genetic improvement strategies

Information was gathered from the ABFA committee, the Darwin Aquaculture Centre, hatchery operators, managers of the main farms with hatcheries and Queensland Department of Primary Industries about existing infrastructure, barramundi biology, industry and farm economics, and hatchery practicalities. This information was processed by the investigators to determine key researchable constraints to barramundi selective breeding and practical options for beginning selective breeding. A computer based bio-economic simulation model for the barramundi selective breeding program was developed. The model was used to estimate genetic and economic improvement likely to result from a basic selective breeding program. Feedback on the Research and Development options and output from the model were obtained at the Annual Research and Development Workshop held by the ABFA in Cairns in February 2009.

We recommend that the breeding program for the barramundi industry be established as an “ex situ” central breeding nucleus (ie. that the breeding occurs using one facility which is isolated from existing barramundi farm sites, and that once the nucleus of breeding families is established, there is no further translocation of animals into the nucleus). Closing the nucleus and isolating it from existing farms reduces the risks of disease being introduced to the breeding families and reduces the risk of genetic improvement being diluted. In addition, operating one central breeding nucleus can be achieved at a lower cost than breeding families across several locations, commercial production imperatives that are normally faced on-farm will not override selective breeding imperatives and the focus purely on selective breeding and supply of genetically improved stock should also yield higher benefits for the industry. Animals from the nucleus would be sent to farms to be grown and tested with each generation of selective breeding, and these animals can be used as an emergency backup to restock the nucleus.

There are a number of practical challenges with selectively breeding this species. Barramundi mature first as males and then differentiate into females, multiple males are normally present in spawning tanks, small numbers of spawning tanks are normally available, larval batch performance varies greatly, females mature after 4 years and so the generation interval is relatively long and barramundi are grown in diverse production environments (saltwater, freshwater, pond, cage and tank).

A number of key researchable constraints to selective breeding were identified.

- The breeding program will need tight control over sexual maturation, sexual differentiation and reproduction to allow planned and synchronised mating of selected broodstock each generation. Coordinated experiments are needed to test how sexual maturation, differentiation and reproduction could be manipulated or induced.

- Genotype by environment interactions if they exist could have a large effect on how the selective breeding program should be run. These interactions need to be measured.

- Few genetic parameters have been estimated for barramundi, however we need to know the extent to which important traits are controlled by genetics and we need to know if selection for one trait will beneficially or adversely affect other important traits.

- We lack understanding about the variability in the genetic contribution of parents to subsequent generations with mass spawning of barramundi and how inbreeding might be affected by this practice in industry breeding programs.

- Finally, for barramundi in particular, whose breeding biology and behaviour poses many challenges for selective breeding, we need to develop and utilise novel technologies (such as cryopreservation of sperm etc) that will assist us to increase genetic gain while reducing inbreeding.

Many of these research challenges could be addressed using the base population of families that are established for initiating the breeding program. With the establishment of this base population, genetic diversity will be maximised, and valuable information about genetic variation and its expression in

different environments will be obtained. This information can be used to guide the formulation of detailed selective breeding plans for subsequent generations.

Even a basic breeding program lacking many of these technologies was demonstrated to be likely to yield a high benefit-cost ratio for the barramundi industry. After 10 years of selective breeding the model predicted benefit-cost ratios of from 11:1 to 18:1, net present value to the investors in selective breeding of from $12-28 million, and internal rate of return on investment of between 42 and 144%. A simple and effective way of selective breeding, while accounting for the transformation of males into females, is to mate each generation's high performing animals (mature males) with the previous generation's high performance animals (mature females that were not mated as males). The simulation model that has been developed for barramundi selective breeding will be a useful tool for predicting the performance of the selective breeding program under new and different scenarios as selective breeding goes forward into the future.

Finally, the hands-on running of a breeding nucleus requires a high degree of specialist training. There is a need for the industry to develop the knowledge and skills necessary for establishing and running the day-to-day practical routines for the breeding program, profiting from the selective breeding business and providing detailed analysis and interpretation of data for selective breeding. The barramundi industry should take advantage of the travel bursaries, education and exchange programs being offered by the Seafood CRC. The industry can use these opportunities to foster skilled and knowledgeable people who can run the breeding program.

2. Acknowledgements

Thanks to Graham Mair Seafood CRC, Jenny Ovenden QDPI, Glenn Schipp DAC, Michael Macbeth QDPI, Jerome Bosmans DAC and Alex Safari Flinders University, who have all provided useful feedback and information for the report. Thanks also to all the barramundi farms and hatcheries who contributed information and provided helpful advice. Thanks also to the South Australian Research and Development Institute (Steven Clarke, Xiaoxu Li) who supported the PI with the development of the project.

3. Background

The establishment of centralised, well designed selective breeding programs has proven a key to the successful development and ongoing viability of major aquaculture industries worldwide. Major world aquaculture finfish species, (eg. Atlantic salmon, rainbow trout, Mediterranean sea bass, carp, catfish and tilapia) are typically serviced by two or more such programs. The first such major breeding program was established for Atlantic salmon in Norway by Nofima (formerly Akvaforsk), and the impact of this program has been well documented. Almost the entire production in Norway, and much of the production in Chile, is from fish derived from this program (eg. those sold by Aquagen). Thodesen et al. (1999) compared the performance of unselected animals to selectively bred Atlantic salmon grown under the same conditions after 5 generations of selection on growth rate. The growth rate of the selectively bred fish was more than doubled (+113%), feed consumption increased by less than 50%, protein retention was 9% improved, energy retention was 14% improved and the feed conversion ratio (feed to gain) was 20% improved. After 7 generations this improvement in feed conversion ratio alone resulted in a saving of $300 million in feed costs each year to the industry in Norway. Typically, in a well designed program, a genetic change of 10-15% per generation can be achieved for a trait like growth rate, and the selective breeding program can yield a benefit-cost ratio of around 15:1 per generation of selection (Gjedrem, 2000).

However, selective breeding programs will have limited benefit if:

- there is limited genetic expertise involved
- small family numbers are used
- traits of no economic value, little genetic basis and difficult to measure are chosen
- there is no solid long-term business plan
- the program is exclusive and the benefits flow to few in the industry
- continued translocation from the wild occurs (introducing disease and diluting out genetic progress) and
- there is inadequate biosecurity and disease surveillance

Barramundi aquaculture is a maturing industry in Australia with the majority of producers based in Queensland, although there is also significant production in SA and NT, and the industry is developing in WA. Annual production is over 3,000 tons per annum with a value exceeding $26 Million (O'Sullivan and Savage, 2009). Most producers in Australia are currently supplied with fingerlings from several major hatcheries. Due to infrastructure constraints and a lack of training in the genetics of broodstock maintenance it is likely that these hatcheries spawn a small number of broodfish, and do not keep pedigree records, with the result that future generations of broodstock will represent a small proportion of the available gene pool. Loss of genetic variance and inbreeding depression effects on fitness are likely to impact negatively on production within just a few generations if these practices continue.

Barramundi (otherwise known as Asian sea bass when produced outside Australia) is now produced by many countries around the world. Competition is increasing and Australian producers need to improve efficiencies while producing a consistent high quality product with a strong brand name. Commercial selective breeding program for the species are being developed elsewhere in South-East Asia. For instance, a well resourced selection program has begun in Singapore that is aimed at stimulating development of a barramundi industry in this country. Therefore it is imperative that Australian producers gain a head start, and benefit early from genetic improvement, by initiating selective breeding ASAP.

In providing a service that the majority of the industry in Australia can benefit from, we need to consider whether breeding program(s) should be run on individual farms (ie. a decentralised model) or whether the industry would be best served by a centralised program. The term “centralised” is used here to refer to a selective breeding program that acts in a coordinated way (ie. as one program with a pool, or nucleus, of breeding animals), uses one facility (centralised breeding facility) and operates as a separate business entity (stand-alone selective breeding entity). Possible advantages of developing a separate centralised program/facility/entity v. individual farm based programs would be that

- the benefit would be realised by more farms, assuming these farms could have access to the selected progeny
- costs per farm would be reduced,
- commercial production imperatives will not override selective breeding imperatives,
- larger numbers of families could be produced,
- faster rates of improvement could be achieved,
- a broader genetic base could be established in the beginning,
- inbreeding depression of fitness would be minimised,
- a strict timetable and plan for selection and production of families could be followed,
- biosecurity could be both addressed on-site and by receiving farms and
- potential for year round spawning could be realised.

The main disadvantage of a separate centralised program is that a dedicated facility would be required with associated costs and a business structure needs to be established clearly identifying stakeholder investment and ownership of IP.

The key issues to address in establishing any selective breeding program are as follows:

1. Detailed methodology for the program (practicality and costs)
2. Breeding objectives and traits
3. Risks and risk management
4. Key researchable constraints (lack of knowledge, technology and methodology)
5. Cost-benefits of alternative models for running the program
6. Commercialisation of the program (investment, pricing, returns to shareholders)
7. Where and how (facilities- public, private, leased or owned)? This is an issue that will need to be addressed outside of this project by the industry.

The majority of these issues can be modeled and the pathway which gives the highest genetic response, greatest economic benefit to industry, and cash flows/returns to the selective breeding company can be predicted. The Seafood CRC through its Breeding for Profit Theme, including the principal investigator (NR) and the CRC’s Theme Leader – Graham Mair, are involved with related projects to scope selective breeding programs for abalone and Yellowtail Kingfish. Models for these selective breeding programs have been developed by NR and can be adapted for the barramundi selective breeding program. DJ has a long-history in barramundi genetic research and has developed the molecular tools to allow genetic audits to be undertaken on hatchery broodfish. He also has experience in the design and conduct of selective breeding programs for prawns, crayfish and pearl oyster species and therefore has a good understanding of the resources and R&D requirements needed to conduct breeding programs.

By addressing these needs outlined above and elsewhere in this proposal the project relates to the

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following Seafood CRC milestones:

1.3.1 New genetic tools and/or appropriate breeding strategies developed for genetic management and improvement of at least two aquaculture species - as the project may lead to the development of a national selective breeding strategy for the species.
1.3.2 Genetic parameters estimated for key commercial traits; genetic improvement programs designed and implemented for at least two aquaculture species - as the project will go some way toward designing a selective breeding program for barramundi and will go some way toward mapping a pathway for the implementation of this program.

4. Need

The Australian barramundi industry (through ABFA) have long appreciated the potential for improving the sustainability and profitability of production through the appropriate implementation of genetic management and improvement of the species. However, previous attempts to develop a coordinated national strategy for this species have not met with success due to a poor understanding of possible models by which a breeding program within the industry can be implemented, the resources required for implementation, R & D impediments and the potential benefits that will arise from a successful breeding program. ABFA views the investment in the Seafood CRC as an opportunity to catalyse efforts towards an industry wide approach to the sustainable and economically viable genetic management and improvement strategy for cultured barramundi.

ABFA prepared a Terms of Reference for the proposed scoping study identifying the following needs and researchable constraints to the development of a barramundi genetic management/improvement strategy:

- lack of a broad, industry wide, understanding of the potential benefits (and risks) associated with genetic management and improvement
- a lack of a clear understanding of the resources (human, economic, infrastructure and genetic) required for the implementation of genetic management and improvement in this species
- gaps in knowledge required for the implementation of genetic improvement including key genetic parameters and economic weights of traits that could be improved.
- limited information on the genetic status of existing hatchery broodstock and thus their potential as founder stocks for genetic breeding programs
- lack of clear guidelines on appropriate genetic improvement strategies

5. Objectives

1. To review existing barramundi-related genetic knowledge to identify relevant research and where the R&D gaps preventing instigation of barramundi breeding programs presently exist (section 7.2).
2. To develop research options that will fill the knowledge gap currently impeding commencement of targeted breeding programs. This will be achieved through consultation with CRC R&D providers and industry (section 7.4).
3. To identify, through direct engagement with industry and R&D providers, the levels of infrastructure and resources that are available to undertake collaborative genetics related R&D
4. Undertake a genetic audit of available hatchery stocks, using established genetic marker systems, to provide a census of captured genetic variation and genealogical relationships among broodstock. This information will be essential for establishing commercial breeding programs, and could be used to identify suitable broodstock for CRC related research, if supported by the commercial company owning the fish.
5. To identify opportunities for additional industry investment in barramundi genetic improvement and related research through consultation with both ABFA and non-ABFA barramundi farmers and other industry groups and the stakeholders in the CRC Breeding for Profit Research Theme.

6. Undertake a prioritisation exercise to identify traits that should be targeted in future R&D and selection programs based on estimation of the economic weights of key traits and consultation with industry members.
7. Conduct a benefit cost analysis of the effective and appropriate implementation of genetic improvement strategies (Section 7.5)
8. Identify ABFA training needs in genetic management and improvement and raise awareness of the potential benefits of genetic improvement to the industry (Section 7.7)
9. Utilising all the information gathered in 1-8 above, identify one or more strategies for the implementation of a nationally coordinated genetic management and improvement program for cultured barramundi. The strategies to be prioritized, and implementation plans drafted, in consultation with ABFA.

6. Methods

6.1 Review existing barramundi-related genetic knowledge (Objective 1):

A literature and internet search was conducted and used to draft a report on the current state of knowledge (and important gaps) of Barramundi genetics. This was presented at the annual ABFA R&D Workshop in Cairns and is presented in this report.

6.2 Develop R&D strategy (Objective 2):

A summary of major risks and gaps to selective breeding were highlighted by the industry as the authors visited farms and hatcheries. Future key research that will be needed to mitigate these risks and close gaps were identified and prioritized.

6.3 Resource availability and requirements (Objective 3):

Visits were made to all major barramundi hatcheries to conduct an audit of potentially available resources, to gather information for the reviews described below and to determine hatchery manager/owners perspectives and willingness to invest in genetic improvement.

6.4 A genetic audit of available hatchery stocks (Objective 4):

An offer will be made to genotype all routinely used hatchery broodstock using a 21 microsatellite parentage assignment suite developed by DJ at JCU to estimate amounts of genetic diversity captured within broodstock hatchery populations within the industry, and to identify genealogical relationships (Frost et al., 2006). In some cases this will require identification of source populations and in these cases samples of hatchery stock will be included (randomly) into a study of the population genetics of Barramundi jointly conducted, in part as a component of this project, by Flinders University and JCU. The information originating out of this audit will be returned to the hatcheries along with a structured broodstock management plan aimed to prevent the occurrence of consanguineous matings within hatcheries. This will in the short-term allow the industry to instantly reduce the rate of inbreeding within their hatcheries.

6.5 Identify opportunities for additional industry investment (Objective 5):

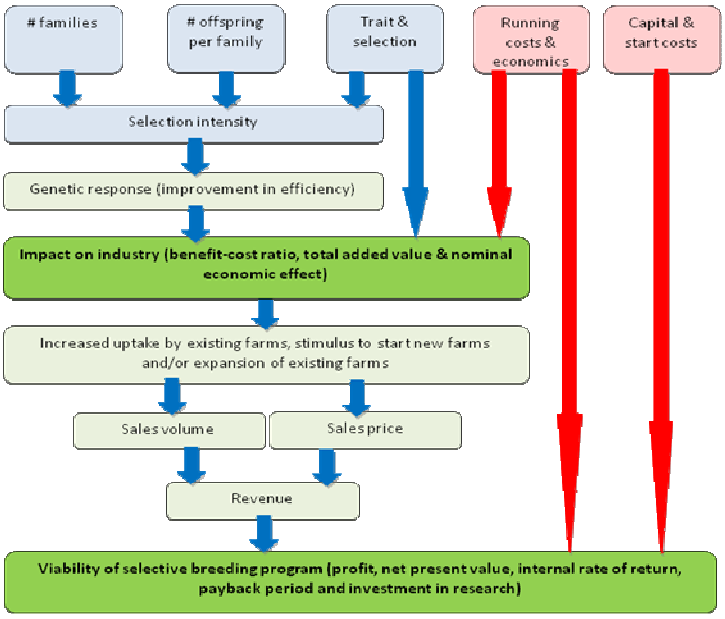
Interviews were conducted with barramundi producers (focussed on hatcheries and including ABFA and non ABFA members) to review options for securing additional investment in genetics R&D and associated benefits and risks.

6.6 Develop economic model and perform benefit cost analysis (Objectives 6 & 7):

The primary data to populate the economic model was obtained from one-on-one discussions that were held with key barramundi producers and hatcheries, Darwin Aquaculture Centre (Glenn Schipp) and Queensland Department of Primary Industries. A number of farm visits and teleconferences were used to review the costs of production and to identify and prioritise key traits for the selective breeding program. This work was done in conjunction with the visits made to determine resource availability and requirements (above). The key findings from the model (including the prioritization of traits based on

the model) were verified in group discussions and at the Barramundi Annual R&D Workshop held in Cairns in February 2009. We estimated the potential economic benefits of the implementation of a genetic management and improvement strategy by incorporating the values derived through these discussions into a bioeconomic model. The bioeconomic simulation model was developed by the PI (NR) and has already been applied to evaluate the impact of new selective breeding technologies on the Atlantic salmon industry in Norway (Robinson and Hayes, 2008) and to evaluate the economics of selective breeding abalone in Australia (Robinson and Li, 2008; Robinson et al., submitted manuscript). The model used for Atlantic salmon in Norway was adjusted by NR to allow its application to the barramundi industry in Australia. This is a basic model that can be used to compare the effects of different breeding strategies when selecting one trait at a time. Once the bioeconomic model was adjusted and populated with data, it was used to help determine the optimum basic strategy for breeding. This was done by evaluating the effects on genetic response, cost-benefit, nominal economic effect and profit per kilo of production when a number of factors are varied (eg. number of families, traits under selection, type and intensity of selection applied, adoption by industry etc). As the effect of selective breeding is accumulative (additional improvement is achieved with each generation), the genetic response and economics were modeled over a number of generations. A brief outline of the model is shown in Figure 1.

Figure 1. Outline of the bioeconomic simulation model used.



The economic part of the model is based on the principle of alternative costs. As an example, when growth rate is considered, the model performs calculations to answer the following questions: How many more fish would we expect to grow in the same growing environment, or how much more efficient would the fish be? What are the economic benefits gained from the increased value of production less increased cost of feed accounting for correlated improvement in FCR? What are the economic benefits from savings in labour costs resulting from the faster rate of production? What are the costs of applying different selective breeding criteria?

The following practical considerations were taken into consideration when adjusting the model for simulating a barramundi breeding program:

- Animals mature as males first, then differentiate into females

- Multiple males are present in a spawning tank
- Most hatcheries have small numbers of spawning tanks
- Most hatcheries use one large tank for larval rearing/hatching
- As larval batch performance can greatly vary it is necessary to select from animals grown together in same environment at the same time (within a cohort)
- Inbreeding should be kept at a low level
- The program should aim for high rate of genetic improvement
- Barramundi are grown in diverse production environments (saltwater, freshwater, pond, cage and tank)

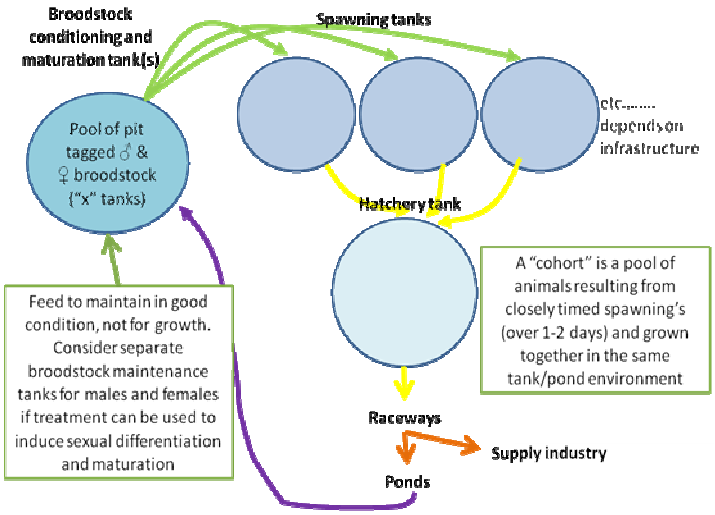


Figure 2. General set up assumed for the basic selective breeding program that was modeled.

A very simple scenario (similar to that used by the Darwin Aquaculture Centre) was modelled for the production of families each generation. We assumed for simplicity that a single breeding nucleus of animals would be established and that:

Step 1. One mature female was transferred into each spawning tank.
Step 2. Two-three males were transferred to each spawning tank.
Step 3. The female in each tank was injected with LHRHa to induce spawning.
Step 4. When spawning occurred, eggs were collected from the spawning tanks and pooled into the hatchery tank (cohort 1 of 5 in a year).
Step 5. Cohorts were set in a separate raceway/pond/tank and performance recorded.
Step 6. All males and females were removed and disposed of from spawning tanks (never re-used).
Step 7. Steps 2-6 were repeated (up to 5 spawning sessions in a year)
Step 8. The best performers were selected. DNA pedigreeing was used to reduce inbreeding.

Two basic alternative mating options were considered as shown in Table 1. These options both account for the current situation, hatchery practices and biology of *L. calcarifer*. A major challenge for selective breeding of this species is that *L. calcarifer* is protandrous, that is animals mature as males first, and then differentiate into females. The challenge is how to present mature males to mature females from same selected generation, as fish will generally be the same gender when chosen as selection candidates. The two options tested were as follows:

Option 1. For Option 1, each generation's males were crossed with the same generation's females. Option 1 therefore assumed that the technology for harvesting and cryopreserving milt from selected individuals (maturing as males at 2 years of age) and for using the cryopreserved milt to reliably fertilise eggs harvested from selected females at year 4 of the same selected generation, was fully developed by the time the first round of selective breeding occurred. This option resulted in a generation interval of 4 years.

Option 2. For Option 2, each generations males were crossed with the previous generations females. No new reproductive technology was needed under this option. The first generation of selected males were crossed with wild caught females. This option resulted in a generation interval of 2 years.

We assumed that males matured at 2 years of age while females matured at 4 years of age. This assumption was based on the experience of DAC using domesticated broodstock (ie. some females matured by 3 years of age) and we assumed that as the breeding program progressed, and as the results from research became known, that we would be able to reliably induce the spawning of males and females of these ages (with no selection for early maturation). We assumed that enough viable eggs would result from these matings to create the relatively small number of progeny required as candidates for selective breeding and to supply the industry with fingerlings.

Table 1. Summary of the two options for mating that were compared.The generation interval was calculated as the number of years from production of families from one selected generation to the next.

	Option 1	**Option 2**
♂s mated with……..	♀s from same generation	♀s from earlier generation
Reproductive technology	Collection and cryopreservation of milt. Fertilisation of eggs with cryopreserved milt	None
Generation interval	4 year	2 year

These are not the only options that are available. They are simple options based on the current state of knowledge and practices for barramundi family production and are based on the current scale of facilities available to the industry for running such a program (such as at the Darwin Aquacuture Centre or at the QDPI Northern Fisheries Centre and Walkamin). More complex options involving new technologies were not considered here, but have been considered as research options. Research project results would allow us to refine these options (improving genetic response, yielding higher benefit for industry and reducing the cost of breeding). Investment in new infrastructure would allow us to upscale the program (ie. produce more families and to grow more families together in the one cohort for selection).

All of the assumptions used in modelling the breeding program are shown in detail in Appendix 3. Major assumptions are described here.

- The selective breeding program we modelled only selected for growth rate. For the actual breeding program other traits could also be considered.
- Production grew steadily from 3,038 tonne by 2% every year.
- One hundred progeny per family were grown out, measured and used as candidates for selection. Broodstock were randomly selected from the 10% best performing progeny each generation.
- We assumed that there was no impact from inbreeding depression on production or fitness.

The facility that was modelled contained four 30,000 litre broodstock conditioning and maturation tanks (each tank capable of holding 30 fish), 10 spawning tanks (giving capacity for 5 spawnings in 1 year for generation of 50 full sibling families), one hatchery tank, raceways for rearing fingerlings and some ponds. The facilities were of a similar scale to that existing at QDPI's Northern Fisheries Centre & Walkamin. The facility was leased for $60,000 per annum. If 100 families were to be produced per generation, we assumed that, in addition to the greater running costs (dependent on number of families and number of fish), $800,000 for one off infrastucture investment costs would be needed to purchase and instal a duplicate larval/rotifer system and additional shed holding 4 broodstock conditioning and maturation tanks with plumbing.

The benefit-cost rato was estimated as total benefits to industry participants divided by total running costs. Total added value as the benefit to farmers per kilo of fish (eg. savings in labour costs, feed, additional opportunities for production due to shorter grow out time). Nominal economic effect on operating income was estimated as the total nominal benefits to the industry participants from growing the improved seedstock less the costs of selectively breeding the improved seedstock. All economic indicators were calculated on an annual basis for the life of the selective breeding program.

The net present value (excess or shortfall in present value terms once finance charges are met) and internal rate of return (indicator of the efficiency or quality of investment) was calculated for the section

of the industry investing in and benefiting from the genetic improvement. It was assumed that 50% of the industry participated and benefited from the breeding program initially and that industry participation slowly increased to 95% by the fourth generation of selective breeding (the fourth generation being reached after 16 years under Option 1 and 8 years under Option 2).

6.7 Identify ABFA training needs in genetic management and raise awareness of the potential benefits of genetic improvement to the industry (Objective 8):

A training needs analysis was conducted as part of the hatchery tour and the workshop, and this was linked with activities under the CRC's education and training program and the Finfish Theme Business plan and its finfish hatchery network which is intended to build a cross sectoral training program in aquaculture genetics.

6.8 Strategy development and industry consultation (Objective 9):

The findings from the above methods were reviewed and summarized in this report and a strategy for industry wide genetic management, improvement and its commercialization was suggested. A preliminary presentation of this strategy was presented and reviewed in the industry workshop held in Cairns in February 2009. At the workshop the findings related to objectives 1-8 were presented, feedback was sought, and here we review and prioritise the proposed strategies.

7. Results/Discussion

7.1 Strengths, weaknesses, opportunities, threats and risks to selective breeding

There are a number of strengths in support of the centralised program in particular, (eg. good opportunities for growth and expansion of such a program) and some weaknesses and threats that need to be taken into consideration and addressed where possible for all selective breeding entities (Table 1). Points in the SWOT analysis that either support or weaken the case for running the selective breeding program as one central breeding facility, in isolation from existing barramundi farm sites, are highlighted with an asterix. We recommend from the risk analysis, and from the advantages outlined in the introduction to this report, that if the industry develops a selective breeding program, they should seek to establish a central breeding nucleus of animals in isolation from existing farms. We explore the pros and cons of this approach further in section 7.5.9 and assume for our calculations in this report that breeding program is run in this way.

Threats and weaknesses have been included in a risk analysis (Table 2) and suggestions for risk management have been made. Some of the key constraints for the breeding program might be overcome through research (ie development of new technologies and methods) and an attempt has been made by the research provider participants in the project to prioritise the research needed (Table 3).

*Table 1. Strengths weaknesses, opportunities and threats identified for barramundi selective breeding entities. Some points in the SWOT analysis depend on the type of entity under consideration. ** indicates where these points relate specifically to a centralised selective breeding entity supplying a large proportion of the industry.*

Strengths	**Weaknesses**
• Industry support & knowledge • Services most of industry** • Large economic benefits for growers** • Stimulus for expansion • Stimulus for new entrants** • Advantage over competitors • Small scale (inexpensive) compared to farm or livestock breeding programs • Multiplier an integral part of program and no additional investment needed to "gear-up" to increase supply • Closed to further entry of new animals leading to reduced risk of disease** • Helps meet increased demand for seafood and reduced volume of supply from wild fishery • Farms save $$ and risk as no need to spawn and mate** • Farms running their own on-farm selective breeding programs save resources (potentially hundreds of thousands of dollars in lost opportunity costs) and benefit from a better (more improved) product** • Future product allows farms to produce more from use of same resources • Genetic improvement and benefits compound with each generation of selection • Flexibility to supply volumes desired by market each year	• Lack of control of sexual maturation, differentiation and reproduction affects selection intensity, evaluation of families grown in same environments, rate of genetic improvement and rate of inbreeding • Lack of basic genetic information (heritability of traits, correlations between traits) affects rate of genetic improvement • Lack of information about family representation in subsequent generations • Size and limits (eg. regulations) on growth of industry • Price farmers are willing to pay for stock • Division in industry () • Industry consists of a small number of farms and the costs of producing and supplying genetically improved seed are essentially the same whether the industry is small or large. Because of this the total benefits-costs, and profitability of the selective breeding entity, are less than for larger aquaculture industries. • Expense (infrastructure and costs) to hold broodstock in tanks • Above points limit possible revenue stream and reduces attractiveness to investors • Succession planning (ie. three staff to operate)

Opportunities	**Threats**
• Merge with other selective breeding companies (national or international) • Share genetic expertise with other selective breeding companies (eg. national organisation) • New or niche markets (?) • Large government owned facilities exist which could potentially be used as selective breeding facilities (eg. DAC, NFC and Walkamin)***	• High AUS$ continues or value of barramundi product slumps • Primary industries rules prevent translocation of improved seed to farms or restrict farms to locally sourced varieties • Translocation between mainland states is restricted by primary industries** • Bad press stemming from competition or green groups (eg. recreational fishing industry or groups concerned about the Great Barrier Reef) • Contamination from pollution, algal blooms or disease • Loss of key hatchery management staff or quantitative geneticists • Genotype by environment interactions mean that selected stock only performs well in a particular environment and not in others • Competitors arise

Table 2. Risk analysis for selective breeding entities. Ratings for A and B. 3=high probability/cost, 2=medium probability/cost, 1=low probability/cost

Threats/risk	**Prob of event (A)**	**Amount it would cost to set things right if it happened (B)**	**Estimate of value for risk (A*B)**	**Managing risk**
Genotype by environment interactions mean that selected stock only performs well in a particular environment and not in others	2	3	6	Assess whether genotype by environment effects are a concern. If so, consider creation of different strains or a multipurpose strain.
High AUS$ continues or value of barramundi product slumps	2	3	6	Accept risk. As improvement is made to the stock that is grown the industry will become more resilient to this risk.
Primary industries rules prevent translocation of improved seed to sea based facilities or restrict sea based farms to locally sourced varieties	2	3	6	Demonstrate strict biosecurity and quarantine measures. Produce infertile stock for growout. Research needed into genetic and other methods.
Contamination from pollution, algal blooms or disease	2	3	6	Contingency for total recirculation. Stock tested on farm can be retrieved in emergency.
Bad press stemming from competition or green groups (eg. recreational fishing industry or groups concerned about the Great Barrier Reef)	2	2	4	Produce infertile stock for growout. Research needed into genetic and other methods. Demonstrate strict biosecurity and quarantine measures.
Competitors arise for the breeding program	1	3	3	Stay ahead (optimised methods, research, protection)

Translocation between mainland states is restricted by primary industries	1	3	3	Demonstrate strict biosecurity and quarantine measures.
Loss of key hatchery management staff or quantitative geneticists	1	2	2	Accept low risk. Provide good working environment (peers, location etc) and national support network for aquaculture breeding programs

7.2 Review of barramundi genetic-related knowledge and research and development strategy

Before efficient breeding programs can be implemented for aquaculture species four prerequisites need to be met (as summarised in Chapter 1.7, Gjedrem, 2005a). These are
1) that the husbandry and reproductive biology of the animal is understood and can be manipulated,
2) the genetic basis for traits (including correlations with other traits) is known,
3) a method to identify pedigree relationships and control inbreeding is in place, and
4) adequate infrastructure and resources are available to support the program, including researchers and extension personnel that are well educated in breeding theory.
Only once all four prerequisites are in place can efficient breeding programs proceed.

One of the primary terms of reference for the Seafood CRC barramundi genetics scoping study was to identify the R&D knowledge gaps in the above four areas that currently impede the Australian barramundi industry developing a breeding program. Consequently, relevant literature relating to barramundi genetics and reproduction was reviewed to establish the current state of knowledge and consultations with industry, researchers, and participants conducting breeding programs for other aquaculture species was undertaken to identify genetics-specific R&D areas that needs be addressed by the industry before they can commence a breeding program. An analysis was also undertaken to gain an understanding of strengths, weaknesses, opportunities and threats that have to be considered for barramundi selective breeding programs in general (Table 2, 3).

7.2.1 Control of sexual maturation, differentiation and reproduction

Rationale

Perhaps the biggest impediment to barramundi selective breeding is the inability to control sexual differentiation, sexual maturation and the consequent inability to spawn/reproduce broodstock in a planned and synchronised way. Ideally, we need to be able to quickly transform half of the broodstock into highly fertile males and half of the broodstock into highly fertile females, select and mate particular individuals together as required and synchronise the timing of production of large numbers of families. Control over these features is essential so that we can achieve a high rate of genetic improvement and a

low rate of inbreeding, and so that the breeding program can be run in an efficient way (minimising running costs and needs for infrastructure).

In barramundi there are two major reproductive problems that impact on breeding programs. The first is that males prematurely change into females under culture conditions. This can mean that fish selected as males change sex into females (or are transitional) before they can be used for breeding, or that some fish never fully mature as males. The second problem has to do with maturation of broodstock. Broodstock can be hormonally induced to spawn using hormones (LHRHa), but there is still variability in maturation and gamete quality, particularly sperm maturation.

Several studies have looked at the problem of sex control in barramundi. Gamage (2001), for example, examined the effect of nutrition on male reproductive development in an attempt to understand if nutritional factors drive male-female sex changes. Here, extreme feeding regimes (ie starvation, re-feeding, low dietary energy) impacted on male reproductive development (ie either decreased or increased male reproductive development), but no clear effects of feeding regime on the transition of male to female gender were observed. Forrester (2001) examined the addition of exogenous oestradiol-17ß (E2) on sex change and demonstrated that treating males with E2 at a dosage > 1.5 mg.kg BW^{-1} initiated degeneration of male testicular tissue and the proliferation of oocytes. After 28 days all males that were treated had either undergone sex change into females, or were transitional. However, this study did not examine if artificially induced sex-changed barramundi were fertile, or produce quality eggs. The reversal of sex from females into males, and the maintenance of males under prolonged reproductive condition, through addition of testosterone or 11-keto-testosterone appears harder to achieve and has not been documented.

The impact of administrating exogenous hormones on male barramundi milt production has also been investigated. Hilomen-Garcia, et al (2002) examined the response of sexually mature male barramundi to injections of luteinizing hormone-releasing hormone (LHRHa) and 17a-methyltestosterone. Their study showed that injections with 20 ug kg^{-1} BW LHRHa without 17a-methyltestosterone led to approximately three-fold higher total expressible milt volume and spermatozoa counts over control fish, indicating a stimulation of spermatozoa production and increase in milt hydration. However, a combination injection of LHRHa and 17a-methyltestosterone led to an overall lower spermatcrit, with the addition of 17a-methyltestosterone suppressing milt hydration. This suggests that the addition of masculinising hormones has a negative effect on milt production and is not a viable option for increasing spawning success.

We need to be able to artificially induce and control sexual maturation in the breeding program without selectively breeding barramundi that mature prematurely in the production environment.

Knowledge gaps

Currently there is a poor understanding of the genetic and environmental mechanisms leading to sex change and maturation in barramundi. Research has nevertheless demonstrated that it is possible to speed up the process of sex-change from male to females through the addition of exogenous hormone implants. What is unknown, however, is whether early sex-changed females are fertile and produce viable larvae and would be useful in a breeding program. The ability to induce an early sex-change from males to females has obvious benefits to a selection program as it will reduce the amount of infrastructure and costs required to maintain broodfish and increase the genetic gain through reducing generation interval. The other major gap in knowledge relates to male spermatozoa maturation.

Benefits to breeding programs

- Control of reproductive cycle, increased spawning reliability and timing.
- Increased fertility
- Production of large numbers of families (higher rate of genetic improvement, control of inbreeding)
- Ability to synchronously produce family material from desired mate pair combinations
- Ability to test and compare the performance of large numbers of families and individuals in the same environments at the same time
- Sex-inversion from male to female which would shorten the generation interval leading to increased genetic response
- Reduced need for broodfish holding infrastructure and reduced broodstock holding costs.
- Increase opportunities for cryopreservation and artificial fertilization

Constraints

- Low fertility, small eggs numbers (although large egg number is not a requisite for a breeding program).
- Male sexual differentiation and sperm maturation
- Shortage of mature males
- Public perceptions over hormonal manipulation

Strategy

Two major research experiments are recommended.

Development of a genetic management and improvement strategy for Australian cultured barramundi

1. Research has shown that it is possible to sex-invert males into females (Forrester, 2001). The commercial feasibility of inducing early male-female sex change through the addition of exogenous hormones should be further scientifically investigated to better understand the influence age and commercial hatchery conditions have on artificial sex-inversion. Furthermore, fertility, egg quality and larval survival from artificially sex-inverted females needs to be assessed. This research is ideally suited to be undertaken at the Darwin Aquaculture Centre, or one of the major participating hatcheries.
2. Research into male spermatozoa maturation (and to a lesser extent female egg quality) – there is still uncertainty about the environmental factors influencing gamete maturation – particularly in males. Consistent male maturation appears to be the single biggest impediment to large-scale breeding programs. Further experiments involving manipulation of nutrition, environment and hormones should be instigated with the end goal of quantifying spermatozoa production and maturation.

Infrastructure

Adequate infrastructure and expertise to undertake research in the two key areas outlined above are available within both the Northern Territory and Queensland Department of Primary Industries and Fisheries. Infrastructure required involves tanks to hold experimental fish and artificially sex-inverted females, larval rearing capacity to determine fertility, and replicated recirculated tank systems to manipulate environmental parameters. A capability to measure sex hormones and gonadal status is also required. Aquaculture animals can be sourced for experiments.

Linkages with other projects

The researchable "Control of sex and reproduction" links into the researchable component "Novel breeding technologies to increase genetic gain". The fertile males and females can be directly used in the ongoing breeding program.

Costings and Timings

Projects that increase our knowledge of sex control and reproduction are expected to take 2-3 years to undertake. Direct costs (exclusive of labour) are anticipated to be between $40 000 – 60 000 p.a.

Possible articipating institutions

QDPIF, NTDPI, CSIRO, Industry participants.

7.2.2 Genotype by environment interactions and their influence on selection for production traits

Rationale

Realisation of genetic potential of an organism is determined by the genes it possesses and their interaction with their external environment. As has been shown for both domesticated animals and plants, often genotypes perform better in one environment than an alternate environment – probably as a consequence of differing selective pressures (so termed genotype by environment (GxE) interactions). Genotype by environment (GxE) interactions appear to be pervasive in agricultural systems (Baker, 1987) and complicate selection for broad adaptation because often genotypes selected for high performance under one suite of environmental parameters fail to perform to their potential when reared in disparate environments (Basford and Cooper, 1998).

GxE interactions when present can manifest in two ways, the first being a scale effect whereby genotypic differences among individuals from different families/populations may be greater in one environment than another, or secondly, where an interaction results in a rank change whereby the best genotype in one environment is not the best genotype in another environment (Gjedrem, 2005b). Those GXE interactions that result in a change of ranking, such that the families that perform best in one environment do not perform well in another environment, are the type of interactions that have serious consequences for the design of the selective breeding program. GxE interactions are highest when the culture system is subject to extremes of environmental stresses, as may be the case for pond (low salinity) and sea cage (high salinity) based barramundi. If genotype by environment interactions are particularly significant this effect may necessitate separate selection programs targeting individual culture systems to be established, or that a multi-purpose strain is created.

Encouragingly for fish aquaculture, many studies with sufficient power for detection have failed to find significant GXE interactions on the realisation of growth. Studies with European seabass (Dupont-Nivet et al., 2008) and rainbow trout (Fishback et al., 2002) for instance, showed that the best performing genotypes in one environment remained the better performing individuals under an alternative environment. However, in these instances selection pressures between culture environments were relatively low, as these authors were comparing performance of genotypes reared in similar culture systems at different locations. A more relevant comparison to that of barramundi may be the study of Eknath, et al (2007), where they found the genetic correlation between growth performance of genetically selected tilapia (*Oreochromis niloticus*) families reared in earthen pond and cage environments to be quite low and variable (0.36-0.82), demonstrating in this case at least, that the divergent culture systems significantly influenced realisation of genetic growth.

Barramundi in Australia consist of at least three major genetic stocks (Kimberley, Top End and East Coast) and are farmed under a diverse range of culture systems, ranging from seacages, ponds, raceways and tank-based recirculation systems. They also are farmed in both freshwater and saltwater and under different thermal regimes. Complex population genetic structure, coupled with regional environmental pressures, makes barramundi a possible candidate for formation of genetically based adaptation. As a consequence, understanding how barramundi genotypes respond to differing culture systems is imperative if an efficient industry-wide breeding program is going to be implemented.

Despite the fact that barramundi are genetically structured, there have been few studies which have attempted to simultaneously quantify the influence that disparate environmental or culture conditions have on the expression of phenotypes. All studies published to date which might be considered relevant have focused on nutrition or environment in isolation of genetics, or population of origin. For example, Katersky and Carter (2007) examined the effect of temperature on feed intake, growth rate and growth efficiency in juvenile barramundi. The findings from this study showed that increasing temperature from 20-39℃ resulted in increases in feed intake and specific growth rate up to a maximum of 33℃, whereafter feed intake and growth decreased. However, this experiment was based on a single genetic strain and it is unknown whether different genetic stocks respond to increasing temperatures in a similar way. In fact, recent research based on evaluating the upper thermal maxima and aerobic swimming performance under temperature stress for two barramundi genetic strains suggest the presence of genetically determined adaptations to temperature that may need to be factored into future industry selective breeding programs (Newton et al unpublished, Edmunds et al, unpublished). Differences of salinity and culture method (pond vs recirculation) have not to date been formally evaluated.

Knowledge gaps

There is presently no knowledge on the impact differing environments will have in the realisation of genetic potential in barramundi. More specifically, it is not known if superior genotypes, whether strain or family based, will maintain their superiority in a range of culture systems, or if culture-specific strains need to be considered. There is particular concern from some farmers that genetic strains may perform differently in Queensland compared to the Northern Territory, or that pond-selected fish will not perform in intensive recirculation systems.

Benefits to Selection Programs

- Knowledge about the reliability of selectively bred barramundi (eg. in terms of growth rate performance) under different culture practices
- Estimation of responses of other correlated traits (ie length-weight) under different culture practices

- Knowledge is needed in considering the design of the breeding program (1 strain, multipurpose strain, culture environment specific strain)

Constraints

- Requirement to produce enough numbers of families, and offspring within families, to detect G X E
- Infrastructure needed to hold broodstock
- Translocation policy
- Co-ordination of hatchery spawnings, farm stockings and collection of data
- Disease management policy
- Co-operation among industry partners

Strategy

Genotype by environment impacts are estimated through examination of the magnitude of genetic correlation between genotype performances in different test environments (Falconer and Mackay, 1996), and as such, such a project would require co-ordination of spawnings to allow the simultaneous production of 20-30+ families and the on-rearing of families under a range of farm-based culture systems. Government aquaculture facilities with grow-out capacity (ie QDPI Walkamin, Cairns, Bribie Is, JCU Townsville) would also be necessary so that systematic data could be collected on growth traits and survival in addition to final harvest data. Due to the pooling of families and rearing within a culture system under a common environment, such a project would also rely on DNA parentage technology to estimate family means and variances at harvest.

Infrastructure and personnel requirements

- Family production - Based on current infrastructure no single hatchery at the present has the capacity to produce 30 barramundi families simultaneously. However, development of novel breeding strategies (see researchable 5) and/or co-ordination among major hatcheries could produce this number of families. Hatcheries would be required to spawn as many families as possible and then transport larvae to a designated central rearing facility (ie DAC, Northern Fisheries Centre) where larvae will be reared until juvenile stocking. Disease certification would be required and translocation issues resolved before shipping to evaluation farms.
- On-rearing juveniles - At least one farm production unit per culture system would be required, with fish under evaluation treated according to normal culture practices of the farm. To fully evaluate the impact genotype by environment interactions might have in the realisation of family growth, families should be evaluated in both freshwater/saltwater ponds, and tank-based aquaculture systems.

Linkages with other projects

Development of a genetic management and improvement strategy for Australian cultured barramundi

R&D examining the impact of GXE can be integrated with projects that estimate genetic parameters (Research project described in section 7.2.3) and composition of production units (Research project described in section 7.2.4) (see Figure 3).

Participating institutions

QDPI&F, NTDPI, James Cook University, Flinders University.

Timelines and Costing

A project evaluating the impact of GXE on production traits will take three years to conduct. This allows the establishment of experimental procedures and co-ordination of fingerling production. Major costs will be incurred in years 1 and 3 and are associated with fingerling production/purchase, disease testing and DNA pedigreeing. At least one project officer will be required to be appointed to co-ordinate research and this may come as in-kind contribution from participating R&D providers or industry. Additional assistance will also be required to help during peak sampling and data collection periods. Direct research costs (excluding labour) related to the project are anticipated to be ~$90 000-$120 000 in year 1, $50 000 -70 000 in year 2, and $100 000 -$120 000 in year 3. These costs are dependent on substantial in-kind contribution from government institutions and industry for infrastructure use and day-to-day culture costs.

7.2.3 Genetic parameter estimation

Rational

Before commencement of a selective breeding program it is prudent to gain an understanding of the fundamental genetic mechanisms determining the expression of a commercially important trait. More specifically, we need to know the extent to which the expression of a trait is determined by the genes inherited from its parents (=heritability of the trait), and we need to know the correlative relationship with other traits of economic interest (=genetic correlation between the expression of two traits). This information will help us to design efficient breeding programs (Falconer and Mackay, 1996).

If a trait has a low heritability, most of the variation seen in a population is primarily due to environmental influences and selection will have very little impact on increasing the proportion of desirable phenotypes within the population. Alternatively, a trait with high heritability will exhibit rapid responses to selection. Estimating the heritability of traits before breeding programs commence is useful, as the estimates obtained can aid in choosing which selection strategy to adopt and assist in predictions of long-term genetic gains.

Similarly, knowing how traits are genetically correlated allows breeding programs to be designed that prevent adverse selection of non-target traits. Often the phenotypic expression of two traits share similar genetic pathways and therefore selection on one trait can indirectly select on the second trait. Depending on whether the two traits are positively or negatively correlated, selection on the primary trait can either result in a correlated improvement in the performance of the second trait, *or* a decrease in its performance. Most growth traits in fish (ie body weight, fish total length, fish depth) are positively correlated and selection for body weight usually results in advantageous selection for longer fish. However, in rainbow trout, selection for increased growth and feed conversion efficiency is negatively correlated with resistance to viral haemorrhagic septicaemia, which indicates that selection purely for growth in this species will increase the susceptibility of cultured animals to this disease (Henryon et al., 2002). Although body weight at harvest is the main objective of current breeding programs in aquatic species, other traits, such as survival, disease resistance, feed efficiency and flesh quality are now being included in order to meet the future demands. Therefore knowledge of how commercial traits correlate is essential to the long-term success of a breeding program.

Within the literature, there is only one study which has estimated heritability of growth traits in barramundi. Wang et al, (2008b) conducted a preliminary analysis into the heritability of various growth traits in 90 day old juveniles and reported heritabilities of 0.22 (±0.16), 0.31 (±0.14) and 0.22 (±0.22) for body weight, total fish length and condition factor, respectively. Although these estimates are based on small numbers of families (n = 19), they roughly approximate those of other fish species where growth

traits tend to have a heritability of 0.3 and the results of Wang et al's (2008) study may be useful information to initially apply to an Australian breeding program. However, no data was provided on the heritability of growth-related traits at harvest sizes, or on how traits are genetically correlated and these will need to be generated before highly efficient breeding programs can be designed and accurately evaluated.

Knowledge gaps

Besides the one study of Wang et al, (2008b), which should be only considered as preliminary, there are no accurate genetic parameter estimates for growth-related traits in barramundi. Within the wider fish literature, however, there are numerous estimates for growth traits for other fish species (ie. Vandeputte et al., 2004; Winkelman and Peterson, 1994) which could be used as a guide until such time as accurate genetic parameters become available for barramundi.

Benefits to selection programs

- Improves breeding program design
- Accurate prediction of genetic gains
- Understand how non-target traits will respond to selection on primary trait

Constraints/Threats

- Requirement to produce enough numbers of families for accurate estimation of genetic parameters.
- Infrastructure needed to hold broodstock
- Adequate representation of families within on-reared cohort. Obtaining accurate estimates of genetic parameters depends on being able to accurately estimate family means and variance for the trait of interest. Due to differential broodstock contribution in mass spawning species like barramundi, often spawning cohorts are dominated by a few families, with the remaining families having small representation. The approach of Michael Macbeth's progeny test which uses artificial fertilisation if it proves feasible may circumvent this problem as it could allow family contributions to be equalised at time of juvenile stocking.
- Disease management

Strategy

Accurate estimation of genetic parameters relies on the production of enough half/fullsib families and subsequent examination of how variation for a trait can be explained by these sib relationships. As a result, like estimating genotype by environment effects, a project to estimate genetic parameters will rely on the simultaneous spawning of 20-30+ families and the on-rearing of juveniles until harvest in a

common environment. At harvest comprehensive measurements need to be taken on growth and other traits. DNA parentage analyses will need to be applied to assign pedigrees to fish.

Infrastructure requirements

- Family production - A hatchery which has the capability of producing 20-30+ half-sib/full-sib barramundi families is required. No hatchery which we visited currently has this capacity using present culture techniques, although the government facility at NFC in Cairns if dedicated to barramundi has adequate facilities to hold the required number of broodfish.
- Rearing ponds/tanks - In its simplest form on-rearing of families could be conducted in a single pond or tank and periodic measurements to obtain interim estimates of growth-related traits can be performed. In its simplest form if translocation and disease issues can not be overcome this research can be conducted on a single farm, or research facility like Walkamin.

Linkages with other projects

R&D to estimate genetic parameters can be integrated with projects to determine genotype by environment interactions (Research project described in section 7.2.2), composition of production units (Research project 4) and development of novel breeding technologies (research project described in section 7.2.5) (see Figure 3).

Costings

As for genotype by environment project

Participating institutions

NTDPI, QDPI, Industry farmers, JCU, Flinders University

7.2.4 Family composition and metrics of populations

Rational

Before selective breeding programs can be accurately modelled and implemented an understanding of how families are represented within cultured populations is essential. This involves identifying how families may perform relative to each other, and more importantly, how family mean and variances are distributed within a population. This knowledge is particularly important if the program is based on DNA parentage determination methods, which due to cost constraints generally restrict genotyping to the extreme tails of a trait's distribution within a population (ie best performing 500 individuals).

Barramundi are semi-mass spawners and as a result common commercial hatchery spawning practices are to hormonally induce 1-2 females in a tank with up to 6 males. DNA parentage studies have demonstrated that each resulting "batch or cohort" of larvae consists of several half-sib families, depending on the number of males/females in the tank and the reproductive contribution of the male fish (Frost et al., 2006; Wang et al., 2008b). The contribution of each broodfish to the cohort is generally skewed, with progeny from one or two male/female pairings dominating the cohort. Throughout early larval culture the contribution of each broodfish to the cohort can increase or decrease depending on the fitness of the family under culture conditions. This can often result in the amount of families being represented in the cohort by the time of juvenile stocking to be significantly lower than that initially spawned. Additionally, in the case of barramundi, where cohorts of fish are heavily graded to prevent cannibalism, it is possible that family compositions can be even further skewed, whereby the slower growing families have a reduced representation in faster growing grades (Frost et al. 2006). These processes of differential survival and size-grading may mean that by the time of harvest family genetic variation is too limited for the efficient conduct of selection programs, or if all families are still represented in the faster grades, that large numbers of fish will need to be genotyped to identify superior individuals from all the families initially stocked.

As well as understanding the family distribution of growth traits, discussions and subsequent literature searches have failed to find information on how sex ratios are represented within aquaculture populations. There is anecdotal evidence that after 2 years of culture, sex-change is occurring early in cultured barramundi and that some fish may even mature first as females instead of males. Early sex change has important ramifications for breeding programs, as it directly affects the design of mating strategies and broodstock management.

Knowledge gaps

Currently there is no long-term data on family variance in growth rates and sex ratio traits (eg. time until male/female sexual maturity).

Benefits to selection programs

- Can be examined using existing farm populations
- Improves breeding program design
- Accurate prediction of genetic gains
- Essential for projects estimating heritability, GXE
- Reduce inbreeding
- May reduce broodstock numbers to be held

Constraints/Threats

- Specific experiment should involve production of 20-30+ families
- As most farms do not process the animals on farm, the sex status is difficult to record based on current practices
- Industry cooperation

Strategy

To acquire this information two strategies are suggested.

1) Rudimentary information on how family variance in growth rates and sex ratio traits (eg. time until male/female sexual maturity)are distributed within aquaculture cohorts could be obtained from genotyping harvested fish that are currently under culture on industry farms. To get an accurate representation of how family diversity is represented within culture units several farms would need to be examined. The strategy here would be to take tissue samples from fish at harvest, collect growth and sex information, and then estimate family means and variances for each trait. The success of this approach, however, will be dependent on how many families are initially represented in each cohort, and genotyping studies at JCU have indicated that this can be very variable and may range from 3 up to 10 half-sib families. The advantage of this approach is that information on family distributions could be obtained relatively quickly.
2) For comprehensive information on family distributions of traits a large-scale breeding experiment is proposed. Here 20-30+ families of barramundi would be produced (could be by simultaneous spawnings by several hatcheries) and stocked into several production units according to standard grading and culture practices. Growth and sex traits would be measured at harvest and DNA parentage analyses used to estimate family means and variances. The advantage of this approach is that this experiment obviously links in with that of estimating genetic parameters and GXE interactions.

Infrastructure requirements

- Strategy 1 – Participating farm production units

- Strategy 2 - Family production - Based on current infrastructure no single hatchery at the present has the capacity to produce 30+ barramundi families simultaneously. However, development of novel breeding strategies (see researchable 5) and/or co-ordination among major hatcheries could produce this number of families. Hatcheries would be required to spawn as many families as possible and then transport larvae to a designated central rearing facility (ie DAC, Northern Fisheries Centre) where larvae will be reared until juvenile stocking and where initial family compositions can be estimated using DNA parentage analyses.

    - On-rearing – Facilities would be required to rear pooled cohort through to harvest. This should involve at least 3 replicated ponds and 3 tank-based production units.

Linkages with other projects

This project directly links with projects to estimate genotype by environment interactions (Researchable described in section 7.2.2) and genetic parameters (Researchable described in section 7.2.3).

Costings

Direct costs (excluding labour)

- Strategy 1 - $40 000
- Strategy 2 - $110 000

Participating institutions

NTDPI, QDPI, Industry farmers, JCU

7.2.5 Novel breeding technologies to increase genetic gains

Rational

Modern breeding plans are a balance between maximising genetic gains, whilst simultaneously minimising rates of inbreeding accumulation. How these two goals are achieved depends largely on the design of the breeding program and its capacity to manipulate the genetic contribution of individuals under selection. The greater control breeding program managers have over the manipulation of genetic contributions, the more sophisticated breeding programs can be, resulting in higher long-term genetic gains and overall sustainability of the program.

The reproductive biology of barramundi poses particular problems to the design and conduct of breeding programs. Spawning behaviour is induced in barramundi in the presence of multiple males, which appears to preclude reliable one-on-one male/female pairings. Therefore at this stage there is a lack of confidence in the capacity to produce the large numbers of families that would be required for a breeding program. Broodstock are also large fish and require significant space and expense to maintain. Finally, under aquaculture conditions male barramundi appear to prematurely mature into females which pose problems in long-term holding and use of broodfish.

Research into mass production of a large number of families in barramundi has been very limited. Current hatchery procedures generally limit the number of spawners to 1-2 females and several males. However, in tanks containing large numbers of broodfish where natural spawning of non-target females has occurred along with hormonal induction of targeted females, there is DNA evidence for up to 9 full-sib families being produced in a single night's spawning (Smith-Keune and Jerry, unpublished data). Wang, et al (2008) also report results from a heritability study where they combined 10 mature males with 10 females and from this mass-mating cohort produced 19 full-sib families (ie all but one male contributed). These two studies show that it is therefore possible to produce large numbers of families in single tank spawnings, however, in both these examples family contributions to the cohort were extremely variable and this may still pose a constraint to further selection programs.

Successful barramundi breeding programs will require good mating control so that adequate numbers and equal contributions of families result. One way this has been achieved in other fish species is through the development of artificial fertilization techniques (ie the Atlantic salmon breeding program where all families are the result of controlled strip spawnings). Two studies have developed sperm cryopreservation techniques for barramundi (Leung, 1987; Palmer et al., 1993), with the study of Palmer et al, (1993) going one step further through actually evaluating the fertilization success of cryopreserved sperm and "stripped" ova. Here hatch rates of 84% were achieved, with 29 dph fingerlings showing no abnormalities. However, sperm for this experiment was collected from wild sperminating males and it is

uncertain given maturation problems in male cultured barramundi whether this approach could be adopted in a large-scale breeding program.

Knowledge gaps

Fertility experiments with cryopreserved sperm and the use of mass spawning broodstock populations have been reported in barramundi and other species(oyster, Adams et al., 2008; Mediterranean sea bass, Fauvel et al., 1998; barramundi, Palmer et al., 1993). These studies demonstrate that it may be possible to have control over the reproductive cycle and/or to produce large numbers of families using limited infrastructure. However, these approaches have not been adopted by Australian industry and their applicability to breeding programs needs to be formally assessed.

Benefits to selection programs

- Control of family contribution
- Enable production of large numbers of families
- Lower infrastructure requirements
- Increase flexibility in breeding program design (ie progeny test model becomes viable)
- Preservation of family genetic resource
- Disease testing of sperm/eggs for nodavirus

Constraints/Threats

- Reliable numbers of mature males
- Reliable numbers of gravid females
- Broodstock handling difficulties
- Mass spawnings may give variability in family number and contribution
- Industry cooperation

Strategy -

Two experiments should be conducted.

1) Evaluation of artificial spawning on a commercial scale. The practicality and efficiency of strip spawning male and female barramundi should be formally evaluated (this could be done in combination with other experiments).
2) Family output from mass spawning cohorts. An experiment whereby 10 female and 10 male broodstock are hormonally induced and mass-spawned should be undertaken. Larvae should be grown till fingerlings and then mating success and family contributions determined by DNA parentage analyses. Concerns about larvae hatch time issues and its downstream effects should be formally evaluated.

Infrastructure

Both these experiments require access to large numbers of broodfish and capacity to rear larvae. These experiments are ideally suited to be undertaken at the Darwin Aquaculture Centre and should involve QDPIF researchers with experience in artificial spawning of barramundi.

Costings

Direct costs (excluding labour)

Strategy 1 - $25 000

Strategy 2 - $35 000-40000

Participating institutions

DAC, QDPI&F, JCU

Other research areas identified

As well as the five major research gaps identified above, our consultations also identified nodavirus testing, breeding for resistance to disease and use of genetic markers linked to traits as possible constraints to breeding programs. They, however, have not been critiqued as possible projects to be conducted within the Seafood CRC for the reasons below.

7.2.6 Nodavirus testing

Threats posed by nodavirus and other communicable pathogens have to be considered for any future breeding program. In relation to nodavirus, current tests often result in misdiagnosis and there is a real need for future research into the development of more accurate diagnostic tests. Several groups globally are currently working with nodaviruses from other fish species and have had similar problems as seen in Australia in developing reliable diagnostic tests. Therefore the development of new nodavirus detection methods does not appear to be easy. The Seafood CRC also will not generally support disease R&D and is unlikely to fund a barramundi nodavirus research project. Funding should be sought outside the Seafood CRC for nodavirus research.

7.2.7 Genetic improvement of disease or parasite resistance

One strategy to reduce the economic impacts of disease in aquaculture is to breed for resistance. However, effective breeding programs targeting resistance to a specific pathogen require both accurate disease diagnosis/challenge testing procedures and the capacity to estimate breeding values based on mean family performance. The later requirement usually dictates that a breeding program is already functioning, because breeding values of broodstock selected for resistance need to be estimated from the mean resistance of their siblings (ie sibling selection). Due to disease transfer risks, selected animals are never actually exposed to the pathogen. Instead, a sample of offspring from each family is normally exposed to (challenged with) the disease and the performance of these animals is used to estimate breeding values for the family As highlighted in 6) above, the Seafood CRC is unlikely to invest in the R&D required to develop accurate disease diagnosis and challenge procedures and funding to develop diagnostic tests should be sourced from other sources.

Fortunately, disease resistance traits often are correlated with growth and survival traits (eg. Table 5.6, Gjedrem and Olesen, 2005) and selection for these later two traits in an early breeding program could result in a correlated positive improvement response to pathogens.

7.2.8 Genetic markers linked to growth traits.

Several studies have actively pursued the identification of genetic markers linked to barramundi growth traits (De Santis et al., 2008; Wang et al., 2006; Wang et al., 2008a; Xu et al., 2006). However, many of the genetic markers so far found have proven to be either unreliable across multiple families, or found in Asian populations and appear to not be present at high frequencies in Australian barramundi (Smith-Keune and Jerry, unpublished). Genetic markers can also not be applied without an existing breeding program on which to base selection decisions (ie. markers provide an indirect means for selecting animals for breeding, and their use may be advantageous for some traits that are otherwise difficult to directly measure on living breeding candidates such as meat quality traits). Because of these reasons R&D into their use in Australian barramundi as part of Seafood CRC research is premature and is not recommended as a primary researchable until the industry commences a breeding program. The seafood CRC is conducting a benefit-cost analysis on the development and use of marker assisted selection for barramundi and other species.

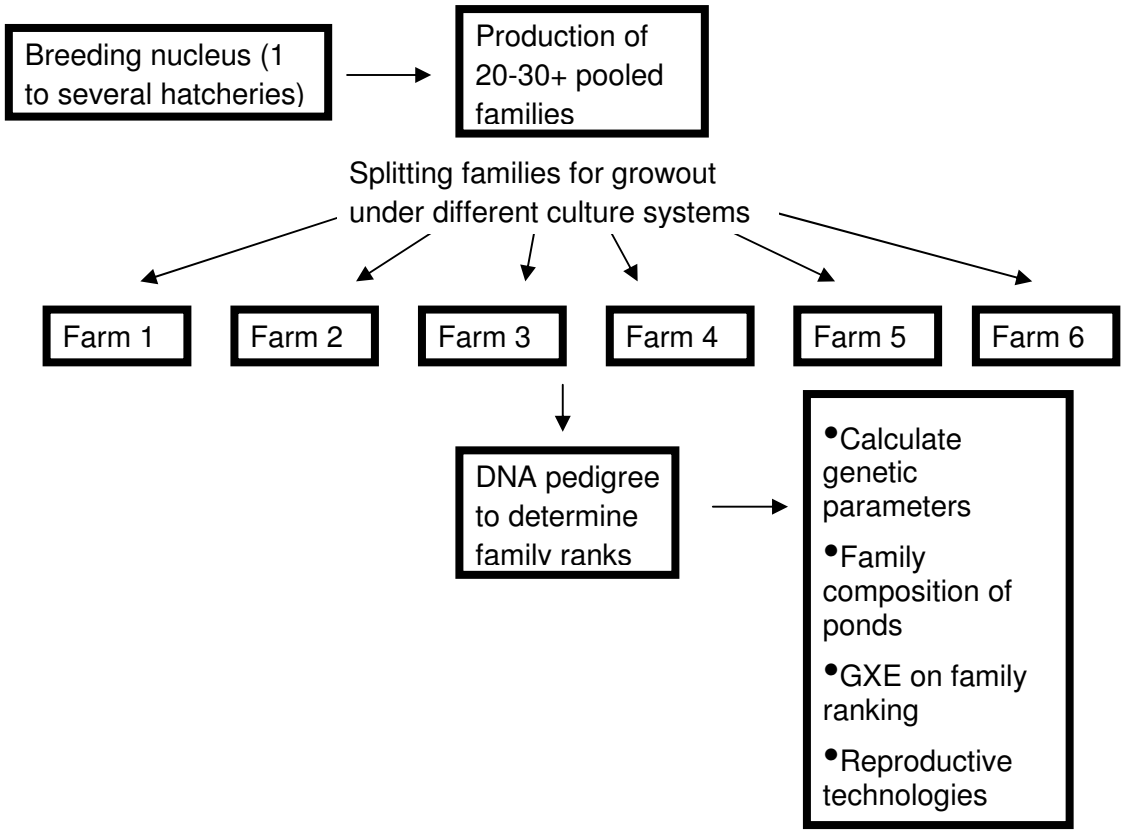


Figure 3. Simple illustration of how one large experiment can fill knowledge gaps in family composition, genetic parameters and genotype by environment interactions.

7.3 Major constraints and threats to R&D "researchables"

7.3.1 Translocation, Natural Population Genetic Structure and Disease

The movement of barramundi within Queensland and between State jurisdictions is regulated as Summarized in the following policy/information documents – Queensland Aquaculture Policy FAMOP015, WA – Fisheries Management Paper 159, SA – Aquaculture Act 2001 – Fact sheet 34/3, NSW Barramundi farming policy 1997, VIC Fisheries Victoria Management Report Series No. 65 and 47, NT Fisheries Act. The national policy on the translocation of aquatic organisms is described in the document "National Policy for the Translocation of Live Aquatic Organisms – Issues, Principles and Guidelines for Implementation, Ministerial Council on Forestry, Fisheries and Aquaculture 1999". In summary, there is no specific policy relating to genetically improved animals and under these policies the translocation of animals would be dealt with on a case-by-case basis.

Translocation/disease policies pose a current constraint on dissemination of genetic material originating from selective breeding programs. However, most policies have contingencies allowing risk assessments to be undertaken on a case by case basis and clarification will need to be sought by the **ABFA executive** from State agencies on the requirements they will have to meet to farm improved barramundi, or barramundi originating from outside current management zones. The number of genetically improved aquaculture species farmed in Australia is slowly increasing and in many cases fisheries management agencies are in the process, or have the capacity under their relevant State Fisheries Acts, to draft species-specific policies regulating the culture of improved animals.

Disease management and the real risk of disease transfer have to be considered in achieving some of the researchable components, and in future breeding programs. Disease management is also covered by the various Fisheries Acts and Discussion papers and each State has clear guidelines on importation and movement of aquatic organisms. As with normal practice, barramundi destined for research projects in various regulatory jurisdictions (ie GXE experiment) or ultimately originating from a breeding program, will have to be health tested/certified free for reportable pathogens. The requirements of these fish should not be considered different from those outlined in current State translocation/disease policies (QLD FAMPR002 – Health protocol for the importation and movement of live barramundi). However, given the complexity of disease detection it is recommended that an extensive multiple testing strategy/quarantine protocol is put in place to lessen the risk of disease transfer and advice from regulatory agencies on additional requirements should be sought from the ABFA executive.

The potential for escaped selectively bred stock, or stock derived from interstate and grown in aquaculture, to have an ecological impact or to affect local genetic structure or diversity has been considered as part of the risk analysis performed for translocation protocols in some states (eg. Victorian Fisheries Management Report Series No. 65 and 45). Some of these risks have been rated as

moderate in some instances (eg. the risk that translocation of domesticated abalone seed to offshore culture systems will result in competition with, and displacement of, wild marine species populations was seen to have a potential moderate likelihood and moderate consequence, whereas disease transfer was seen to have higher likelihood, consequence and potential risk, Victoria Fisheries Management Report Series No. 45).

7.3.2 Infrastructure/Resources

Most of the researchable questions outlined in this R&D document require the production of large numbers of families, and this resource could essentially be used as the base population for the breeding program. Our survey of government and industry infrastructure available for breeding experiments showed that collectively enough resources exist to produce the number of families needed for the selective breeding program. It was noted that it may even be possible that major hatcheries and/or government facilities could produce near the number of families required with adequate commitment of resources to a program. We envisage that a facility with 10 x 30-50t tanks and the capacity to do multiple spawning and larval rearing would be necessary to produce the required families under current spawning rituals. However, if novel breeding approaches can be proven and applied the required amount of infrastructure is reduced dramatically. Until such time available infrastructure is a major constraint to the progression towards answering the R&D questions outlined.

Like the breeding program itself, grow-out experiments will require commitment of at least one culture production system from a number of industry farms, as well as utilization of tank and pond infrastructure at government research facilities. QDPI&F has ideal facilities both at Walkamin and Bribie Island that would be appropriate to run many of the growth experiments with replication.

7.3.3 Broodstock availability

Based on discussions with all of the major hatcheries producing barramundi seedstock, we estimate that there are less than 500 captive-held broodfish within the industry. A breakdown on sex ratios was not available. Collectively this is sufficient to produce the required number of families for many of the R&D projects outlined. However, not all fish mature at the same time and along with genetic diversity, disease and translocation issues the number of broodfish available to a R&D program will be a constraint. One solution to this problem may be to invest in obtaining additional mature wild broodfish that can be conditioned prior to commencement of the R&D program to boost available numbers. This may also help circumvent issues associated with IP held in current hatchery broodstock.

7.3.4 Maturation

See (3) above. Control of maturation and sexual differentiation is crucial if we are to make genetic improvement in our selective breeding program. This is because the extent of genetic improvement achieved (and our ability to control inbreeding) relies on being able to:

i. reliably choose which individual will mate with which other individual
ii. produce large numbers of the matings outlined in (i)
iii. reliably synchronise the generation of families so that we can assess the performance of families, and individuals within families, that are grown in the same environments at the same time

Reliable synchronised production of large numbers of families from desired mate pairs requires being able to mature, differentiate and induce synchronised spawning of male and female broodfish on demand. If there is little control, and we must rely on identification of individuals that are at the appropriate stage of maturation and sexual orientation, genetic improvement will be compromised. A major constraint is that it is difficult to identify sufficient numbers of mature male fish in particular. This problem could be addressed through R&D into sex control and maturation, or through novel breeding technologies such as cryopreservation and artificial fertilisation.

7.3.5 Industry cooperation/commitment

The development of a successful genetic R&D program for barramundi will require substantial long-term commitment, cooperation and coordination between R&D providers and industry. Sharing of information and coordination of activities/resources will also be required.

7.4 Key researchable constraints for selective breeding programs

Rationale

For a barramundi selective breeding programs to be effective information and reliable maturation and mating systems are required. The key researchable constraints to the implementation of a barramundi breeding program are listed below, along with their priority.

Consultation with farmers, hatchery personnel and researchers led to the identificationof 7 major gaps in knowledge which are presently hindering the instigation of a barramundi breeding program.

Table 3. Key researchable constraints for selective breeding programs. Ratings - 3=high, 2=medium, 1=low; Priority – A=high, B=medium, C=low

Constraint/opportunity	**Research/development needed**	**Benefit to SBP (A)**	**Likelihood of success (CRC life) (B)**	**Urgency (C)**	**Cost of research**	**Priority (A*B*C)**	**Notes**
1. Control of sex and reproduction (early induction, accurate timing, gamete maturation, hormonal regulation, cryopreservation, artificial spawning)	1. Basic reproductive physiology, genetic/environmental regulation of sex change, possibilities of manipulation 2. Feasibility of large scale artificial spawning 3. Cryopreservation of gametes	3	2	3	$40-60K	A+	Efficient selective breeding programs need to have control of reproduction. An inability to house large numbers of broodstock, prevent sex change, and improve male maturation are impeding the design and conduct of breeding programs.
2. Genotype by environment interactions	Relative performance of family genotypes reared in the diverse types of systems used in barramundi culture	3	3	3	$310K	A	GXE interactions have potential to prevent realisation of genetic gains from selection. We need to

							understand their magnitude and factor any effects into the design of future breeding programs
3. Genetic parameter estimation	Obtain estimates of trait heritabilities, genetic correlations and common environmental effects	3	3	2	$310K	A	Essential for accurate planning of breeding programs. However, could be estimated from other R&D projects in other species and could be estimated using data from the families produced as part of the breeding program.
4. Novel breeding programs	Evaluate different mating schemes and selection plans	3	3	2	$40-100K	B	Important to the ultimate success of breeding programs, but will need to factor in research outcomes from other R&D to allow planning.
5. Composition of grades	1) Family representation at harvest (means, variance). 2) Distribution of sex at harvest	3	3	3	$25-40K	A	Basic information on composition of ponds, etc lacking. Essential to have this information before the breeding plan can be fully developed.
6. Nodavirus detection	Detection, elimination and development of management	2	2	2	-	C	Whilst variability in current detection methods, tests

and management	procedures						already exist which could be applied in a breeding program. Unlikely disease work will be funded within CRC
7. Genetic improvement of disease or parasite resistance	1) Development of disease challenge test systems (culture, infection methods, survival analysis). 2) Heritability of disease resistance, correlations with other traits.	3	2	1	-	C	Which diseases/ parasites? Do challenge test systems exist for other species? Success depends on heritability. Challenging to implement.
8. Genetic markers for quality/ production/ disease traits	QTL genome scans Integration of MAS.	3	2	1	-	C	Quality and disease resistance traits are slow to improve by conventional means and can be correlated with production traits (negatively or positively). Markers have potential but few resources available for barramundi. The Seafood CRC will assess the benefits/costs in a related project. Strategic issue that will become important once breeding begins.

7.5 Some options for a basic selective breeding program for barramundi

For the basic selective breeding options considered in this section we have assumed for the sake of simplicity that a single breeding nucleus would operate in isolation from any other farming activities. An evaluation of the merits of having one versus many selective breeding programs is given in Section 7.5.9.

7.5.1 Maximising genetic diversity in the founding population of animals

The initial founding population could be established using both mature wild caught animals and existing broodstock held on farms (providing good records are available on the wild source and breeding of the latter). This would be done in a way that maximises genetic diversity and gives the best opportunities for sustainable genetic improvement of all traits (eg. as proposed by Macbeth M. personal communication: Holtsmark, 2007; Holtsmark et al., 2006; Holtsmark et al., 2008a; b).

One of the advantages of Option 2 is that additional genetic variation can be injected into the founding population with the 1st generation of selective breeding (when selected 2 year old males are mated with mature (>4yr old) wild/domesticated females). In establishing the base population, the families created can be used to carry out the research that is proposed in the above sections. This will provide valuable information about the traits that can be incorporated into subsequent generations of the breeding program and will help with the formulation of a detailed plan for selective breeding.

7.5.2 Minimising risk of infection with disease and dilution of genetic improvement

After production of either the first generation of families under option 1, or the second generation of families under option 2, it was assumed that the breeding nucleus would be closed to the entry of new animals to minimise the risk of introduction of disease. A strict quarantine regime would be carried out when the breeding program is established. Regular disease testing, veterinary visits, monitoring and reporting would be carried out for the breeding nucleus. Each batch of fingerlings sent to the industry would be certified according to its disease status. Results would of all testing would be published by the testing agency.

It is assumed that both options for running the selective breeding program would involve some on-farm performance testing. But the animals sent for performance testing on farm would never return to the breeding program (their siblings would be held in ponds at the breeding nucleus facility, conditioned in tanks at the breeding nucleus facility and used for mating). This would however provide information on the performance of barramundi in different environments and would act as a backup in case of disaster affecting the breeding program.

7.5.3 Testing on-farm performance

Performance testing on-farms (Figure 4) is important for the selective breeding program in three ways:

1. the animals sent out to farms from each family can be used as backup broodstock in case of disaster (eg. disease),

2. if the best performing families in one environment (eg. freshwater ponds) are not among the best performing families in other environments (eg. saltwater tanks) a decision must be made whether to breed multipurpose or separate strains suited to the different environments, and
3. the results from the ranking of families in the different environments can be used to guide selection decisions made in the breeding nucleus.

Fingerlings from the breeding nucleus would be distributed to several farms in different environments and using different systems for grow out (Figure 4). Information about the performance of families in these environments would be used to inform decisions for breeding in the nucleus.

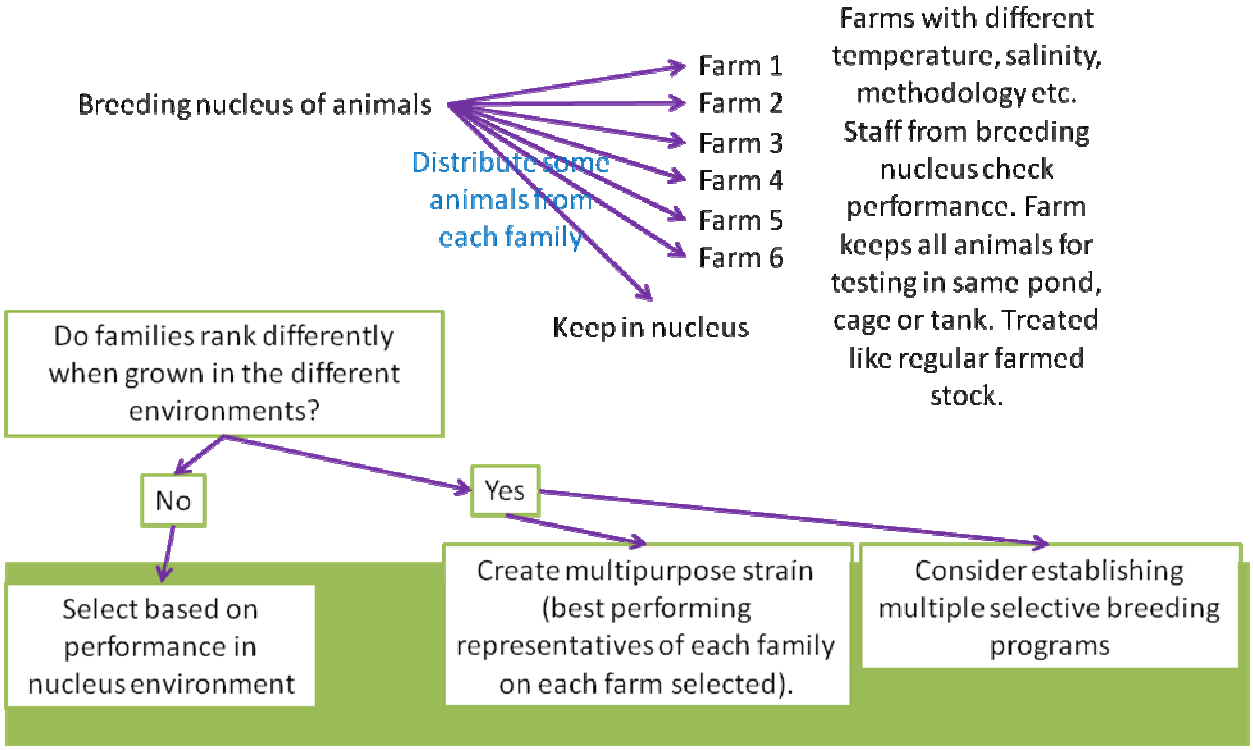


Figure 4. The complexity of the selective breeding program will be dependant on results from the on-farm test regime and G X E experiments.

7.5.4 Costs of the options modelled

The average running costs per year for a breeding program using 50 or 100 families under Options 1 or 2 are summarised in Table 4. The "other overheads" category includes costs for water reticulation, aeration, waste treatment, office maintenance, office stationary, office equipment rental, vehicle rental and fuel costs, depreciation, power, oxygen, chemicals, health certfification and return on capital. More detailed itemised costs are shown in Appendix3.

Table 4. Average running costs per year (entire costs per generation of breeding, averaged over the generation interval under each option) and one-off infrastructure investment costs for a selective breeding program run under the various options

Item	**Option 1**		**Option 2**	
Number of families	**50**	**100**	**50**	**100**
Facility lease	$60K	$60K	$60K	$60K
Feed and labour	$184K	$311K	$222K	$521K
DNA pedigreeing	$1K	$2K	$2K	$4K
Quantitative genetics	$30K	$30K	$30K	$30K
Other overheads	$201K	$254K	$342K	$402K
Total running costs	**$476K**	**$657K**	**$656K**	**$1,017K**
Additional infrastructure (one off)	-	$800K	-	$800K

7.5.6 Genetic improvement predicted

The model assumed it would be possible to select across and within families. If 10 tanks are used, selection across families is limited to those families that fall into the same cohorts (ie fish grown in the same environment). If more tanks were available (allowing more families to be pooled into the one cohort) this would improve the power of selection and lead to higher rates of genetic improvement.

Figures 5 and 6 show the genetic improvement in growth rate predicted by the model under the different options (measured as weight in kg at 2 years of age). Under option 1 the model predicts around a 12.5% improvement in growth rate with each additional initial generation of breeding. This is a similar order of improvement to that realised for Coho salmon (Hershberger et al., 1990), Atlantic salmon (Gjerde and Korsvoll, 1999), tilapia (Rye and Eknath, 1999) and rohu carp (Mahapatra et al., unpublished manuscript) of 10, 14, 15 and 30% per generation respectively (multiple studies showing response to selection for growth rate summarised in Gjedrem and Thodesen, 2005).

A higher more sustained response is achieved when 100 families per generation are used as the basis for selection rather than 50 families per generation (Figure 5). The genetic benefits from using more families are that there is a higher rate of genetic improvement, reduced inbreeding depression and that higher levels of genetic variation are captured and sustained by the breeding program.

When males are mated with previous generation females (under Option 2), there are genetic advantages for the program (Figure 6). This is because Option 2 results in a shorter generation interval (hence a greater rate of genetic improvement over the initial years) and as more genetic variation can

be captured to initiate the breeding program (first generation selected males would be mated with wild females, adding genetic variation for the founding stock). However, the improvement made by selecting each generation's best males is diluted somewhat by mating with the previous generation's best stock of improved females.

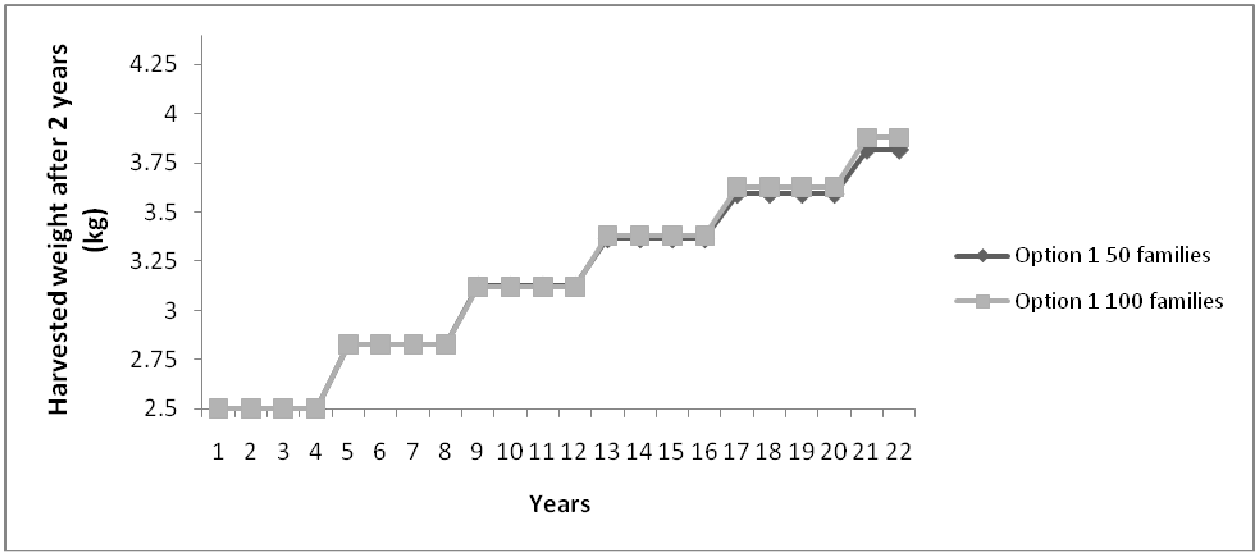


Figure 5. Mean response in growth rate (kg at 2 years of age) for Option 1 selecting from 50 or 100 families per generation.

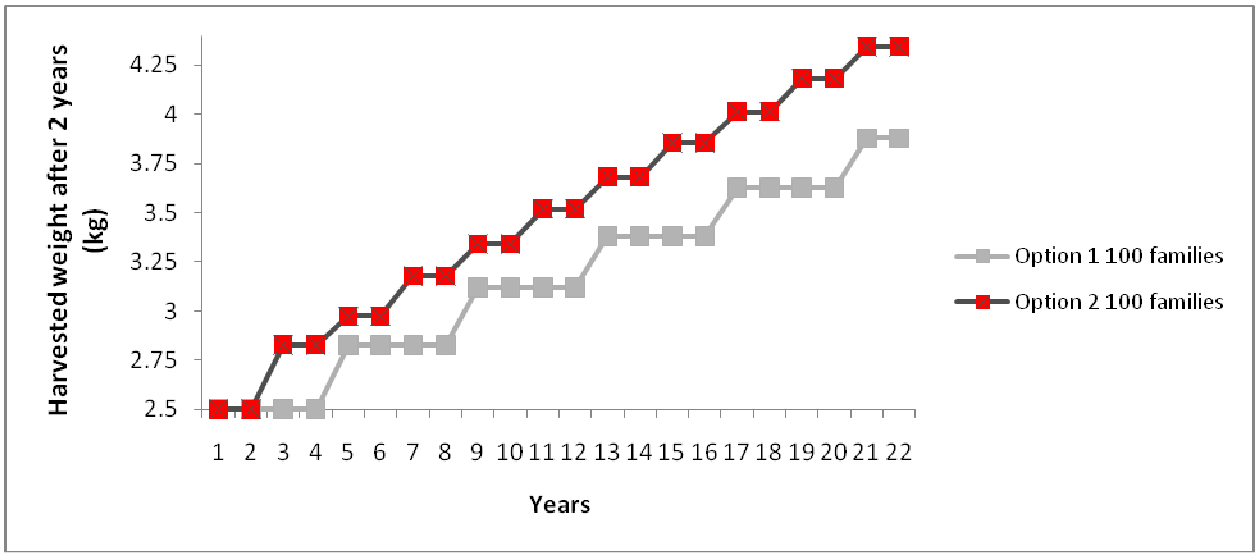


Figure 6. Mean response in growth rate (kg at 2 years of age) for Option 1 and 2 selecting from 100 families per generation.

7.5.7 Benefit-cost analysis

In the initial years of the program the highest benefit to cost ratio is achieved under Option 2 using 50 families (Figure 7). After 9 or so years the highest benefit to cost is achieved under Option 1 using 50 families. This suggests that it might be best to begin the breeding program using Option 2 (mating selected males with previous generation selected females) in order to get a "head start", and to transfer to Option 1 in subsequent generations. An advantage of doing this is that research to induce the early differentiation and maturation of females can be carried out during these initial generations, and the results can be adopted and introduced to the breeding program in subsequent generations. This research could allow a shortening of the generation interval under Option 1 (assumed in the model to be 4 years) and add to the genetic improvement, and hence economic benefit, achieved over the years under this option.

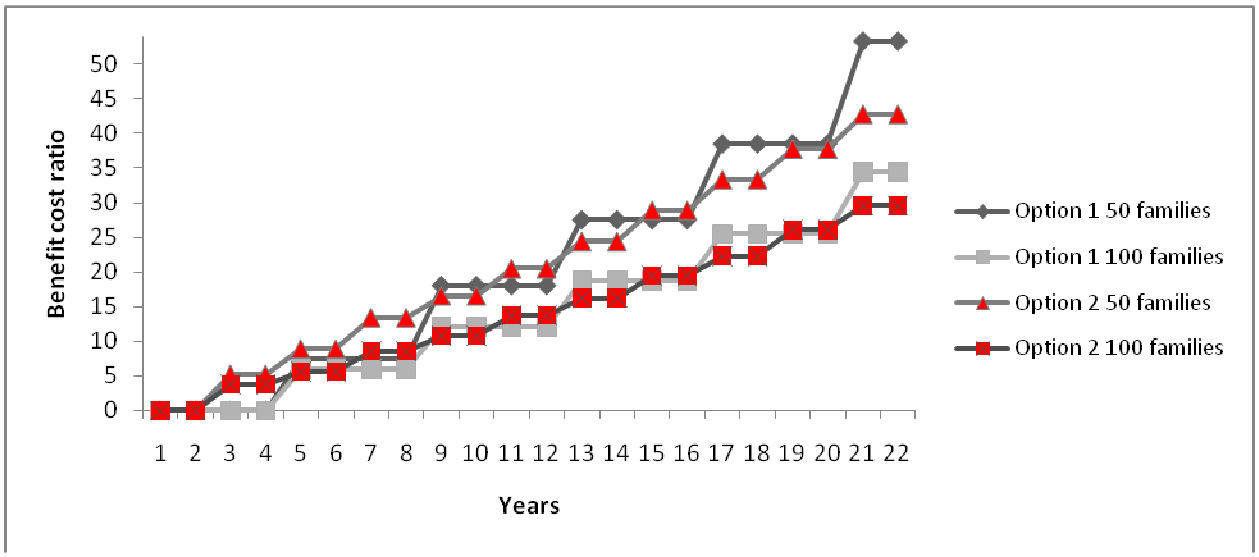


Figure 7. Benefit cost ratio for option 1 & 2 using either 50 or 100 families.

The benefit-cost ratios estimated are high. Selection for growth rate in the Atlantic salmon breeding program has been estimated to have resulted in a benefit-cost ratio of 15:1 (Gjedrem, 2000) while the benefit-cost ratio of vaccination for Atlantic salmon has been estimated at 14:1 (Thorarinsson and Powell, 2006). Compared to vaccination, genetic improvement is accumulative, so that higher benefit-cost ratios would be achieved with subsequent generations of selective breeding.

7.5.8 Commercialisation of the selective breeding program

We recommend that the selective breeding program is run as a commercial entity. We should consider selective breeding as a potential "enabler" that allows the farmer to increase his revenue stream by gaining access to the benefits that come from, for instance, stock that has a faster growth rate. We have already seen in the above sections what those benefits could be for the barramundi industry in Australia if there were a single selective breeding program supplying a large proportion of the industry. If the industry does not invest in some way in genetic improvement, then there is no opportunity for individual farmers to realise these future benefits.

For the mainland Australian abalone industry there has been interest by a number of farms to establish a joint venture to run a selective breeding company. The Seafood CRC has proposed for the abalone industry that a sliding scale of investment cost per farm tonnage is adopted, and that the investment cost is set depending on the total level of industry participation in the selective breeding company. A similar model for investment could be adopted by barramundi farms that wish to invest in and benefit from selective breeding.

For barramundi, the highest net present value for investment and internal rate of return on investment in 5 years was found using 50 families under Option 2 (Table 5).

Table 5. Cash flow, additional capital costs, net present value (millions of dollars) and internal rate of return under Option 2 for breeding programs producing 50 or 100 families each generation

Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
50 families																						
Cash Flow	-0.7	-0.7	2.8	2.8	5.2	5.2	8.1	8.1	10.2	10.2	12.8	12.8	15.3	15.3	18.3	18.3	21.2	21.2	24.1	24.1	27.4	27.4
Additional capital costs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NPV					6.4					28.8					57.5					87.5		
IRR					129%					144%					144%					144%		
100 families																						
Cash Flow	-1.0	-1.0	-2.8	2.8	4.7	4.7	7.7	7.7	9.9	9.9	12.9	12.9	15.4	15.4	18.7	18.7	21.6	21.6	25.4	25.4	28.9	28.9
Additional capital costs	-0.8																					
NPV					4.8					26.1					55.2					86.1		
IRR					52%					72%					74%					74%		

7.5.9 One versus many selective breeding programs

The more people that join and participate in a selective breeding program, the less the investment costs for each participating farm, and the greater the benefits that will be realised from the overall program. Put simply, each selective breeding program that is initiated by the industry will have approximately the same running costs providing each is aiming to generate the same rate of genetic improvement. Therefore on a cost-benefit basis it is difficult to justify the existence of more than one program. If we assume that a single program is initiated, it would be possible to do so using one facility or a number of facilities. But as indicated in earlier sections, there are a number of merits in establishing and operating a single breeding nucleus of animals in isolation from existing farms:

- Participating or investing farms in the program could be certain that the selective breeding program won't be dependent on the fortunes or misfortunes of a particular farm (that which

holds the selectively bred stock) and that commercial production imperatives will not override selective breeding imperatives. This would likely result in

- the benefit being realised by more farms, assuming these farms could have access to the selected progeny
- reduced costs per farm,
- a strict timetable and plan for selection and production of families could be followed,
- larger numbers of families,
- a broader genetic base established in the beginning,
- faster rates of improvement,
- reduced inbreeding depression of fitness,

In addition to these benefits, a dedicated facility for selective breeding could mean that

- biosecurity could be better addressed on-site
- potential for year round spawning could be realised.

The main disadvantage of a separate centralised program is that a dedicated facility would be required with associated costs and a business structure needs to be established clearly identifying stakeholder investment and ownership of IP. Providing these issues are addressed, it would be advantageous to establish one dedicated selective breeding program consisting of a single breeding nucleus of animals isolated from barramundi farming activities.

7.6 Conclusion from modeling basic selective breeding for barramundi

In conclusion, the basic selective breeding programs that were modeled were predicted to have high benefit-cost ratios for industry and to provide high net present value and internal rate of return on investment for industry after 10 years of selective breeding (Table 6). Highest benefit-cost ratio was under Option 1 with 50 families, while the highest total added benefit was under Option 2 using 100 families. However Option 2 using 100 families would have the highest running costs and capital start up costs. Internal rate of return on investment was highest under Option 2 using 50 families. The computer model created for Barramundi can be adjusted and re-run as necessary to test alternative options as the selective breeding program progresses in the future.

Table 6. Snapshot of predicted economic effect in year 10 under different selective breeding options.

	Option 1		Option 2	
Number of families	**50**	**100**	**50**	**100**
Benefit-cost ratio	18:1	12:1	17:1	11:1
Total added benefit / kg production	$1.08	$0.99	$1.36	$1.37
Nominal economic effect operating income	$8.1M	$7.0M	$10.2M	$9.9M
Capital cost (one off)	-	$0.8M	-	$0.8M
Total costs/yr (undiscounted)	$0.5M	$0.7M	$0.7M	$1.0M
Net present value	$13.9M	$12.3M	$28.8M	$26.1M
Internal rate of return	68%	42%	144%	72%

7.7 Training needs in genetic management

Selective breeding is highly technical so that even the hands-on running of a breeding nucleus requires a high degree of specialist training. The business of selective breeding is relatively new in Australia and there is much to be learnt from experiences overseas. Australian breeders could learn a lot from the experience of Norwegian breeding companies, and from the experience of Nofima (formerly Akvaforsk) who have been providing genetic and breeding research services directly to breeding programs around the world for more than 30 years and who have established programs for Atlantic salmon, rainbow trout, Atlantic cod, Mediterranean sea bass, tilapia and other species that are now commercialised and recognised internationally.

The Seafood CRC is developing an education and training program with Nofima and is offering bursaries to industry and scientists to cover travel costs. The education and training program is aimed at individuals or organizations that are:

- considering starting selective breeding programs or supplying industry with hatchery stock
- analysing data and making decisions about the selection and mating of selectively bred stock
- involved with the day-to-day operation of a nucleus or hatchery (includes hatchery managers)
- trying to run a profitable selective breeding or hatchery business (eg. managers or owners of companies such as Australian Seafood Industries PL for Pacific Oyster or Saltas PL for Atlantic Salmon)

Individuals/organisations in these interest groups have overlapping needs to some degree:

a) Need for owners, managers, geneticists and hatchery staff in Australia to be put in touch (network) with those having similar responsibilities in other sectors within Australia, as well as with international players in the selective breeding scene, so that they can benefit from the experience of existing research organisations and businesses.
b) Australia's reputation, isolation and environment could make it an attractive source of selectively bred stock internationally. Therefore there is an opportunity for some breeding programs to supply overseas producers with genetically improved stock. But there is a need to

understand how such a business might operate and how risks can be managed.

c) Need for those analysing data and making decisions about the selection and mating of selectively bred stock, and involved with the day-to-day operation of a nucleus or hatchery, to be informed of the latest technical developments overseas and how developments might be adopted to benefit Australian breeding companies and aquaculture industries.
d) Need for improving basic knowledge about establishing, running and profiting from selective breeding.

The program will develop workshops, industry master classes and bridging programs and implement exchange programs where:

a) managers of selective breeding companies and/or hatchery managers work for a short period with managers in a similar role from the breeding companies operating in Norway,
b) scientists could take a paid sabbatical to work on a project in Norway or vice versa and
c) students working on Seafood CRC related projects could do part of their research for their thesis topic in Norway and Australia.

The ABFA should take advantage of these opportunities to develop the skills of a core group of people in the industry in order to help get their selective breeding program underway.

8. Benefits and adoption

Refer to detailed results presented above in Sections 7.5.4 to 7.5.8. With an initial adoption rate of 50% growing to 95%, and production growing at 2% per year from current levels of around 3,038 tonne, after 10 years of selective breeding we predict a benefit-cost ratio of greater than 10:1, total added benefit per kg of barramundi produced of more than $1 and nominal economic effect on operating income of over $7 million per annum.

9. Further development

To realize the benefits from genetic improvement the industry needs to make a substantial investment and commitment to establish projects that address researchable constraints and to begin the selective breeding program itself.

Many of the costs that are highlighted in this report could be offset by travel bursaries, government grants, industry levies, FRDC, research institute contributions and the Seafood CRC. The CRC will give priority to research that aims to alleviate constraints affecting a number of its partner industries. Some of the research areas we have identified in this report (eg. control of sexual maturation, differentiation and reproduction) could be carried out as part of a larger research project across two or more different sectors (eg. barramundi and yellow tail kingfish). Also, the research projects of high priority could be linked together (project described in section 7.2.1 with that described in section 7.2.5 and projects described in section 7.2.2 and that described in section 7.2.3 with project described in section 7.2.4) making a total of two large projects (utilizing the same family resources etc). In carrying out one or more of these research projects, the initiation of the breeding program (production of families) could also begin.

To begin a single cooperative selective breeding program, the industry needs to rally together a core group of farmers/hatcheries to form a partnership to form the new business entity. The industry also needs to take advantage of the training program and bursaries being offered by the Seafood CRC in order to further develop the skills and knowledge needed for running a selective breeding program. Initial issues that need clarification include where the breeding nucleus will be based, what biosecurity, quarantine and health monitoring will be required to allow translocation, and what equity each partner will have in the program. A simple investment scenario that the mainland abalone industry is considering is that farmers invest according to estimated production requirements (eg. a rate depending on thousands of seedstock required). For participating barramundi hatcheries, equity in the business might depend on time or facilities donated to the breeding program.

10. Planned outcomes

The principal outcome from this scoping study will be an Australian barramundi industry implementing a coordinated and structured R&D strategy for genetic management and improvement of cultured stock. The industry can utilise the outputs of this project to make informed decisions on how to proceed using the resources available to them within and without the Seafood CRC towards industry based genetic improvement of this species). The report clearly identifies a recommended strategy for genetic

improvement of barramundi stocks, the resources required to implement the strategy and the critical researchable constraints that should be addressed through ABFA's investment in the Seafood CRC's research (through the "Breeding for Profit" Research Theme). The beneficiaries of these outcomes will be the ABFA and its members through future investment in genetic improvement and its associated benefits. Barramundi production is rapidly growing overseas and Australian producers are facing increasing economic pressure to improve efficiency of production and to maintain high product quality standards. With each generation of selective breeding the Australian industry will benefit from improved efficiency of production, which will result in increased competitiveness and sustainability.

11. Conclusion

This study has identified and prioritized the research and steps that need to be taken to establish a sound program for genetic improvement of barramundi farmed in Australia. Strengths, weaknesses, opportunities and threats associated with selective breeding have been analysed, a risk analysis performed and suggestions for risk management made. Barramundi genetic knowledge and constraints to barramundi genetic improvement have been reviewed. A research and development strategy, linking research topics into larger collaborative projects, has been developed to address these issues. Some basic options for selective breeding have been modeled and the benefit-costs compared. The models predict that even under these basic options that selective breeding would be profitable and of high benefit to the industry. The model that has been developed will be a useful tool that can be refined and used to assist ongoing planning and to help make continuous improvements in efficiency and profitability for any selective breeding program that is established by the industry. Recommendations for commercialization of a selective breeding entity have been presented, and the model has been used to predict cash flow, net present value and internal rate of return on investment for some basic breeding options. Training through Seafood CRC programs in day-to-day operation of selective breeding programs, profiting from selective breeding and more detailed technical aspects of selective breeding have been suggested. Finally, some ways in which the expenses associated with selective breeding can be offset by taking advantage of government grants, training bursaries and other funding sources have been suggested. The study predicts that the continuously improved seedstock supplied by an industry wide selective breeding program for Australian barramundi should stimulate expansion, raise profitability and improve international competitiveness of the industry. This study therefore provides a compelling case for government to fund the industry, and for industry to invest, in order to help develop the genetic improvement program and in order to help the industry to overcome constraints to genetic improvement.

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13. Appendix 1. Intellectual property

All of the information generated from this scoping study should be made publically available to the industry. The bioeconomic model software program that was used in this project remains the property of Nofima Marine in Norway.

14. Appendix 2. Staff

Principal Investigator Dr Nick Robinson. Co-investigator Dr Dean Jerry. Other research investigators, Graham Mair, Alex Safari, Jenny Ovenden, Glenn Schipp, Jerome Bosmans and Michael Macbeth .

15. Appendix 3. Detailed assumptions used in modelling the breeding program

2.5	average target weight at slaughter (kg)
2	average time until slaughter (years)
0.3	heritability for growth rate
0.117	starting population variance for genetic effect
0.273	starting population variance for environment effect on phenotype
2.5	starting population mean for genetic effect (kg)
0	starting population mean for environment effect
0.90	quantile not to be selected for phenotype
8.5	price of feed for grow out (in AUS$) per kg
1.6	total feed conversion ratio for grow out
0.37	market value (AUS$) per fish at time before set out in ponds/tanks
0.18	improvement made in feed conversion ratio with every improvement in growth rate
0.20	possible proportion of animals that would need to be DNA fingerprinted and tagged for walkback selection
20	number needed to be tested on farms (per family and per farm)
6	number of farms where animals will be tested
100	cost transport of fingerlings per farm for testing ($)
100	cost transport of fingerlings to farms for sale ($)
0.25	proportion of animals lost during grow out phase (after tagging)
0.05	proportion lost from settlement to tagging (raceways at DAC)
0.45	proportion lost from spawning to settlement (hatchery tank DAC)
0.45	cost of power needed to grow in ponds per kg of production ($)
18331	cost of labor needed per cohort of fingerlings in raceways (including excess fingerlings for sale) ($)
13498	cost of labor needed per cohort in the hatchery (ie. spawn and fertilise according to preferences for mating) ($)
28438	Cost of other overheads to run tanks per cohort of fingerlings in raceways including excess for sale (DAC) ($)
7496	Cost of other overheads to run tanks per cohort in the hatchery (SEE DAC COSTS) ($)
1000	Annual costs of special permits needed ($)
104203	Any other additional costs (per annum) that will be incurred because of the selective breeding program (eg. additional energy costs for temperature control?) ($)
22464	feed costs of keeping 65 broodstock in holding tanks (elite broodstock chosen for mating after growout, ie 1 years holding cost per generation) ($)

56831 labor costs of keeping 65 broodstock in holding tanks (elite broodstock chosen for mating after growout, ie 1 years holding cost per generation) ($)
70983 other running costs needed to keep 65 broodstock in holding tanks (elite broodstock chosen for mating after growout, ie 1 years holding cost per generation) ($)
7467 cost of feed (rotifers, artemia, algal, feed) needed per cohort in the hatchery ($)
8574 price to feed fingerlings per cohort (in a raceway) including excess fingerlings for sale ($)
0.5 total number of fingerlings needed to give 1 kg of production
60000 cost of leasing a facility (per year) ($)
0.07 Rate of discount over the length of one period (eg. 0.07 = 7%)

In order to calc cost of DNA fingerprinting for walkback selection
100 mean number of fingerling progeny produced per mating
20 standard deviation in the number of fingerling progeny produced per mating
20 DNA fingerprinting cost per animal ($)
100 number of times to repeat simulation