

### EX POST BENEFIT/COST ANALYSIS

### Three projects on:

Genetic Diversity of Tasmanian Salmon

Project No.: 1992/152

## Genetic Diversity in Tasmanian Atlantic salmon

and

1995/080 and 1996/347

# Microsatellite variation and identification of a Y-chromosome marker in Atlantic salmon

Prepared for the FRDC

June 2001

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#### 1. Introduction

This report carries out ex-post cost/benefit analysis on three Tasmanian Atlantic salmon research projects carried out by CSRIO Marine Research, Hobart. The three projects are analysed together as all are concerned with examining genetic diversity in Tasmanian Atlantic salmon. In addition to the issue of genetic diversity, two of the projects also attempted to develop a molecular genetic Y-chromosome marker in Atlantic salmon.

#### 2. Background

The Australian salmon aquaculture industry began with the importation of salmon ova to New South Wales from Nova Scotia, Canada. Over the period 1963-65, approximately 100,000 Atlantic salmon ova were imported. Based on spawning procedures of the Nova Scotian hatchery, an effective population size of 72 was calculated. These imported ova allowed the establishment of a landlocked population in New South Wales (Gaden hatchery) which was maintained through natural spawnings supplemented with hatchery stockings. Hatchery records from the late 1970s to early 1980s indicate that several hundred broodstock were used each year in the hatchery. Therefore losses of genetic variation were considered minimal. Between 1984 and 1986, approximately 100,000 ova were imported into Tasmania to initiate the Tasmanian stock. The first Tasmanian broodstock were available in 1988, and for that and each subsequent year, several hundred males and females are used as broodstock.

Maintenance of genetic variation in broodstock is important in order to prevent the negative consequences of inbreeding – such as deformities and reduced resilience to stress and disease. Knowing that there is a reduction in genetic variation of the stock allows for action to be taken to prevent further inbreeding. This can be done either by importing new genetic material as either frozen semen or frozen fertilised eggs (both requiring quarantine for up to 2 years) or developing selective breeding programmes where only individuals of known pedigree are used as broodstock. In addition understanding the level of genetic diversity present in a non-native aquaculture population is important to ensure sufficient diversity exists upon which to base a selection program.

The impetus for the first FRDC funded genetic diversity project came from the incidence of jaw deformities in some farmed Tasmanian salmon in the early 1990s. At this time, industry was uncertain whether the deformity was the result of declining genetic variation or environmental factors. The first project (92/152) was aimed at assessing whether there was decreased genetic variation in the Tasmanian population compared with that found in the Nova Scotian parental stock.

The two subsequent projects continued the research on genetic diversity using a new genetic technique and included research to identify a Y-chromosome marker. The rationale for the Y-chromosome research was related to the industry-preferred practice of farming females. Females mature slower than males, and since fish are culled just prior to maturation, harvesting females means higher yields.

To produce all female strains, the standard breeding protocol had been to androgenise fry using hormones in order to produce all male fry - both XX males (whom, without androgenisation would be female) and XY males. Crossing the XX males with normal XX females would then produces all female progeny (Figure 1)

#### 2.1.1 Figure 1 Production of all female salmonids



A major obstacle in the production of all female populations had been the difficulty in distinguishing XX males from 'regular" males that have a Y chromosome. Mistakes can be made and contamination (in terms of using the milt of XY males) can occur. The identification of a Y-chromosome marker that could identify XY males easily,

say from a fin clipping would eliminate the need to sacrifice animals to produce all female progeny and reduce costs associated with contamination events.

#### 3. Objectives

The objectives for all three of the research projects are as follows:

#### 3.1 Project 92/152: Genetic Diversity in Tasmanian Atlantic Salmon

• To identify the level of decline in genetic variation in Tasmanian stock of farmed Atlantic salmon.

# 3.2 Project 95/80: Microsatellite variation and identification of a Y-chromosome marker in Atlantic salmon

- To develop DNA microsatellite techniques in Atlantic salmon and to apply these to:
  - A comparison of levels of microsatellite variation in farmed Tasmanian salmon and the parent stock in Nova Scotia; and
  - ii Locating a male-sex (Y-chromosome) marker for broodstock management.
- To develop non-lethal and non-destructive DNA extraction techniques to enable genetic analysis of valuable and non-replaceable individuals.

# 3.3 Project 96/347: Microsatellite variation and identification of a Y-chromosome marker in Atlantic salmon

• To locate a Y-chromosome marker in Atlantic salmon by applying a range of molecular genetic techniques.

• To establish the rate of change in genetic variation in Tasmanian Atlantic salmon by comparing the genetic (microsatellite and allozyme) variation in progeny from 1993 year-class parents with that present in the 1989 year-class parents and the parental Nova Scotia population.

#### 4. Main Research Findings

The first FRDC project (92/152) focused on establishing whether the jaw deformity could be attributed to the genetic health of the Tasmanian population. The two subsequent projects (95/80 and 96/347) expanded research to not only include monitoring of genetic diversity but also pursue the development of genetic markers.

#### 4.1 Genetic variation

Project 92/152 compared genetic variation in Tasmanian and Canadian populations using two (allozymes and mitochondrial DNA) molecular genetic methods. The research found that there was no evidence of reduced genetic variability in the Tasmanian population. The project concluded that there was a need to regularly monitor genetic variation.

Projects 95/80 and 96/347 continued this research, and tested for genetic variation using microsatellite markers, a potentially more sensitive method. The research showed that there was a loss of genetic variation in the River Philip sample compared to the Australian samples. However, this was a comparison with a 1990s River Phillip sample. The 'correct' comparison would have been to compare samples from River Phillip from the 1960s with Australian samples in order to determine whether there had been genetic drift. However at the time the projects were being carried out, this was not possible, as the technology was not available to obtain DNA from archived historical samples (now this technology is available). Furthermore, when comparing the Tasmanian samples with the Gaden samples (which were a much smaller population) the Gaden sample showed greater genetic variation than the Tasmanian sample. This could not be explained fully.

Nevertheless, despite these possible sampling difficulties, it was concluded that there appeared to have been a small overall loss of genetic variation in the Tasmanian Atlantic salmon population. This was suggestive of a bottleneck (low breeding numbers) in the Australian Atlantic salmon population early in its introduction.

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However in later years, due to hatchery practices in Tasmania, it was concluded that current broodstock numbers provide relatively large effective population sizes. It was also noted that a number of assumptions were made for the analysis of effective population size, such that changes in these assumptions might lead to different interpretations of results. It was concluded that the local population had a relatively high level of genetic diversity despite evidence that there was a small decrease, but that continual monitoring was required to assess whether current effective population sizes were sufficient to maintain this level of genetic diversity.

#### 4.2 Y-chromosome marker

A number of molecular techniques have been designed for identification of genetic differences of specific genes. Six of these techniques were considered by the second and third projects (95/080 and 96/347) and the preferred method selected was Representational Difference Analysis (RDA). This is a technique designed to isolate sequences present in one DNA sample (i.e. male or Y chromosome specific markers) relative to another DNA sample.

The research failed to identify a Y-chromosome marker for Atlantic salmon (a similar result to a number of other international laboratories using different techniques). However modifications were made to the RDA protocols that may in the future be useful for the investigation of a sex specific DNA fragment in Atlantic salmon, or for searching other species for specific gene markers.

#### 5. Cost/Benefit Analysis

There are two major components of net economic benefit in cost/benefit analysis – producer's surplus and consumer's surplus. In the case of the aquaculture projects being considered, producer's surplus is a measure of net economic benefits created for the farming sector. Although a simplified explanation, producer's surplus can be thought of as additional profits generated. In addition, if the research findings induce increases in production and employment, then to the extent that previously unemployed labour is employed, the associated wages would also be included as a benefit in producer's surplus.

Consumer's surplus is a measure of net economic benefits to consumers. For example, if a research project induces an increase in product supply that in turn results

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in a decrease in prices on the domestic market, then domestic consumers would be better off. Consumer surplus is simply a measure of this improvement in consumer well-being.

In simple terms, to undertake benefit/cost analysis, it is necessary to estimate all economic benefits that flow from the research findings. Benefits are then compared to the financial cost of research, plus any economic costs that are required to capture the benefits.

#### 5.1 Costs

Table 1 displays the total research costs for all three projects. The total costs amount to just under \$550,000, of which the FRDC contributed 43%.

Project No.	FRDC	Other	Total	
92/152	20,500	0	20,500	·····
95/180	104,632	100,944	205,576	
96/347	113 ,479	209,472	322,951	
TOTAL	238,611	310,416	549,027	

#### **Table 1: Costs of Research Investment**

#### 5.2 Benefits

There are two sources of benefits associated with these projects: benefits related to information gathered on the genetic diversity of Tasmanian Atlantic salmon stocks and benefits that flow from the identification of a Y-chromosome marker.

#### 5.2.1 Benefits from information gained on the genetic diversity of the stock

As mentioned earlier, in the early 1990s there was industry concern that jaw deformations were a sign of inbreeding. Since the research found little evidence that supported a genetic-diversity problem, the benefits of the research are not obvious. The information gathered from the research did not increase industry profits (and producer's surplus), nor were consumers made better off (via an increase in consumer's surplus). Essentially, the research information did not result in any direct action.

However, the fact that there was not a genetic problem was not known until after the research was completed. Prior to the completed research, there was a probability that there was indeed a genetic problem. At the time, the expected benefit of the research was the probability that there was a problem multiplied by the benefits of knowing there was a problem. To pursue this issue further, the following section examines the question: What if there had been an underlying genetic problem and the research had not been funded?

# 5.2.2 What if there had been an underlying genetic problem and the research had not been funded?

Without research findings indicating that there was a genetic problem, farmers would have continued to invest in salmon farming throughout the 1990s. Salmon farming has been a growing business in Tasmania. In 1991, Tasmanian salmon production stood at \$39.6 million in 1991 and \$63.6 million in 1997. Employment in 1995 was estimated to be 570 full-time equivalents, with 2,000 jobs generated by direct and indirect employment<sup>1</sup>. Eventually, the genetic problem would have surfaced; however, there would have been an interval before the problem became obvious in which additional farming investment would have taken place. Inbreeding was unlikely to have caused a catastrophic problem within one or two generations, but was more likely to have had a marginal but increasing impact (Pheroze Jungalwalla, pers. comm).

As the genetic problem would have eventually showed up, the cost of not undertaking the research is related to the unprofitable investments made in farming during the period before the genetic problem surfaced.

It is worthwhile examining this in more detail. If the research had been undertaken and indicated a <u>severe</u> genetic problem at the end of 1992 then some of the existing broodstock would have been sold or destroyed. Therefore it would have been necessary to import new genetic material (fertilised eggs or sperm presumably after an import risk assessment on the introduction of disease, specifically *Aeromonas salmonicida*. These imports would have to be quarantined in a special facility. No such facility exists, and the estimated cost of construction is in the range of \$5 - 7

<sup>&</sup>lt;sup>1</sup> ABARE. 1999. Salmon Imports into Australia – Bilateral Market Penetration.

million. Quarantine periods would probably be in the range of up to two years (Jeremy Carson, pers. comm). Without new broodstock, there would have been little investment in farms over the 1993-94 period.

Without the research, the underlying genetic problem would not have been known in 1992 and farming investment would have continued. For the sake of argument, assume that the genetic problem became obvious at the end of 1994. Then the cost of not undertaking the research would be the profits that would not be forthcoming from investments made over 1992-94. In other words, the scarce resources that went into the investment in farming over 1992-94 would not show any economic return. And the unrealised profit is a measure of the lost economic opportunity that resulted from the farming investment (i.e., the investment resources could have gone into other sectors of the economy to generate economic returns).

If one assumes that the 1992-94 investments were expected to pay off in terms of profits over the 1995-97 period, then the cost of not having undertaken the research would be the 'wasted' investment, which can be very roughly measured by the unrealised profits over 1995-97. Revenue over 1995-97 was approximately \$180 million, and assuming a before-tax profit rate of 20%, this equates to profits of \$36 million.

No information is available on the probability of a severe genetic problem, however it is possible to work backwards and calculate the probability that would make the FRDC's funding a break-even investment. Specifically, a 0.66% probability of a severe genetic problem would have produced benefits equivalent to the FRDC's investment. This is calculated as follows.

For the investment to break-even, the expected benefit of the research, which is equal to the probability of a genetic problem (unknown) multiplied by the profit benefit (\$36 million), is equal to the <u>total</u> cost of the research (\$542,000). Therefore the unknown probability of a genetic problem that would set benefits equal to research costs is equal to 1.5% (542,000/36 million)<sup>2</sup>. As FRDC contributed just under \$239,000 to total research costs, a probability of 0.66% of a severe genetic problem

 $<sup>^{2}</sup>$  It is important to note that social adjustment costs related to unproductive investments would act to lower the size of the probability of a genetic problem that is required for the research investment to break-even.

would would likely justify the investment made by the FRDC, whilst a probability of 1.5% would justify total research investment. All of these numbers are very rough and so are some of the time periods selected; however the basic idea is to get a feel for the value of the research. Clearly, if the probability is higher than 1% then expected benefits are greater than FRDC costs. The question of the probability distribution of a severe genetic problem (given the population background of the Tasmanian stock) is one of conservation biology not economics, and consequently we have nothing to say on the issue.

It would be useful if all proposals included a simple estimation of the likely benefits and costs. This is especially important when industry itself is not willing to undertake this research itself.

#### 5.3 Cost savings associated with the identification of a Y-chromosome marker

Cost savings associated with the identification of a Y chromosome marker are savings in the costs of labour in hatcheries for killing XX males to obtain the milt and savings associated with contamination of all female stocks with XY males. Without the Y chromosome marker, XX males have to be killed in order to obtain their sperm for fertilising XX females. The Y-probe would be more efficient because the fish would be anatomically intact and would not have to be killed prior to use. As a result, animals could be used repeatedly or, at the very least, previously used animals could be retained as back-ups in case of mortality in later year classes. The labour savings and associated costs are negligible.

An additional benefit is that contamination of all female stocks would be zero. Under the current system, a farmer buys<sup>3</sup> what (s)he thinks are all female fish for stocking in sea cages and may only find out that some of these are in fact XY males (contaminated stock) once the males reach maturity i.e. before the females. These fish have no sale value as it is too expensive for the farmer to select them out and they are used as trash fish (Pheroze Jungalwalla, Harry King pers. comm). Current testing by hatcheries yields a 2% contamination rate; a Y probe would mean 0% contamination of all female XX stocks.

<sup>&</sup>lt;sup>3</sup> There is no difference in price for male and female smolts.