Promoting Marine Finfish Aquaculture in NSW

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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Abbreviations

DPI: Department of Primary Industries

FCR: Food Conversion Ratio

MARL: Marine Aquaculture Research Lease

MWSAS: Marine Waters Sustainable Aquaculture Strategy

USC: University of the Sunshine Coast

YTK: Yellowtail Kingfish (*Seriola lalandii*)

Executive Summary

Huon Aquaculture and NSW DPI are working together to establish NSW's first Yellowtail Kingfish (YTK) farm. Using a recently approved Marine Aquaculture Research Lease (MARL) and an existing farm site off Port Stephens, NSW, we have begun to establish NSW largest fish production facility, with the capacity to hold up to 2000 tonnes standing stock of YTK. To prepare for farming operations Huon and DPI have worked collaboratively with the University of the Sunshine Coast to better understand and protect the genetics of local kingfish stocks while developing the necessary genetic resources to establish a broodstock population with sufficient diversity to meet production and breeding needs and develop a selective breeding plan for YTK.

Background

NSW currently imports approximately 85% of its seafood and needs a substantial increase in investment and production, most notably, major new marine based aquaculture development is required (Bond University, 2012). However, the development of marine aquaculture in NSW is constrained by the lack of approved lease sites and hampered by the lack of demonstrated operational viability.

A Marine Waters Sustainable Aquaculture Strategy for NSW (MWSAS), under State Environmental Planning Policy 62 – Sustainable Aquaculture (SEPP62) is being canvassed to streamline investment pathways while promoting sustainable seafood production. It is envisaged that a MWSAS would describe the approvals process, best practice system design and operation, and then identify areas thought to be suitable for future development.

The development of a MWSAS was promoted by DPI through two key projects to underpin aquaculture investment in marine waters of NSW:
• An application for a 20 hectare marine finfish aquaculture research lease (MARL) off Port Stephens, and
• An application for 50 hectares of commercial shellfish (Mussels, Scallops, Pearl oysters) aquaculture leases in Jervis Bay.

In 2013, NSW Fisheries gained permission from Planning NSW to establish the MARL off Port Stephens and began negotiations seeking a commercial "research partner". At the same time, funds for research project were obtained from the Seafood CRC that were to be used to provide background information that would be necessary for the operation of the lease and that would inform other potential proponents in the future. In particular the original objectives were to undertake preliminary environmental monitoring of the lease site and then monitor environmental and performance metrics during the first year of farm establishment.

Monitoring of the lease site commenced in September 2013; however, several factors complicated lease development and monitoring, and curtailed any attempt to establish farm baseline conditions. To prevent the unnecessary expenditure of further time and funds, monitoring work halted awaiting the resolution of two variables: the research partner and the MARL location.

In 2014, a research partner was selected, Huon Aquaculture, who subsequently acquired another existing marine farm site off Port Stephens ("Pisces" Lease). The proponent then sought advice from Planning NSW on the potential to move both the MARL and Pisces lease to a deeper water location. While this is expected to have overall improved social and environmental outcomes, monitoring and reporting at the existing locations was called into question. Advice from Planning NSW was sought and application to move the leases was prepared (and has recently been approved). This however precluded the completion of the remaining milestones for the original project.

While the delay prevented the project continuing on its existing path, it did not prevent further significant preparatory work to be undertaken to assist kingfish industry development in NSW. We proposed to use remaining funds to prepare the necessary genetic resources to enable NSW DPI to establish a broodstock nucleus with sufficient diversity to meet production and breeding needs and develop a selective breeding plan for YTK in NSW.

Aims/objectives

In collaboration with the University of the Sunshine Coast, and with support from the Seafood CRC and FRDC the revised project objectives sought to:

- Assess genetic diversity of the current NSW DPI YTK broodstock
- Compare diversity of Wild and F1 YTK stocks.
- Compare the diversity of the captive population with that of the wild in NSW (samples would be collected in a fashion to support proposed larger scale national and international kingfish population assessments.
- Monitor group spawning dynamics to better understand individual contributions to larval batches.
- Review of available molecular information on the status of local (NSW) wild and captive YTK broodstock
- Assess facilities available for YTK broodstock maintenance and breeding
- Evaluate production demands and identify breeding objectives to best focus selection efforts
- Identify/design a data management system for data preparation and analyses
- Prepare a 5 year breeding plan for NSW YTK

Outcomes

Wild NSW YTK samples from captive broodstock populations appear genetically diverse, and are unrelated to each other (no siblings were detected). Samples from different locations in NSW were not genetically different from each other. NSW samples could not be distinguished from the other Australian samples tested and are very different from those in the Northern Hemisphere.

Four broodstock YTK tanks are held in NSW, comprising two wild and two F1 (first generation of wild) sets of broodfish. The genetic diversity within tanks, and the genetic relationships among tanks were documented using either genetic samples of the actual broodfish or deduced from genetic samples of their offspring. There are now adequate data to make conclusions and give advice concerning the number of broodstock tanks and type of operations required to support a YTK genetic program with inbreeding kept below 1%.

For a sustainable genetic program to operate and service an industry initially of 2,000 tonnes, numbers of fish and tanks at NSW DPI PS needs to be increased by at least several fold. Floor space and technical experience is available for this type of expansion.

It is considered likely that genetics will make contributions to profit, initially by improving growth, appearance (condition index) and survival, and indirectly through correlated selection response, improved food conversion ratios, using genetic models based on mass selection and pedigree management post selection (aka “classical design”). Other traits, for example flesh quality/muscle fibre density will be assessed for their genetic basis with a view either to improve them or to operate so they are not degraded by inadvertent selection. Assessments, probably ongoing, will be made of the commercial value of ‘omics and epigenetics to augment the step by step, generation by generation, classical methods of genetic improvement exploiting additive genetic variance.

A pathway for web based data acquisition and storage was deployed. The data from the Kingfish website will be retrieved to estimate breeding values for individual fish in the pedigree and perform mate allocation.

Road maps for a 5-year breeding program were charted. One model most closely considered is analogous to that proposed in CRC project 2008/703 and was deployed already in SA (Knibb et al. 2015). This model previously has proved to yield substantial selection response in both land tanks and sea cages. Because of a) its track record, b) modest cost, c) feasibility, and d) genetic sustainability, it was considered here in most detail. Other models, varying from low budget and low infrastructure (intraspecific hybrids) to a full within and between family selection (salmon model), were also considered. Some “add ons”, e.g. genomics and epigenetics were also considered.

The information gathered during this project has been of particular value in assuring the local community, managers and policy makers of the environmental sustainability of the proposed activities. That the stocks being used for fingerling production are of local origin have the diversity required to begin production without posing a threat to the genetics of wild populations in the event of escape or spawning during production. Further the increased understanding of the spawning behaviour of YTK and the development of a plan to ensure diversity is maintained as production proceeds provides some additional assurance that the genetics of wild populations will be protected into the future. From an Industry perspective, guidance has been received with respect to the additional stock and infrastructure that will be required to progress toward a selective breeding program.

Keywords

Yellowtail Kingfish, *Seriola lalandii*, Genetics, Breeding

Introduction

In 2013, NSW Fisheries gained permission from Planning NSW to establish a Marine Aquaculture Research Lease (MARL) off Port Stephens and began negotiations seeking a commercial research partner. The initial project was designed to provide background information that would be necessary for the operation of the lease and that would inform other potential proponents in the future. In particular the objectives were to undertake preliminary environmental monitoring of the lease site and then monitor environmental and performance metrics during the first year of farm establishment.

Monitoring of the original lease site commenced in September 2013 and good progress was made (see previous milestone reports), however, several factors have complicated lease development and monitoring, and curtailed any attempt to establish farm baseline conditions. To prevent the unnecessary expenditure of further time and funds, we halted work awaiting the resolution of the following two variables.

In 2014, in what was a positive development, the research partner acquired the other existing YTK farm site off Port Stephens ("Pisces"). The proponent then sort advice from Planning NSW on the potential to move both the MARL and Pisces leases to a deeper water location. While this is expected to have overall improved social and environmental outcomes, ongoing monitoring and reporting at the existing locations was called into question. Advice from Planning has been received and an application to move the leases is being prepared. This however precludes the completion of the remaining milestones in this project before June 2015.

While the delay was unfortunate it did not prevent further significant preparatory work being undertaken to assist YTK industry development in NSW. Accordingly, we proposed the use of the remaining funds to prepare the necessary genetic resources to enable NSW DPI to establish a broodstock nucleus with sufficient diversity to meet production and breeding needs and develop a selective breeding plan for YTK in NSW.

Objectives

The revised objectives of this project were to:

- Assess genetic diversity of the current NSW DPI YTK broodstock (approx. 60 fish)
- Compare diversity of Wild and F1 YTK stocks.
- Compare the diversity of the captive population with that of the wild in NSW (samples would be collected in a fashion to support proposed larger scale national and international kingfish population assessments).
- Monitor group spawning dynamics to better understand individual contributions to larval batches.
- Review of available molecular information on the status of local (NSW) wild and captive YTK broodstock
- Assess facilities available for YTK broodstock maintenance and breeding
- Evaluate production demands and identify breeding objectives to best focus selection efforts
- Identify/design a data management system for data preparation and analyses
- Prepare a 5 year breeding plan for NSW YTK

Methods, Results & Discussion

The outcomes of this research have been prepared in the form of two separate reports and have been provided to all current industry participants for their consideration.

Report 1 – Kingfish genetics: genetic diversity in NSW hatchery and wild samples

CONTRIBUTORS: Wayne Knibb, Abigail Elizur, Stewart Fielder, Wayne O'Connor, Brooke McCartin, Jane Quinn

Abstract

To support a future breeding program for Yellowtail Kingfish in NSW, investigations were conducted on the genetic variation in samples from wild and captive animals.

Wild NSW samples, as measured by DNA microsatellite loci, appear genetically diverse, and are unrelated to each other (no siblings were detected). Samples from different locations in NSW were not genetically different from each other. NSW samples cannot be distinguished, wrt DNA microsatellite alleles, from SA samples, but the Australian samples are very different from those in the Northern Hemisphere.

Four broodstock YTK tanks are held in NSW, comprising two wild and two F1 (first generation of wild) sets of broodfish. The genetic diversity within tanks, and the genetic relationships among tanks were documented using either genetic samples of the actual broodfish or deduced from genetic samples of their offspring. There are now adequate data to make conclusions and give advice concerning the number of broodstock tanks and type of operations required to support a YTK genetic program with inbreeding kept below 1%.

Presently, the total genetic resources, with respect to captive wild fish and their lineages, are less than 40% that required for a sustainable genetic breeding program under ideal conditions of capturing all the variation and linages from one generation to the next. However, data indicate that most of the available genetic resources are lost between generations, with evidence that as few as a single male and single female parent contribute to given generations. Under current procedures, then, the genetic infrastructure needs to be multiplied by an order magnitude to be sustainable, or, more realistically, procedures changed to more effectively capture the genetic variation and lineages across generations.

Not every animal, sire or dam, spawned consecutively every day, for example, on some days a single sire spawned. However, over several or many days, most of the tank's broodstock seem to contribute to the spawn, so that taking eggs or larvae over several days, or doing repeated hatchery runs from same brood tank at different times, coupled with pedigree management, could help maximize the amount of genetic variation and lineages passed generation to generation.

However, even when we pool all available data and all animals genotyped, i.e. hundreds of animals, we still observe a gradual generation by generation loss of genetic diversity. Pedigree knowledge, and pedigree management ensuring the passage of different lineages over generations, notwithstanding issues of family breeding values, may stop the substantial loss of variation over generations.

Introduction

There is interest in NSW to develop commercial Yellowtail Kingfish (YTK, *Seriola lalandi*) aquaculture. Genetic improvement will support the profitability of the business, but for genetic

improvement to continue generation after generation and be sustainable, a minimum effective population size (of about 50) is required otherwise inbreeding will subtract from profitability. A first step in planning for genetic sustainability is to document the available genetic resources in NSW Fisheries. Accordingly, investigations were conducted to document the genetic variation in samples from wild and captive NSW YTK samples.

Project Objectives

1. What is the genetic structure of the NSW captive broodstock.
2. What are the spawning dynamics of the captive broodstock, and specifically, how many males and females contribute to each spawn.
3. What is the population structure of NSW and other Australian YTK populations?

Methods

Broodstock Tanks at Port Stephens

NSW DPI indicated there are 4 broodstock tanks (22,000L):

- 2 tanks stocked with wild caught animals (7 and 9 fish respectively of mixed sex)
- 2 tanks stocked with first generation hatchery-reared fish (10 and 8 fish respectively)
- Tank ID's are 1, 5, 6 & 8.

Larvae and egg samples received at USC from NSW Department of Primary industries

Broodstock finclips and tubes of larvae and of eggs were received from NSW Department of Primary Industries (DPI; TABLE 1).

For the present study, some eggs or larvae samples were taken from multiple different spawns on different dates from the same tank (although for a given spawn, samples were placed into up to four tubes with 70% ethanol).

Table 1 Register of broodstock, larvae and egg samples received from NSW DPI

Sample ID	Location (Tank #)	Tissue type	Date of Spawn	Date Collected	Number of tubes
YTKL1	1	Larvae	19/03/2015	24/03/2015	1
YTKL2	1	Larvae	20/03/2015	24/03/2015	1
YTKL3	1	Larvae	22/03/2015	24/03/2015	1
YTKE1	1	Eggs	27/03/2015	27/03/2015	4
YTKE2	1	Eggs	3/04/2015	3/04/2015	4
YTKE3	1	Eggs	1/04/2015	1/04/2015	4
YTKE4	1	Eggs	21/07/2015	21/07/2015	4
YTKE5	6	Eggs	19/08/2015	19/08/2015	4
YTKE6	6	Eggs	28/07/2015	28/07/2015	4
YTKE7	6	Eggs	27/08/2015	28/08/2015	4
YTKE8	6	Eggs	9/09/2015	9/09/2015	4
YTKE9	1	Eggs	24/02/2015	24/09/2015	4
T5FCF1	5	Finclip	N/A	22/09/2015	1
T5FCF2	5	Finclip	N/A	22/09/2015	1
T5FCF3	5	Finclip	N/A	22/09/2015	1
T5FCF4	5	Finclip	N/A	22/09/2015	1
T5FCF5	5	Finclip	N/A	22/09/2015	1
T5FCF6	5	Finclip	N/A	22/09/2015	1
T5FCF7	5	Finclip	N/A	22/09/2015	1
T5FCF8	5	Finclip	N/A	22/09/2015	1

Sample ID	Location (Tank #)	Tissue type	Date of Spawn	Date Collected	Number of tubes
T5FCF9	5	Finclip	N/A	22/09/2015	1
T5FCF10	5	Finclip	N/A	22/09/2015	1
T6FCF1	6	Finclip	N/A	23/09/2015	1
T6FCF2	6	Finclip	N/A	23/09/2015	1
T6FCF3	6	Finclip	N/A	23/09/2015	1
T6FCF4	6	Finclip	N/A	23/09/2015	1
T6FCF5	6	Finclip	N/A	23/09/2015	1
T6FCF6	6	Finclip	N/A	23/09/2015	1
T6FCF7	6	Finclip	N/A	23/09/2015	1
T6FCF8	6	Finclip	N/A	23/09/2015	1
T8FCF1	8	Finclip	N/A	22/09/2015	1
T8FCF2	8	Finclip	N/A	22/09/2015	1
T8FCF3	8	Finclip	N/A	22/09/2015	1
T8FCF4	8	Finclip	N/A	22/09/2015	1
T8FCF5	8	Finclip	N/A	22/09/2015	1
T8FCF6	8	Finclip	N/A	22/09/2015	1
T8FCF7	8	Finclip	N/A	22/09/2015	1
T8FCF8	8	Finclip	N/A	22/09/2015	1
T8FCF9	8	Finclip	N/A	22/09/2015	1
T8FCF10	8	Finclip	N/A	22/09/2015	1
T8FCF11	8	Finclip	N/A	22/09/2015	1
T5FCDF1	5	Finclip	N/A	23/09/2015	1
T5FCDF2	5	Finclip	N/A	23/09/2015	1
T5FCDF3	5	Finclip	N/A	23/09/2015	1
T5FCDF4	5	Finclip	N/A	23/09/2015	1

Wild caught samples received at USC from NSW DPI

Additional samples (eye stalks) were received which were taken from wild animals found along the NSW coastline (TABLE 2), presumably from unrelated wild individuals (i.e. unrelated to each other and unrelated to those in the above Section). There are no gender data for most of the eye tissue samples.

Table 2 Register of wild samples received from NSW DPI

Sample ID	Location sampled	Tissue Type
YTKEYF1	Auginish Reef	Eye tissue
YTKEYF2	Auginish Reef	Eye tissue
YTKEYF3	Auginish Reef	Eye tissue
YTKEYF4	Auginish Reef	Eye tissue
YTKEYF5	Auginish Reef	Eye tissue
YTKEYF6	Auginish Reef	Eye tissue
YTKEYF7	Sth Maroubra	Eye tissue
YTKEYF8	Sth Maroubra	Eye tissue
YTKEYF9	Sth Maroubra	Eye tissue
YTKEYF10	Sth Maroubra	Eye tissue
YTKEYF11	Sth Maroubra	Eye tissue
YTKEYF12	Sth Maroubra	Eye tissue
YTKEYF13	Nth Maroubra	Eye tissue
YTKEYF14	Nth Maroubra	Eye tissue
YTKEYF15	Nth Maroubra	Eye tissue
YTKEYF16	Nth Maroubra	Eye tissue

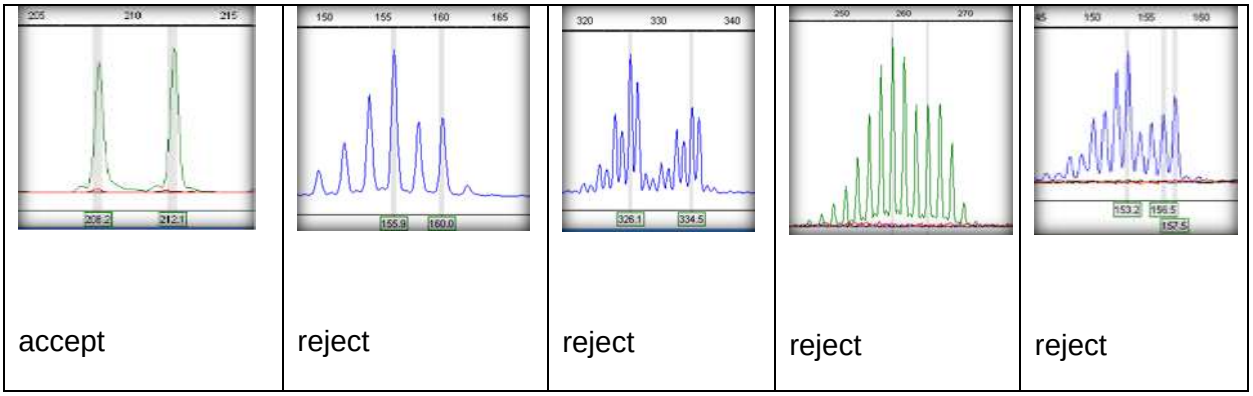
Sample ID	Location sampled	Tissue Type
YTKEYF17	Maroubra	Eye tissue
YTKEYF18	Maroubra	Eye tissue
YTKEYF19	Montague Island	Eye tissue
YTKEYF20	Montague Island	Eye tissue
YTKEYF21	Montague Island	Eye tissue
YTKEYF22	Montague Island	Eye tissue
YTKEYF23	Montague Island	Eye tissue
YTKEYF24	Montague Island	Eye tissue
YTKEYF25	Montague Island	Eye tissue
YTKEYF26	Montague Island	Eye tissue
YTKEYF27	Montague Island	Eye tissue
YTKEYF28	Montague Island	Eye tissue
YTKEYF29	Montague Island	Eye tissue
YTKEYF30	Montague Island	Eye tissue
YTKEYF31	Montague Island	Eye tissue
YTKEYF32	Montague Island	Eye tissue
YTKEYF33	Coffs Harbour	Eye tissue
YTKEYF34	Coffs Harbour	Eye tissue
YTKEYF35	Coffs Harbour	Eye tissue
YTKEYF36	Tura	Eye tissue
YTKEYF37	Point Perp	Eye tissue
YTKEYF38	Point Perp	Eye tissue
YTKEYF39	Point Perp	Eye tissue
YTKEYF40	The Murk	Eye tissue
YTKEYF41	The Murk	Eye tissue
YTKEYF42	The Murk	Eye tissue
YTKEYF43	Malabar	Eye tissue
YTKEYF44	Deadmans	Eye tissue
YTKEYF45	Deadmans	Eye tissue
YTKEYF46	Longre	Eye tissue
YTKEYF47	Macquarie Lighthouse	Eye tissue
YTKEYF48	Jibbon	Eye tissue
YTKEYF49	Bronte Beach	Eye tissue

Microsatellite markers

Nine to 17 microsatellite loci were used to construct the pedigrees. A detailed description of the development of six markers (YTK001, YTK002, YTK008, YTK011, YTK017 and YTK019) from YTK transcriptome sequences can be found in Whatmore et al. (2013). A further eight loci were subsequently developed using similar methods. In addition, twenty five published microsatellite primer pairs were assessed for parentage assignment suitability. Three loci were deemed optimal; Sdu21, Sdu32 and Sdu46 (Renshaw et al. 2006, 2007). All chosen markers showed consistent PCR amplification, polymorphism and clarity of electrophoretic signatures.

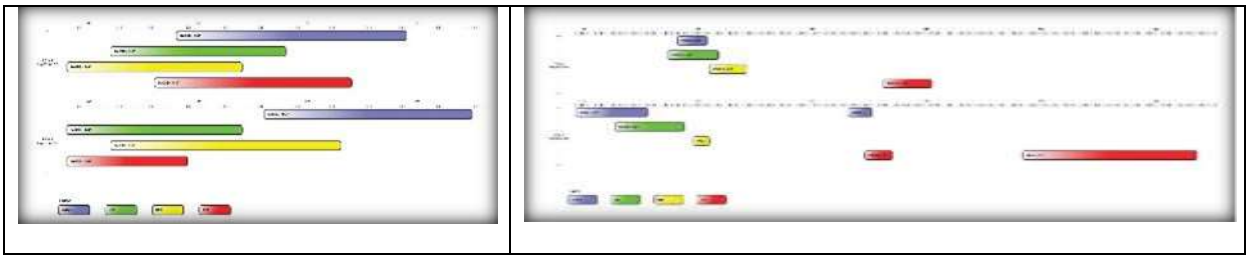
Examples of acceptable and unacceptable DNA microsatellite loci electropherograms (Figure 1) show that we restrict loci to those that are a. machine scorable (do not require human reinterpretation and thus errors), b. tend to not have any stutter, c. are polymorphic, d. amplify well, repeatably and reliably, e. able to be paneled, given size and amplification characteristics. USC is the only group worldwide that have developed such a volume of high quality DNA microsatellite loci for Kingfish.

Figure 1 High and low quality DNA microsatellite loci



We have developed four PCR multiplex pools by which several microsatellite loci can be simultaneously amplified at reduced cost (FIGURE 2).

Figure 2 Example of two DNA microsatellite panels for Kingfish



Genotyping

Microsatellite primer pairs were assigned to multiplex PCR pools using Multiplex Manager software v1.2 (Holleley & Geerts, 2009) and forward primers end-labelled with fluorescent dyes (NED, PET, VIC or FAM). Forward and reverse primers for each multiplex pool were combined in 10x primer mixes using approximately 0.2 µM of each primer, with final volumes dependent upon PCR product fluorescence intensities.

Total genomic DNA was extracted from larvae and caudal fin clips using DNeasy Blood and Tissue kits (Qiagen, Germany) following the manufacturers protocol. DNA amplification was achieved using Qiagen Multiplex PCR PLUS Kits (Qiagen, Germany) in 13.5 µL reactions, each containing 1.25 µL of 10x primer mix, 6.25 µL of Multiplex PCR Master Mix, 2.75 µL of RNase free water, 1.25 µL of Q-Solution and 2.0 µL of approximately 20 ng template gDNA. Amplification was performed using an Eppendorf Mastercycler nexus (Hamburg, Germany) with cycling conditions as follows: initial denaturation at 95 °C for 15 min, followed by 35 cycles of 95 °C for 30 s, 57 °C for 90 s, and 72 °C for 30 s; with a final extension at 68 °C for 30 min.

PCR products were separated by capillary electrophoresis on an AB 3500 Genetic Analyser (Applied Biosystems) at the University of the Sunshine Coast. Fragment sizes were determined relative to an internal lane standard (GS-600 LIZ; Applied Biosystems) using GENEMARKER v2.4.0 software (SoftGenetics; State College, USA) and double-checked manually. Individuals with low or missing peaks were amplified and genotyped a second time.

Parentage assignment was completed using COLONY software v2.0.5.0 (Jones & Wang, 2010) with confidence scores of above 95%.

Where sex is unknown

In some cases sex of the broodstock is unknown. For these cases, where there are offspring, we are able to detect two groups of parents, but don't know which group is female and which is male, so future ground truthing of our pedigree may reverse the present gender assignment for blocks of animals. How did we identify two blocks?: without sex information we experimented with various

strategies to define the sex; eg initially all the tank 6 broodstock were considered as potential dams in a COLONY run (COLONY is a software program that assembles pedigrees from genoytpes, see Jones and Wang, 2010); then any identified dams in the first run (note, equally they could all be sires, the software can only split into two groups and doesn't know sex per se) were re-entered as dams in a second run with any "not identified" dams in first run entered as potential dads in the second run.

Where no genotypes for parents were available

There were no samples from tank 1 broodstock. Only hypothetical broodstock parents could be assigned for the tank 1, based on the larvae and eggs produced from this tank.

Results

The wild NSW samples

Forty-nine wild samples from various sites along the NSW coast and 11 wild broodstock (from tank 8) were genotyped with up to 17 loci. No full sibling pairs were detected using the "COLONY" software, which is consistent with the animals being wild caught.

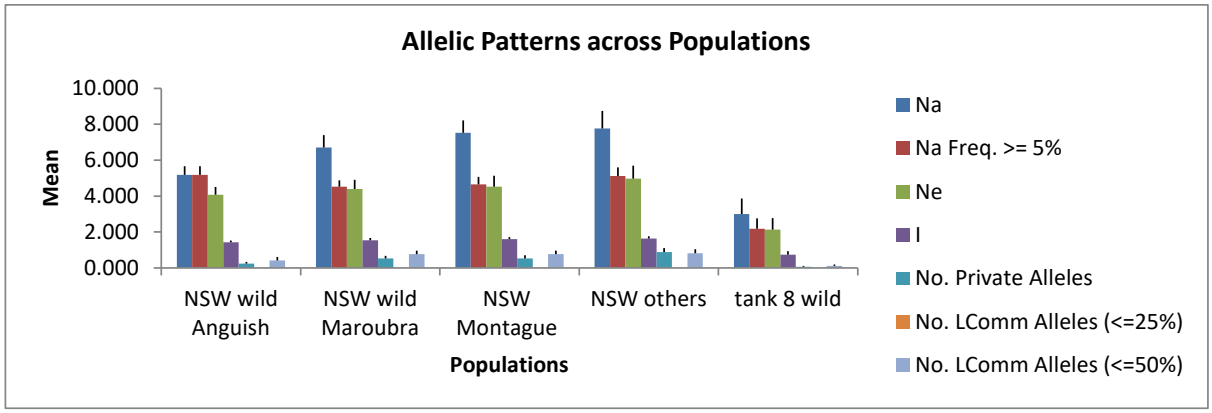
Negligible differences among wild NSW samples

Superficially, the allele patters at 17 DNA microsatellite loci were very similar for each of the five groups of wild samples (FIGURE 3, FIGURE 4).

Figure 3 Allele frequencies at 17 microsatellite loci for wild YTK groups in NSW

Note: tank 8 only have 9 loci assessed, the rest had 17.

Figure 4 Summary allelic statistics for five NSW wild YTK sample sets

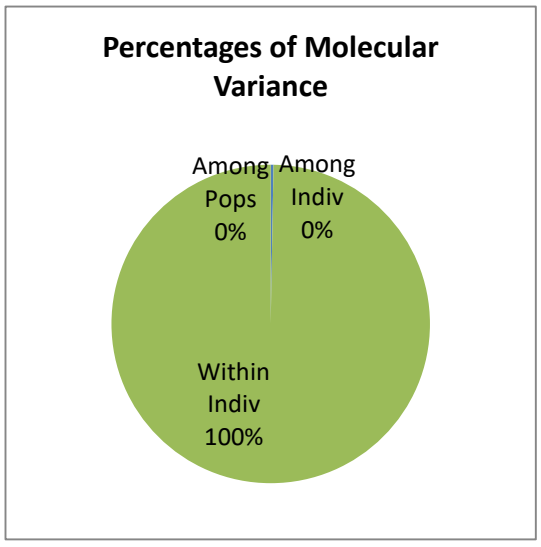


Na = No. of Different Alleles
Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%
Ne = No. of Effective Alleles = 1 / (Sum pi^2)
I = Shannon's Information Index = -1* Sum (pi * Ln (pi))
No. Private Alleles = No. of Alleles Unique to a Single Population
No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations
No. LComm Alleles (<=50%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations

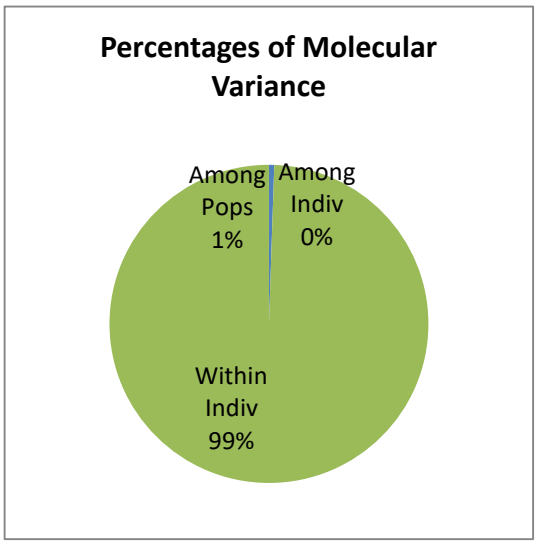
Differences among groups of samples (groups taken from FIGURE 3, FIGURE 4) were negligible and not statistically significant (AMOVA, P>0.001, FIGURE 5, A and B).

Figure 5 Analyses of molecular variance for wild NSW samples

A. 5 populations, 9 loci



B. 4 populations (exception tank 8), 17 loci



Genetic distances among populations were negligible considering Nei's genetic distance (FIGURE 6, FIGURE 7).

Figure 6 Nei's genetic distances among 5 NSW wild YTK sample sets considering 9 loci

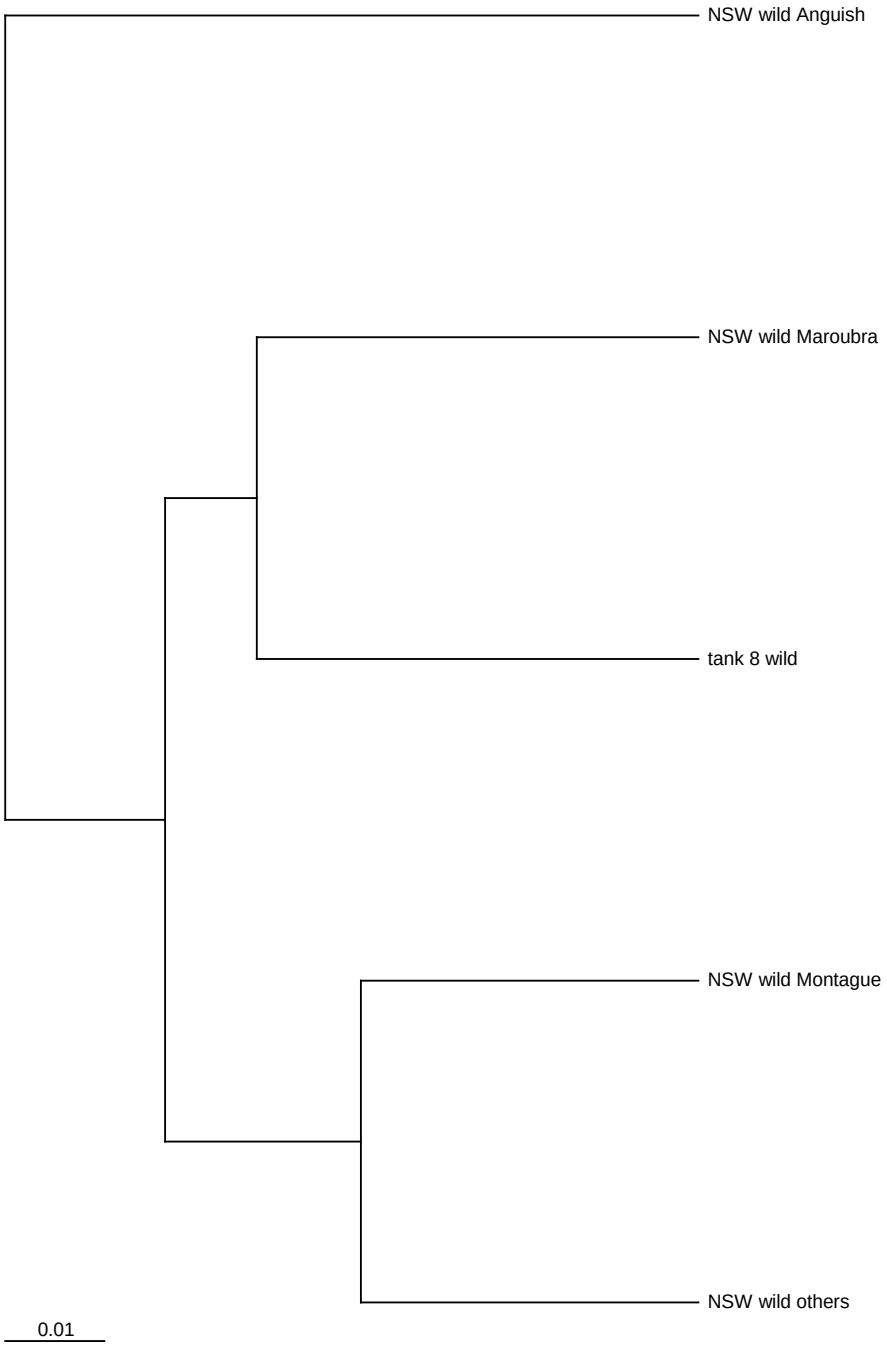
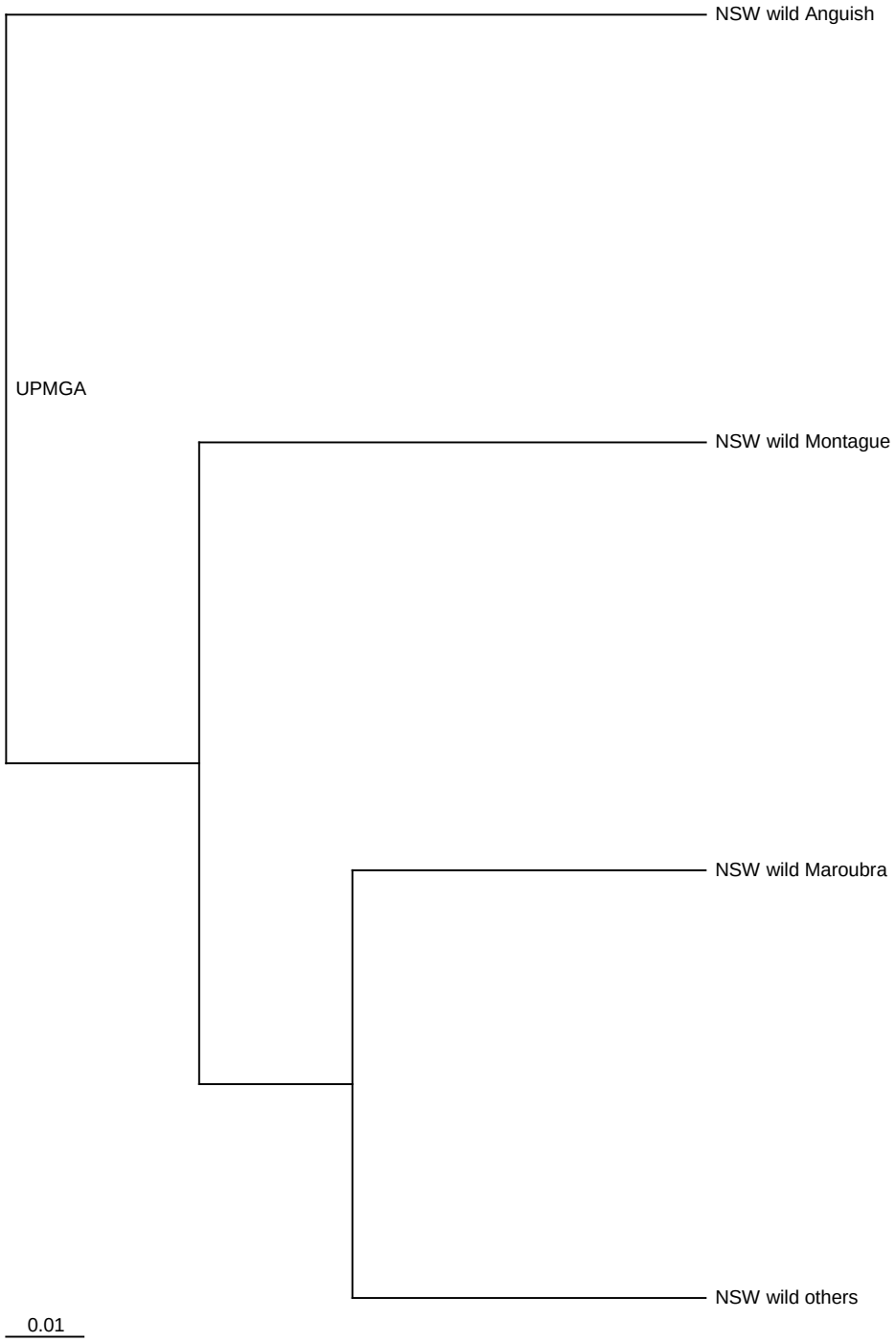


Figure 7 Nei's genetic distances among NSW wild YTK samples (excluding tank 8) for 17 loci

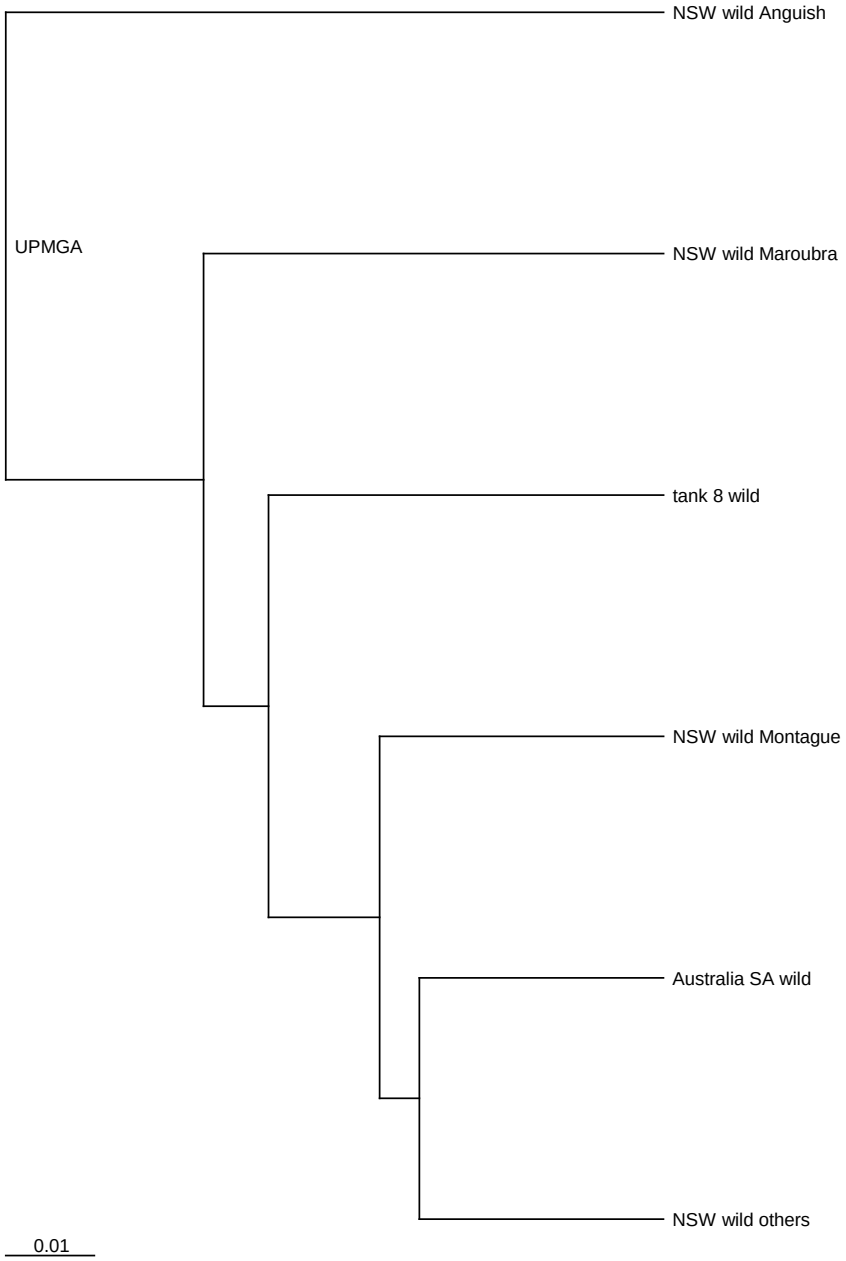


Relationships between the wild NSW and those interstate

UPMGA biogeographic tree

The South Australian Wild population was not distinguished from the NSW populations using Nei's genetic distance (FIGURE 8).

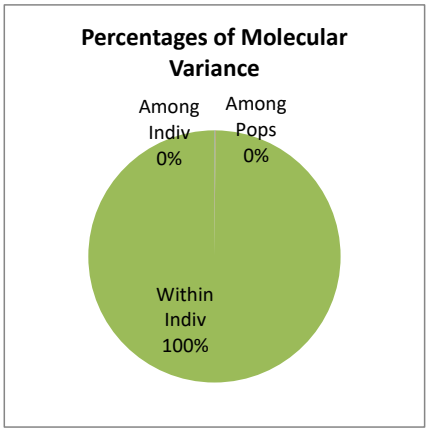
Figure 8 UPMGA tree of Nei's genetic distances



Analysis of molecular variance

Differences among the pooled SA samples and the pooled NSW samples (so there were two groups to compare) were negligible and not statistically significant (AMOVA, P > 0.001, FIGURE 9).

Figure 9 Analysis of molecular variance, pooled SA vs pooled NSW



Plots of allele frequencies of pooled SA and pooled NSW wild samples

Superficially, the allele patters at 9 DNA microsatellite loci were strikingly similar for each of the pooled NSW wild and pooled SA YTK samples (FIGURE 10, FIGURE 11).

Figure 10 Allele frequencies at 9 loci for the pooled NSW wild and pooled SA YTK samples

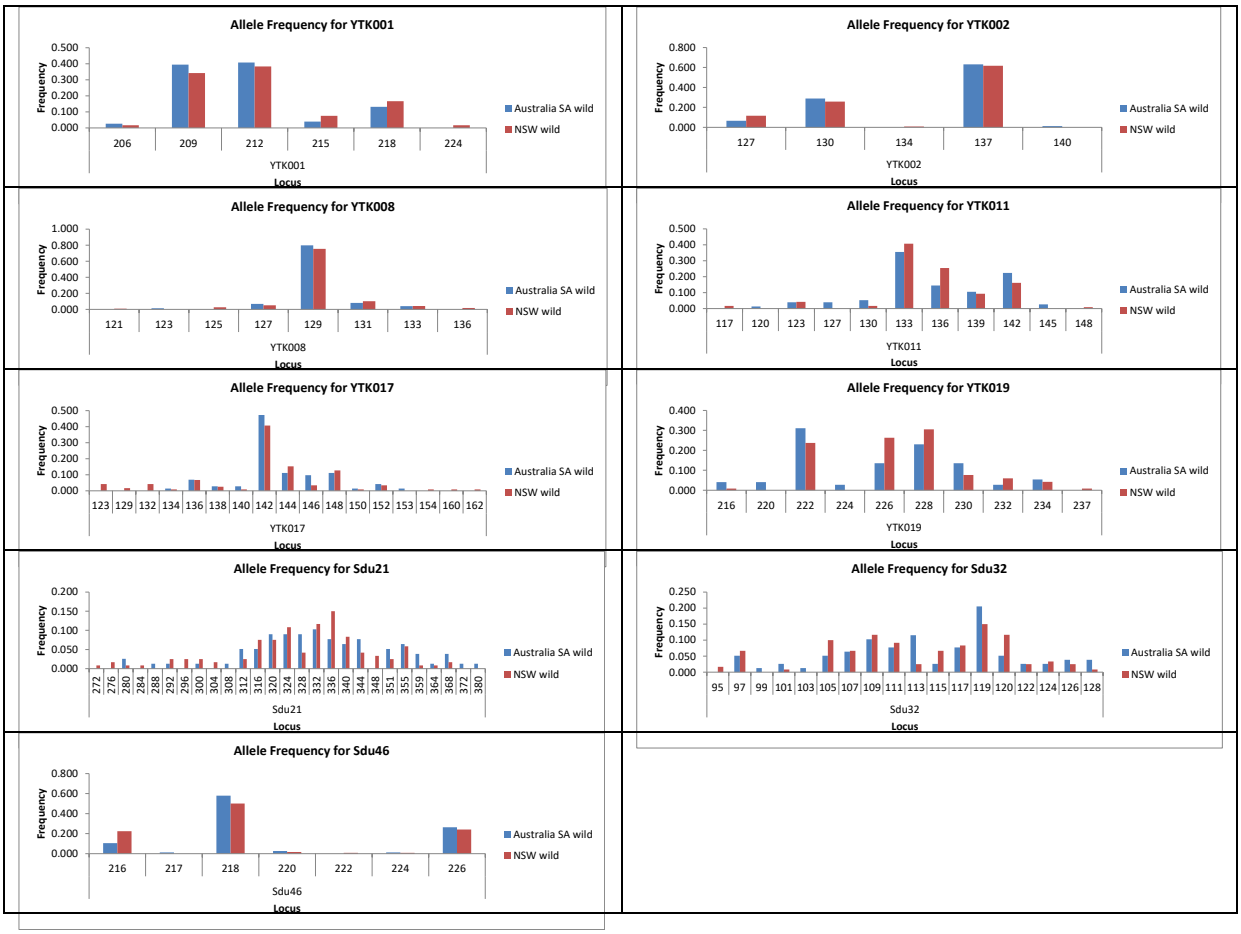
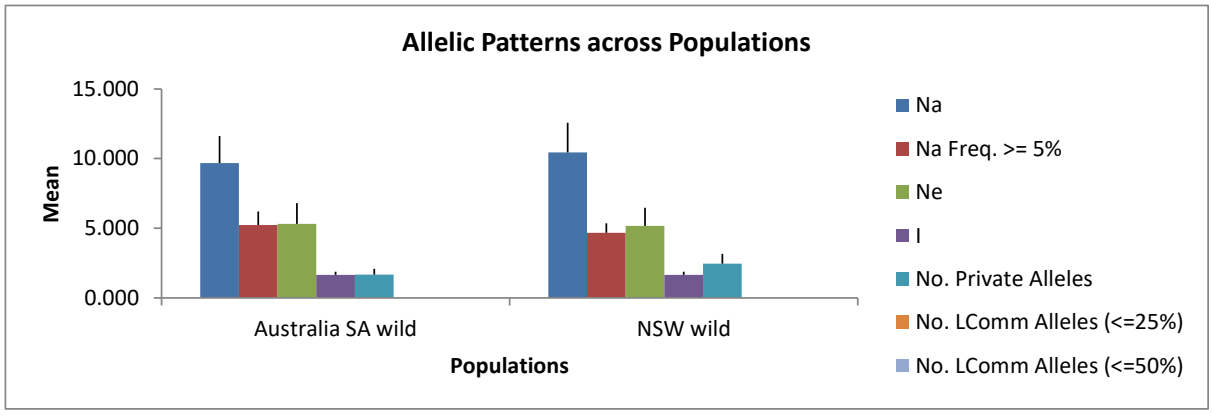


Figure 11 Summary of allelic statistics for the pooled NSW wild and pooled SA YTK samples



Na = No. of Different Alleles
Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%
Ne = No. of Effective Alleles = 1 / (Sum pi^2)
I = Shannon's Information Index = -1* Sum (pi * Ln (pi))
No. Private Alleles = No. of Alleles Unique to a Single Population
No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations
No. LComm Alleles (<=50%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations

Relationships between the wild Australian and overseas samples

Differences among populations (Mexico wild 1, Mexico wild 2, Australia SA wild pooled, NSW wild pooled) were fairly major and statistically significant (AMOVA, P < 0.001, FIGURE 12, also see the UPMGA trees of Nei's genetic distances, FIGURE 13, FIGURE 14).

Figure 12 Analysis of molecular variance, Australia vs Mexico

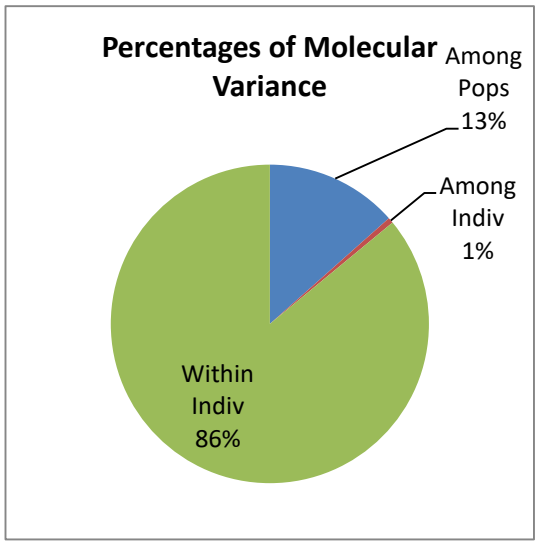


Figure 13 UPMGA tree of Nei's genetic distances, Australia vs Mexico

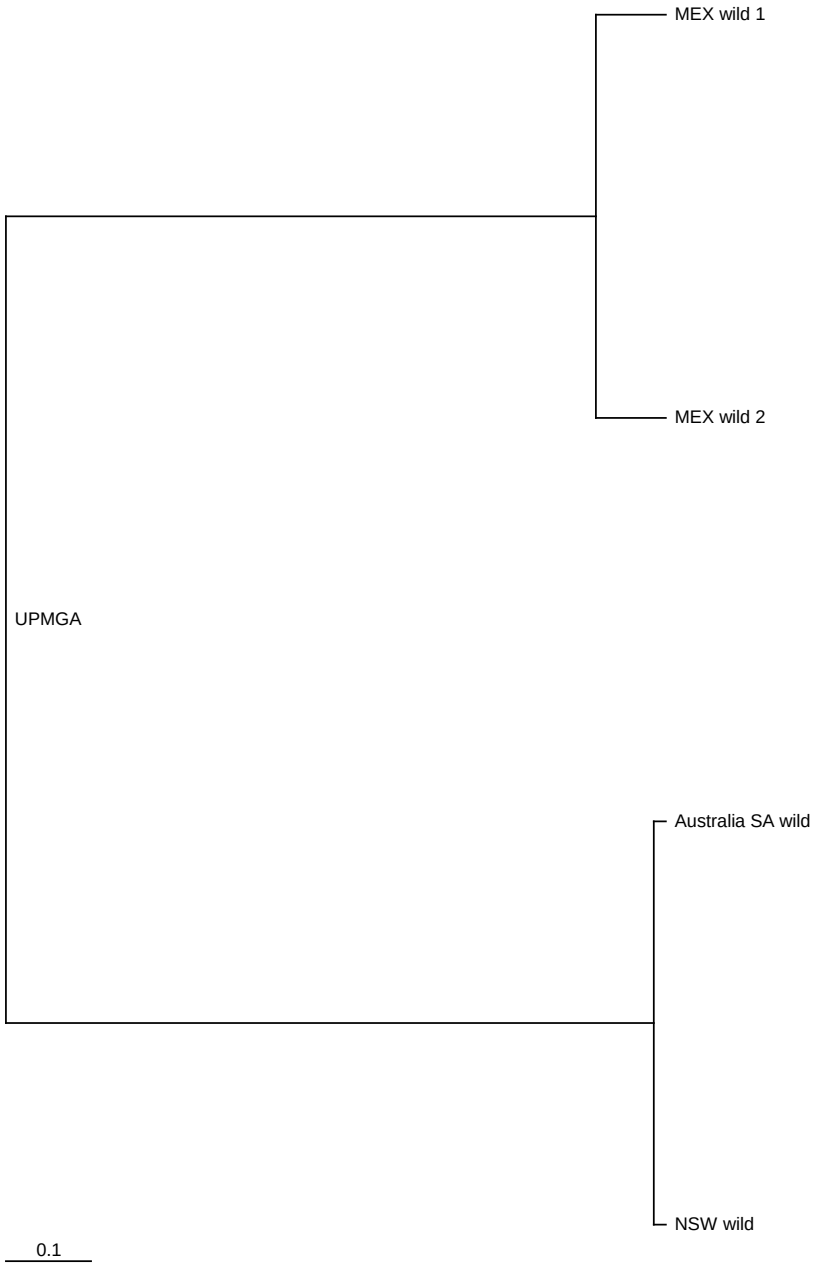
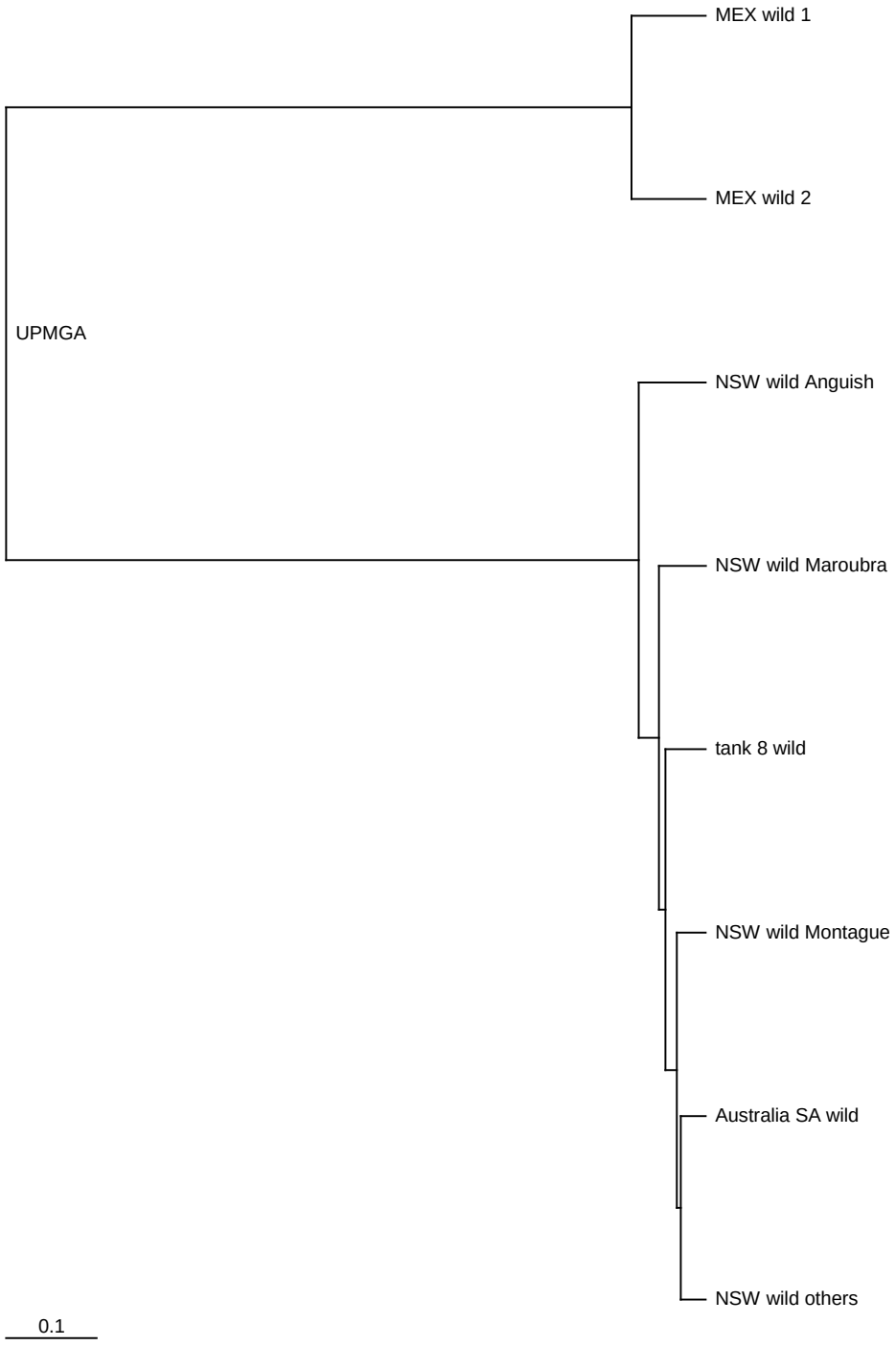


Figure 14 UPMGA tree of Nei's genetic distances, Australia vs Mexico

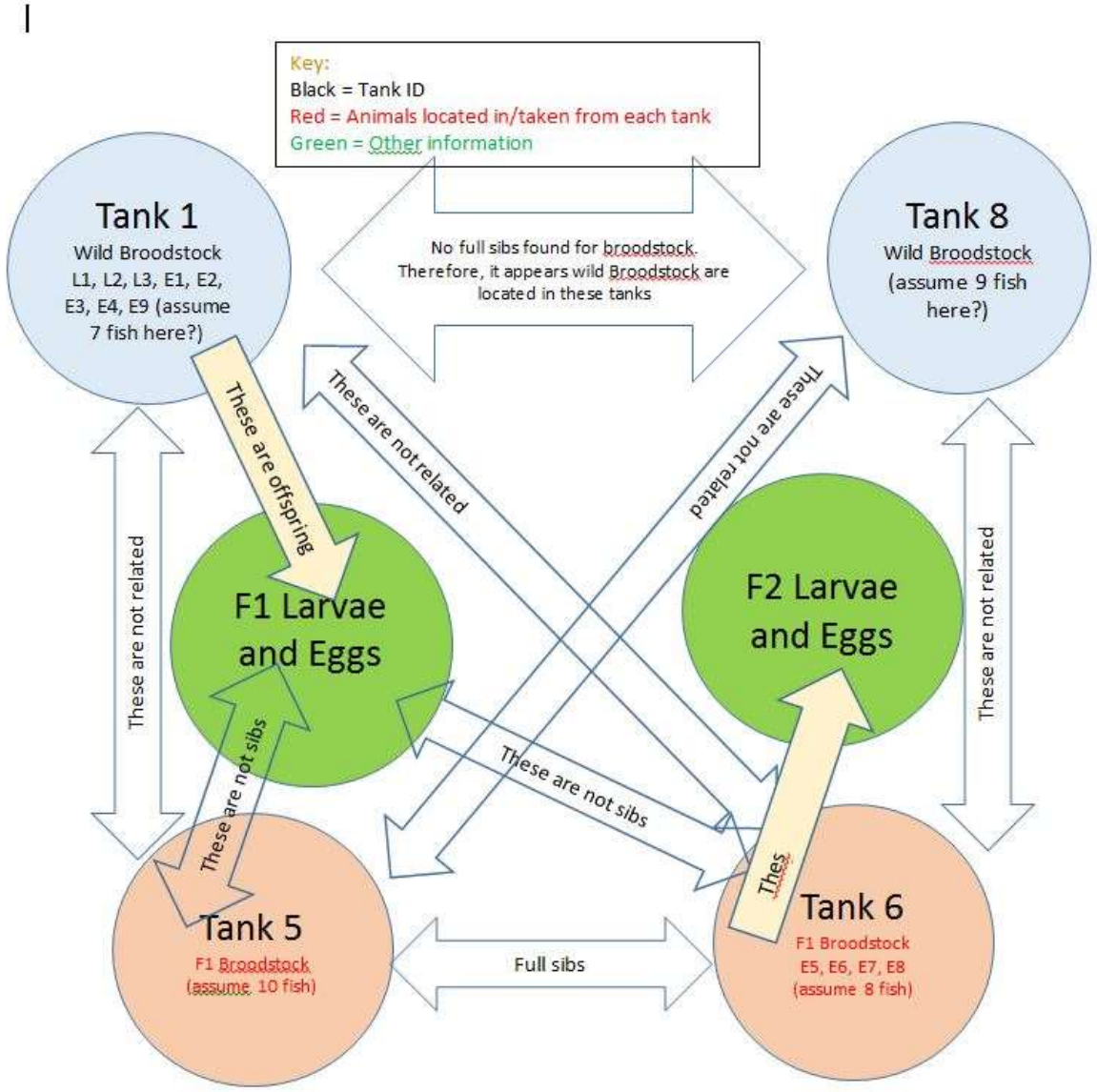


Assessment of stock in various tanks and their genetic relationships

Full siblings were found in tanks 5 and 6. Therefore, tanks 5 and 6 are deemed to be stocked with F1 fish, and tanks 1 and 8 stocked with wild caught broodstock. Further analysis was conducted to determine if the parents of the F1 broodstock in tanks 5 and 6 could be located. These parents were not identified in the tank 8 broodstock, nor were they related to offspring form tank 1, see diagram below. The full pedigree of the NSW fish is given in Attachment 2.

Tank 5 and 6 animals descend from a single dam, and in nearly all cases from a single sire (just two fish have a [single] sire different from the common one). So even though the F2 (offspring of tank 6 parents) arise from some few different parents, they all derive from a single grand sire and single grand dam (see pedigree in Attachment 2).

Figure 15 Genetic relationships among tanks in NSW



Nature of sequential spawning over days

Number of fish that spawned in broodstock tank one

Samples of eggs and larvae were taken from tank one at different times/ spawning events (TABLE 3). 166 larvae and eggs were assigned to parents in tank 1 (TABLE 4, TABLE 5) (since the actual broodstock in this tank were not fin clipped and genotyped, the parental assignments were hypothetical).

Table 3 Sample labels and dates (all 2015) for tank 1

Batches	19/03	20/03	22/03	27/03	1/04	3/04	21/07	24/09	Grand Total
YTKE1				24					24
YTKE2						24			24
YTKE3					26				26
YTKE9								56	56
YTKE4							32		32
YTKL1	43								43
YTKL2		27							27
YTKL3			23						23
Grand Total	43	27	23	24	26	24	32	56	232

In column 1, “E” refers to egg, and “L” to larvae

A total of three hypothetical fathers were inferred (TABLE 4). On any given day, only one or two sires participated in the spawning. So, for example, if a commercial batch was taken on 01/04/2015, only one sire would have contributed and the genetics program may have lost 66% or more of the available diversity from sires from that tank.

Table 4 Contributions of sires to spawns in tank 1 when sampled on different days in 2015

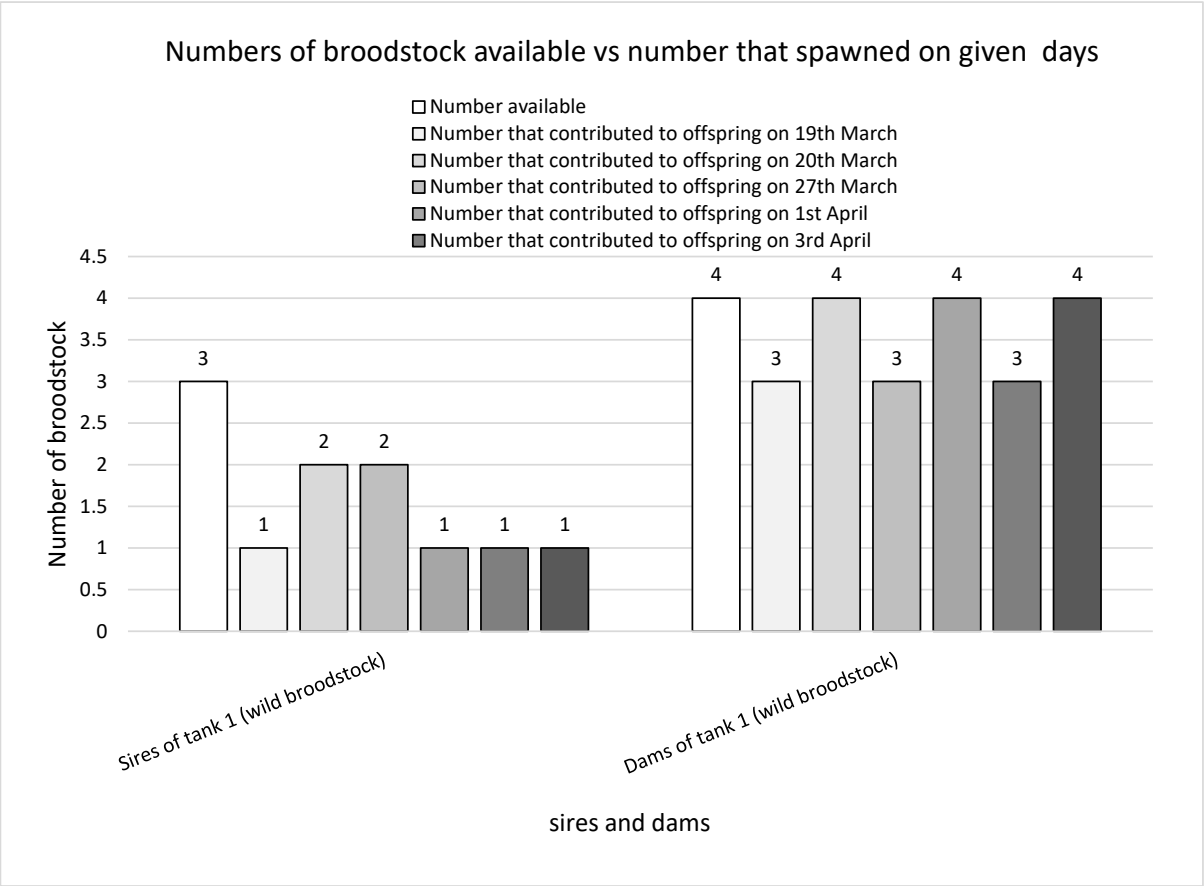
Hypothetical sire	19-03	20-03	22-03	27-03	01-04	03-04	21-07	Grand Total
*1		12		17		23		52
*2	26		14	2				42
*3		13	9		20		30	72
Grand Total	26	25	23	19	20	23	30	166

A total of four hypothetical dams were inferred (TABLE 5). On most days, all the inferred females spawned, although usually, on a given day, only two or three contributed to most of the spawning.

Table 5 Contributions of dams to spawns in tank 1 when sampled on different days in 2015

Hypothetical dams	19-03	20-03	22-03	27-03	01-04	03-04	21-07	Grand Total
#1		9	1	9	7	14	8	48
#2	19	6	8	6	2	7	3	51
#4	1	1	8	4	2		1	17
#6	6	9	6		9	2	18	50
Grand Total	26	25	23	19	20	23	30	166

Figure 16 Diagrammatic representation of numbers spawning vs numbers available in tank 1



Number of alleles represented from sequential spawning of tank 1

The total number of alleles available from each separate spawn day (TABLE 6) is less than that if we pool all spawns together (47 over all loci), which in turn is less than that found in the wild (94 over all loci). Presumable the latter difference is a reflection of the limited total number of broodstock in tank 1.

Table 6 Number of allele in sequential samples from tank 1

Locus	N individuals approximate	YTK001	YTK002	YTK008	YTK011	YTK017	YTK019	Sdu21	Sdu32	Sdu46	All alleles over all loci
NSW wild	60	6	4	7	8	16	8	23	16	6	94
Tank 1 F1 eggs 27-7	30	4	2	3	5	3	3	7	6	3	36
Tank 1 F1 eggs 27-3	20	4	2	3	4	2	4	8	6	3	36
Tank 1 F1 eggs 23-3	24	3	2	2	4	2	3	7	5	3	31
Tank 1 F1 eggs 22-3	20	4	2	3	5	3	3	7	6	3	36
Tank 1 F1 larvae 19-3	25	3	2	1	4	3	3	5	5	3	29
Tank 1 F1 larvae 20-3	27	4	2	4	5	3	3	8	7	3	39
Tank 1 F1 larvae 22-3	23	4	2	3	6	4	4	8	6	3	40
Tank 1 all F1	160	4	2	4	6	4	4	12	8	3	47

Number of fish that spawned from broodstock tank six

Samples of eggs were also taken from tank 6, but at different times/ spawning events (TABLE 7).

A total of four hypothetical fathers were inferred (TABLE 8), and only on one day out of four were all four detected as contributing to the spawning. Overall, one male (designated T6FCF6) contributed to 85% of the F2 offspring. Different dams spawned on different days, so eggs from a single day would not capture all the possible genetic variation of the tank (TABLE 9).

Table 7 Sample labels and dates (all 2015) for tank 6

Batches	28-07	19-08	28-08	09-09	Grand Total
YTKE5		31			31
YTKE6	24				24
YTKE7			56		56
YTKE8				24	24
Grand Total	24	31	56	24	135

All samples were eggs

Table 8 Contributions of sires to spawns in tank 6 when sampled on different days in 2015

Sires	28-07	19-08	28-08	09-09	Grand Total
*4	1	1	3		5
*5	3				3
T6FCF4	1	3	2		6
T6FCF6	15	27	14	23	79
Grand Total	20	31	19	23	93

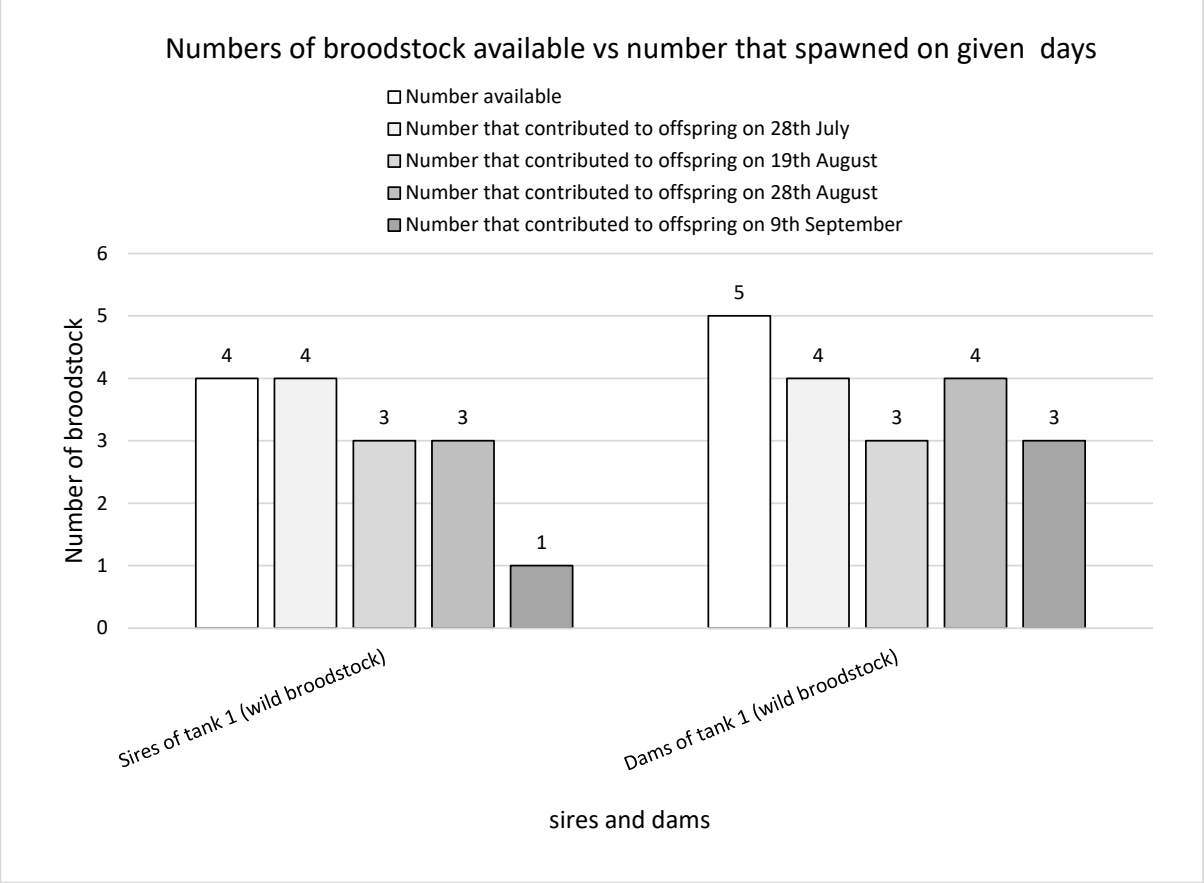
Animals with an * are hypothetical

Table 9 Contributions of dams to spawns in tank 6 when sampled on different days in 2015

Dams	28-07	19-08	28-08	09-09	Grand Total
#10	1		2		3
#12			2		2
#9	11	10	5	9	35
T6FCF1	2	3		2	7
T6FCF5	6	15	9	12	42
Grand Total	20	31	19	23	93

Animals with a # are hypothetical

Figure 17 Diagrammatic representation of numbers spawning vs numbers available in tank 1



Number of alleles represented from sequential spawning of tank 6.

The total number of alleles available from each separate spawn day (TABLE 10) is the same or less than that if we pool all spawns together (31 over all loci), which is turn is less than that found in the wild (94 over all loci). Presumable is latter difference is a reflection of the limited total number of broodstock in tank 6, and that the broodstock in tank 6 are F1.

Table 10 Number of allele in sequential samples from tank 6

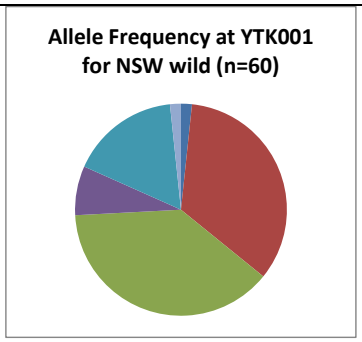
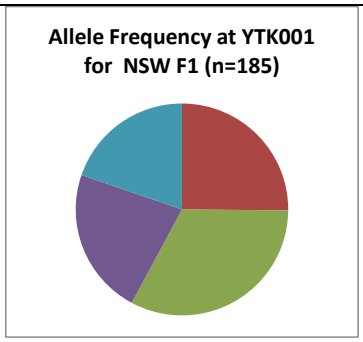
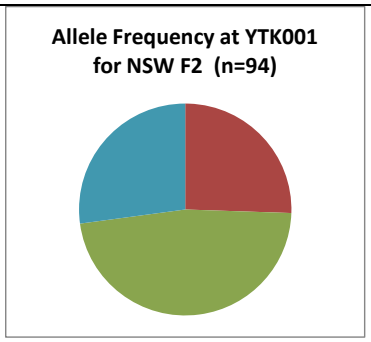
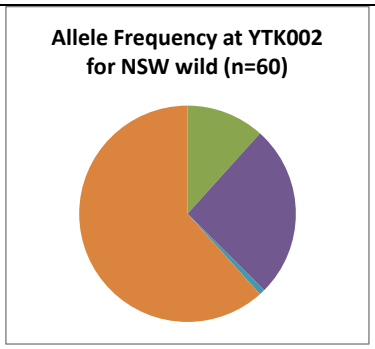
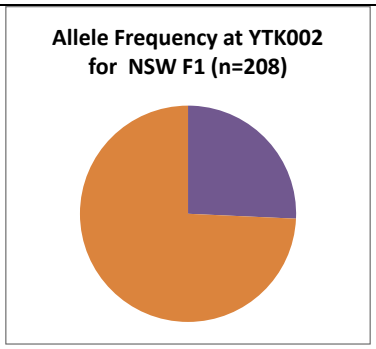
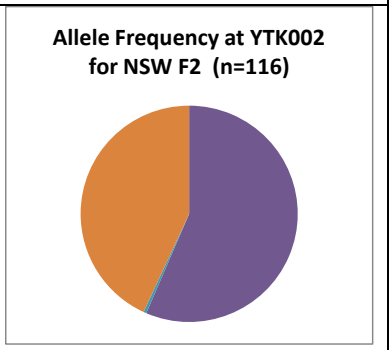
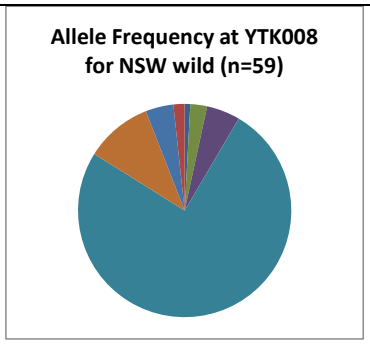
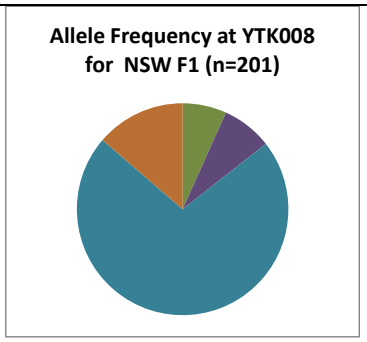
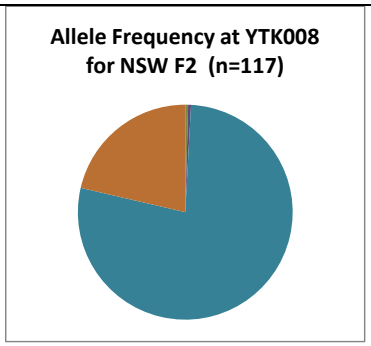
Locus	N individuals	YTK001	YTK002	YTK008	YTK011	YTK017	YTK019	Sdu21	Sdu32	Sdu46	All alleles
NSW wild	60	6	4	7	8	16	8	23	16	6	94
tank 6 F1 broodstock	8	3	2	2	3	3	4	6	5	3	31
Tank 6 F2 eggs 19-8	31	3	2	2	2	2	3	4	4	3	25
Tank 6 F2 eggs 28-7	24	3	2	2	2	2	3	4	4	3	25
Tank 6 F2 eggs 27-8	20	3	3	4	4	2	3	4	5	3	31
Tank 6 F2 eggs 09-9	24	3	2	2	2	2	3	4	4	2	24
Tank 6 all F2	90	3	3	4	4	2	3	4	5	3	31

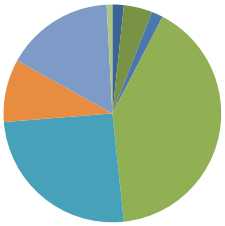
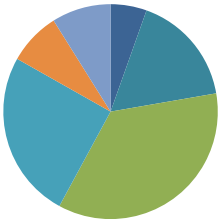
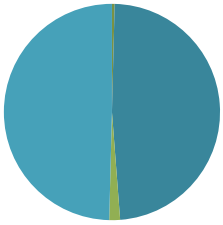
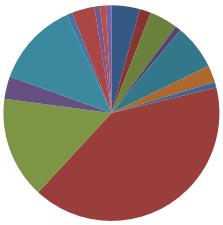
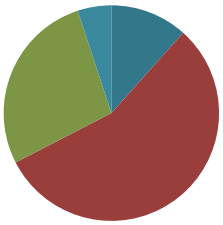
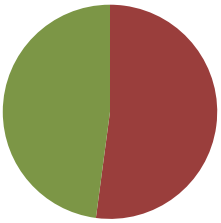
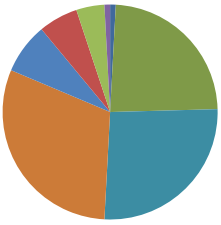
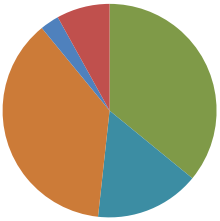
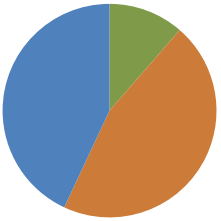
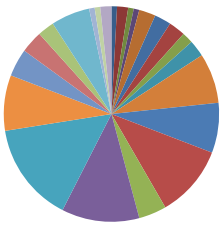
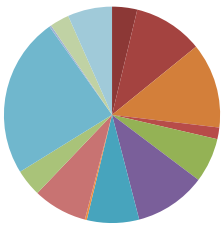
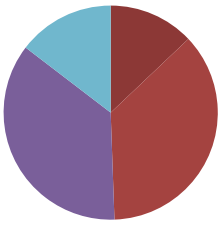
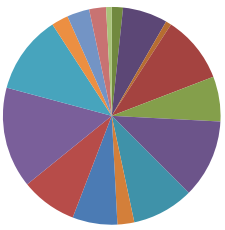
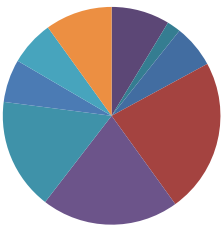
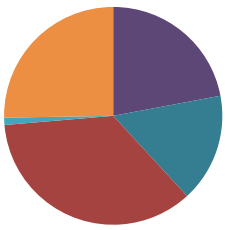
Gradual loss of allelic diversity over generations in NSW kingfish

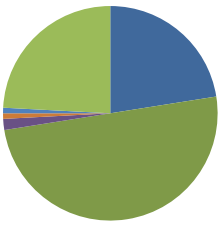
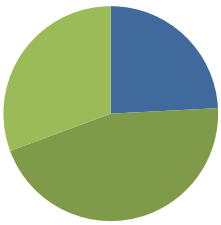
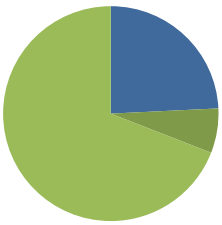
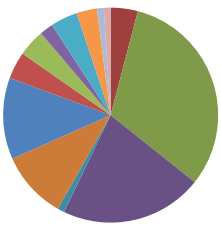
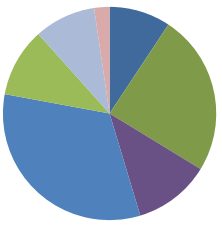
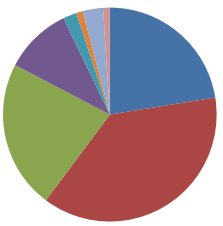
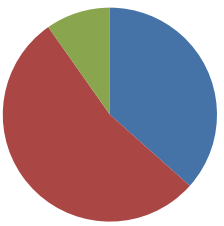
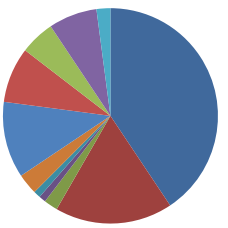
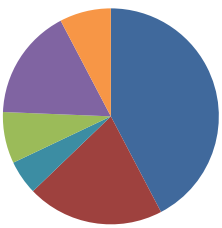
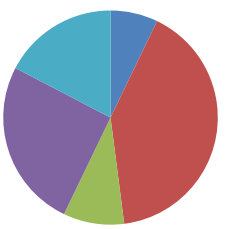
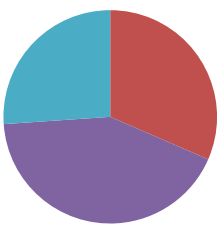
The potential for loss of alleles due to failure of all broodstock to spawn on given days was assessed in the previous sections. Here we determine if there is still loss of diversity over generations when we assume every F1 and F2 sample provided to USC was available in a future pedigree (FIGURE 18).

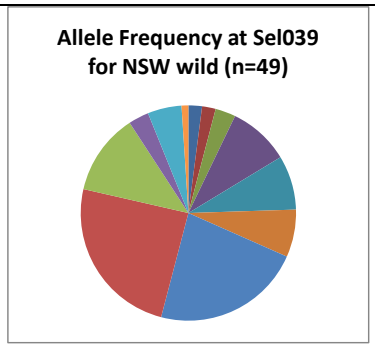
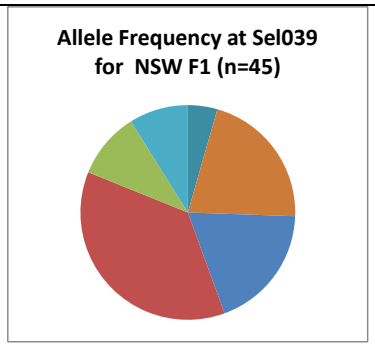
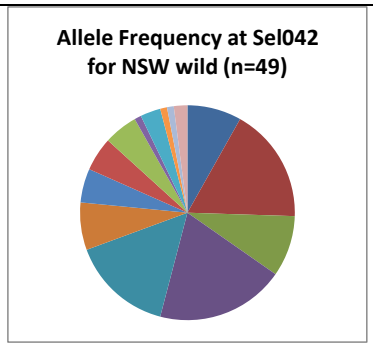
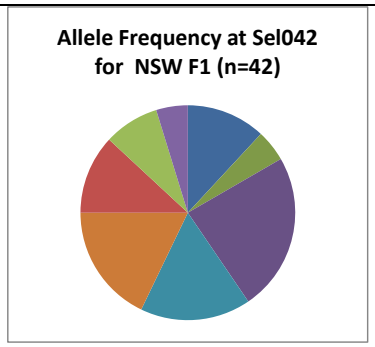
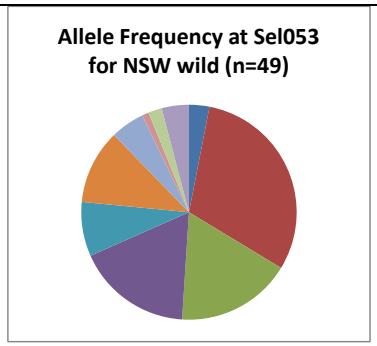
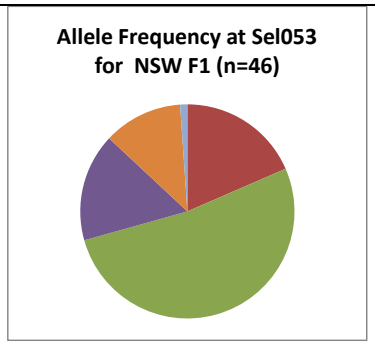
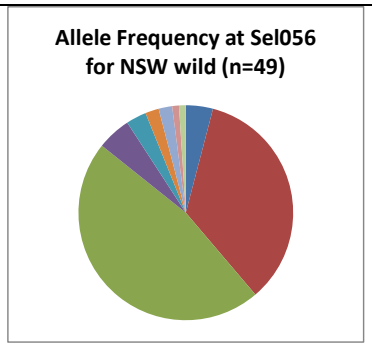
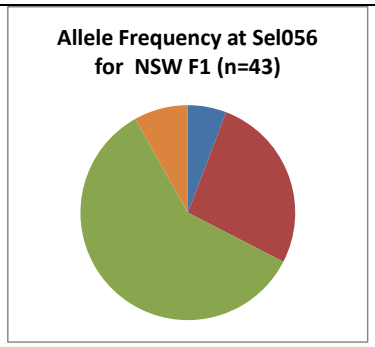
Typically, the first alleles to be lost are the rare alleles (see example of YTK017, Sdu21), but by the F2 even common alleles are being lost. Eventually without pedigree management, it is anticipated the NSW captive stock will become monomorphic for all alleles at all loci, even when moderately large number of offspring are taken.

Figure 18 Allele frequencies among pooled wild, F1 and F2 NSW Kingfish

Loci	All NSW wild samples combined	All NSW F1 samples combined	All NSW F2 samples combined
YTK001			
YTK002			
YTK008			

YTK011	Allele Frequency at YTK011 for NSW wild (n=59) 	Allele Frequency at YTK011 for NSW F1 (n=202) 	Allele Frequency at YTK011 for NSW F2 (n=124) 
YTK017	Allele Frequency at YTK017 for NSW wild (n=59) 	Allele Frequency at YTK017 for NSW F1 (n=198) 	Allele Frequency at YTK017 for NSW F2 (n=119) 
YTK019	Allele Frequency at YTK019 for NSW wild (n=59) 	Allele Frequency at YTK019 for NSW F1 (n=174) 	Allele Frequency at YTK019 for NSW F2 (n=79) 
Sdu21	Allele Frequency at Sdu21 for NSW wild (n=60) 	Allele Frequency at Sdu21 for NSW F1 (n=173) 	Allele Frequency at Sdu21 for NSW F2 (n=89) 
Sdu32	Allele Frequency at Sdu32 for NSW wild (n=60) 	Allele Frequency at Sdu32 for NSW F1 (n=196) 	Allele Frequency at Sdu32 for NSW F2 (n=93) 

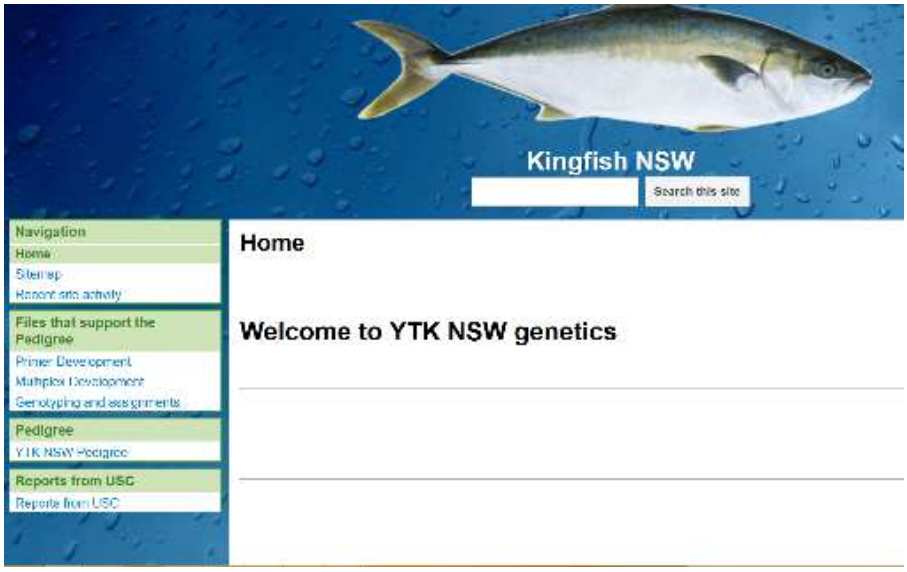
Sdu46	Allele Frequency at Sdu46 for NSW wild (n=60) 	Allele Frequency at Sdu46 for NSW F1 (n=186) 	Allele Frequency at Sdu46 for NSW F2 (n=97) 
Sel032	Allele Frequency at Sel032 for NSW wild (n=49) 	Allele Frequency at Sel032 for NSW F1 (n=43) 	
Sel035	Allele Frequency at Sel035 for NSW wild (n=49) 	Allele Frequency at Sel035 for NSW F1 (n=41) 	
Sel036	Allele Frequency at Sel036 for NSW wild (n=48) 	Allele Frequency at Sel036 for NSW F1 (n=39) 	
Sel037	Allele Frequency at Sel037 for NSW wild (n=49) 	Allele Frequency at Sel037 for NSW F1 (n=46) 	

Sel039			
Sel042			
Sel053			
Sel056			

Where are all the data stored

Date are stored on USC hard drives (which are backed up every day) and on a web site. Access to the web site is available for approved personnel on the project.

Figure 19 Screen shot of NSW YTK genetic web site



Discussion

NSW wild populations

The wild NSW samples seem moderately diverse in terms of DNA microsatellite alleles, with an average of about 6 alleles per loci. Samples from different sites were not different from each other in terms of microsatellite alleles, nor were the NSW samples different the South Australian samples (as also found by Miller 2011). The evidence does not refute the hypothesis that Kingfish are a single breeding stock along the east coast of Australia and around into the Great Australian Bight, so from the perspective of genetics, and notwithstanding health, regulatory and commercial considerations, fish and genetic material could be shared between NSW and SA. On the other hand, the Northern Hemisphere Mexican samples are quite different from the Southern Hemisphere NSW and SA samples, and indeed Northern vs Southern Hemisphere differences are also being reported elsewhere in the literature (Martinez-Takeshita 2015).

Genetic resources in NSW

DNA and physical records seem to agree that there are two tanks containing wild broodstock, each tank with less than 10 animals per tank. There are also two tanks each with less than 10 broodfish that apparently are ‘F1’ (offspring of wild animals); these fish are related to each other, but not to the above mentioned wild fish. The origin of tank 5 and 6 fish is uncertain. Tank 5 and 6 animals descend from a single dam, and in nearly all cases from a single sire (just two fish have a different sire from the common one). So even though the F2 (offspring of tank 6 parents) have some few different parents, all F2 derive from a single grand sire and single grand dam.

For a long term sustainable genetic program, about 50 parents are needed each generation. So NSW presently has less than 40% of the resources (wild equivalents) needed at the first generation, even assuming all animals can be spawned (which apparently is not the case). Indeed, available information on the various groups of F1 brood fish, eggs and larvae suggest they derive from just a couple or just a few wild parents.

The ratcheting down of the number of independent spawning lineages over generations is visually and strikingly evident by the dramatic loss of alleles over generations (wild, to F1, to F2), even when we pool all available F1 and F2 samples.

In light of this information, we can conclude that present practices are not genetically sustainable. Potential solutions and workarounds will be explored in the next report by USC and DPI.

Participation of broodstock over sequential days

Many broodstock do not spawn sequentially every day, but spawn intermittently. So if spawns are collected over several days or more, more genetic variation would be captured than if eggs were sampled on just one day before rearing the samples in the larval rearing tanks, and this was shown to be the case empirically by tabulating the number of different alleles available on different days and over several or more days.

Taking eggs on just one day could rapidly and substantially reduce the genetic diversity and effective population size of the nucleus, for example, on some days just a single male spawned in tank 1.

Were there differences among families in survival of offspring

While this is a very important commercial aspect of genetic differences among families, data are currently not available to test this.

Report 2 – Kingfish genetics: genetic planning and operations

CONTRIBUTORS: Wayne Knibb, Stewart Fielder, Wayne O'Connor, Nguyen Nguyen, David Whyte

Abstract

This is a report from USC to

1. Review of available molecular information on the status of local (NSW) wild and captive YTK broodstock
2. Assess facilities available for YTK broodstock maintenance and breeding
3. Evaluate production demands
4. Identify breeding objectives to best focus selection efforts
5. Identify/design a data management system for data preparation and analyses
6. Prepare a 5 year breeding plan for NSW YTK

For a sustainable genetic program to operate and service an industry initially of 2,000 tonnes, numbers of fish and tanks at NSW DPI PS needs to be increased by at least several fold. Floor space and technical experience is available for this type of expansion.

It is considered likely that genetics will make contributions to profit, initially by improving growth, appearance (condition index) and survival, and indirectly through correlated selection response, improved FCR, using genetic models based on mass selection and pedigree management post selection (aka "classical design"). Other traits, for example flesh quality/muscle fibre density will be assessed for their genetic basis with a view either to improve them or to operate so they are not degraded by inadvertent selection. Assessments, probably ongoing, will be made of the commercial value of 'omics and epigenetics to augment the step by step, generation by generation, classical methods of genetic improvement exploiting additive genetic variance.

A pathway for web based data acquisition and storage was deployed. The data from the Kingfish website will be retrieved to estimate breeding values for individual fish in the pedigree and perform mate allocation.

Road maps for a 5-year breeding program were charted. One model most closely considered is analogous to that proposed in CRC project 2008/703 and was deployed already in SA, and published (Knibb et al. 2015). This model previously has proved to yield substantial selection response in both land tanks and sea cages. Because of a. its track record, b. modest cost, c. feasibility, and d. genetic sustainability, it was considered here in most detail. Other models, varying from low budget and low infrastructure (intraspecific hybrids) to a full within and between family selection (salmon model), were also considered. Some "add ons", e.g. genomics and epigenetics were also considered.

Introduction

There is interest in NSW to develop commercial Yellowtail Kingfish (YTK, *Seriola lalandi*) aquaculture. Genetic improvement, if feasible and cost effective, will support the profitability of the business.

Genetics is about "cutting the coat according the cloth", and there are many considerations and factors that can directly and indirectly impact on genetic planning. The strategy followed here was to step through the order of tasks set out in the original tender documents, trying on one hand to provide concrete planning while on the other to scope some of the more feasible various possibilities, risks and benefits and likely returns.

While the overall thrust of this report was to advocate, at least initially, genetic plans that worked elsewhere, i.e. were feasible over generations and cost effective, there is substantial opportunity and scope to augment the basic plans, or indeed to attempt novel approaches.

Tender items

Review of available molecular information on the status of local (NSW) wild and captive YTK broodstock

This section reviews data from the Final report "Kingfish genetics: genetic diversity in NSW hatchery and wild samples" and interprets them in light of possible future genetic programs in NSW.

Key point 1: NSW and SA samples are not genetically different

The genetic distances among wild NSW samples and SA are so close that the offspring of NSW and SA stock are more different from their parents than NSW is to SA. Accordingly, notwithstanding biosecurity, genetic material could be exchanged between NSW and SA.

Key point 2: NSW hatchery samples have only captured part of the allelic diversity in the wild

There are two tanks with wild broodstock at NSW DPI PS, only one of the tanks was genotyped. Direct comparison between this one and the wild samples shows that less than 50% of wild alleles are present in the one tank. Even considering both "wild" tanks, when/if samples are genotyped, it is likely only part of even the common alleles in the wild has been captured. So, simply on the basis of capturing wild diversity as measured by DNA microsatellite polymorphisms, more wild fish should be brought into captivity. This concurs with another method to consider the size of the founding nucleus, that of effective population size, also see following.

Key point 3: NSW has about 15 wild Kingfish animals or lineages on hand, whereas it may need a minimum of 50

There are approximately 15 wild fish on hand at NSW DPI PS. The F1 fish derive from just one or several wild sire and dam lineages so do not add substantially to the number of wild lineages counted in the two wild tanks. An acceptable rate of inbreeding per generation is 1%. If inbreeding of 1% corresponds to 0.5% inbreeding depression and loss of performance, this loss can be more than compensated by forward genetic gain. Moreover, at 1% inbreeding rate, the loss of additive genetic variation is slow and compensated to some extent by the recruitment of new variation via mutation. Since inbreeding is calculated as $1/2N_e$, 50 fish would suffice to achieve an inbreeding rate of 1%, but on the condition that there are equal contributions of the animals and a 1:1 sex ratio. Both these requirements probably are not met, indeed, the F1 mature fish all seem to share the same single mother, and monitoring spawning over days suggests animals, both sires and dams do not always spawn every day. Thus for some type of mass selection program, with pedigree management retrospectively fitted onto the selected animals, a substantial ramp up in the number of wild *spawning* fish is required. Selection models that don't require such numbers, but response is limited to just several generations, are considered infollowing sections.

Conclusion from diversity studies

The present breeding systems may suit production but are probably inadequate to support a closed multi-generation stock (breeding across generations), as they will generate high rates of inbreeding in the long term. Inbreeding can be avoided by a. only using wild stock, b. use some type of group crossing (following Sections), c. judicious crosses of F1s (at least for one or two generations), d. introduce many more wild fish to the nucleus and spawn them to produce F1.

Assess facilities available for YTK broodstock maintenance and breeding

Knibb and Fielder inspected the facilities at PSFI.

The existing floor space, water supply and treatment options, and the experience of the technicians is able to support the “classical” selection program, which is the minimum program that is “genetically” sustainable over generations and years.

However, around 10 new 30,000 litre tanks dedicated to Kingfish are not yet available, but are probably needed.

Egg collection technologies and systems are in place and with minor upscaling can support the genetics program, at least for the first few years. Likewise for food chain production and perhaps larval rearing (possibly more tanks are needed).

There are many small-scale experimental systems that support a large variety of commercial experimentation that may be required for the genetics.

Facilities to on-grow a moderate number of offspring right through to commercial size could be needed if fish are not to be returned from the sea, and these facilities with their own biosecurity features are not yet developed.

From the above Sections, there needs to be between a threefold and tenfold scale up, or so, of the present number of wild fish, assuming the selection model is mass selection followed by pedigree assignment (aka “classical design”).

Evaluate production demands

Assuming an initial target of 2,000 tonnes annual production. Only one or so brood tanks are needed to supply this amount of production. Any more tanks or infrastructure would be to support either security of supply or the genetics program. Even if the industry should expand substantially, the bottleneck probably is not the number of existing tanks if production of juveniles is the only criterion.

The 2,000 tonne scale of industry can perhaps be used to estimate maximum sustainable investment point for genetics in the longer term.

Generation 1. Assume a 5% return, and no extra cost for feeds due to increased FCR = 200 extra tonnes = $2,000,0000 (@$10 a kg) or = $1,000,000 (@$5 a kg profit). These numbers would be per annum. If 30% of extra is returned to genetics (as seems to the model for NZ king salmon genetics), then the costs of FIGURE 25 and FIGURE 26 are probably covered. Generation 2, assume 10% return and so on.

Identify breeding objectives to best focus selection efforts

In discussions with David Whyte of Huon Aquaculture and DPI PS Staff a diverse range of breeding objectives (goals or outcomes) and selection criteria (technical methods to achieve industry goals) were discussed. Some items are mandatory, others are important under due diligence and avoiding inadvertent future problems that could be caused by selection.

Table 11 List of objectives and selection criteria

Objectives	Selection criteria	
Profit	Growth rate	Faster growth means more fish for the same infrastructure or shorter production times;
	Survival	High survival, which in turn is inadvertently selected by choosing living fish as future broodstock, or, if family selection is done, families with high survival, will be selected. Selection response could be faster or more reliable

		with the latter (which costs more).
	FCR	See following.
	Other traits and selection criteria	There are other traits where knowledge is limited, e.g. heritability of susceptibility to gill flukes and perhaps others diseases of relevance in NSW. We can collect data on these and see if they should be added into a selection index. There are also emerging new ways to measure traits, such as 'omics, and new issues in genetics such as epigenetics. The relevance of these emerging technologies should be assessed on a continuing basis and where approach and cost effective, deployed.
Fish that present well	Appearance, lack of deformity, high condition index	All selected broodstock will have no deformities (while heritability is low, it is not zero); selected fish can also have higher condition index which can, depending on requirement, improve appearance or presentability.
Fish suitable for culture		
Low FCR	Growth rate OR experimental assessment of families	Usually faster growth and domestication (calmness) correlates with improved FCR. We can monitor this correlated selection response by comparing wild and selected stock in experimental systems or at sea.
No undesirable changes	None	Traits such as muscle fibre density will be monitored

Identify/design a data management system for data preparation and analyses

In principle, records that combine pedigree information with trait measurements can add to genetic knowledge, inform about the genetic basis of traits, inform about breeding values, genetic relatedness, genetic performance and rank animals for selection, need to be collected, collated and stored in an organized fashion. Other data assets, such as spawning records, are informative, e.g., spawning egg volumes may inform about the number of females participating in the spawning, but are not strictly genetic data, and are not considered here. Geneticists may wish to access these husbandry and production data but probably will not collect and manage them.

The following will set up an initial stream to acquire, transfer, store and analyze genetic data. It is advisable where possible the commercial partner and NSW PS keep their own records, hard copies or excel spreadsheets for insurance against loss.

Genotyping, assignments and pedigree spreadsheet

Note: to access these sites, either request access from WRK using your current email, or, ask for a domain email address and password (which will allow you to enter data using your iphone).

Data include genotypes, records of samples and finclips (from NSW DPI PS), analysis of genotypes in "Colony" software and are posted at
https://sites.google.com/a/aquagenes.com/kingfish-nsw/genotyping-and-assignments

These data provide a variety of snapshots of the total NSW Kingfish pedigree.

These snapshots are compiled into a continually growing and updated overall pedigree file for NSW held at https://sites.google.com/a/aquagenes.com/kingfish-nsw/ytk-nsw-pedigree

Data acquisition - broodstock

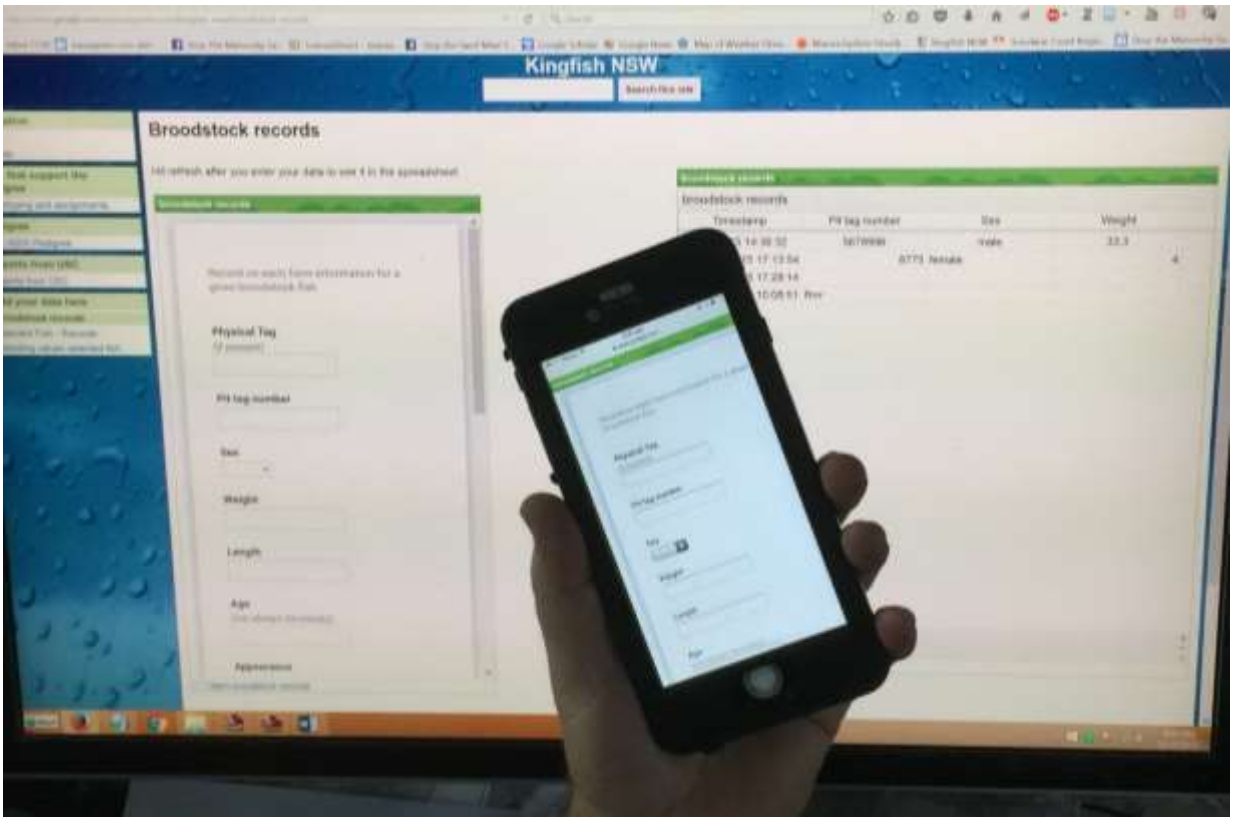
Whenever broodstock data are available, they can be entered into a web page at https://sites.google.com/a/aquagenes.com/kingfish-nsw/broodstock-records

This site will write to a spreadsheet at https://docs.google.com/spreadsheets/d/1mY0oyBl4ZEQjNybpvy-eBq1mnwYGHpPwjOLnIpf1OvI/edit#gid=1976020003&vpid=A2

Access will be controlled either by email accounts provided by USC or your own email address.

Data can be entered either by computer or iphone.

Figure 20 Data can be entered by web page or iphone



Data acquisition – selection at sea

Records of fish selected at sea can be entered here:
https://sites.google.com/a/aquagenes.com/kingfish-nsw/selected-fish---records

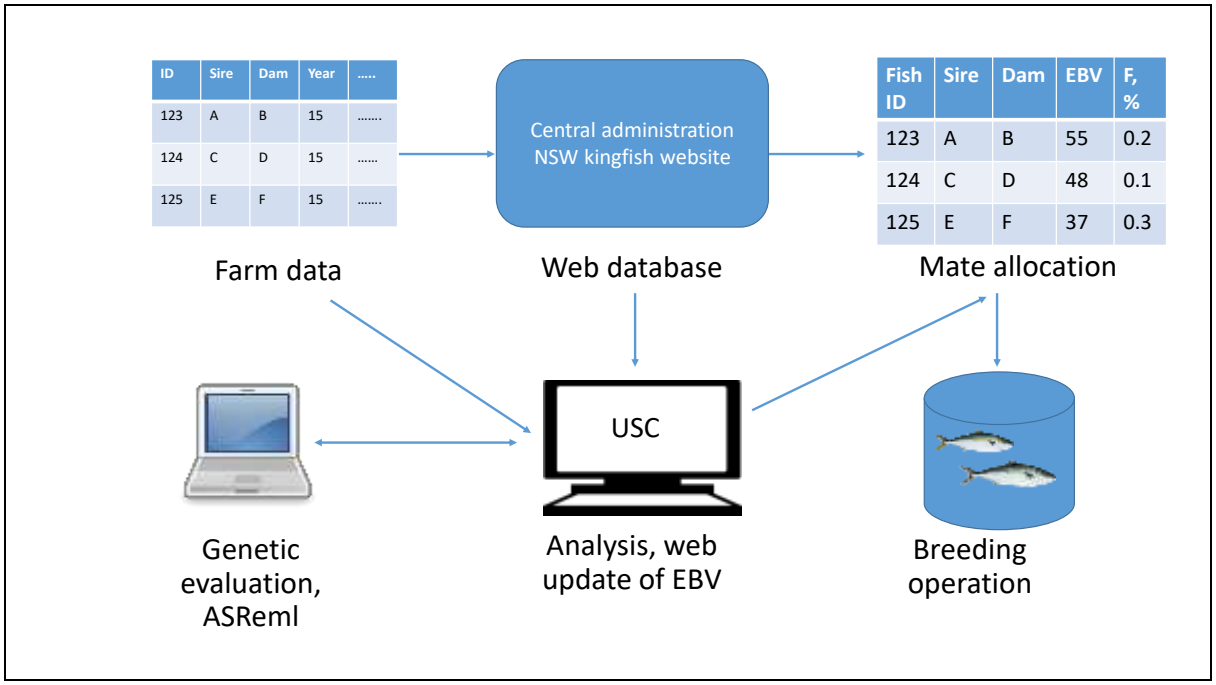
Data acquisition – one off experiments to estimate genetic parameters

A modified form from Section 0 can be deployed when the specifications of the traits to be measured are known.

Estimation of breeding values

A flow of the data analysis to obtain estimated breeding values (EBV) for individual animals in the pedigree is presented in FIGURE 21. The data from the NSW Kingfish website will be retrieved and checked with general statistical packages such as SPSS, SAS or R. They are then transformed in a suitable format to estimate breeding values using ASReml. Mate allocation will be conducted using separate specialized packages: EVA or MateSel. A mate list including EBV of parental stocks and potential inbreeding level of their offspring will be published online.

Figure 21 Genetic evaluation system and mate allocation



Prepare a 5 year breeding plan for NSW YTK

USC in the tender proposed:

It is likely USC will reflect on its past unique experience of breeding and selecting Kingfish over three generations under commercial conditions, and formulate plans for NSW that:

1. **Makes most efficient use of existing facilities**
2. **Plans to have less than 1% inbreeding per generation**
3. **Achieves at least 10% genetic gain per generation for growth**
4. **Maximizes the rate of genetic gain per year**
5. **Minimizes the cost of the program in relation to genetic gains**

We will use these headings to guide the development of a breeding program. But also note that there are a very substantial list of items that can be considered in designing a genetic breeding program (see following list for some examples), these matters can be considered at any time to modify basic breeding designs foreshadowed in this tender.

antagonistic genetic correlations
cost benefit ratio
domestication and inadvertent selection
existing current staff
existing facilities
fertility of broodstock
generation interval
genetic protection for the investment
inbreeding
mate choice system.
methods to estimate breeding values
methods to estimate genetic parameters (heritabilities, and genetic correlations)
number of families
number of family members
number of generations

optimal selection index and selection weights
rate of genetic improvement and return to industry
security and robustness of program
size of the industry
software and data base systems to manage the program
unequal contributions of families in mass spawning

Make most efficient use of existing facilities

The present numbers of breeding animals and brood tanks at NSW PS can provide fingerlings for research and probably for some not insubstantial level of production. However there are too few broodstock to support a "classical" Kingfish breeding program as published by Knibb et al. 2014 without unacceptably high levels of inbreeding in the long term and unacceptable costs. However there are a number of short term approaches that could avoid inbreeding yet still make some selection response over at least one or a few generations. Genetic material produced by both of these options can, maybe, at some future point feed into the "classical" selection program as published.

Select but avoid mating relatives for a generation or so

The "classical selection" plan is followed but using existing fish and infrastructure, and tanks are configured with ensuing F1 so that no brothers or sisters are present in the same tank, thus offspring (F2) of selected F1s can be produced on a reasonably large scale. Perhaps even some selected F3s could be produced that derived from unrelated F2s, but it is most unlikely any future non-inbred animals could be produced. This plan could yield some commercial outcome (5-10% gain for growth per generation) while resources are built up for a more sustainable program.

Cross between two (or more) tanks

Each wild tank at NSW DPI PS can be spawned to produce F1, which can be on grown and selected to yield two *unrelated* cohorts. Each selected cohort is then crossed with the other to yield selected but unrelated F2 on a scale sufficient for production. To go further with this approach, each F1 cohort, which will comprise of related animals, need to be bred, and on grown and selected. This can be done on small scale to reduce loss from inbreeding, which will occur for the F2 pure lines. (Inbred) F2 from line A is crossed with (inbred) F2 from line B. However, the capacity of each "pure" line or cohort to respond to selection gradually diminishes. This type of breeding plan is set out in more detail in the Appendices, and performs reasonably well for several generations, and also avoids the cost of any genotyping. However the performance of this design plateaus after several generations.

Plan to have less than 1% inbreeding per generation

Previously plans were described to avoid inbreeding using existing facilities, but these options are suitable only for one or few generations because of either eventual inbreeding or loss of forward selection response.

Using the "classical selection model"

The published Kingfish selective breeding program will require at least 50 independent sires and dams, ideally at a one to one sex ratio, and all contributing to the next generation. While this number may be phased in over many years, the phasing in strategy can actually delay the implementation of a sustainable program and represent under some models a loss of delivery from genetics. At present stocking densities and size of Kingfish at NSW DPI PS, up to 10 (20 ton or 30 ton) tanks may be required to hold and spawn 50 animals. Certainly reducing the size of the broodfish to 4-6kg and increasing stocking densities can reduce infrastructure, but capacity is still needed to hold and mature selected fish, to hold reserves, to manage cohorts and to compensate for variable spawning over days, so probably the estimate of 10 tanks holds even with

improvement in efficiencies in other ways and is not an overestimate. Floor space seems available, and the cost of tanks is not substantial in light of other considerations.

It is probable that only a proportion of the wild fish will ever spawn, so more than 50 may be needed to be screened. Fertility in captivity of the F1 may be greater than that of the wilds.

There is little commercial gain in increasing the Ne beyond 50, at least for medium to small scale aquaculture production and over several generations (FIGURE 8, FIGURE 9).

There are also costs of genotyping associated with this type of selection plan, but costs can be contained by genotyping only after selection, so only one to several hundred fish are genotyped.

Using "cohort" selection

If ~20 tanks could become available, then a type of rotational mating can be developed where each generation the males from one tank are exchanged with those from another. Each tank can be considered equivalent to a family, and depending on some factors such as the family variances in their contributions to the next generation, inbreeding can be constrained to about 1 %. There are a few problematic logistical issues with this design. If genotyping on a large scale is to be avoided, then the progeny from each tank needs to be raised and selected separately, or at least raised until they can be branded somehow. This translates into multiple hatchery and production runs per generation, which may suit some industry configurations. Planning and procedures with a rotational breeding design is simple, and the cost of genotyping can be avoided.

Achieve at least 10% genetic gain per generation for growth

Assuming inbreeding can be managed to acceptable levels, so the program is sustainable, the next priority is to consider the rate of selection response either in units of separate traits or in $ terms when traits are combined into some type of index selection. For species where given pair matings can be arranged, selection response (or actually breeding values) can be considered jointly with genetic relatedness to optimize response for given levels of inbreeding, but this is not possible for Kingfish, a group spawning species.

Selection response (R), at its simplest considering one trait, and theoretically, is equal to the heritability of the trait multiplied by the selection differential (average difference between the mean of the cohort and the selected mean), or

$$R_1 = h_1^2 S_1$$

where

R_1 is the predicted response
h_1^2 is the heritability
S_1(selection differential) = $\bar{x}_1$ - $\bar{x}_2$

where

$\bar{x}_1$ is the mean of the sampled unselected parents
$\bar{x}_2$ is the mean of all the selected parents

also

$$R_2 = i h_1^2 \sigma_P$$

where

R_2 is also the predicted response
i = selection intensity and = 2.06 for a selection intensity of 5%
σ_P is the phenotypic standard deviation of unselected parents

So, initially, we can consider two main terms that determine selection response, namely heritability and selection intensity or differential.

Selection intensity

The proportion selected relates to logistical costs of screening animals. The relationship between the proportion selected and selection intensity is not linear, especially below about 10% selection intensity (FIGURE 22). Previous calculations on Kingfish show only relatively small predicted difference between selection intensities of 1% to 10% (FIGURE 29, FIGURE 30), although even small genetic differences may have substantial $ value. The "classical" published selection procedures for Kingfish use about **5-7%** selection intensity (FIGURE 23 – the average of the selected is the top 5-7% the average of a random group). With mass selection it is feasible to achieve selection proportions of around 5%. If *both* the within and between family selection is desired, then, assuming 25 families are retained, then one would need 50 families to achieve a *between* family selection intensity of 50%, and so on, that is the logistical costs to do effective *between* family selection accelerates as the targeted selection intensity on families increases.

Figure 22 Relationship between intensity and proportion selected

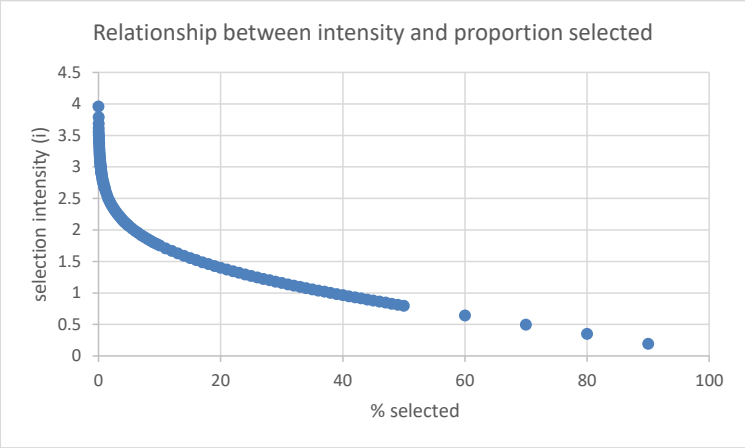
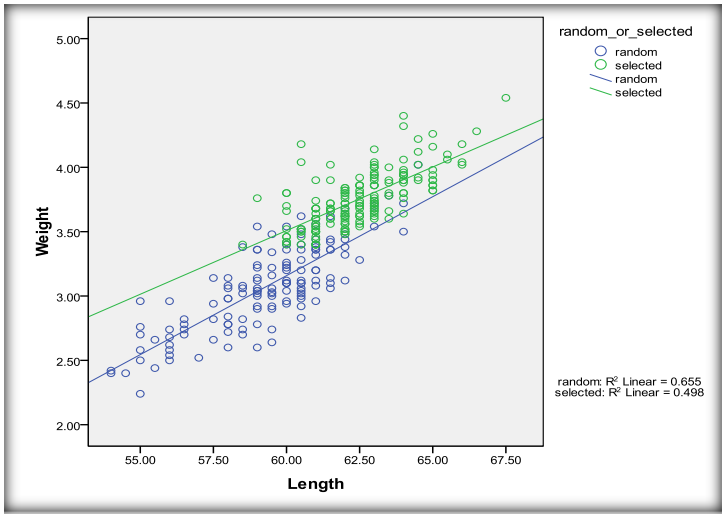


Figure 23 Regressions of weight on length for selected and random YTK



(from the round of selection that produced the SA F_1 parents)

Heritability

The heritability is the proportion of observed phenotypic variation for a trait that is due to additive genetic variation. Counter intuitively perhaps, the heritability for traits can vary, and maximization of the heritability can increase selection response. We now have various estimates for the heritability for weight in Kingfish, and it is around 0.3 under what are probably stable production systems and diets. Variable and stressful production, diseases, poor diets will tend to reduce the heritability and thus selection response.

The heritability for weight in sea cages may be different for that in tanks; indeed traits may be slightly different (due to slightly different sets of alleles, and the genetic correlation could be less than 1). So in a sense, the heritability, or at least the selection response is maximized by selecting fish grown under commercial conditions (which most likely is sea cages). The caveat is that fish retuning from commercial conditions may also bring diseases back into the hatchery.

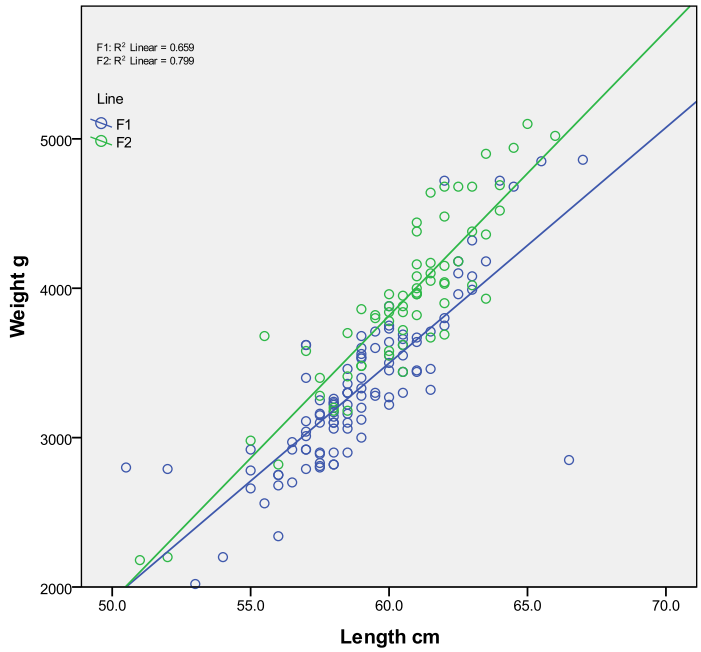
Heritabilities estimated using families can be very high (approaching 1). However, to exploit this we need to do selection among families, which would require several times scale up of resources required for the “classical” Kingfish selection option. Also the variances among families tend to be less than that among individuals.

Worked example

The following is a worked example from the CRC public report Kingfish genetics: Commercialisation strategies Project No. 2013/700. The predicted response for weight is around 6.9%. The actual response observed in tanks was 20% (Knibb et al. 2016), and that observed in sea cages was 10%. The favourable discrepancies could be due to unmeasured domestication selection (Knibb et al. 2016), and/or some type of magnification of genetic differences during communal rearing of selected and non-selected fish.

There are other aspects that may increase the per generation value of selection. First, FCR is expected to be reduced in selected fish from experience in other cases, and preliminary data for Kingfish support this possibility (extra growth of the selected fish required no extra feed). Second, as evident from FIGURE 23 and FIGURE 24, selection response was also achieved for condition index which may have $ value under various circumstances. The formal use of selection indices for multiple traits (weight, CI, fat content, texture, flukes, disease and or survival, maybe deformity, fibre density) would likely see the commercial return achieve 10% per generation.

Figure 24 Regressions of weight on length for the F_1 and F_2 fish



Maximizes the rate of genetic gain per year

The above sections have for the most part considered genetic response per generation. However, for commercial outcomes it is desirable to increase the genetic response per year. A practical way to do that is to breed fish as soon as possible (i.e. as young as possible), and it should be possible to target the breeding of 3 year old males and 4 year old females. Some previous

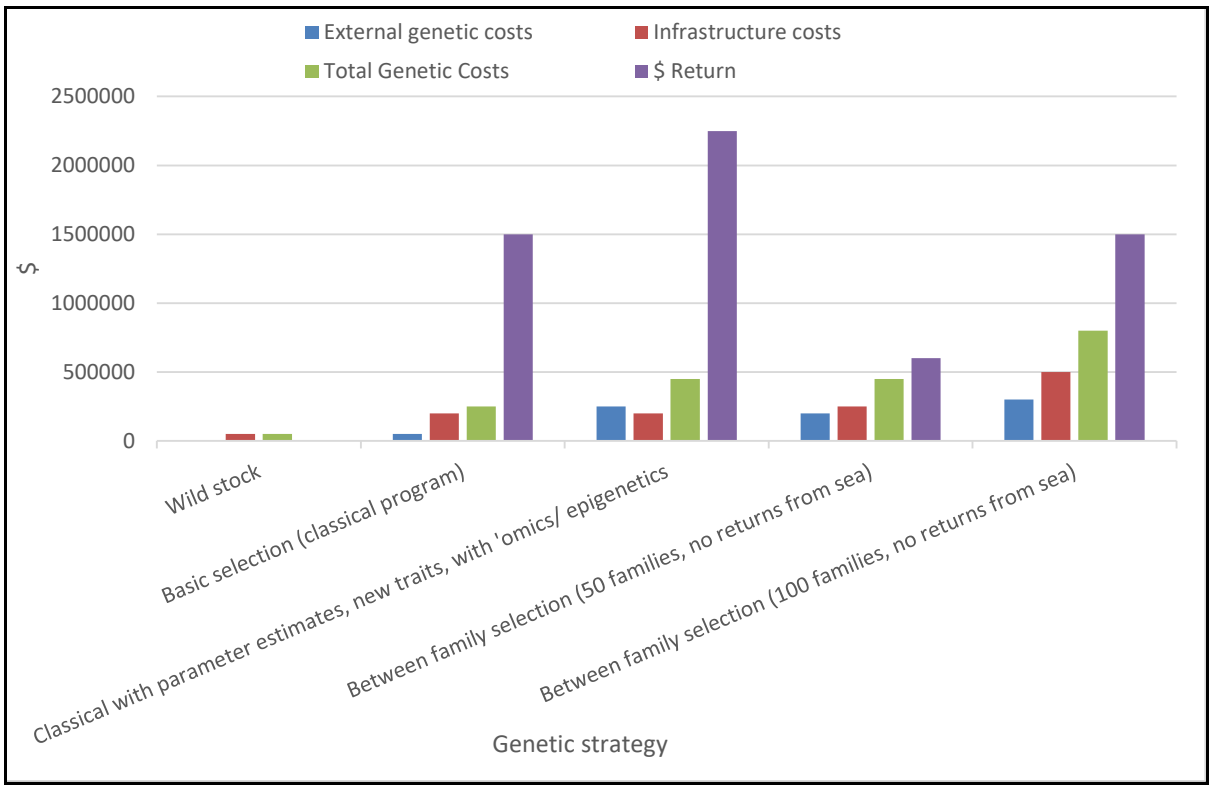
estimations (CRC Project 2008/703) suggest halving the generation time surpasses selection at 1% intensity and all other genetic approaches (FIGURE 29). This aspect of the breeding program should be prioritized.

Minimizes the cost of the program in relation to genetic gains

Scope of genetic options

There are quite a number of distinct possible approaches to genetic improvement and/or prevention of rapid inbreeding. Some notion of these approaches (what they could include, their value and their costs) is set out in FIGURE 25, which while more conceptual than $ accurate, gives some sense of the CBRs of different strategies. The best CBRs, coupled with modest cost, come from the classical designs which, coupled with feasibility, was why this strategy was adopted in SA (non-sustainable genetic selection approaches mentioned in Sections 0, 0 are not considered here). Family based selection, when fish are assessed but not returned from the sea, yeilds poorer CBRs but greater biosecurity. Combined within and between family selection options were not graphed but would see improved CBRs but risk biosecurity unless individual selection could be based on some type of ‘omic data (i.e. fish are not returned from sea, yet fish are still selected within families by ranking with ‘omic information).

Figure 25 Broad comparison of different genetic strategies



Assumptions: 2000 tonne industry, 5% genetic gain per generation
External genetic cost are those outside of NSW DPI, e.g. to USC for genotyping
Infrastructure costs are tanks, technical support to maintain fish
Total genetic costs are the sum of the above two lines.
Estimates are also based on a single generation, cost benefit ratios will improve over generations as genetic gains are cumulative and in principle the costs for a given generation can be recovered again and again over each future year of production.

Reduce genetic costs

Considering the *classical option*, costs can be reduced if genotyping can be avoided. Perhaps husbandry technologies/logistics can be developed to spawn single pairs of Kingfish, whose offspring can be grown seperately until they can be tagged. It may be however, that the costs of husbandry and physical tagging is greater than the costs of genotyping.

Increasing the number of fish in a given tank that contribute to the next generation will have a substantial contribution to reducing overall costs. Greater tank densities, and smaller broodfish can also reduce overall costs.

Apart from technical strategies, costs can be reduced through nationally funded research projects that focus on commercially relevant science.

There may also be some commercial options to reduce costs, such as collaborating with existing Kingfish producers.

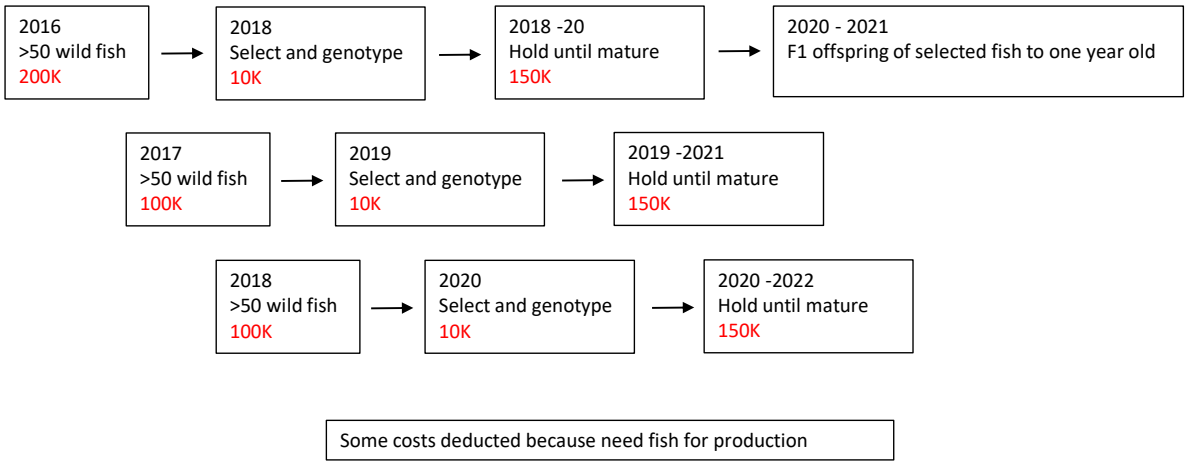
While the above considers options to reduce cost, these strategies need to be coupled with estimates of return. Most genetic options have favorable cost benefit ratios, especially as the scale of the industry increases and the genetic outcomes accrue over generations. There are also stategies for budgeting genetic programs other than those considered above, for example, apparently in NZ King salmon, a percent of the benefit obtained from genetics (30%?) is allocated for future genetic work.

Overveiw of 5 year plan

A stylized 5 year selection program (FIGURE 25) shows that the bulk of the initial costs in a classical program are associated with setting up the large numbers of wild fish, housing and reproducing them. Unfortunately, no commercial gain is achieved in the five year span.

To this basic design, we can add commercial research on determining the heritabilities and genetic correlations, possibly epigenetics and 'omics (see FIGURE 25).

Figure 26 Simplified diagrammatic five year plan



legend: numbers in red are $ cost, very approximte. Arrows link either between years or generations

Consider synergies with other existing YTK programs

Here synergy is understood to mean collaboration as opposed to commercial interpretations such as buying stock.

According to public domain reports and publications (CRC reports, Knibb et al 2016), the number of independent broodstock lineages in SA is about 35, which is less than the minimum of 50 needed for a sustainable program, although the SA genetic program has reached F3, with fish in production that are the offspring of selected parents and grandparents. For reasons of biology, it is not likely all 35 independent lineages can be continued through to the F3, so inbreeding will reach levels higher than desired. This is especially so since realized inbreeding so far was avoided by mating completely unrelated individuals (Knibb et al. 2016), but by at about F4, this avoidance is longer possible. Moreover, the realized inbreeding at F4 will be higher than if animals had been randomly mated from G0 (there is a price to pay by avoiding any realized inbreeding for a few generations).

Should NSW initially progress with the "classical" Kingfish genetic design, even with 10 tanks, it may also eventually, due to reasons of biology, have a shortfall in the number of lineages in the pedigree to keep inbreeding at less than 1% per generation. If the SA and future NSW Kingfish pedigrees were managed jointly, then it is conceivable that inbreeding jointly can be kept to less than 1%. Moreover, should just males from SA be crossed with NSW females, then the arising cohort will have zero inbreeding.

There are also security issues, not just the matter of disease transmission interstate, but of losing the whole genetic program in a given state through some type of accident, disease or disaster. Should SA and NSW operate in some collaborative way, then one provides security for the other. This becomes increasingly important the more generations of selection that are completed. So if SA lose their breeding nucleus, it would take six or seven years, or up to a decade to rebuild, and

in the interim, production costs would increase by 20% perhaps erasing profit until F2s and F3s were again available. If the genetic lineages were also held in NSW, SA could rebuild immediately.

USC is currently collaborating scientifically with Mexico (the Centro de Investigación Científica y de Educación Superior de Ensenada, CICESE) on Kingfish genetics. Mexico has a new Kingfish industry, supplying into the US market. It is possible, even likely, Kingfish will become an international species in aquaculture, and as a young and new species there is much to learn how to best produce them. As evident historically for the sea bream industry in the Mediterranean, each country initially learnt different things (e.g. France discovered how to avoid failure of swim bladder inflation in sea bream). At some point, there was strong scientific collaboration among scientists and countries leading to advancement across the whole industry. The same possibility holds for Kingfish, whether for open sea cage technology (where Mexico seems to have a lead) or for Kingfish DNA markers (where Australia has a lead). In practical terms, Mexico has sent sexed DNA Kingfish samples to Australia to assist in the development of sex specific markers for Kingfish. Furthermore, USC is collaborating worldwide with scientists on Kingfish, and is currently undertaking a worldwide analysis of the genetic differences of Kingfish using DNA microsatellite loci, mitochondrial DNA sequencing, and partial genome sequencing. In the future, it is likely scientists internationally will join together to develop a deep sequence reference genome for Kingfish.

Discussion

It is possible to cut the coat according to the cloth

To a large degree, genetics can cut the coat according to the cloth, and the cloth comprises quite of few different considerations. There are options to commence genetic improvement with just the existing two tanks of wild fish, and to do so without genotyping. But these interim types of programs all have some type of “use by” date (usually a few generations). But perhaps of more significance, these interim measures can delay the implementation of more sustainable genetic approaches, and by delaying things, may actually cost more than they provide in the longer term.

Suggest start with the classical genetic program for Kingfish

The published and currently operating genetic approaches in SA, considered here as the classical approach, requires about 10 tanks and 50+ wild fish, and genotyping. This approach has been shown to be feasible, reliable and achieves substantial industry outcomes at modest cost, and all things considered could also be the benchmark for NSW. However, even if NSW starts its genetic program immediately, it will return nothing (commercially) for five or more years.

The classical approach is not without some weaknesses and limitations. Fish are grown and selected at sea, and fish returning to the hatchery may represent a biosecurity risk, or not. It is problematic to select on traits such as flesh quality as the classical selection is done on living fish. Upgrading the classical program to family based selection will start to address some of these issues but while adopted for salmon, may represent considerable logistical hurdles, at least initially for Kingfish, so a phased approach with respect to family selection may be sound.

Operating procedures including logistical requirements, methods of data acquisition and analysis procedures to run the “classical” selection program are detailed.

Understand the role and utility of “game changers”

Potential “game changers”, should they reach commercial feasibility and application, are emerging technologies, such as genome wide trait association studies, epigenetics and so on. Estimation of breeding values by some type of chip means the possibility of mass selection on living fish for traits normally requiring their sacrifice. It also means new possibilities to run within and between family selection perhaps in a cost effective and biosecure way. The acquisition of much of these data is now not prohibitively costly, and can be done beside the acquisition of other data known to

be of commercial value, namely the heritabilities and genetic correlations of a variety of traits. So, as the NSW program gets going, it is conceivable that one of the first major experiments will be matching the classical trait data to pedigree fish, and coupling those data to ‘omic and epigenetic information.

Implications and Recommendations

The information gathered during this project has been of particular value in assuring the local community, managers and policy makers of the environmental sustainability of the proposed activities. That the stocks being used for fingerling production are of local origin have the diversity required to begin production without posing a threat to the genetics of wild populations in the event of escape or spawning during production. Further the increased understanding of the spawning behaviour of YTK and the development of a plan to ensure diversity is maintained as production proceeds provides some additional assurance that the genetics of wild populations will be protected into the future. From an Industry perspective, guidance has been received with respect to the additional stock and infrastructure that will be required to progress toward a selective breeding program. The Implications of various designs for that breeding program are discussed at length in Appendices 1 & 2.

Extension and Adoption

Both reports compiled within this project were provided to our collaborative partners in the current Growing a profitable, innovative and collaborative Australian Yellowtail Kingfish aquaculture industry: bringing 'white' fish to the market - RnD4Profit-14-01-027. These partners include the two existing YTK farming companies (Cleanseas, South Australia, and Indian Ocean Fresh, Western Australia)

Both DPI and Huon are currently considering the implications of the need to increase the size of broodstock populations. DPI is actively acquiring YTK broodstock from local wild populations while concurrently expanding its broodstock holding facilities. The intention is to dedicate all current holding facilities at the PSFI (presently shared with Mulloway and Australian Bass) to YTK and to double the number of broodstock held (N>50). Independently, Huon is investigating the establishment of its own production facilities with a broodstock holding capacity.

A broodstock data acquisition program has been developed that permits data to be entered either by computer or iphone. Data include genotypes, records of samples and finclips (from NSW DPI PS), analysis of genotypes in "Colony" software and are posted at https://sites.google.com/a/aquagenes.com/kingfish-nsw/genotyping-and-assignments

These data provide a variety of snapshots of the total NSW Kingfish pedigree, which are compiled into a continually growing and updated overall pedigree file for NSW held at https://sites.google.com/a/aquagenes.com/kingfish-nsw/ytk-nsw-pedigree

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Appendices

Appendix 1 Classical breeding program

The classical Kingfish breeding program as published by Knibb et al. 2016 consists of group spawning the maximum number of potential sires and dams from as many tank as possible simultaneously and on-growing the mixed progeny until commercial size. The top 5-10% of fish are selected by size but also conformation (tending to be above the weight on length regression line). Selected animals are genotyped and future group matings designed to maximize the number of ancestral sire and dam lineages and avoid realized inbreeding.

This "classical" program is the only one known to have delivered genetic gain in land tanks and at sea for Kingfish.

Appendix 2Elements from CRC Project 2008/703 Development of a Genetic Management for Temperate Marine Finfish

This previous CRC report provides still relevant information about, and comparison of, a variety of selection procedures and issues.

2.1 Inbreeding management

Inbreeding (depression) may not only subtract, but can over-ride any genetic improvement. Inbreeding can also reduce the rate at which genetic selection response can occur, due to loss of genetic variation.

At its simplest, the rate of inbreeding = $1/2N_e$ where N_e is the number of breeding individuals, assuming, among other things:

- same sex ratio
- all fish are treated as belonging to the same single population, not subgroups
- all fish are breeding and parents mate randomly and have equal chances contributing to the next generation
- there is no artificial or natural selection between particular progeny groups (which are used as future broodstock)
- selection within cohorts (cages) doesn't favour particular families

That is, it is only random sampling differences of a finite population size that generates the inbreeding. Note: selection, particularly intense selection and index selection will accelerate inbreeding rates over that from other causes.

However, the above assumptions are usually violated, and N_e may be only a fraction of actual N (actual number of individuals). A worse case is where eggs are taken to the hatchery result from the spawning of only one female (and fertilization by one male) from each of the tanks. In the case where there are e.g. 5 tanks, this will contract the N_e to 5 females and five males, leading to inbreeding at the rate of 5% per generation, assuming there is no preferential selection between the mating groups during growout, harvesting and selection for broodstock, etc.

2.1.1 The economic cost (for Kingfish) of inbreeding (based on various assumptions)

Assumptions:

- Initial production is 1,500 tonnes and fish are sold for $10 a kg.
- production will increase by 20% per annum for the next few years (compounding), thereafter 7.5% per annum.
- base line selection response per generation at 5% (half that published by us for sea cages

and 25% that observed in tanks)

Economic gain is set at half that of genetic gain since there are extra costs of feeds, shipping and so on. There are some situations where the economic gain may be higher than 50% of the genetic gains, especially where there are major savings in infrastructure, or fish reach market early to attract a premium price, or when FCRs are improved, and so on. The 2.5% economic gain increases yearly due to compounding production every third and additive (cumulative) selection response (increasing by 2.5% every five years). But the new selection response itself is assumed to be the same each generation, and the total response is the new 5% added to all the previous gains. Here, we only consider for a given programmed level of production, how present and past genetic selection contributes to production above that programmed for that given year.

Direct cost through loss of performance: A 5% inbreeding rate per generation could translate into a 2% inbreeding depression rate for weight every harvest, and increase per generation or every 5 years (using data from pig weight). Importantly however, not one, but many traits are affected by depression, including fecundity, health, survival. The ratio of inbreeding to inbreeding depression is less than 1, and may range from .5 to .1 or lower. However, as most traits are negatively impacted by depression, we have assumed 1% inbreeding translates into 1% economic loss through direct depression over a range of commercially important traits.

The direct economic costs of depression are yearly (per year), once the first inbred fish are grown and sold, and the cost compounds every genetic generation because of the additional inbreeding, and also increases due to the growth of the industry. At 5% inbreeding level (N_e of 10), under the above assumptions, the losses from inbreeding eliminate the selection gains at every year and every time point (FIGURE 27).

Indirect (but real) cost through reduction of selection performance:
Selection response tends to be linearly related to population size (for small population sizes), and the theoretical maximum selection response is directly related to $2N_e$ (twice the effective population size). Here, we make the crude assumption that 1% inbreeding reduces selection response per generation by the same percentage (i.e. 1%), so a 5% inbreeding rate will reduce selection response in the first generation to 95% of that without inbreeding. This cost is due to loss of genetic variation and opportunity for selection response, and is a cost additional to that arising directly from inbreeding depression (above). This indirect cost increases due to the accumulation of inbreeding from one generation to the next, so becomes more important in later generations. This cost also increases with increases in production. Forward selection response still occurs, but slows with generations and loss of variation. Note: more comprehensive methods exist for estimating this indirect effect of inbreeding (Volume 2: Evolution and Selection of Quantitative Traits Lynch and Walsh).

Cumulative losses over years from inbreeding, direct and indirect, are not presented, but clearly are much larger than the yearly costs presented in the following graphs.

Based on the above assumptions, and considering the theoretic background, control of inbreeding may a most important issue at this stage in the genetic program.

Figure 27 Loss from inbreeding (in $ per fish)

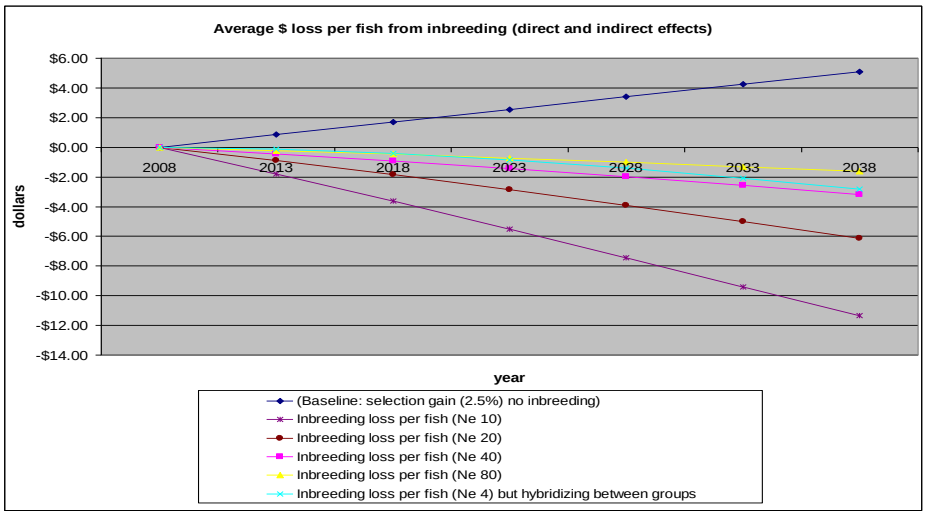
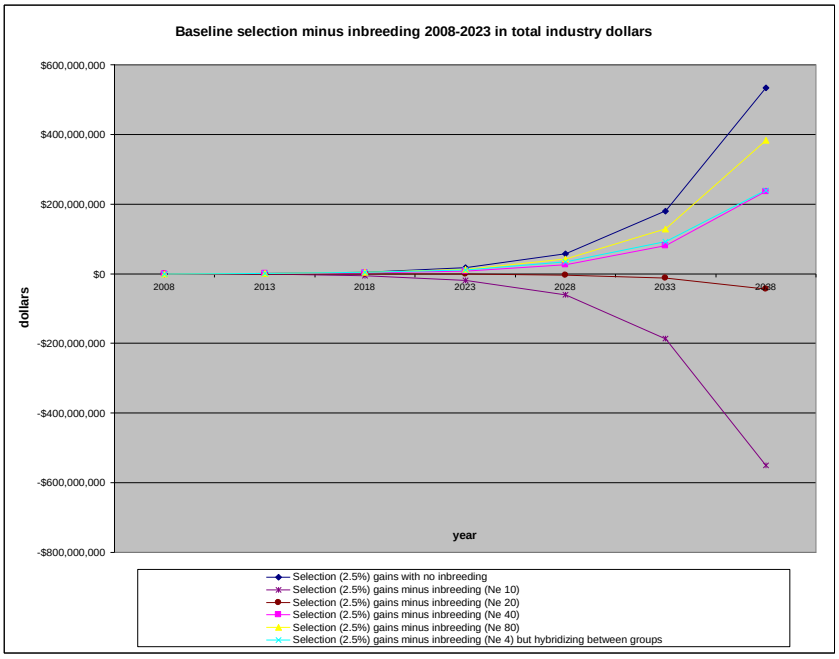


Figure 28 Selection minus inbreeding in total industry $



2.1.2 N_e of 10

In practical and dollar terms, the inbreeding cost per fish at N_e 10 is approximately $2 per fish per generation, accumulating over generations (FIGURE 27).

2.1.3 Husbandry measures to manage inbreeding (Ne 20)

There are various husbandry approaches that can be deployed to mitigate inbreeding rates. They can be deployed almost immediately, without the need for new research or outsourcing of expertise and technologies, and with little disruption to present activities:

- Maximise the number of females and males that contribute to a given batch of eggs for the hatchery. Do this by using relative large egg collections on one given day (where you calculate according to egg volume that most or all females participated) and observing mating behaviour from more than one male, and the amount of milt in the water is considered to have come from a number of males. This will increase the effective population size, and reduce the variance of family size.

- Try to roster **all** the different broodstocks through the hatchery runs and record their progress through the system. This will increase the effective population size and the records will help choosing future broodstocks in a manner to increase effective population size and reduce the variance of family size.
- Record average survival percentages in the hatchery, so subsequently low surviving hatchery runs can be avoided wrt providing future broodstock.

If we assume the effective population size is approximately 20, and inbreeding will occur at the rate of 2.5% per generation. Under these assumptions, the economic loss from this level of inbreeding is slightly more than that made from forward selection (FIGURE 27). Even so, this method could be used for a generation or two if there are other urgent priorities (notwithstanding the loss of net genetic diversity for future selection response).

In practical and dollar terms, the inbreeding cost per fish at N_e 20 is approximately $1 per fish per generation, accumulating over generations (FIGURE 27).

2.1.4 Increase broodstock numbers to manage inbreeding (Ne 40)

With industry expansion, broodstock numbers are expected to double (both tanks and number of individuals). Assuming husbandry procedures as above, and N_e is 40, then inbreeding would be 1.25% per generation. Under these assumptions, selection gains are made at about half the rate possible without inbreeding (FIGURE 27).

Note: there may also be some option to use younger animals as broodstock in order to grow the effective population size (selection implications of bigger Ne are considered later). Young animals, however, may compromise production, and, without more accurate tagging systems (in order to tack which animal is spawning), perhaps would not help reduce inbreeding rates (under the present systems).

The next couple of sections explore whether there are any future in-house and practical options to reduce the cost of inbreeding.

In practical and dollar terms, the inbreeding cost per fish at N_e 40 is about $0.5 per fish per generation, accumulating over generations (FIGURE 27).

2.1.5 Manage mating groups separately (Ne 4+ Hybridizing)

In terms of practical in-house options, one further issue to discuss is whether it is desirable to manage each of the broodstock tanks separately, or jointly as one mating group.

Here we consider each broodstock tank is as one mating group. The descendants of a given tank eventually replace their parents in a given tank. N_e is estimated to be 4 per tank/ mating group, and under this structure there will be relatively rapid inbreeding within each mating group. It would not be an option to use the uncrossed progeny of each mating group for commercial production as this would lead to very large and very rapid economic losses, and this is <u>not</u> the genetic strategy here.

The strategy proposed here depends on a function of probability, where a given mating group will become inbred for recessive deleterious alleles different from those of another mating group. This happens as recessive deleterious alleles are usually quite rare, and there is a very small probability that two mating groups will become inbred for the same deleterious rare allele.

Crossing between two mating groups should effectively remove all the <u>direct</u> economic losses from inbreeding (reset inbreeding to zero) as each rare deleterious allele is now masked by the dominant wild type allele. Some believe, there may also be some heterosis between the crosses (although most see this as simply recovery from inbreeding). Although there is no major <u>direct</u> production cost

from inbreeding, the inbreeding within lines diminishes the long term rate of genetic improvement to one third that possible without inbreeding, giving rise to an indirect cost.

The advantage of this system is that it requires at a minimum only two tanks, or just two mating groups. More tanks and more crosses could be used to investigate if there were some elite combinations (this topic not considered here due to small number of crosses that can be evaluated, and impractical logistics of forming dozens of crosses, although it is a genetic method used in selection of corn).

There are some important disadvantages of this method. Each mating group has to be replaced every five years by its own descendants. This is a cost and complexity in terms of extra cages, management, and less production from inbred lines (although one would try to limit the production to the least necessary). There is also the logistical complication of arranging the broodstock crosses between mating groups to supply the outbred progeny for the commercial production, although this is not considered to be a critical cost. Depending on how many mating groups were kept, this genetic method could require more hatchery runs than present. These additional costs are not considered in this section of the project report.

The main advantage of this method is the reliable provision of consistently outbred animals for production, and relatively simplicity of operation if only two or few mating groups are used. This is a method for consideration when the company wishes to stop using wild animals that may contain diseases, yet doesn't want at this time more complicated genetic programs.

The main disadvantage may be the long term slowing of selection response. As will be shown later, this type of inbreeding management actually leads to some of the better rates of selection response in the first few generations, but thereafter response rapidly slows relative to other selection options.

In practical and dollar terms, the inbreeding cost per fish when hybridizing is negligible for direct inbreeding effects, and, initially, negligible for indirect costs (FIGURE 27).

2.1.6 Manage as one mating group but initially prevent mating of relatives (Ne 40+)

This option is similar to that in Ne 40, but delays any inbreeding for the first few generations.

Here, progeny from each mating tank are mated with the progeny from a different mating group (eg. offspring males mated from tank 1 mated with offspring females from tank 2, and so on). With 10 tanks, inbreeding as expressed in production can be kept at zero for three generations by ensuring mating groups have no common ancestors (e.g. the descendants of crosses between tank 1 and tank 2 are mated to the descendants of tank 3 and tank 4). Eventually, however, there are no more unrelated groups to cross, and inbreeding will occur. Also, the average per generation rate of inbreeding in the long term is unaffected by this procedure (which means that at generation 3 there is a large jump in an individual's inbreeding coefficient).

This genetic option offers deferment of the direct cost of inbreeding until later generations. The attractiveness depends on present cash flows, and an understanding that future costs directly from inbreeding are going to be higher than present due the increased growth in the industry.

This completes the options entailing use of in house management and husbandry methods. The following section explore how inbreeding can be further reduced, and if this can be done in a cost effective manner.

2.1.6 Monitor the existing methods for rate of inbreeding with DNA tools (Ne 40++)

This is an option in the sense that is requires a different budget and methodology and has a different outcome. Also note that here DNA markers are for identification of members of families and as one type of measure of overall diversity. The DNA markers here are NOT for identification of traits (which is a subject outside the mandate of this report).

Broodstock are assessed each generation for their DNA microsatellite (and perhaps mitochondrial haplotypes) and the resulting measures of allelic diversity can "ground truth" whether any of the above inbreeding management methods are meeting their goals. This will enable an early notification to management if methods are not meeting goals, as opposed to waiting until inbreeding noticeably impacts production.

There is very little additional cost over that of section 2.1.4. One can assume $50 a sample, and at most, a hundred samples a year, i.e. total cost around a few thousands of dollars. This work can be done by up-skilling staff to collect the samples and ship to a high through-put company (cost is several thousands of dollars), or involve an Australian external provider to do the DNA preparations and analysis (cost would around $10,000 to $15,000 to cover laboratory dislocation and staff time).

There is little disruption to the broodstock as they can be sampled anytime and during routine handling, or when selected.

External partners are not critical (there is no urgency when the data are provided, different groups can provide the same service, the data are probably not seen as confidential).

The main attractiveness of this investment is to ground truth internal management of inbreeding, and provide an early warning if inbreeding management is not meeting specified goals.

2.1.8 Monitor and maximise Ne under existing methods with DNA tools (Ne40 +++)

Note that here DNA markers are for identification of members of families and as one type of measure of overall diversity. The DNA markers here are NOT for identification of traits (which is a subject outside the mandate of this report).

Here the option of section2.1.7 is extended to consider 30-50 samples from each commercial run arising from a given broodstock group, and samples analysed for their DNA microsatellite variation. This option will not only monitor inbreeding performance as for section 2.1.7 but also permit detection, discarding and replacement of those offspring groups that have little variation, or appear to come from restricted number of parents. Thus this procedure will contribute, probably in a meaningful way, to maximising effective population sizes, and ensuring the in house husbandry options (2.1.4) actually meet their specifications regarding management of inbreeding. If goals are being met, and no genetically restricted groups are being detected, then investment in this present option can be discontinued.

The total cost of this procedure would be 50 samples per group x 10 groups per year x $30 per sample, or about $15,000 if the work is done in house by up-skilled staff, and samples are processed overseas. An Australian local agency could also do the work, but this would add another $20,000 (approximately) to the costs for staff time.

2.1.9 Family tracking with DNA markers

2.1.9.1 "20 of so" families (Ne80)

If the offspring of mass spawning events can be identified as belonging to specific families (or rather, specific spawning males or females), then it is possible to do broodstock selection so that an equal number of offspring per family (actually per male and female parent spawning parent) are chosen for the next generation. That is all families (spawning parents) are selected. Selection is within families, ranking individuals within each family. There is no between family selection, so even poor performing families are used. For this very special condition where all families (actually spawning parents) are replaced, and equal numbers per families are selected, the variance of family groups is zero (there is no sampling variance), and the effective population size (N_e) is doubled to become 2N. The selection outcome of this special situation is discussed more fully in section 0.

So, building on the option in section 2.1.4 where 2 males and 2 females spawned per tank, then by recovering equal representation of these in the next generation would increase N_e from 40 to 80, and inbreeding reduces to about .6% per generation.

The extra investment in tracking would yield about 50% more genetic gain than the option of section 0, under the above assumptions. The cost of full pedigree tracking would be between $20,000 to $100,000 (reasons for the variation follow). If we assume a price of $60,000 per annum, the benefit cost ratio, according to the extra genetic gain from N_e of 40 to N_e of 80 is 3:1 in 2008, 10:1 in 2011, 26:1 in 2014 and 565:1 in 2029, assuming no additional cost for doing the actual selection (see later). One flaw with this approach is that at the next generation, some families may be lost (i.e. when only two of the three females in a tank spawn. Hopefully some husbandry workarounds can be developed in time, otherwise this problem could impact the long term viability of this option.

Note: the range of prices given for the pedigree arises because the cost is sensitive to the variation of family groups, so if one female or one male dominates, relatively more samples need to be processed in order to find all the families, and then find sufficient numbers in order to impose "within family selection" (see later section on selection). There is also the uncertainty of how many half sib family groups are produced, and how much this complexity will cost to analyse, although a mitigating factor will be the low number of parents in a given spawn, and also, the factor that the generation of half sib families should enrich the number of family and perhaps speed selection response, and permit an even more favorable BR ratio (see later). These issues are not seen as critical as they do not disturb the basic calculations and positive BCRs.

In practical and dollar terms, the inbreeding cost per fish at N_e 80 is about 20-30 cents per fish per generation, accumulating over generations.

2.1.9.2 "Many" families

The previous section has achieved an acceptable long term rate of inbreeding section (0), and while the addition of more families could further reduce inbreeding (if all families are selected), the chief value of adding more families is that it now permits the culling of poor performing ones. That is, now one can deploy both within and between family selection, and this is discussed more fully in section (2.3.1.1.4). However, once between family selection is used, the required fish holding infrastructure and DNA assignments can multiply several fold. For example, to achieve a selection intensity of 10% within family selection with acceptable inbreeding, about twenty or so families are needed (N_e = 160), but a hundred or more families would be needed to achieve the same selection intensity between families yet maintain acceptable inbreeding rates (after selection, N_e = 40). Even a 50% selection intensity between families requires quadrupling the resources scoped in section 0.

Due to economy of scale, the extra cost of graduating to a between and within program is probably less than 10x the $60,000 referred to in section 0, and may be around $200,000 pa.

2.1.10 Full pedigree and family tracking with visible markers

An alternative approach to tracking with DNA markers is to track with visible markers. This requires separate spawning, hatchery runs and fingerling production of single family groups. This in turn would require either stripping each of the 20 females, or setting up 20 tanks each with a single female and single male. Fingerlings could then be tagged and grown communally.

While the cost of such an operation would be in the order of one hundred thousand to several hundreds of thousands of dollars (based on staff times, extra infrastructure, and drawing on comparable experience from leading the Israeli marine fish breeding program), it would achieve a positive BCR by 2014. This option also has an advantage of providing very large numbers of offspring per family for future selection, although, if selection intensity is about 10%, large numbers are not required.

The are some disadvantages of this option (personal opinion, based on experience in operating a comparable marine fish genetic breeding program in Israel):

- the separate rearing of the family groups introduces a common environment bias to the families, reducing the future accuracy of selection (and reducing selection response).
- the technologies for stripping and for single pair matings in tanks is unproved, and may have a major commercial impact if attempted without prior work up (if it is ever possible to work up).

The second disadvantage is the most serious. Stripping a tank of sea bream usually terminates spawning or severely suppresses the spawning of the whole tank for weeks or months, or even the whole season (Israeli experience). Also, given the precise timing of daily spawners, it was usually only possible to find one or three animals out of 20 or so ready for stripping.

On the grounds of discounting options that require operation of unproved technologies, like stripping for this section, this option (2.1.10) is not at present recommended (again, personal view, based on Israeli experience). This criterion of recommending only proved technologies is applied fairly and consistently to other section (see sections on section generation time reduction, and reduction in FCR).

This concludes the section on inbreeding, where a reasonable or acceptable level of inbreeding has been achieved at various cost levels. Obviously the addition of more tanks will add value. The following selection models will relate to these methods to manage inbreeding.

2.1.11 Summary/Recommendation on inbreeding

The present breeding systems may suit production but are probably inadequate to support a closed multi-generation stock (breeding across generations).

Extra tanks will be acquired in the course of industry expansion, which coupled with use of large spawns for the hatchery, will reduce inbreeding to levels of little consequence over a few generations. This option is not consistent with very long term maintenance of the stocks nor with fast rate of selection progress.

A simple method of hybridization (simple both in infrastructure and management) is outlined to achieve zero inbreeding over many generations, but this method does not support fast long term selection gain.

Acquiring pedigree information, even from the present system, will result in acceptable long term inbreeding rates, and support a range of selection options. However, there is a yearly ongoing cost of $60,000 to 200,000 pa.

Should stripping technologies be developed, then these recommendations can be reviewed (commercial stripping technologies were not developed for seabream despite ongoing attempts).

All options would be favorable in term of benefit cost ratios under the climate of rapid industry expansion.

Overall recommendation depends of the present business model, present cash flows, and confidence of rapid industry expansion. A high confidence could favour the later options, a lower confidence the former.

2.2. Background Considerations for selection options

Before tabling and comparing the performance of some selection options (0), some important factors in selection response, namely, selection intensity, quantum gains from selection, generation time, and correlated response to selection are illustrated using, hopefully, industry relevant examples.

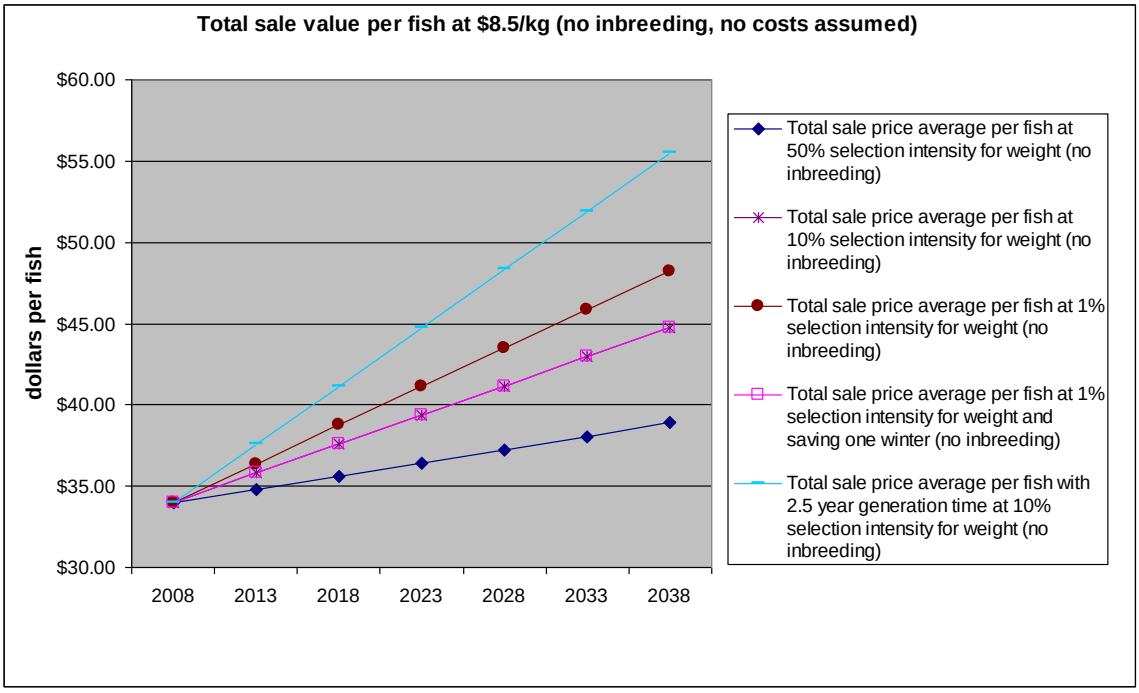
Some general assumptions: For the moment, it is assumed there is no inbreeding. It is also assumed there are no sex effects, although in reality, it is expected that sex differences will exist for growth, GSI and so on. These differences will require the following to be adjusted at some point for sex effects, and for selection to be performed on separately on each sex. Also, unless stated, it is generally assumed that selection does not accelerated inbreeding, although this assumption is violated for some types of selection. Last it is assumed there are no unaccounted adverse correlated selection responses unless explicitly stated or reference.

Some specific assumptions: Initially assume a two year production cycle, of 730 days, for 4kg, or 5.48 gms per day. The economic value of increasing every 1kg by the same harvest date could be about $8.50 (minus the usual costs of say $6 a kg). The heritability for weight is assumed to be .3, and the standard deviation to be .4.

2.2.1 Selection intensity

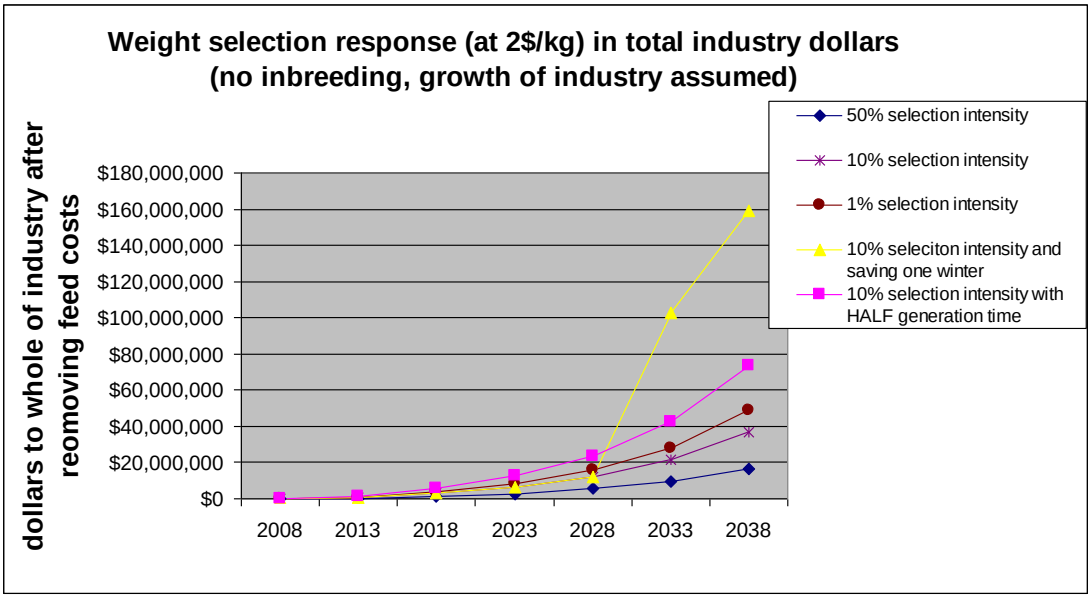
Using the example of selection for one trait, namely weight on individuals, it is evident that increasing selection intensity over a range of 50% to 1% does increase the sale value of the fish (sale value is calculated as an average of $8.50 per kg, starting with 4kg fish in 2008, and every kg improvement valued at $8.50) , but the rate of improvement is not directly proportional to intensity, and after about 10% intensity, there are diminishing returns (in terms of response) from screening the large numbers to achieve very intense selection pressure (FIGURE 29). Perhaps an intensity of about 5-10% represents a reasonable tradeoff between gain, and cost of selection. Note: at this stage the costs of feeds and inbreeding are not considered.

Figure 29 Selection responses at different selection intensities



These selection gains, ignoring inbreeding, are translated into industry gains (FIGURE 30). Gains, as oppose to sale values, are valued at $2 a kg, or the profit per kg of flesh after removing feed costs.

Figure 30 Selection response in total industry dollars



2.2.2 Quantum gain: Saving the cost of a winter growth

If we take a very long term view (next 20 plus years), we can see that when selection has achieved, say 1kg of response per fish (so fish reach 5kg in the time they normally reached 4kg), then the production costs may actually go down steeply, say to $4 a kg (present values) due to the saving of one whole season of winter growth. At 10% selection intensity, this occurs at generation 4 (i.e. 20 years hence although gradual steps towards this threshold would have made at each generation, this can be inferred from FIGURE 29). Once the costs go down, there is a very favorable genetic response not only in terms of the extra kilograms of flesh produced at lower costs, but the lowering of costs for the whole of the production (FIGURE 30). This could be an important economic feature of the selection program, and attract more interest in speeding the rate of selection response for growth (so the winter saving is achieved earlier).

If we use a 1% selection intensity, as opposed to the 10% considered presently, then the saving of the winter season would happen 3 years earlier (say in 2028 rather than in 2033). Even so, there is still a long time to wait for this winter saving, and the following considers a method to shorten this wait.

2.2.3 Reducing generation time

Present generation times are assumed to be 5 years for females and 5 years for males. If we use 2 year old males, and 3 year old females, the generation time is halved (FIGURE 29). Halving the generation time also accelerates other aspects of selection response. Indeed halving the generation time and selecting at only 10% intensity surpasses selection response at 1% intensity with the usual generation time (of five years), and surpasses all previous methods in value per fish prior to considering inbreeding.

2.2.4 Correlated selection response for growth

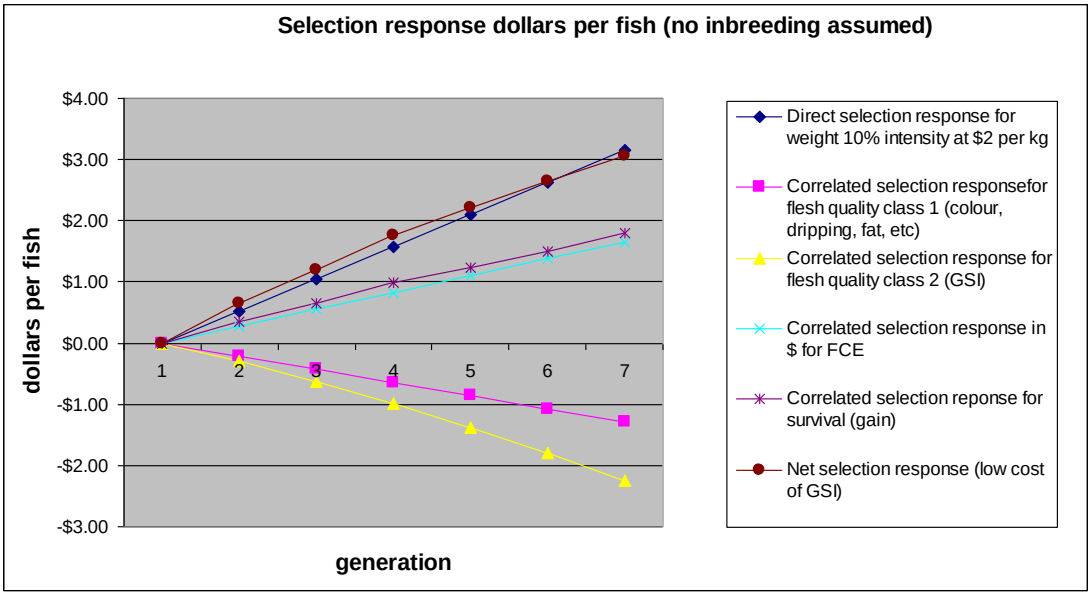
Some of the genes and alleles that contribute to growth will also influence other metric traits, due to an effect of genes known as pleiotropy (one gene affects many traits). This effect may be strong or weak, beneficial (economically) or adverse. The following serve as examples as to what one may expect when selecting just for growth, regarding correlated responses on other traits, and through this exercise, develop procedures to maximise economic outcomes from pleiotropy.

2.2.5 Correlated response for flesh quality – category one

Flesh quality comprises many traits, such as colour, fat content, drip loss, fibre length (or shortness), and so on. The genetic heritabilites of flesh quality traits tend to be low, although some correlations with weight, such as fat, can be high. To expedite the analysis, the above flesh quality traits are grouped into one category (flesh quality, category one). Flesh quality (category one) is assumed to have a heritability of .2, a negative genetic correlation of .2 with weight at market size (i.e., assumes fat, drip loss and short fibres increase with selection for weight), a mean of 5, a phenotypic standard deviation of .5 (10% of mean), and a value is $5 per unit (i.e. the lowest 5% in terms of flesh quality would attract an economic penalty of $5 out of the average of $34, or reduce the profit by 50%).

Under the above assumptions, there is a long term economic penalty of a dollar or so per fish from unfavourable correlated response on flesh quality (category 1) after selection for weight (FIGURE 31).

Figure 31 Selection response now considering negative correlations with quality traits



2.2.6 Correlated response for flesh quality – category two (precocious maturation)

It is assumed that at harvest 20% of the fish have a GSI of 20%, and 80% have a GSI of 2%, so GSI appears as a discontinuous trait. On one hand, this may represent the outcome of an underlying liability scale, and amenable to such an analysis (not done here, but done in section 0). On the other hand, GSI could represent two different, albeit correlated traits, one giving the “2%” effect, the other giving the “20%” effect (also not considered here). Here, for convenience, GSI is treated as continuous trait, and at some point, for statistical correctness, this percentage scale should be normalized.

Elsewhere, genetic correlations between weight and sexual maturation or maturation indices tend to be positive in fish, but show large range, e.g. from 0 to 0.6. Sexual maturation is usually associated with a loss in flesh quality. Here we take the correlation between weight and sexual maturation to be 0.24, and the heritability of GSI to be 0.21 (using data from Atlantic cod). The economic cost of the maturation is twofold, both reducing kgs of flesh, and reducing flesh quality. For convenience, the value of GSI taken as negative one times the GSI percent in terms of the average value of a fish (1% GSI = 1% of the value of an average fish). The long term effect of this correlated selection response is to reduce the value of an average fish by a couple of dollars (FIGURE 31).

Maturation at harvest size (about two years) would be undesirable (as calculated above) but should this early maturation at two years be correlated with maturation at 3 years, then early maturation could assist in reducing the generation time, something that is highly desirable in terms of reducing

generation interval (section 0). Hopefully the genetic correlation between maturation and two years and that at three years is much less than unity, permitting response to delayed maturation at two years, but there after rapid onset of maturation. Also, hopefully, there are non genetic means to promote the onset of sexual maturation at 3 years, so selection could focus on suppressing maturation at age 2 years.

For the present, however, maturation is treated as an economic cost and side effect of selection for growth. (Note: there was some industry discussion that the faster growing fish were not sexually mature, and it is conceivable that maturation will slow growth, so it could be that the above assumption of a positive genetic correlation with negative economic consequences is incorrect, ie, there is a negative genetic correlation between growth and GSI).

2.2.7 Correlated response for feed conversion efficiency

There appears to be relatively little data on feed conversion efficiency (or ratio) in fish. Here we assume the genetic correlation with weight is positive 0.2, and the heritability is also 0.2. We assume the mean FCE is 50% (2kgs of feed for 1kg of fish), the standard deviation is 20%. It would take 8kg of feed, costing $16 (at $2 a kg) to produce a 4kg fish. One percent of this is $0.16, which is the economic value assumed for one percentage FCE. The long term consequence of correlated selection response for FCE under the above assumptions adds only a dollar or so to the value of an average fish (FIGURE 31).

Note: for statistical correctness, this percentage scale should be normalized.

2.2.8 Correlated response for survival and disease resistance

Survival is a discontinuous trait (dead or alive). But it is assumed that the underlying genetic variance for survival is continuous which is termed the “liability for survival” (i.e. it is not a Mendelian trait). It is assumed that standard deviation of the liability is 1. If 80% survive usually (say from 20 g to market size, then the mean survival liability is calculated to be +0.842 (converting percentages into a liability value, Appendix Table A, Falconer and Mackay 1996). The heritability and genetic correlations (on the liability scale) with weight are assumed to be low, i.e. 0.15 and 0.15 respectively (based on ranges in the literature). Very approximately, the cost of a fish that didn’t survive is guestimated to be 50% the cost of fish that did survive to market size, as death could occur at any time from 20g onwards. A 1% death rate is assumed to cost .5% of the $34 for an average fish. Selection response is first calculated on the liability scale, then back-transformed into the percentage survival scale to estimate the economic costs.

Under the above assumptions, the longer term effect of correlated favorable response to survival after selecting on growth rate is to add a dollar or so to the value of an average fish. The rate of response for survival slows as survival approaches 100% (FIGURE 31).

At this stage there are insufficient data to guesstimate the genetic correlation between growth and gill flukes, and care should exercised when getting into guesstimates as the literature indicates that both positive and negative genetic correlations exist for different diseases and fish species. While it may seem reasonable to consider a positive correlation, as one may see that slow growth associated with lots of flukes (this is a phenotypic correlation), the genetic correlation could be completely opposite, namely a high genetic predisposition for growth is at the cost of increased susceptibility to diseases. It is easy to be misled!

Future analyses can consider related traits such as “days survival”, parasite load, flukes, etc.

2.2.9 Correlated response for deformities, Fillet percent and body shape

There are insufficient data to generalize about the genetic correlation between growth and fillet percent, deformities or shape, although the latter do often have moderate to high heritabilities. Note: this section is now superseded with publication of Whatmore et al. 2013.

Overall, the summing across all the correlated responses, it appears that the sum of favorable and unfavorable correlated selection response cancel each other out (FIGURE 31), but this conclusion involved a lot of assumptions.

Indeed, the outcome is sensitive to assumptions. For example, revisiting GSI, we may argue that because GSI impacts both in terms of loss of flesh weight and loss of flesh quality, then the cost per one percentage of GSI is not $0.34 as used above (0), but $0.68. This increased cost is sufficient to wipe out all the forward selection gains, even before inbreeding is considered! Hence, in practical and dollar terms, the long term effect of selection could be to reduce the value of a fish by $3.00. So depending on which assumptions are used, there could be **a strong argument for at least record keeping of all traits**, if not a system where a **number of traits can be selected simultaneous**. This simultaneous selection could **not only prevent** the inadvertent loss detected here (under the expensive assumptions for GSI), but actually provide **selection response** to improve, or hold constant, some of the adverse traits.

There is a strong case for ongoing research and understanding of correlated effects.

2.2.10 Summary/ recommendations on background considerations

Selection intensity: between 10 and 5% may be a reasonable tradeoff for individual selection and less for between families.

Saving one winter: the economic benefit is highly desirable, but the long generation time delays tremendously realising the benefit

Generation time: reducing generation time could contribute as much or more to speeding rate of selection response than hundreds of thousands of dollars spend in increasing the number of families.

Correlated selection response: it is import to monitor, if not actively select a range of traits, as ignoring this area could limit or prevent forward selection response.

2.3 Selection options

Here we consider first some simple, then some more complex selection models to gain some insight into the areas most sensitive to economic outcomes and investment. Selection intensity is assumed to be at 10% unless otherwise stated. Inbreeding (**cumulating per generation)** is usually assumed to equivalent to that from N_e40 (ie. 1.25% per generation), but for within family selection, it is assumed to be equivalent to N_e80 (ie 0.625% per generation). Selection response from within family selection is assumed to be 70% of that from individual selection.

2.3.1 Select on the living

Unfortunately, selection on living animals can limit the traits that can be selected. Those traits that can be selected without sacrificing include, perhaps, weight, shape, general appearance, deformities, general vigor, external colour. In the future, for Kingfish, specialized diagnostics may enable the measurement of fat content, and GSI, but they are not considered here. The examples used here are weight and appearance.

2.3.1.1 Select on single traits

2.3.1.1.1 Selection for weight using individual selection

Business goal: more profit, greater profitability
Production goal: animals faster to market (“days early to market”)
Selection criteria: weight of animals when the group mean has reached market size
Genetic parameters: section 2.2.

Selection responses after selecting individuals (individual selection) for weight (assuming an animal is worth $8.50 per kg), before and after accounting for inbreeding and feed costs (assuming costs are $6.50 per kg of flesh) are given in FIGURE 32, FIGURE 33, FIGURE 34 respectively (given as the red line with star). Positive gains appear to be made after subtracting inbreeding (inbreeding is set at 1.25% cumulating each generation). Interestingly, and of some commercial relevance, all selection gains based on individual selection for one trait are eliminated once both inbreeding and feed costs are considered (FIGURE 34).

Figure 32 Selection response not considering inbreeding or feeds

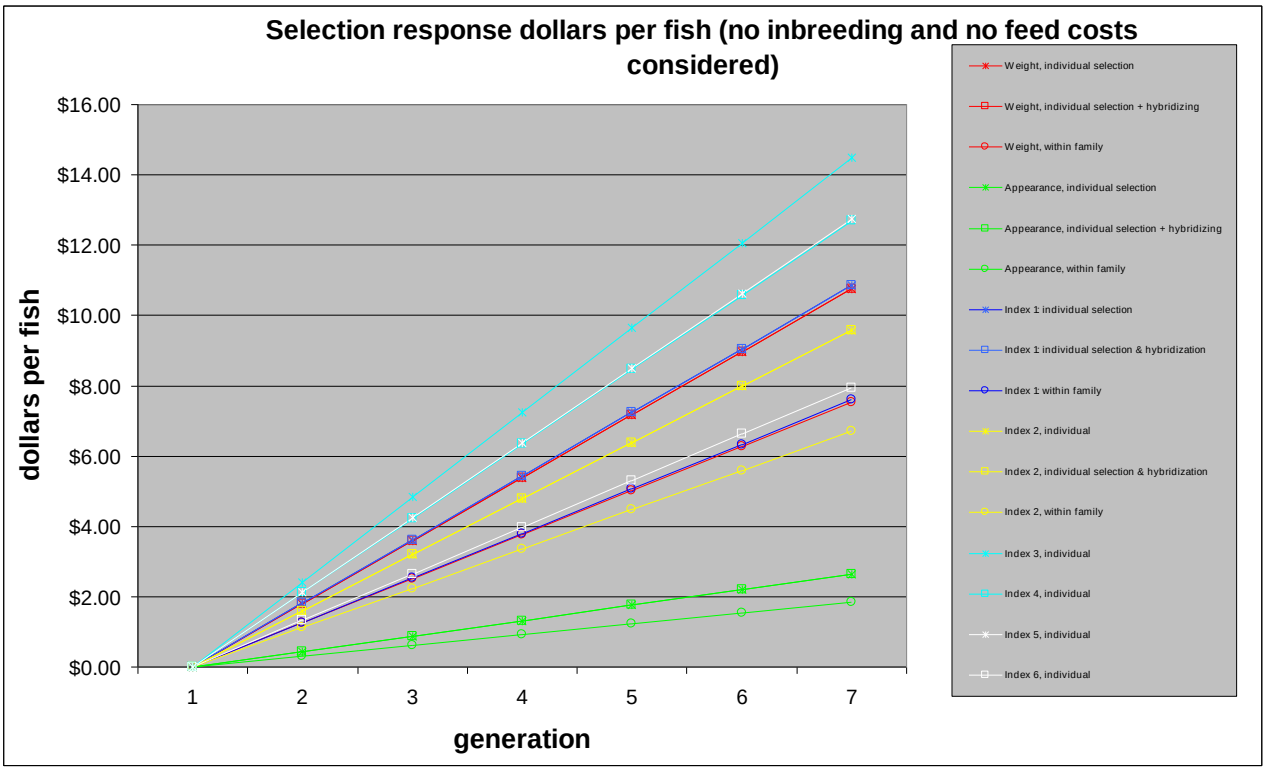


Figure 33 Selection response considering inbreeding

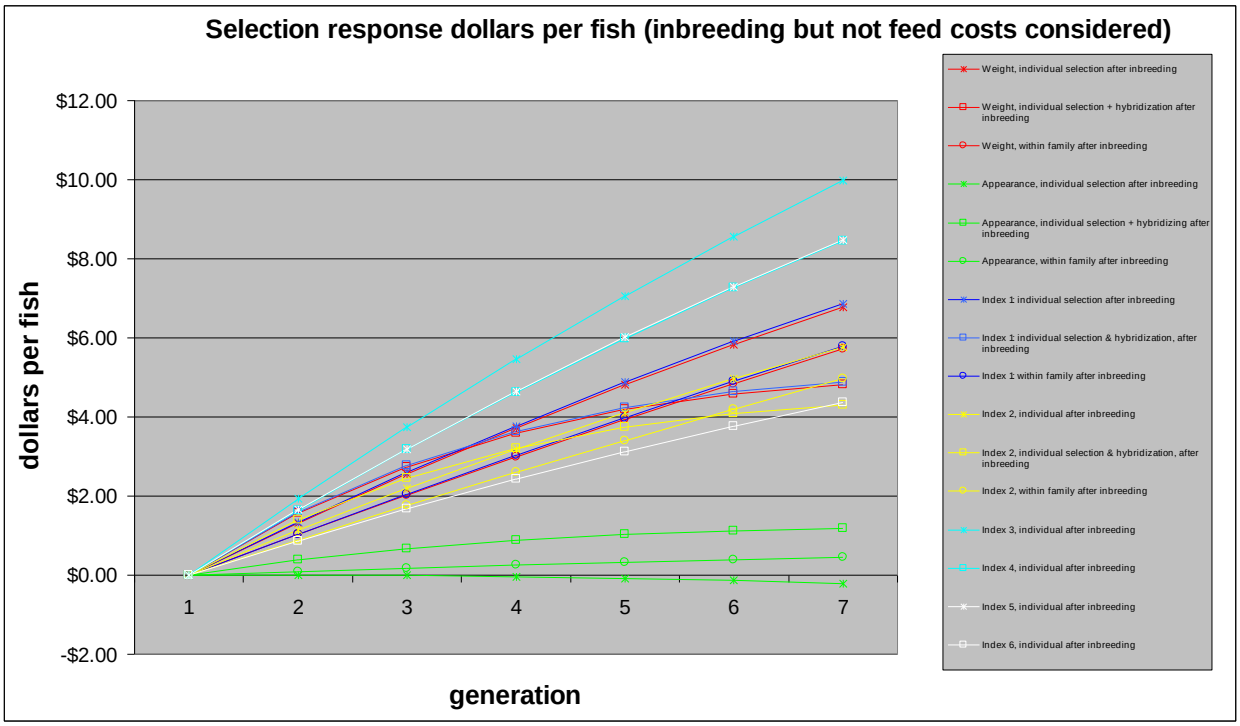
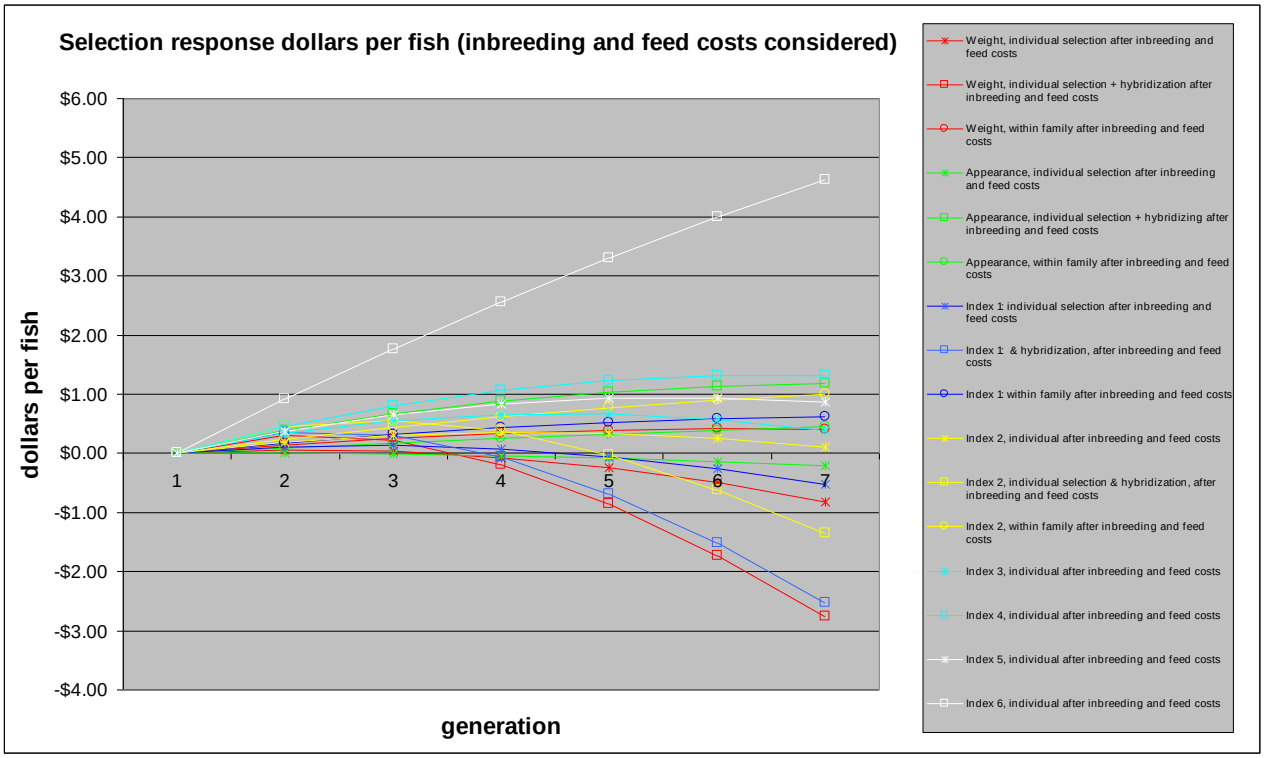


Figure 34 Selection response considering inbreeding and feed costs



2.3.1.1.2 Selection for weight using individual selection + hybridization

The inbreeding option given in section2.1.5 considered hybridizing between groups for the commercial production. Selection under this system is actually initially (for a couple of generations) superior to individual selection, and after considering inbreeding or inbreeding plus feed costs. After two generations, response from individual selection surpasses that from small demes and hybridization.

2.3.1.1.3 Selection for weight using within families

A previous section on inbreeding illustrated how to reduce the inbreeding to less than one percent per generation (2.1.9.1). This was achieved by effectively replacing each family (or spawning parents) with equal numbers of offspring per generation, but at an extra cost of using DNA markers. Here we explore the nature of selection response obtained by this special set of conditions, after some introduction and basic explanation of within family selection (immediately following). A fair amount of detail is given on within family selection (immediately following) as this type of selection I expect will be short-listed as a genetic option due to low infrastructure costs, low inbreeding, and moderate selection gains.

2.3.1.1.4 Advantages and disadvantages of within family selection

There are four basic types of selection, namely selecting on individuals (ranking individuals over the whole population), selecting between families (ranking family mean values), selecting within families (rankings individuals within each family), and selecting on the combined between and within rankings (combined or index selection). With the mating inbreeding management design of section (2.1.9.1), the selection choice is limited to just within family selection, as the other choices would lose families from the breeding group, and this would cause excessive inbreeding.

When environmental and maternal effects cause large differences between families (as can happen for average size of piglets), then within family selection will outperform between and individual selection, as both the latter are confounded by the non genetic effects. For the mating plan envisaged for section (2.1.9.1), offspring are the same age and have the same environment, so there are not expected to be large environment and maternal differences between families. However, if the same mating design as 2.1.9.1 is achieved using physical tagging, as scoped in section 2.1.10, then the Kingfish families are produced separately, and reared separately until physical tagging. Here there will be large environmental inputs into the differences between families, which would favour within family selection in terms of rate of response.

Probably the most important practical advantage of within family selection is that it minimises the infrastructure required to operate a genetic program. First, as there is no between family selection, the family numbers can be kept to a minimum (say 20 or so, rather than 50-200), second, by selecting on each family, the effective population size is doubled (2.1.9.1).

Last, there is a possibility that the rate of inbreeding from the selection process itself is lower than other methods (Falconer and Mackay 1996), although this process has not been considered for any selection method in the present report.

Selection response depends on how much of the total variation is due to differences between families (t, the intraclass correlation coefficient), on n, the number of measures within a family, and on the relatedness of the sibs (full or half sibs, etc). For full sibs, and as n becomes large, and assuming about half the variation is due to family differences, then within family selection is about **70%** as effective as individual selection (Falconer and Mackay 1996, pp237), and reducing the differences between families to only 20% of the total variation still leaves within selection about 50% as effective as individual selection.

2.3.1.1.5 Analysis

Using the assumptions above (2.3.1.1.1), within family selection is superior to both individual and individual + hybridizations in the later generations once inbreeding and feed costs are considered.

2.3.1.1.6 Selection for appearance using individual selection

“Appearance” is used as an example of a trait that would not cost extra (presumably) in the way selection for weight costs extra feed (unless compensated by improved FRCs).

Appearance is considered on a percentage scale, commencing at a mean of 50, with a standard deviation of 5. (Note, percentage scales should be normalized). The heritability is assumed to be 0.2, and the $ value one percentage unit assumed to be $0.25.

Selecting individuals just for appearance would see a dollar or so response, but this would be lost by inbreeding (FIGURE 33, FIGURE 34). There are no extra production costs assumed for appearance.

2.3.1.1.7 Selection for appearance using individual selection + hybridization

Selecting for appearance under the hybridizing program actually seems to be a superior method (FIGURE 34), due to the loss of value of other traits once feed costs are subtracted. It should be further considered whether this inbreeding and selection method will also be superior for other single traits apart from appearance and growth.

2.3.1.1.8 Selection for appearance within families

After accounting for inbreeding (there are no additional costs), within family selection was a little superior to individual selection, but not to hybridization.

2.3.1.2 Select on two traits simultaneously

Theoretically, the rate of selection response can be accelerated by improving the economic and breeding value assessment of an animal. The following explore, in increasing steps of complication and resources, options to so improve estimations of economic breeding values.

2.3.1.2.1 (Index 1) Individual selection

The optimal (economic) index (using public domain UNE xls programs from Prof. Werf untitled "mtindex_desgains") for both traits (weight and appearance) together is, when a value of $8.50 per kg is placed on 1kg of flesh:

Index 1

TRAIT	own	dam	sire	full sibs	half sibs	progeny
growth	2.513	-	-	-	-	-
appearance	0.030	-	-	-	-	-

ie
I (index) = 0.694 weight + 0.044 appearance
(for individuals, expressed as deviations from the population means).

The selection response at selection intensity of one (about 38%) is calculated to be $1.03, or $1.81 per generation at selection intensity of 1.755 (ie 10%).

The value of Index 1 is only marginally superior to that for individual selection (it is very similar), and is also unprofitable in the long term. Unprofitability seems to be arise due to the high economic weight placed on the kgs of flesh.

2.3.1.2.2 (Index 1) Individual selection + hybridization

Selection and hybridization for Index 1 produced relatively superior short term gains, but was not a viable long term option (FIGURE 34).

2.3.1.2.3 (Index 1) Within family selection

Within family selection for Index 1 was superior to individual selection Index 1, and also to hybridization in the longer term, once inbreeding and feed costs are considered, but still it was not very valuable.

2.3.1.2.4 (Index 2) Individual selection

Index 1 valued kgs of flesh at $8.5 a kg, which gave a high loading to selection on weight. However part of the $8.50 comprised feed costs, and while this was later subtracted from the genetic response, the initial loading remained high. Accordingly, a new index was constructed where the kgs of flesh was valued at $2.50 a kg, or the expected value after removing production costs. This tended to reduce the index weightings on kgs of flesh.

Index 2

TRAIT	own	dam	sire	full sibs	half sibs	progeny
Growth	0.694	-	-	-	-	-
appearance	0.044	-	-	-	-	-

The selection response from Index 2 at selection intensity of one (about 38%) is calculated to be $0.37, or $0.65 per generation at selection intensity of 1.755 (i.e. 10%).

Index 2 was superior to Index 1, after accounting for inbreeding and feed costs. Still, the index is not valuable, although its value would increase if feed costs are reduced.

2.3.1.2.5 (Index 2) Individual selection + hybridization

Selection and hybridization for Index 2 produced relatively superior short term gains, and outperformed Index 1 hybridization, but was not a viable long term option.

2.3.1.2.6 (Index 2) Within family selection

Within family selection for Index 2 performed relatively well once inbreeding and costs were considered, but its net value was still low, probably due to the high costs. If costs can be reduced, as illustrated (FIGURE 30), selection response will accelerate.

2.3.1.2.7 (Index 3) Individual and relative selection

In this section of "selecting on the living" there is a further option to improve accuracy of selection and rates of selection response using living animals. It involves measuring the same traits, namely weight and appearance, but from additional animals, namely living parents and siblings.

Principle: Measurements and information on a candidate's living relatives (parents, brothers, sisters) in addition to the candidate can increase the accuracy of predicting the breeding value of the candidate, especially when the traits have low heritabilities (as more measures of relatives are taken, the closer the average approximates the true breeding value). Increased accuracy will ultimately speed the genetic selection response in terms of dollars per year.

Example: Here we build on the two trait (weight and appearance) selection example of section given previously where we selected on the phenotype (physical appearance) of the candidate, but accessing phenotypic information on the (live) parents, and live 15 full siblings, and live 15 half siblings. The same genetic assumptions as previously are used, and the "mtindex_desgains" program is used to calculate the indexes and responses. We assume that the effect common to full sibs (c^2) other than additive genetics is 0. The genetic correlation between weight and appearance is assumed to be 0, and the phenotypic correction assumed to be 0.1.

The optimal (economic) index (using "mtindex_desgains") for both traits together is:

Index 3

TRAIT	own	dam	sire	full sibs	half sibs	progeny
growth	1.741	0.412	0.224	3.937	1.277	-
appearance	0.022	0.009	0.006	0.107	0.04	-

This index performs well until feed costs are considered (FIGURE 34), and should feed costs be reduced, this could be an attractive index.

2.3.1.2.8 (Index 4) Individual and relative selection ($2 per kg)

This is similar to index 3, except the economic value per kg of flesh is downgraded to $2.50 a kg, which reduces the relative importance of selecting for weight in the index. Feed costs, however are still considered.

Index 4

TRAIT	own	dam	sire	full sibs	half sibs	progeny
growth	0.482	0.118	0.065	1.149	0.380	-
appearance	0.032	0.010	0.006	0.110	0.04	-

Once both inbreeding and feed costs are considered, index 4 outperforms index 3 (because less emphasis is place on growth and therefore there are less costs of feeds). This advantage over index 3 probably would change once feeds prices decline, or FCEs increase.

Select on the living and the dead (sacrificed animals)

Principle: if there is family information, then one can sacrifice brothers and sisters, and new traits like fat content, flesh colour, can be added to the selection index. These traits could not be considered without family information as the candidate would have to be sacrificed to get this information.

2.3.1.3 (Index 5) Select for more than two traits simultaneously

Example: Here we build on selecting for weight and appearance as immediately above, but also reintroduce some of the traits previously considered in section 2.2.4, namely, flesh quality (categories one and two) and FCE . Some other traits considered previously, including survival are not considered here due to computational convenience and lack of data. To get information on flesh quality (categories one and two) we need to sacrifice some relatives (say 5 full sibs and 5 half sibs), but not the candidate animal for selection nor its parents. We keep the same genetic parameters as used previously, but all other correlations (both phenotypic and genetic) between the traits not previously listed are set at 0 for convenience.

Table 12 Parameters of traits and number of relatives

						Number of records					
Trait	Name	Units	Stand. Dev	heritability	$ value	own	dam	sire	full sibs	half sibs	progeny
1	body weight	kg	0.40	0.3	$2.50	1	1	1	15	15	0
2	appearance	percentage	5.00	0.2	$0.25	1	1	1	15	15	0
3	Flesh quality 1	10 units	0.50	0.2	$5.00	0	0	0	5	5	0
4	Flesh quality 2	percentage	7.91	0.21	-$0.34	0	0	0	5	5	0

Table 13 Correlations (phenotypic above, genetic below diagonal)

	body weight	appearance	Flesh quality 1	Flesh quality 2
body weight	1	0.10	0.00	0.00
appearance	0.00	1	0.00	0.00
Flesh quality 1	-0.20	0.00	1	0.00
Flesh quality 2	0.24	0.00	0.00	1

Index 5

Trait	own	dam	sire	full sibs	half sibs	progeny
body weight	1.279	0.335	0.209	3.243	1.167	-
appearance	0.025	0.009	0.006	0.107	0.04	-
Flesh quality	-	-	-	1.457	0.637	-
Flesh quality	-	-	-	-0.101	-0.044	-

Index 5 performs reasonably well prior to accounting feed cost (FIGURE 33, FIGURE 34).

2.3.1.3.2 (Index 6) Select for more than two traits simultaneously
This is similar to Index 5, except 1kg of flesh is valued at $2.50, which downgrades the weighting of weight in the index.

Index 6

Trait	own	dam	sire	full sibs	half sibs	progeny
body weight	0.022	0.043	0.053	0.478	0.282	-
appearance	0.035	0.010	0.006	0.110	0.04	-
Flesh quality	-	-	-	1.543	0.654	-
Flesh quality	-	-	-	-0.108	-0.045	-

Index 6 performed the best of all options once feed costs were removed.

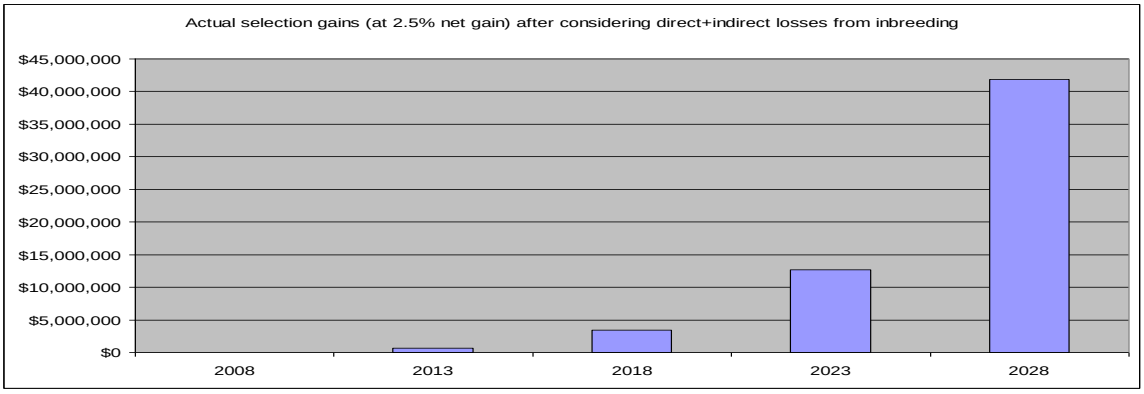
2.3.2 Summary/ recommendations on selection

The overall performance of the selection models was modest, delivering a maximum economic return of 5-6% prior to removing costs and inbreeding. However, this upper result was consistent with the initial assumptions that the long term rate of economic benefit would be about 2.5% ().

After accounting for inbreeding (but not costs of feeds etc.), the upper ranges for selection responses were about 5%. However, these responses came from index selection using information from relatives. Once information from relatives is used, between family selection needs to be applied, which, arguably, would require dozens of families, dozens of tanks, and hundreds of thousands of dollars. If we set the limit of investment at or about the present infrastructure, and perhaps with some DNA tagging (under $100,000 investment), then we can achieve a selection response of about 2% (before subtracting costs). This level of selection response also can be obtained almost without any investment (using hybridizations), but long term response is compromised.

After considering costs, there was only one viable selection option (namely Index 6), which delivered about a 2% return after accounting for inbreeding and feed costs. The success of Index 6 was due to the low (actually negative) weighting of weight, which saved the feed costs. While it may be true, given the feed costs, that selection should focus on non weight traits, it does seem counter intuitive, and it may be more reasonable to focus on reducing feed costs and FCRs, using both husbandry and genetic means (this issue can be further refined using the above tools and accompanying excel file). The high cost of running index selection using information from relatives is also a concern, particularly when coupled with the low 2% rate of return. However, the original inbreeding options (2.1) did consider an economic return of 2.5%, and from this predicted, for inbreeding at less than 1%, gains over half a million dollars considering the whole industry by the first generation, gains that grow to millions as the industry expands (FIGURE 35). Obviously the benefit cost returns could be enormous, provided there was confidence the industry would grow.

Figure 35 Actual selection gains considering all inbreeding costs



2.4 Overall discussion for the appendices/attachments

The initial assumptions, and conservatism of suggesting a 2.5% economic return (annually, and increasing every generation) seem to be supported from the analysis of genetic options above. Even so, it is acknowledged that this report tended to be conservative in considering selection gains, and there could be grounds for higher gains than those indicated here. However, as it stands, the options seem to suggest a relatively heavy investment is required at this stage to secure modest gains of about 2% per generation. Taking a buoyant view of industry expansion, and a climate of high investor confidence, then, with some risks, the heavy investment could return very favorable cost benefit returns in the long term. In part, this is because the genetic gains are expressed each year, not generation, they accumulated each generation, the value of genetics grows with the size of the industry, and, after costs and the selection are done, the gains can represent “something for nothing” provided inbreeding is managed. The genetic improvement should also stimulate some growth of the industry (i.e. make farming the species more efficient and profitable), which in turn will lead to greater economic benefits.

Options are also provided to go into a “holding mode” on genetic improvement and to implement a policy of “doing no harm”. That is, some basic options are developed which minimize the economic losses from closing the breeding nucleus (breeding multiple generations in captivity) through hybridizations, or balance the losses with small genetic gains using within family selection. No, or negligible costs are associated with these options. The benefit from closing the nucleus could be genetic gains from unintentional or domestication selection, which perhaps are not insignificant, at least for the first few generations. There are also important biosecurity benefits from closing the nucleus.

That is, there seem to be two broad options: a rather hefty investment with hefty long term gains, or a holding pattern.

Caveats:

It is not impossible that some non-genetic improvements, notably improved FCE and a reduction in the generation time, could substantially improve the economic outcomes from even the budget genetic options, so this matter is of some importance, and perhaps should be reviewed annually.

Some alert is raised about the possibility of adverse correlated selection responses. All these traits should be monitored if not selected, and these correlated responses determined.

References

Falconer, D.S. and Mackay, T.F. 1996 Introduction to Quantitative Genetics, Ed 4. Longmans Green, Harlow, Essex, UK.

Appendix 3 Selection intensity vs proportion selected

Table 14 Selection Intensity vs proportion selected

% selected	selection intensity (i)		% selected	selection intensity (i)		% selected	selection intensity (i)
0.01	3.959		1.0	2.665		20	1.400
0.02	3.789		1.2	2.603		21	1.372
0.03	3.687		1.4	2.549		22	1.346
0.04	3.613		1.6	2.502		23	1.320
0.05	3.554		1.8	2.459		25	1.271
0.06	3.506		2.0	2.421		26	1.248
0.07	3.465		2.2	2.386		27	1.225
0.08	3.428		2.4	2.353		28	1.202
0.09	3.396		2.6	2.323		29	1.180
0.10	3.367		2.8	2.295		30	1.159
			3.0	2.268		31	1.138
0.12	3.316		3.2	2.243		32	1.118
0.14	3.273		3.4	2.219		33	1.097
0.16	3.235		3.6	2.197		34	1.078
0.18	3.201		3.8	2.175		35	1.058
0.20	3.170		4.0	2.154		36	1.039
0.22	3.142		4.2	2.135		37	1.021
0.24	3.117		4.4	2.116		38	1.002
0.26	3.093		4.6	2.097		39	0.984
0.28	3.071		4.8	2.080		40	0.966
0.30	3.050		5.0	2.063		41	0.948
0.32	3.030		5.5	2.023		42	0.931
0.34	3.012		6.0	1.985		43	0.914
0.36	2.994		6.5	1.951		44	0.896
0.38	2.978		7.0	1.918		45	0.880
0.40	2.962		7.5	1.887		46	0.863
0.42	2.947		8.0	1.858		47	0.846
0.44	2.932		8.5	1.831		48	0.830
0.46	2.918		9.0	1.804		49	0.814
0.48	2.905		9.5	1.779		50	0.798
0.50	2.892		10.0	1.755		60	0.644
0.55	2.862		11	1.709		70	0.497
0.60	2.834		12	1.667		80	0.350
0.65	2.808		13	1.627		90	0.195
0.70	2.784		14	1.590			
0.75	2.761		15	1.554			
0.80	2.740		16	1.521			
0.85	2.720		17	1.489			
0.90	2.701		18	1.458			
0.95	2.683		19	1.428			
1.00	2.665		24	1.295			