Genetic selection for amoebic gill disease (AGD) resilience in the Tasmanian Atlantic salmon (*Salmo salar*) breeding program

Richard S. Taylor, Peter D. Kube, Brad S. Evans and Nicholas G. Elliott

Project No. 2011/771

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August 2015

ISBN: 978-1-4863-0455-4

The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Program. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

Australian Government
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Non-Technical Summary

The main health issue affecting Atlantic salmon marine aquaculture in Tasmania is amoebic gill disease (AGD), a parasitic disease which causes extensive gill pathology. AGD is treated by proactively bathing fish in freshwater, based upon regular assessment of the intensity and frequency of gross gill signs ("gill score") in each caged population. However, the process of densely crowding fish and pumping into fresh water is estimated to cause up to 5% handling mortality over a production cycle or significant loss at a single transaction. This is both an animal welfare and a production issue which may be reduced through genetic selection for improved handling resilience and improved fish handling protocols.

The primary components of the Salmon Enterprises of Tasmania (Saltas) selective breeding program (SBP) breeding objective are improved growth and increased AGD bathing interval. Despite ongoing genetic progress in reducing bath frequency, the need to regularly crowd and treat fish remains. The aim of this project was to determine if there is genetic variation associated with AGD handling resilience, and if so, to identify appropriate and cost-effective trait measures that could be utilised by the breeding program.

Using a high density ramped velocity swim challenge as a surrogate measurement trait for the handling resilience trait, low heritability of handling resilience was consistently recorded across three year classes of fish and over a range of AGD expression. There were moderate genetic correlations between the results from fresh water (hatchery) swim challenges and subsequent marine swim challenges on the same population at low (close to normal commercial) AGD expression, indicating that testing and selection could be applied directly to potential broodstock based upon results of swim challenge at a young age.

Any selection for resilience would need to be countered by possible impact on selection for key traits within the overall breeding objective. Measures of AGD handling resilience were largely independent of AGD resistance (gill score), suggesting opportunity to select for both traits without adverse effects. Strong adverse genetic correlations with fish size (growth or condition factor) indicated by the swim challenge may be an artefact of the challenge but preclude the inclusion of the swim challenge trait within the overall breeding objective. Current genetic progress for AGD resistance and fish growth is likely to minimise the number of handling events required in a production cycle and thus reduce AGD handling losses. The economic value of AGD handling mortality is unclear and requires validation before modelling of the cost-benefit of inclusion of resilience in selection decisions can occur.

Despite significant genetic variation for heart and gill morphology measures, these were discounted as useful selection traits because they are unrelated to the swim challenge trait. However, these data will stand as a useful comparison of cardiorespiratory traits in future generations of the SBP.

Objectives:

(a) Determine the genetic variation for AGD handling resilience, and the opportunity for genetic improvement.
(b) Establish the level of genetic variation for cardiovascular traits and the association with AGD handling resilience
(c) Examine cost-effective and non-destructive resilience selection traits that can be applied to freshwater broodstock.

Outputs

- Development of a high density swim challenge tank that can be used in the freshwater hatchery or on a floating pontoon at sea.
- Development of a swim challenge protocol.
- Genetic parameters of high density swim challenge in freshwater and marine conditions across a range of fish sizes and AGD expression.
- Genetic correlations against key selection traits (AGD gill score and fish size).
- Genetic and phenotypic parameters of cardiovascular traits from the Saltas SBP population and assessment of relationship to AGD handling resilience, AGD gill score and fish size (weight and condition factor).
- Recommendations of future potential research areas

Publications

Taylor, R.S., Kube, P.D., Evans, B.S., Elliott, N.G., 2014. Genetic variation of handling resilience of Tasmanian Atlantic salmon affected by amoebic gill disease (AGD). in: Hermesch, S., Dominik, S. (Eds.), Breeding Focus 2014 - Improving Resilience. AGBU University of New England, Armidale, NSW, pp. 101-113.

Acknowledgements

We thank the Australian Seafood CRC and the Tasmanian Salmonid Growers Association for their support of this project.

The work could not have occurred without the support of Saltas in providing fish from the breeding program, staff assistance and testing facilities in the hatchery and marine trial cages. Tassal provided marine infrastructure and invaluable help with fish husbandry, handling and data collection at sea. The authors gratefully acknowledge the following individuals or groups for various forms of assistance throughout this project, and apologise to anyone we have inadvertently omitted.

- Mark Hilder, Chloe English and Yvonne Sheehan at Saltas.
- Dave Cameron, Josh Fielding, Geoff Dobson, Shane Coleman, Dave Young and the dive crew at Tassal Operations (Dover).
- Ben Maynard, Scott Cooper, Mina Brock, Andrew Wood, Matt Sherlock, Rob Gregor and Robert Kay Elias Polymeropoulos and Anna Do of CSIRO (Hobart).
- Michael Mitchell (Mitchells Plastic Welding) for construction of the swim tank.

1 Introduction

1.1 Background

Intensive animal farming systems offer efficient productivity through increased stocking density and control of nutrition and key environmental conditions. However, animals in intensive farms are inevitably subjected to a range of stressors and pathogens which may rapidly lead to disease expression. Disease has a direct impact upon animal health and welfare and has substantial economic impact due to mortality, production loss and monitoring and treatment costs. Disease is estimated to account for over 20% of turnover in livestock farming in developed countries (Bishop & Woolliams, 2014). There are a range of responses that an animal may exhibit to minimise the impact of an infectious agent, these can be characterised as resistance, tolerance, robustness and resilience. Resistance and tolerance both describe health responses, where resistance is the ability to inhibit or reduce pathogen establishment or replication while tolerance is the ability to maintain performance by counteracting the damage that established/replicating pathogens can inflict (Raberg et al., 2007). The terms 'robustness' and 'resilience' both refer to the ability to remain productive despite disease challenge, where robustness is the ability to resist change and resilience is the ability to react to change. Robustness and resilience may be used interchangeably when underlying coping mechanisms are unknown. For example, Knap (2005) describes robustness as "the ability to combine a high production potential with resilience to stressors". Albers et al. (1987) defined disease resilience as the ability of a host to maintain a reasonable level of productivity when challenged by infection.

Aquaculture production of Atlantic salmon in Tasmania is significantly impacted by amoebic gill disease (AGD). The disease is initiated by attachment of trophozoites of the marine ectoparasite *Neoparamoeba perurans* (Young et al., 2008), the presence of this amoeba on the gills causes localised host tissue reactions including hyperplasia, hypertrophy and lamellar fusion that express grossly as raised white spots and patches. Although gross gill examination does not confirm the presence of the parasite, Tasmanian salmon farmers use a simple non-destructive "gill score" to regularly assess the intensity and frequency of AGD signs in a random subsample of fish from each caged population, which is expressed as an ordinal scale from "clear" to "heavy"(Taylor et al., 2009b). The gill score is used to assist scheduling of freshwater bathing treatments, with each pen of fish requiring up to 13 baths (Tassal Group Limited, 2009) during a 15 month marine production cycle. Proactive freshwater bathing at low average gill score has ensured that direct losses are

minimised, yet despite establishment of commercial fish handling protocols, AGD associated mortality routinely occurs during operational bathing (Kube et al., 2012). Handling related mortality may range from a few fish to, in worst cases, over 5% of the population in a single transaction. Average cumulative mortality due to AGD handling is estimated at 5% over the course of a production cycle (D. Kiemele[1], pers. comm.).

The key features of commercial AGD treatment are that fish with a range of AGD pathologies are continually crowded for transfer into hyperoxic freshwater, where they are held for 2 hours or more. Crowds generally last for 45 to 90 minutes with oxygenation or aeration generally applied. Mortalities may occur in the crowd or be discovered immediately following the freshwater bath. It is generally observed that losses are higher at high average gill score, although individual fish of low gill score can succumb. The link between gill score and handling loss has previously been documented when AGD affected fish are anaesthetised (Taylor et al., 2009b). Observation of commercial bath mortalities indicates that some fish of low gill score can be susceptible at bath handling events, while other fish are resilient despite having a high gill score. In this study we consider 'AGD handling resilience' as the ability of fish to withstand acute handling stress when challenged by AGD.

A range of respiratory and cardiovascular effects of chronic AGD are reviewed by Powell et al. (2008) yet it is currently unclear how AGD causes fish to die. Crowding causes a cascade of stress effects involving cortisol release, respiratory function, osmotic regulation and energy metabolism (Iwama et al., 1997). Crowding, handling and potentially exhausting Atlantic salmon as part of management practices such as freshwater bathing are likely to lead to increases in lactate and the development of an extracellular acidosis (Powell & Nowak, 2003). The mechanisms that allow fish to deal with the physiological demands of AGD during periods of acute handling stress are not known, but are likely to be linked to cardiovascular health and stress-coping styles. Indeed, concerns are raised in Europe that the high prevalence of heart deformities in farmed salmon is both a major production and welfare issue, with stress-induced mortality during routine production procedures being linked to heart deformities (Poppe et al., 2003; 2007; Claireaux et al., 2005; Rodger & Mitchell, 2011). The consensus of opinion is that the high level of salmon cardiac problems reported in Europe is a direct consequence of the conditions experienced in intensive aquaculture. Following several generations of selection for production traits, it is also reported that there is significant genetic variation for epicarditis,

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pericardial fat and heart symmetry of Norwegian salmon (Olesen et al., 2009; Shehzad, 2009). Fish with epicarditis may be more susceptible to stress and disease (Johansen & Poppe, 2002). Our preliminary (unpublished) work had indicated significant genetic variation of heart morphology in Tasmanian salmon, but the functional significance of heart morphology in relation to AGD handling resilience is unknown.

Improvement of host response to infectious challenges by genetic selection and genomics is widely recognised to be a valuable complement to conventional disease control in livestock (Doeschl-Wilson et al., 2012). In aquaculture, selection against disease impact is normally focussed upon improving 'disease resistance' directly expressed as survival (Houston et al., 2010; Odegard et al., 2011). The relative contribution of host resistance and tolerance is generally unknown. Selection for resistance may result in eradication or reduction of pathogens from a population but could encourage evolution of higher virulence. Tolerant hosts are likely to limit damage but may harbour pathogens (Doeschl-Wilson & Kyriazakis, 2012). Approaches to breeding for livestock robustness and resilience may vary, breeding for resilience to nematode infection in sheep has been reported (Albers et al., 1987; Bisset & Morris, 1996) and has been successfully trialled in commercial breeding over several years (Morris et al., 2010). Gunia et al. (2013) demonstrated that selection for resilience (measured as blood packed cell volume) could improve the overall breeding strategy for Creole goats. The need to include 'robustness' traits in breeding goals for poultry was reviewed by Star et al. (2008). To date there are few published examples of robustness or resilience traits included in aquaculture breeding objectives. However, the largest salmon breeding company in Norway (Aquagen AS) includes robustness traits such as survival, heart/circulatory performance (swim test) and stress tolerance (AquaGen, 2006).

The Salmon Enterprises of Tasmania (Saltas) selective breeding program (SBP) was established in 2004 (Elliott & Kube, 2009). The main breeding goals are to improve fish growth and reduce AGD bathing frequency through increased AGD resistance. Each year, a marine test population of tagged individuals of know pedigree is exposed to a field challenge with reiterative rounds of natural AGD infection and bathing. Fish that are tested at sea are not used for breeding, but breeding values are calculated for potential broodstock (which are held in freshwater) based upon their genetic relationship to the marine tested animals. Using gill score as the selection trait, moderate levels of genetic variation of AGD resistance have been measured (Taylor et al., 2007; 2009a; Kube et al., 2012). As a gross measure of disease pathology, gill score conflates resistance to *N.perurans* with host tolerance to the presence of the parasite, the relative contribution of these two responses is unknown (Kube et al., 2012).

1.2 Need

Reducing mortality is economically important to production and has animal welfare and sustainability implications for the industry. The aim of this project is to determine whether AGD bath handling losses can be decreased through genetic selection for 'AGD handling resilience'. This work will compliment current efforts to breed for resistance to AGD within the SBP and may assist the industry in improving handling protocols for AGD affected fish.

1.3 Objectives

The overall objectives of this project were to:

(a) Determine the genetic variation for AGD handling resilience, and the opportunity for genetic improvement.
(b) Establish the level of genetic variation for cardiovascular traits and the association with AGD handling resilience
(c) Examine cost-effective and non-destructive resilience selection traits that can be applied to freshwater broodstock.

1.4 Scope of this report

The initial plan was to test these objectives across two year classes with a 'go/no-go' decision milestone after the first year.

This report presents the work undertaken each year to address the above objectives as separate and stand alone sections.

Section 2 describes the early development of the high density swim challenge protocol (as a surrogate for handling resilience) applied at sea to two year old fish following previous AGD exposure (objective a). Hearts were removed from the tested fish in order to document heart morphology traits that may be related to previous AGD history and handling resilience (objective b). Although refinement of the swim challenge was required, Seafood CRC approval was provided to proceed to the second year of experiments.

Section 3 describes a study to compare freshwater handling resilience against marine handling resilience of AGD affected fish (objective a). The aim was to assess whether the freshwater test is representative of marine testing. If so, the test could be readily applied in the hatchery at lower cost, with the opportunity to run the test on potential broodstock at a young age (objective c). Due to excessive losses related to anaesthesia post exercise in the second year challenges, these trials were repeated

and extended in a third year (Section 4) to cover a broad range of AGD expression (objectives a and c).

Section 5 is a summary and overall discussion of the project

1.5 Animal Ethics

Animal ethics approval was granted through the Department of Primary Industries, Parks, Water and Environment - Animal Ethics Committee on 2nd March 2012. Reference for this approval is “Genetic selection for Amoebic Gill Disease (AGD) resilience in the Tasmanian Atlantic salmon (*Salmo salar*) breeding program (DPIPWE AEC Project 20/2011-12)”

2 Are cardiorespiratory traits related to handling resilience?

2.1 Aim

2.1.1 Cardiorespiratory morphology and abnormalities

The size and shape of the gills and heart are a reflection of the lifestyle of fish species (Gray, 1954; Poupa & Lindstrom, 1983; Tota & Gattuso, 1996) and variation may occur due to environmental adaptation (Tiitu & Vornanen, 2002). There is increasing evidence that domestication of fish contributes to changes in a number of fitness-related traits (Dunmall & Schreer, 2003; Jonsson & Jonsson, 2006). Farmed fish may develop suboptimal heart shape or increased levels of cardiac deformity (Mercier et al., 2000; Poppe et al., 2003; Claireaux et al., 2005; Olesen et al., 2009) due to inadvertent genetic selection or the combined effects of environmental stressors (temperature, nutrition, disease, etc.) which can lead to fish kills in routine commercial handling conditions (Brocklebank & Raverty, 2002). According to Poppe et al. (2003), fish that succumb during crowding and grading and transportation are typically large fish in good condition, reflecting that their cardiac capacity has been sufficient when unexposed to physical challenge. Changes in cardiac structure and cardiac failure may follow periods of elevated blood pressure or hypertension (Poppe & Taksdal, 2000; Gamperl & Farrell, 2004; Takle et al., 2006). Such hypertensive effects are typical of AGD affected fish (Powell et al., 2002b; 2008; Leef et al., 2005b; 2007). AGD history has previously been associated with cardiac changes at a phenotypic level (Powell et al., 2002a). Therefore, the aim of this trial was to examine genetic parameters of heart shape and cardiac deformities in the Tasmanian Atlantic salmon population and their possible relationship to handling resilience (as measured by response to a swim challenge and AGD history).

Gill area is generally held to be proportional to the aerobic capacity of fish and other aerobic indicators such as growth rate and maximum body size (Gray, 1954; Hughes, 1966; Beamish, 1978; Wegner, 2011). The surface area and blood-water barrier thickness determines the morphological potential for gas exchange (Wells & Pinder, 1996). Published methods to measure cross sectional gill surface involve laborious dissection of individual gill filaments and measurement of gill lamellae spacing and area (Hughes, 1966; Stevens & Devlin, 2000). Reliable quantitative data for gill area are limited because the analysis is exacting and subject to errors that become compounded by differing calculation methods. These methods are not suitable for

gathering data from large numbers of animals required for quantitative genetic estimation.

A variety of methods of assessing heart morphology have been reported in the literature. These are essentially based upon the observation that 'healthy' salmonid ventricles have a tall symmetrical pyramidal shape that enables them to pump blood effectively, asymmetrical or rounded hearts reflect lower fitness (Poppe et al., 2003). Measures employed include height to width ratio on the cranio-ventral or dorsal view (Poppe et al., 2003; Pombo et al., 2012; Claireaux et al., 2005), the relative angle of the corners of the ventricle (Claireaux et al., 2005; Pombo et al., 2012) and ventricular roundness (Shehzad, 2009; Fraser et al., 2013; Fraser et al., 2014). The bulbous arteriosus regulates pressure between the ventricle and the gills, in healthy fish this organ should be at a low angle in relation to the vertical midline of the ventricle (Poppe et al., 2003; Pombo et al., 2012; Fraser et al., 2013). Misalignment of the bulbous arteriosus appears to be a direct consequence of altered heart shape and will aggravate heart overload and restrict cardiac output during energy demanding situations (Poppe et al., 2003).

There is generally a positive correlation between body mass and heart mass (Shehzad, 2009), though cardiac index can be expected to be lower in larger fish (Pombo et al., 2012). Cardiac remodelling in response to environmental stress may include an increase in relative heart mass (Powell et al., 2011, 2012). Relatively large hearts should indicate improved pumping capacity and relative fitness. Indeed, Anttila et al. (2014) reported that parr tested as 'good' swimmers had relatively larger ventricles (17.4%) when compared to 'poor' swimmers.

A range of heart deformities are described in the literature, the aetiology of these conditions is often unknown but may include rearing environment, nutrition, stress and genetics. These conditions may often go unnoticed until mortality events occur. Poppe et al. (2007) described high mortality related to myocardial necrosis of harvest sized Atlantic salmon during transport. Arteriosclerosis lesions which occlude the vascular lumen are described in migrating wild Atlantic salmon by Farrell (2002). Aplasia of the septum traversum (which separates the heart and gut cavities) is detailed by Poppe et al. (1998), there is evidence that triploids are more susceptible to this developmental problem and that it is more prevalent above 8°C in the hatchery phase (Fraser et al., 2014). With the advent of higher energy fish-feeds and increased harvest size considerable levels of pericardial fat can develop (Poppe et al., 2003; Gamperl & Farrell, 2004). It is likely that pericardial fat is related to fish size or condition factor, but previous evidence from the Tasmanian SBP indicates that body fat depots can be independent traits (Do, 2013). As a 'lifestyle disease' pericardial fat is likely to affect physiological scope. Pericardial fat may be confused with

epicarditis, which is a focal inflammation of the pericardium or myocardium (Johansen & Poppe, 2002). Shehzad (2009) described pericardial fat in 89% and epicarditis in 42% of fish in a genetic study of 2,736 Atlantic salmon in Norway.

2.1.2 Development of swim challenge

Our preliminary work in 2008 (Taylor & Kube, unpublished) to develop a high capacity swim challenge for the SBP had utilised a small round tank of $1m^3$. This provided a test to establish the value of swim-challenge and produced consistent trends with approximately 50% of tested fish becoming fatigued within each 120 minute trial, but flow characteristics of the challenge chamber were uneven. Therefore, a key objective of the current project was to develop a high capacity swim challenge that could provide a relatively consistent laminar flow to minimise flow variation within the challenge chamber.

Ideally selection traits should be (a) relevant, i.e., they have to reflect commercial conditions and relate to the objective trait (improved handling resilience), (b) simple, i.e., they have to be understandable for users and cost-effective, (c) sensitive, i.e., they have to react to changes in the system, (d) repeatable, i.e., different measurements must lead to the same outcome. Due to the value of test animals or potential brood fish, it is also preferable that selection traits are non-destructive. Experimental tests of swimming capacity have been used in many species of fish as a quantitative assessment of performance linked to fitness (Beamish, 1978). Evidence suggests that salmonids are performing near their maximum oxygen consumption and maximum cardiac output when swimming at their critical swimming speed, though it is clear that fish spend little of their life at this speed (Jain et al., 1997). Therefore forced swimming is a useful experimental method to establish the metabolic limitation of a fish. Critical swimming speed or oxygen uptake is generally lower in stressed or sick fish (Tierney & Farrell, 2004; Wagner et al., 2003). A number of authors have developed swim challenges that can be applied to large groups of fish, Castro et al. (2013) applied a water velocity test to 41 g fish (100 per group, approximately 50 kg/m3) in a ring shaped tank to categorise fish as ‘poor’ and ‘good’ swimmers. Anttila et al. (2014) screened swimming performance of 16 batches of 200 salmon parr (stocking density approximately 38 kg/m^3) as ‘poor’, ‘moderate’ and ‘good’ swimmers by increasing water velocity until 30% remained. Veiseth et al. (2006) tested post crowding recovery of 4.8 kg salmon (25 per batch, 30 kg/m^3 stocking density) in three water velocities in a large D ended tank. A stepped velocity test of large batches of smolt is routinely used to assess cardiac fitness by Aquagen AS, with ‘winners’ retained in the ’robust’ breeding nucleus (AquaGen, 2006). These tests are performed at lower stocking density and scale than could typically be expected during commercial fish handling. In Tasmania, fish are typically

grown at <10 kg/m^3 and freshwater bathed at around 40 to 50 kg/m^3. The stress responses of fish may vary with crowding intensity, time and frequency of exposure (Wedemeyer, 1976; Carey & McCormick, 1998; Basrur et al., 2010; Gatica et al., 2010b). Gatica et al. (2010a) modelled commercial well-boat transport at >107 to 244 kg/m^3. Veiseth et al. (2006) crowded fish at >200 kg/m^3. Fish handling guidelines presented by the RSPCA (2012) do not specify densities, but they recommend crowd intensity based upon a fish behaviour scale 1-5. Therefore, we have developed a high density crowd challenge which utilises high stocking density and ramped water velocity.

2.2 Methods

2.2.1 Preparation of fish

This trial was done using part of the 2010 year class of the Saltas SBP. The challenge group represented 178 full-sib families (i.e. 101 paternal and 96 maternal half-sib families) and the mating design was a 2 x 2 factorial (where each male was crossed with two females and each female with two males). At the eyed eggs stage, an equal number from each family were combined to a communal tank for hatching and swim-up in order to ensure a common rearing environment. In March 2011, a group of these fish were individually tagged with a passive integrated transponder (PIT) tag and a small caudal fin-clip was dissected for eventual genotyping and parentage determination. Following six weeks under lights (22L:2D), 2303 smolt (mean weight 131 g) were input to a 10 X 10 m (800 m^3) sea cage at Tassal Operations Pty Ltd. (Meads Creek, Dover) lease on 16th May 2011. The pen was lit (three 400 W submerged lights) until late November in order to minimise the early onset of maturation. The fish were fed to satiation with commercial (Skretting) diet throughout the production cycle. Routine SBP procedures to measure genetic parameters of AGD resistance and growth were undertaken for this early input group, at each round of infection AGD was managed to an advanced average gill score and fish were assessed prior to freshwater bathing. Three rounds of AGD gill scoring and bathing (termed AGD1, AGD2 and AGD3) were completed by March 2013. On 27th March 2012 a fourth AGD measure (AGD4) was recorded at low average gill score and overtly maturing fish were removed from the population. The fish were then left unbathed in order to encourage the disease to progress prior to the planned swim challenge in mid-April 2012. Due to a continued low expression of AGD by late April (only 18% of fish gill scored at 2 or above), it was decided to bath the fish to 're-zero' AGD expression on 26th April 2012, one week before the swim challenge. This allowed the swim challenge performance to be measured against previous gill score history.

2.2.2 Swim tank

A 3.6 m x 1.4 m 'D' ended polyethylene tank (Figure 2.1), total volume 3.2 m^3, test volume 2 m^3 was developed for this work. Water flow was provided by two 8 hp fire-pumps, each providing 2 m^3/min through multiple reducing nozzles. Laminar flow was encouraged by two stainless steel screens comprising of 25 x 25mm stacked cells (200 mm horizontal length). Inclined netting barriers at the downstream end of each chamber allowed fatigued fish to be gathered. The tank was housed on a 10 m x 4m barge tied alongside the production cage. Fish could be swum in both sides of the tank because there were no impellers or moving parts within the tank that could damage animals. The swim-tank was tested over several days in April and manifolds adapted to optimise water flows. Final testing at sea occurred on 1st May with a batch of 75 fish run through the system. At this stage it was evident that the maximum achieved flows (maximum 0.7 m/sec) were inadequate to exhaust all fish over a 2 hour challenge but that exhaustion of approximately 50% of the fish could be reliably achieved in this time.

2.2.3 Swim challenge

Swim challenges occurred on four days (3rd, 4th, 7th and 8th May 2012) with two 120 minute trials conducted each day. Each challenge consisted of approximately 146 fish (73 per side, total 8 challenges for 1,094 fish). The aim was to record 'time to fatigue' for each animal, the elapsed time from the start of the challenge until an individual became fatigued and was held against the downstream collection screen. Tank side (A or B) was recorded for each animal.

The swim-tank was prefilled and allowed to circulate at low velocity (0.1 m/sec) with pumps set to idle. A small batch of fish were crowded by seine net in the 10 x 10m cage, dip-netted and counted to the swim chambers. Once the required number of fish had been transferred, the seine net was removed to minimise stress effects upon fish remaining in the cage. The fish in the swim tank were allowed to acclimatise for five minutes before water flows were adjusted to maximum flow (approximately 0.7 m/sec, 1.3 body lengths/sec). Fish that became fatigued were collected on the mesh barriers to be removed and recorded via a tag reader. The tag record included a time-stamp, allowing time to fatigue to be calculated. At the end of each challenge (120 minutes) the pumps were stopped and water level dropped before anaesthetising (17 ppm Aqui-S) and recording fish that survived the swim challenge and the PIT tag ID of each 'survivor' was recorded.

Figure 2.1: Layout of swim trial on floating pontoon, showing fish cage with jump fence lowered to transfer fish. Water is pumped to circulate water at velocity through two straight fish-challenge sections. PIT tag ID and time to fatigue is recorded before fish are placed in anaesthetic bath for measurement.

Once recorded, the fish were killed by a lethal dose of anaesthetic (100 ppm Aqui-S) and measured for weight and fork length. Gills and hearts were removed and preserved in 4% neutral buffered formalin for eventual digital imaging and processing. Gonads were inspected to record sex and maturation.

Condition factor of each fish was calculated as:

$$CF = (\text{Weight (g)} \times 100)/\text{length (cm)}^3 \text{ (Fulton, 1904)}$$

Attempts to measure gross abnormalities such as missing septum traversum (the wall between the cardiac and gut cavities), inverted heart (situs inversus) and heart adhesions were abandoned due to no or low incidence and the difficulty of accurate observation and recording in a field setting.

2.2.4 Cardiorespiratory traits

Prior to processing preserved hearts, the presence of pericardial fat and epicarditis was scored (Table 2.1). The atrium was then removed and each heart was photographed from the lateral view (Canon 450D camera) with the bulbous

arteriosus attached. The bulbous was then removed and the ventricle photographed from the dorsal view. For scaling purposes each heart was kept at a consistent distance from the camera by mounting it on a pin in a small overflow water bath (the sample number label for each image contained a standard calibration scale which enabled scale checking). Finally, the ventricle was thoroughly squeezed and blotted on tissue paper before weighing (Table 2.1).

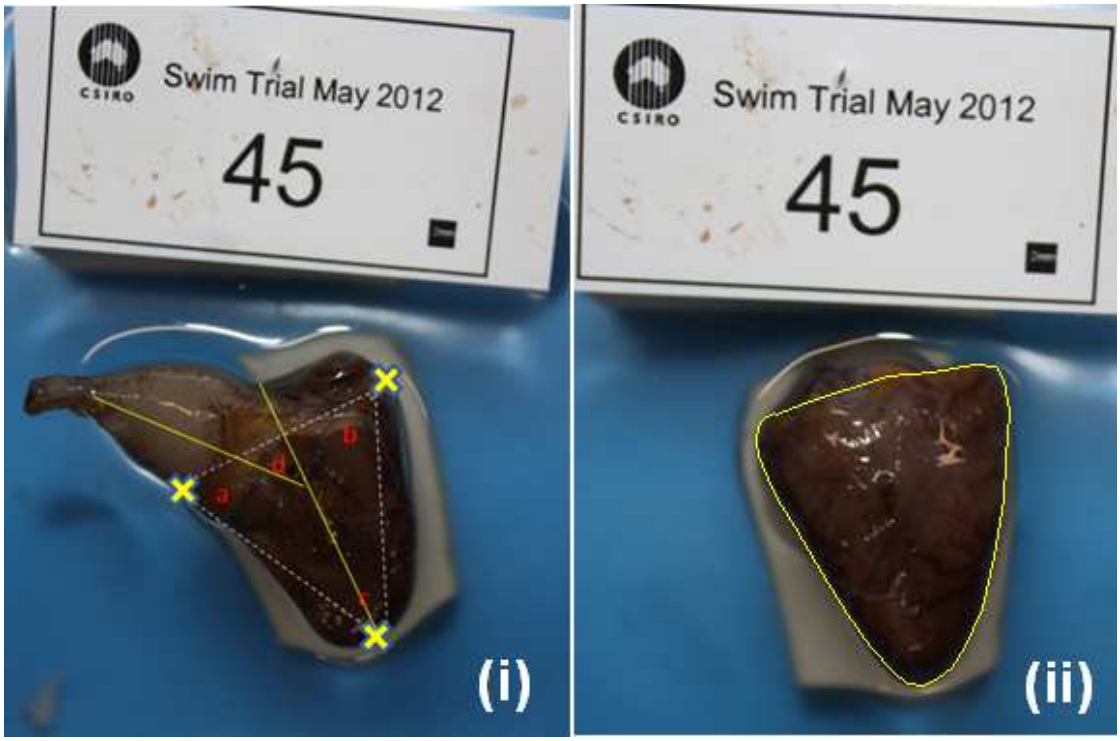

Figure 2.2: Cardiac morphometry - (i) Lateral view (atrium removed) showing corner angles (A, B, C) after Pombo et al. (2012) and the angle of the bulbous arteriosus (angle D) from the ventricular mid-line (Poppe et al., 2003).(ii) Dorsal view of ventricle with perimeter outlined.

Image analysis was performed using 'Image-J v. 1.45s' software. From the lateral view the corner angles were measured (Pombo et al., 2012) and the angle of the bulbous arteriosus (Poppe et al., 2003) (Fig. 2.2.i). From the dorsal view the perimeter length and area were measured to define roundness (Fig. 2.2.ii and Table 2.1), where a roundness of 1 indicates a perfect circle, roundness of 0 is a straight line.

Because published methods to assess gill area are not suitrd to studies of large numbers of animals, a rapid measurement of gill volume (water displacement) was developed whereby the complete formalin fixed gill block was weighed while suspended in fresh water. This was expressed as a ratio of gill displacement to body weight as a simple estimate of relative gill surface area.

Table 2.1: Measurements of formalin fixed salmon ventricles and gills.

Trait	Description	Measurement	Reference
LatA	Ventricle lateral angle a	Image analysis angle a (see Figure 2.2(i))	(Pombo et al., 2012)
LatB	Ventricle lateral angle b	Image analysis angle b (see Figure 2.2(i))	(Pombo et al., 2012)
LatC	Ventricle lateral angle c	Image analysis angle c (see Figure 2.2(i))	(Pombo et al., 2012)
LatD	Bulbous arteriosus angle d	Image analysis angle d (see Figure 2.2(ii))	(Poppe et al., 2003)
VRatio	Ventricle height:width ratio	Ventricle height (mm)/ventricle width (mm)	(Poppe et al., 2003)
VRound	Ventricle dorsal roundness	Roundness = 4π (area)/(perimeter)^2	(Fraser et al., 2014)
VWt	Ventricle wet weight (g)	Weight (g)	(Shehzad, 2009)
VSI	Ventricular somatic Index	Ventricle mass (g)/body weight (g)*100	(Fraser et al., 2013)
Epicard	Epicarditis	Ordinal scale 0 (none) to 2 (conspicuous)	(Shehzad, 2009)
VFat	Ventricle Pericardial fat	Ordinal scale 0 (none) to 3 (conspicuous)	(Shehzad, 2009)
Gill index	Index gill displacement:body weight	Gill displacement (g)/ body weight (g)*100	-

2.2.5 Statistical analysis

Genetic parameters of the swim trial data were estimated using residual maximum likelihood methods in the ASReml statistical package (Gilmour et al., 2009). A multivariate analysis was performed using starting values from preliminary univariate analyses. The mixed animal models tested were (a) Time to fatigue applied to the observed continuous variable (0 to 120 minutes). Survivors were censored after 120 minutes and have been scored as having fatigued at that time. (b) Analysis of gill score at each AGD measure (AGD1, AGD2, AGD3 and AGD4) applied to the ordinal gill score variable (0 to 5) which is treated as a continuous trait. (c) Analysis of cardiac morphological traits (ventricle angles a,b,c,d and weight) applied to the observed continuous variable. (d) Analysis of ventricle weight and ventricle index applied to the observed continuous variable. (e) Analysis of epicarditis and pericardial fat applied to the ordinal score variable (0 to 2 and 0 to 3 respectively). (f) Analysis of relative gill displacement index applied to the observed continuous variable. The terms in the fitted model were:

$$Y = \mu + challenge + \text{assess} + sex + family + a + \varepsilon$$

where Y is a vector of measured values for all fitted traits, μ is the mean for each trait, *challenge* is the fixed effect of challenge run (1 - 8) which includes the effects of day (1 – 4) and time (a.m. or p.m.) for each challenge, *sex* is the fixed effect of sex (M, F or unknown), *family* is the random effect of parental interaction, a is the random animal additive genetic effect and ε is the random residual effect.

Heritability was estimated as the additive genetic variance as a proportion of total phenotypic variance. Genetic correlations were estimated using the additive genetic components of covariance estimated by the linear model.

2.3 Results

2.3.1 Swim challenge and AGD

Table 2.2: Summary statistics for swim time, weight and condition factor at May 2012 swim challenge and gill score at four preceding bathing events.

Trait	Description	N	Mean	SD	CV	Min	Max
Swim	Time to fatigue (mins) May 2012	1087	88.4	36.5	41%	7	120
AGD1	Gill score (first infection) July 2011 (0-5)	1959	1.87	0.90	48%	0	4
AGD2	Gill score (second infection) Oct 2011 (0-5)	1071	2.01	1.05	52%	0	5
AGD3	Gill score (third infection) Jan 2012 (0-5)	2028	1.47	1.09	74%	0	5
AGD4	Gill score (fourth infection) Mar 2012 (0-5)	1741	0.98	0.59	60%	0	4
Weight	Weight (Kg) May 2012	1087	2.33	0.65	28%	0.91	4.85
CF	Condition Factor (%gcm^3) May 2012	1087	1.32	0.22	17%	0.78	1.89

1094 fish were swum over eight separate challenges (seven challenges with both sides of the tank filled and one challenge using a single side), of which 1087 were of known pedigree (Table 2.2). Average start density per side was 169.6 kg/m^3 (range 159.9 to 179.5 kg/m^3). Overall, 50.7% of the fish became fatigued but this varied between challenge runs (range 38.2% - 62.0%, Fig. 2.3). Due to the high throughput of pumped water (one tank volume every 50 seconds), oxygen levels remained within normal ambient limits (mean = 83.6% saturation, range 70 – 92%). Temperatures averaged 15.1°C (SD 0.23°C).

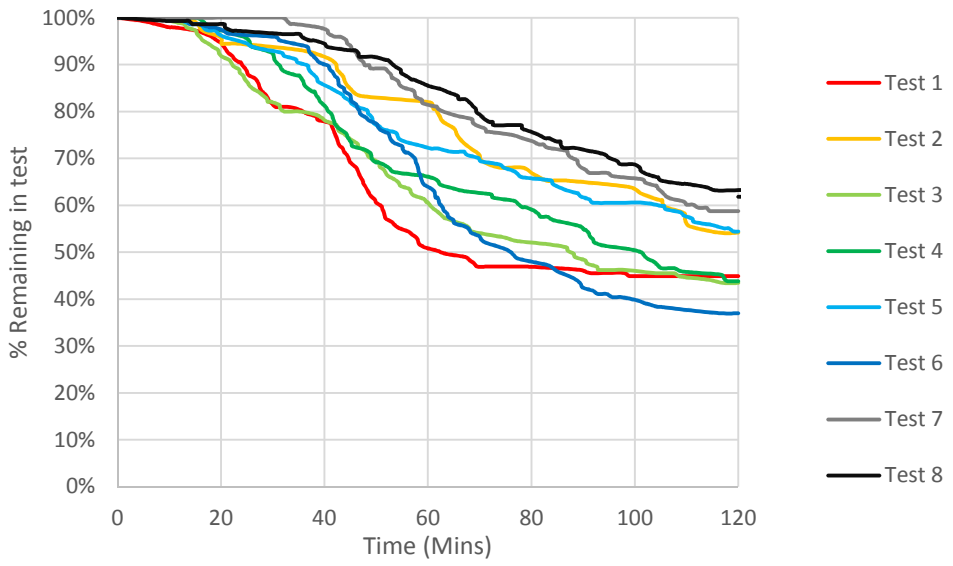

Fig. 2.3: Kaplan-Meier survival curves for swim challenge across eight separate challenge runs. Each challenge is presented as the sum of both tank sides.

Genetic variation of gill score was significant across the first three infection rounds (AGD1 to 3, Table 2.3), which were allowed to express to a moderate to high average score. However, there was no significant genetic variation at the fourth infection (AGD4) which was measured at low average gill score (0.98). The pattern of relationships between gill scores at different AGD infections (Table 2.4) generally conformed with patterns previously seen in larger studies of the Tasmanian population (Kube et al., 2012). The first infection (AGD1) was moderately correlated with later infections (r_g = 0.64 and 0.52) and the second and third infections (AGD2 and AGD3) are strongly correlated (r_g = 0.82). There was no genetic variation for AGD 4 and, therefore, no correlations were estimable for this trait.

Table 2.3: Variance components and heritability for swim time, weight and condition factor at May 2012 swim challenge and gill score at four bathing events. Standard errors are shown in parentheses.

Trait	Additive genetic σ^2_a	Family σ^2_f	Residual σ^2_e	Heritability h^2
Swim	237.5 (85.9)	3.5 (17.6)	1021.6 (71.3)	0.19 (0.06)
AGD1	0.24 (0.05)	0.00 (0.00)	0.59 (0.17)	0.29 (0.05)
AGD2	0.53 (0.15)	0.06 (0.05)	0.51 (0.23)	0.48 (0.11)
AGD3	0.36 (0.09)	0.03 (0.03)	0.81 (0.19)	0.30 (0.07)
AGD4	0.00 (0.00)	0.00 (0.00)	0.35 (0.03)	0.00 (0.04)
Weight	0.10 (0.03)	0.00 (0.00)	0.31 (0.02)	0.25 (0.06)
CF	59.0 (19.5)	6.98 (6.95)	116.9 (11.9)	0.32 (0.09)

Swim time for the recently freshwater bathed fish in the marine swim challenge was found to be a heritable trait (h^2 = 0.19±0.06). Genetic correlations between swim

time and gill score at all AGD infections were not significantly different from zero (Table 2.4). This suggests that genetic variation of handling resilience of recently bathed fish is an independent genetic trait to AGD resistance. There was a moderate negative relationship between swim time and condition factor (r_g = -0.58, r_p = -0.31) indicating that high condition factor fish become exhausted faster than lower conditioned fish. There was a negative phenotypic correlation between swim time and fish weight (r_p = -0.15), however this is influenced by condition factor which is highly and positively correlated with weight (r_g = 0.67, r_p = 0.77). With condition factor set as a covariate on swim time, the relationship between swim time and fish weight becomes positive (r_p = 0.24±0.04, data not shown) indicating that large fish of lower condition will tend to become exhausted later in high density swim challenge.

Table 2.4: Genetic (below diagonal) and phenotypic (above diagonal) correlations between swim time, gross gill scores, weight and condition factor. Standard errors are shown in parentheses. Values in bold are significantly different from zero ($p < 0.05$).

Trait	Swim	AGD1	AGD2	AGD3	AGD4	Weight	CF
Swim	-	0.01 (0.03)	0.05 (0.05)	-0.07 (0.04)	**-0.11 (0.03)**	**-0.15 (0.03)**	**-0.31 (0.03)**
AGD1	-0.09 (0.18)	-	**0.23 (0.04)**	**0.13 (0.03)**	0.01 (0.03)	**0.09 (0.03)**	0.02 (0.04)
AGD2	0.12 (0.19)	**0.64 (0.11)**	-	**0.33 (0.03)**	**0.08 (0.03)**	0.00 (0.05)	0.04 (0.05)
AGD3	-0.05 (0.19)	**0.52 (0.12)**	**0.82 (0.08)**	-	**0.20 (0.03)**	-0.02 (0.04)	0.02 (0.04)
AGD4	0.00 (0.00)	0.00 (0.00)	0.88 (0.75)	0.00 (0.00)	-	0.02 (0.03)	0.02 (0.04)
Weight	-0.26 (0.18)	0.17 (0.17)	0.12 (0.17)	0.02 (0.17)	0.00 (0.00)	-	**0.77 (0.01)**
CF	**-0.58 (0.14)**	0.13 (0.17)	0.13 (0.17)	0.00 (0.17)	0.00 (0.17)	**0.67 (0.09)**	-

2.3.2 Cardiorespiratory morphology and abnormalities

Heart shape at age 24 months (Table 2.5) was generally within ranges of healthy Atlantic salmon (Poppe et al., 2003), with ventricular height:width ratio averaging 1.11 (14.6% of records were at or below 1.00) and an upright bulbous arteriosus (mean 36.7° from the ventricular axis; 13.7% of hearts had a bulbous angle greater than 45°). The majority of fish (58%) were clear of epicarditis and few (10%) were recorded with heavy levels. Pericardial fat deposits were recorded in most fish (83%) with 22% assessed with high fat levels. There were no significant affects of maturation and sex recorded, probably because overtly mature fish had been removed from the population in March 2012.

Heart traits were all of low to moderate heritability (Table 2.6). As could be expected when measuring an essentially triangular shape, genetic and phenotypic correlations were high and negative between angle A and the other two lateral ventricular 'corners' (B and C) – where angle A is large, angles B and C tend to be smaller (Table 2.8). The higher heritability (and lower residual variance) of angle A suggests that it is the most accurate descriptor of ventricular shape change on the lateral view. Angle

A showed a high negative genetic correlation with ventricular roundness, indicating a less round heart shape. Angle C was positively correlated with roundness, a larger base angle corresponding to a rounder heart. Ventricular weight and the ventricular somatic index (VSI) were moderately heritable (h^2 0.34 and 0.32 respectively). The VSI shows a moderate but marginal genetic correlation with the bulbous angle, indicating that hearts that are large in relation to fish weight tend to have a greater (suboptimal) displacement from the ventricular axis. Increase in relative heart size also tends to higher pericardial fat (r_g = 0.38). Epicarditis and pericardial fat levels appear to be closely related (r_g = 0.84). From these results it appears that heart characteristics could be changed through selection decisions, but the potential functional outcomes of changing heart shape are unclear because there were no significant correlations between swim time and heart traits.

Table 2.5: Summary statistics for ventricle morphometry, external fattiness score, epicarditis score and gill index at age 24 months (see Table 2.1 for description of traits).

Trait	N	Mean	SD	CV	Min	Max
LatA	1082	66.04	7.22	11%	29.70	93.90
LatB	1082	61.93	6.32	10%	37.20	95.80
LatC	1082	52.02	5.57	11%	32.10	101.10
LatD	1082	36.75	8.75	24%	11.80	91.80
VRatio	1082	1.11	0.12	11%	0.79	2.49
VRound	1084	0.78	0.07	9%	0.39	0.99
VWt	1072	1.83	0.51	28%	0.75	4.05
VSI	1072	0.79	0.11	14%	0.51	1.50
Epicard	1086	0.52	0.67	130%	0.00	2.00
VFat	1086	1.06	0.62	58%	0.00	2.00
Gill index	1087	1.66	0.29	18%	1.04	2.78

There were no significant genetic correlations between gill score at each measure and ventricle traits (data not shown). This suggests that previous gill score history does not have long-term effects upon heart parameters. There was, however, a moderate positive relationship between bulbous angle (LatD) and gill score at first infection (r_g = 0.44±0.16, data not shown). Gill score at first infection also showed a low phenotypic correlation (0.12±0.04) with VSI. There was a marginally significant and positive genetic correlation between gill score at the second infection (AGD2) and epicarditis (r_g = 0.42±0.21). Gill score had been highest at this measure which may explain some resulting cardiovascular changes.

Table 2.6: Variance components and heritability for ventricle morphometry, external fattiness score, epicarditis score and gill index at age 24 months. Standard errors are shown in parentheses.

Trait	Additive genetic σ^2_a	Family σ^2_f	Residual σ^2_e	Heritability h^2
LatA	14.39 (3.43)	0.00 (0.00)	36.89 (2.43)	0.28 (0.06)
LatB	9.14 (3.1)	0.53 (1.21)	30.11 (2.04)	0.23 (0.07)
LatC	4.99 (1.73)	0.08 (0.11)	26.18 (1.69)	0.16 (0.05)
LatD	11.31 (3.9)	0.00 (0.00)	65.61 (4.07)	0.15 (0.05)
VRatio	0.31 (0.12)	0.02 (0.05)	1.11 (0.09)	0.22 (0.08)
VRound	0.10 (0.03)	0.00 (0.02)	0.39 (0.03)	0.20 (0.06)
VWt	0.09 (0.02)	0.00 (0.00)	0.18 (0.02)	0.34 (0.08)
VSI	0.38 (0.09)	0.00 (0.00)	0.80 (0.07)	0.32 (0.07)
Epicard	0.05 (0.02)	0.00 (0.00)	0.40 (0.02)	0.12 (0.04)
VFat	0.06 (0.03)	0.01 (0.01)	0.31 (0.02)	0.15 (0.07)
Gill Index	0.02 (0.01)	0.00 (0.00)	0.07 (0.00)	0.19 (0.06)

Gill displacement was allometrically related to fish weight (r_p = 0.80 ± 0.01, data not shown). When expressed as a ratio trait (gill index) to account for fish size there remains a negative phenotypic correlation with fish weight (r_p = -0.66, Table 2.7) indicating that larger fish have relatively lower gill volume. Gill index is heritable (h^2 = 0.20, Table 2.6) but was not genetically correlated with swim time (Table 2.8) despite a moderate phenotypic correlation (r_p = -0.30). Gill index showed no genetic correlation with heart traits though there were low size related phenotypic correlations with heart traits, particularly VSI (r_p = 0.35) and epicarditis (r_p = -0.16)

Table 2.7: Genetic (r_g) and phenotypic (r_p) correlations of fish weight and condition factor against cardiorespiratory traits. Standard errors are shown in parentheses. Values in bold are significantly different from zero ($p < 0.05$).

Trait	Weight r_g	CF r_g	Weight r_p	CF r_p
LatA	-0.23 (0.17)	0.04 (0.17)	**0.11 (0.03)**	**0.15 (0.03)**
LatB	0.22 (0.19)	0.01 (0.19)	0.02 (0.03)	-0.03 (0.04)
LatC	0.14 (0.20)	-0.07 (0.2)	**-0.15 (0.03)**	**-0.16 (0.03)**
LatD	-0.05 (0.21)	-0.03 (0.21)	-0.01 (0.03)	-0.03 (0.03)
VRatio	-0.01 (0.19)	0.06 (0.2)	0.01 (0.03)	0.03 (0.04)
VRound	-0.02 (0.19)	-0.05 (0.2)	0.01 (0.03)	0.01 (0.03)
VSI	-0.02 (0.17)	-0.01 (0.17)	**-0.21 (0.03)**	**-0.21 (0.04)**
Epicard	-0.13 (0.22)	0.08 (0.22)	**0.14 (0.03)**	**0.17 (0.03)**
VFat	-0.15 (0.20)	0.08 (0.22)	**0.13 (0.03)**	**0.15 (0.03)**
Gill Index	**-0.66 (0.11)**	**-0.72 (0.12)**	**-0.70 (0.02)**	**-0.71 (0.02)**

Table 2.8: Genetic (below diagonal) and phenotypic (above diagonal) correlations between swim time, heart morphometric measures, ventricular fat, epicarditis and gill index. Standard errors are shown in parentheses. Values in bold are significantly different from zero ($p < 0.05$).

Trait	Swim	LatA	LatB	LatC	LatD	VRatio	VRound	VSI	Epicard	VFat	Gill Index
Swim	-	-0.05 (0.03)	**0.09 (0.03)**	-0.03 (0.03)	0.01 (0.03)	0.06 (0.03)	**-0.09 (0.03)**	-0.05 (0.03)	-0.01 (0.03)	0.04 (0.03)	**-0.08 (0.04)**
LatA	**-0.45 (0.19)**	-	**-0.67 (0.02)**	**-0.54 (0.02)**	**0.20 (0.03)**	**0.17 (0.03)**	**-0.18 (0.03)**	0.19 (0.03)	0.00 (0.03)	**0.12 (0.03)**	**-0.11 (0.03)**
LatB	0.35 (0.21)	**-0.85 (0.09)**	-	**-0.28 (0.03)**	**-0.42 (0.03)**	**0.10 (0.03)**	**-0.10 (0.03)**	-0.13 (0.03)	0.00 (0.03)	**-0.07 (0.03)**	0.04 (0.03)
LatC	0.39 (0.22)	**-0.63 (0.16)**	-0.01 (0.22)	-	**0.21 (0.03)**	**-0.33 (0.03)**	**0.35 (0.03)**	-0.10 (0.03)	0.00 (0.03)	**-0.07 (0.03)**	**0.09 (0.03)**
LatD	-0.01 (0.23)	0.13 (0.20)	-0.39 (0.19)	0.27 (0.22)	-	**-0.09 (0.03)**	**0.07 (0.03)**	**0.10 (0.03)**	0.00 (0.03)	0.01 (0.03)	-0.01 (0.03)
VRatio	0.02 (0.22)	**0.71 (0.14)**	-0.34 (0.22)	-0.79 (0.13)	-0.17 (0.22)	-	**-0.78 (0.01)**	-0.01 (0.03)	**-0.08 (0.03)**	-0.04 (0.03)	-0.01 (0.03)
VRound	0.01 (0.22)	**-0.56 (0.16)**	0.16 (0.21)	0.75 (0.12)	0.30 (0.22)	**-0.94 (0.06)**	-	-0.03 (0.03)	0.04 (0.03)	0.00 (0.03)	-0.01 (0.03)
VSI	0.03 (0.19)	**0.35 (0.15)**	**-0.35 (0.17)**	-0.13 (0.20)	**0.41 (0.19)**	0.09 (0.19)	-0.11 (0.19)	-	-0.06 (0.03)	**0.09 (0.03)**	**0.35 (0.03)**
Epicard	-0.10 (0.25)	-0.12 (0.21)	-0.16 (0.23)	**0.46 (0.23)**	0.13 (0.25)	-0.36 (0.21)	0.23 (0.23)	0.21 (0.22)	-	**0.22 (0.03)**	**-0.16 (0.03)**
VFat	-0.05 (0.24)	0.11 (0.20)	-0.23 (0.21)	0.10 (0.24)	0.10 (0.24)	-0.06 (0.23)	-0.18 (0.23)	0.38 (0.19)	0.84 (0.16)	-	**-0.11 (0.03)**
Gill Index	0.22 (0.21)	0.17 (0.19)	0.01 (0.20)	-0.32 (0.21)	0.00 (0.23)	0.04 (0.21)	-0.04 (0.21)	0.22 (0.19)	-0.01 (0.24)	0.00 (0.23)	-

2.4 Discussion

This initial study of handling resilience of marine reared fish from the SBP (as measured by a high density swim-challenge of fish one week after freshwater bathing) indicates that time to fatigue is heritable and is likely to respond to genetic selection. Although survival is closely linked to gill score (Taylor et al., 2009a; Kube et al., 2012) , our results suggest that handling resilience at low AGD expression is independent of AGD resistance. This indicates that current selection for AGD resistance will not change handling resilience during routine commercial fish handling at low gill threshold.

From the genetic and phenotypic correlations of condition factor against swim time, and the phenotypic correlations of weight and swim time, it is evident that fish size (weight and condition) is an important factor deciding time to fatigue during high density exercise. The effects of fish size on swimming capacity and metabolic rate are well known across many species (Brett, 1964, 1967; Plaut, 2001). In laboratory flume tests researchers tend to overcome these restrictions by choosing fish of similar size or by adjusting data for fish size, a 'good' swimmer is thus a fish that performs well for its size. Likewise, it is known that fish of higher condition factor are poorer swimmers, this may reflect a 'blocking effect' of cross sectional area within a swim flume (Beamish, 1978; Hammer, 1995) or reduced fitness. In a phenotypic study of rainbow trout swimming performance, Claireaux et al. (2005) reported 5% higher condition factor in 'poor' swimmers and noted that high condition factor is linked to rounded heart shape. However, in our study there was no significant correlation of condition factor with heart roundness. There was, however, a low phenotypic correlation of VSI with condition factor and weight (r_p = -0.21) indicating that relative heart weight is lower in larger and higher conditioned fish. There is also a phenotypic tendency for increased heart fat and epicarditis in larger and higher conditioned fish.

For the farmer, the practical implication of fish size effects in handling tolerance is that handling procedures should be gentler for populations of higher condition factor, where crowd times should be minimised.

Correct interpretation of the genetic correlation between condition factor and time to fatigue is important for sound selection decisions. In applying results from high density swim challenge breeders need to be aware of the influence of fish size and condition in swim tests. This effect could perhaps be managed by grading fish into size groups but this is likely to bias family representation in each grade because weight and condition are heritable, thus differences between challenge runs would be difficult to separate from genetic effects. Another option is to fit size effects as

covariates in the model, doing this in our data indicated minimal effect on heritability of swim time which was slightly reduced to $r_g = 0.15 \pm 0.06$ (data not shown). Including size effects on swim time also had negligible impact upon genetic correlations with AGD or cardiorespiatory traits (data not shown). The potential breeding consequence of the reported genetic correlation of condition factor to swim time is that selection for improved handling resilience may have a negative impact upon condition factor. Reduced condition factor is linked to increased variation in processing traits (Do, 2013). Therefore, analysis of breeding scenarios for handling resilience needs to include weighting for body size and condition.

This study introduced measurement of gill volume by displacement as a proxy for three-dimensional gill area. This methodology was not validated against published methods of estimating gill area (Hughes, 1966) but offers a rapid method suitable for genetic trait estimation of large numbers of animals. As previously reported for classical estimates of gill surface area (Palzenberger & Pohla, 1992; Wegner, 2011), gill volume scales positively with body mass but is proportionally lower as fish size increases. There was no link between gill index and swim time which, on the face of it, does not suggest that oxygen exchange across the gill is a limitation during normoxic crowding conditions. However, area (or volume) does not fully reflect oxygen transfer efficiency because gill function is properly explained as the relationship between perfusion and ventilation, permeability, diffusion distances and the oxygen carrying capacity of the blood (Palzenberger & Pohla, 1992).

The relationship between cardiac morphology and function has been extensively studied in teleosts. Fish species that live a sedentary lifestyle tend to have saccular hearts designed to move large stroke volume at low heart rate. Active fish like salmonids have a typically pyramidal ventricle built for pumping small volume at high rate and high pressure. Heart morphology is plastic and may change with life cycle and in response to physiological demand. Significant differences in heart shape and fat deposits in the epicardium have been shown to exist between triploid and diploid Atlantic salmon (Fraser et al., 2015) and between farmed and wild fish (Poppe et al., 2003; Pombo et al., 2012) suggesting a link between cardiovascular health and performance. The only study to link heart shape to swimming ability (Claireaux et al., 2005) categorised low numbers of rainbow trout as ‘good swimmers’ and ‘poor swimmers’ and linked performance to ventricle shape (using echo-Doppler imaging), good swimmers were shown to have significantly higher/more triangular heart shape (length:width ratio 1.01 vs 0.88, $p < 0.05$) concluding that a more rounded ventricle led to reduced performance. It is assumed that rounder or less triangular hearts are less efficient at pumping blood, a difference that may not be critical until fish are subjected to acute stress such as occurs during commercial crowding and pumping operations. Our data (Table 2.4) indicates that the hearts from Tasmanian Atlantic

salmon tend to be within the ranges of healthy (or wild) salmon described by Poppe et al. (2003), who reported that farmed Atlantic salmon hearts had a mean ventricular height:width ratio of 1.05 (wild 1.15) and a bulbous alignment of 40.2 (wild 33.4). Shehzad (2009) found average ventricular roundness of 4.5 kg farmed salmon at 0.73 (±0.03 SD) and pericardial fat averaging 1.33 (±0.66 SD), the lower pericardial fat level (mean 1.06) in our study is likely explained by the smaller size of our fish (2.3 kg). Shehzad (2009) reported a 42.4% prevalence of epicarditis which is similar to our observation (41.7%), however, average epicarditis score (0.43±0.50 SD) was lower than we have presented for Tasmanian fish (0.52) despite the size difference. It is unclear whether this higher level of epicarditis is a feature of commercial Tasmanian populations because it has been derived from an experimental population that was repeatedly exposed to AGD above normal commercial thresholds, therefore this result requires further validation. The Shehzad study found no genetic correlation between epicarditis and pericardial fat, whereas our results indicate that the two traits are closely linked (r_g = 0.84), this may indicate error in distinguishing the two traits by gross examination.

There was a moderate genetic correlation between ventricular angle A and swim time, this may suggest that heart symmetry is important to heart function and that measures of lateral shape may be warranted in future studies. However, due to the high standard error of the genetic correlation between these two traits and the lack of strong correlations between swim challenge and other cardiac traits the pursuit of non-destructive measurement of heart shape (e.g. using veterinary echo-Doppler imaging) on potential broodstock was not warranted for the second phase of the project.

Fish in the SBP test cohorts are allowed to develop a high phenotypic expression of AGD to achieve gill score distributions that are higher than normally experienced by commercial populations (Kube et al., 2012; Taylor et al., 2007; 2009a; 2009b). Heart shape and is known to be highly plastic in response to environmental challenges such as disease (Gamperl & Farrell, 2004). Powell et al. (2002a) compared Tasmanian salmon hearts from commercial cages with a history of ‘heavy’ and ‘light’ AGD and found that there were no differences in heart somatic index between the two groups. Fish with a history of ‘heavy’ AGD were found to have a higher height to width ratio (i.e. less round) and a thicker compact myocardium layer which the authors attributed to an exercise effect related to the higher dorsal aortic blood pressure of AGD affected fish. A similar link between increased cardiac workload, increased heart size and more triangular shape was reported following experimentally induced anaemia in rainbow trout (Simonot & Farrell, 2007) and in Atlantic halibut *(Hippoglossus hippoglossus)* (Powell et al., 2012). However, heart shape effects are not always reported, for example Powell & Gamperl (2015) showed

that Atlantic cod (*Gadus morhua*) responded to microsporidian gill parasite *(Loma morhua)* infection by altering metabolic scope without changing ventricle shape. Our data did not suggest significant phenotypic correlations between gill score history and heart shape (roundness or ventricular angles), though there were weak phenotypic correlations (data not shown) between VSI and gill score at first infection (0.12±0.04) and second infection (0.10±0.05). Moderate genetic correlations between gill score at first infection and the angle of the bulbous arteriosus (0.44) and between gill score at second infection and the level of epicarditis (0.42) suggest that selection for AGD resistance could impact heart characteristic, however, these estimates are likely to be biased due to the hypertensive effects of AGD on the circulatory system and subsequent cardiac responses of our measured animals. These findings should be further validated by comparing genetic parameters of heart shape in fish not previously exposed to AGD.

Overall this work supported the hypothesis that handling resilience (as measured by a high density swim challenge) at sea is under a degree of genetic control and will respond to selection. Cardiac traits are also heritable and may be related to previous AGD exposure. However, there were few useful links between handling resilience and cardiac traits to pursue. The concept of non-destructive imaging of hearts of potential broodstock to support breeding decisions appears to have little justification at this stage.

3 Initial comparison of freshwater and marine handling resilience

3.1 Aim

The operational plan of the Saltas SBP involves freshwater spawning and nursery, with tagging and DNA fingerprinting at 12 months of age, followed by a cohort split into freshwater and marine cohorts for ongrowing and trait assessment (Elliott & Kube, 2009). The central concept of confining potential broodstock to freshwater for the entire production cycle is to maintain biosecurity. The breeding values estimated from siblings in the marine cohort (AGD resistance, marine growth, maturation and harvest traits) are applied to the brood fish at a family level, but without the use of genomic tools it is not possible to predict the best breeding animals 'within family'. Some traits estimated directly from the potential broodstock (growth and maturation) are used to inform the breeding goal and can be applied within family. There is potential to add non-destructive testing of the freshwater cohort to support breeding decisions for improved resilience.

This phase of the project examines whether a simple high density swim challenge applied at 12 months age (pre-smolt) in freshwater can be used to predict handling resilience at sea, as measured by a marine swim challenge.

3.2 Methods

3.2.1 Preparation of fish

The 2011 YC families were produced by 2 x 2 factorial mating design at SALTAS Wayatinah in May 2011 and reared separately until the eyed egg stage where equal numbers of eggs per family were combined to a common environment for hatching and freshwater nursery. In early June 2012, the fish were weighed (mean = 111 g, SD = 31.5 g), PIT tagged and fin clipped for parentage assignment. In mid-July 1859 individuals (representing 198 families from 103 sires and 103 dams) were subjected to a freshwater swim challenge (Section 3.2.3). The fish were subsequently transferred to an 800 m^3 sea cage at Tassal (Dover) on 7th August 2012, fed to satiation on commercial diet and managed to advanced natural AGD expression prior to a marine swim challenge (Section 3.2.4).

3.2.2 Swim tank

Due to difficulties with achieving high and consistent water flows with the initial (May 2012) swim trials, the swim tank was modified to house an electric outboard (48 V Torqeedo, 8 hp equivalent) and an upstream stainless steel screen comprising of 25 x 25mm stacked cells (200 mm horizontal length). This configuration reduced the tank to a single 0.9 m^3 challenge section but produced higher and controllable water velocity (Fig. 3.1). Outboard speed is easily set by a wattage regulated throttle, thus water velocity can be controlled using pre-determined calibrations (Appendix 1).

3.2.3 Freshwater swim trial

Fish were swum in two challenges on 18th and 19th July 2012. For each challenge, a batch of approximately 1000 fish (121 kg/m^3) was transferred to the swim tank and allowed to equalise to the tank environment at low water flow (approximately 0.5 body lengths/sec [bl/s] for 30 minutes). Oxygen levels were monitored and controlled to 100% saturation at ambient water temperature (mean = 6.1, SD = 0.6°C). At the start of the challenge water flow was set at a low rate of approximately 1 bl/s and then increased every 30 minutes by a further 0.5 bl/s until 3-3.5 bl/s had been achieved at 120 minutes. Due to issues with failing circuit breakers at higher amperage there were a number of stoppages at around 1000 W power. Therefore the challenges were extended to allow 50% of the population to become fatigued (300 minutes and 225 minutes for challenge 1 and challenge 2). Time to fatigue was recorded for each individual; animals were readily scanned without anaesthesia and returned to a recovery tank. Following the freshwater swim challenges there was minimal mortality (1.5%).

Figure 3.1: Freshwater swim trial at Saltas Wayatinah showing electric outboard (left side of picture) and fish challenge section with downstream collection screen (right side)

3.2.4 Marine rearing and swim challenges

Following marine input, the swim cohort was monitored fortnightly for AGD development. By early November a sufficient range in AGD gill scores was available for the first marine swim trial to begin (gill scores 0 to 5 were distributed 6.2%, 37.1%, 36.5%, 16.0%, 3.8% and 0.5% respectively). The swim tank was installed on a pontoon and tested on 1st November, the electric outboard was powered by a 250 m underwater cable (240 V) stepped down to 48 V. An Oxyguard Atlantic oxygen control system was installed to automatically maintain oxygen levels within the tank at 90 to 110% saturation.

For each challenge, the swim-tank was prefilled and allowed to circulate at low velocity (0.15 m/sec) with the outboard set at 50 W. A small batch of fish were crowded by seine net in the cage, dip-netted and counted to the swim chambers. Once the required number of fish had been transferred (initially 460 fish, then lowered for subsequent challenges as detailed below) the seine net was removed to minimise stress effects upon fish remaining in the cage. The fish in the swim tank were allowed to settle at low flow for 30 minutes (approx. 0.5 bl/s) after which the velocity was rapidly increased to 1.5 bl/s and then 2 bl/s 30 minutes later. Due to power cable limitations (amperage) the system could not be run above 1200 W,

therefore challenges were limited to approximately 2.5 bl/s. As fish began to fail, the upstream screen was moved down by 10% of the challenge chamber length for every 10% reduction in fish remaining in the challenge, thus achieving a consistent crowd (Fig. 3.2). The upper screen was not dropped below 70% of the length (30% remaining), allowing 0.6 m to remain between the last fish and the downstream collection screen. This was intended to mimic commercial crowding, where the crowd net is tightened to maintain an even flow of fish through the fish transfer pump. Temperatures averaged 13.7°C (SD 0.53°C).

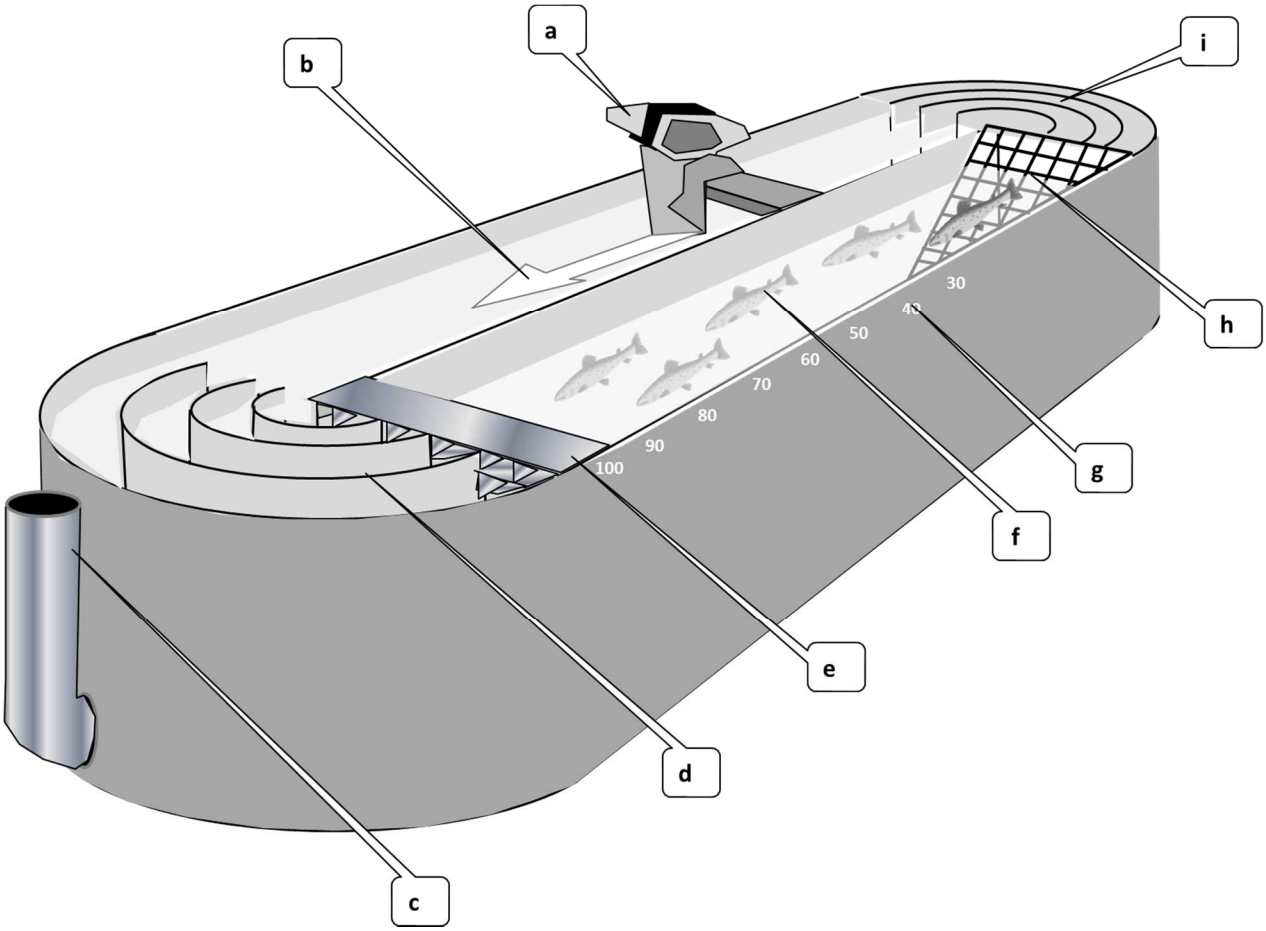

Figure 3.2: Schematic layout of swim tank showing (a) 48 v/8 hp outboard motor (b) water flow direction (c) standpipe to control water level (d) concentric end walls (e) stacked cell crowding screen (f) fish in test section (g) calibrated scale for crowding screen to regulate fish density (h) failed fish collection screen (i) position of flowmeter and oxygen control systems.

For the first challenge (6th November), the concept was to test batches of fish at high stocking density (460 fish at 485 g, 247 kg/m^3) so that the entire population could be measured over four challenges. As each fish became fatigued, it was anaesthetised and measured (gill score, weight and length) before being returned to an oxygenated recovery net. After 120 minutes, the flow was slowed to a minimum and water level drained so that anaesthetic could be applied to the remaining (unfatigued) fish. Unfortunately, many fish did not recover from anaesthesia. Therefore, it was

decided to limit the number of fish to be tested in subsequent challenges, so 410 fish were transferred without challenge (measured and gill scored, but not swum), leaving approximately 940 fish to challenge. On the second day, the number of fish in each challenge was reduced (around 310 fish per challenge, 167 kg/m^3) and the number of fish in anaesthetic at any stage was reduced. However, recovery of fatigued fish from anaesthesia was still poor. An overall handling mortality of 56.9% was reported to the AEC for the two days. All remaining fish were bathed on 8th November.

Due to the low numbers surviving swim challenge and anaesthesia it was decided in consultation with the AEC that these fish should not undergo swim-challenge at the height of summer. Therefore, at the second AGD infection and bathing on 22nd January 2013 (AGD2), remaining fish were simply gill scored and bathed. Due to a prolonged period of high water temperatures in March and non-specific mortality, there were not enough fish left by April 2013 to warrant a second marine swim challenge.

3.2.5 Statistical analysis

Freshwater and marine data was analysed with ASReml (Gilmour et al., 2009) and involved fitting multivariate linear mixed animal models. These were (a) %Swim time, where time in swim is normalised to 0 to 100%**.** Fish that were still swimming at the end of each challenge were censored at 100%**,** (b) weight and condition factor (CF = weight/length^3) in freshwater and at the first marine measure, (c) gill score at the first (AGD1) and second (AGD2) marine measures. The terms in the fitted model were:

$$Y = \mu + challenge + assess + family + a + \varepsilon$$

where Y is a vector of measured values for all fitted traits, μ is the mean for each trait, *challenge* is the fixed effect of challenge run (1 -2 in freshwater, 1 – 4 in marine), *assess* is the fixed effect of gill score assessor at AGD1 and AGD2, *family* is the random effect of parental interaction, a is the random animal additive genetic effect and ε is the random residual effect.

Heritability was estimated as the proportion of additive genetic variance as a proportion of total phenotypic variance. Genetic correlations were estimated using the additive genetic components of covariance estimated by the linear model, phenotypic correlations were estimated using overall variation components.

3.3 Results

Table 3.1 : Summary statistics for swim category (freshwater and marine), weight and condition factor at November 2012 and gill score at two bathing events.

Trait	Description	N	Mean	SD	CV	Min	Max
Swim1	% Time to fatigue Freshwater (0-100%)	1833	81.09	24.52	30.2%	2.0	100
Swim2	% Time to fatigue November 2012	1329	80.80	22.40	27.7%	11.7	100
AGD1	Gill score (first infection) November 2012	1705	1.75	0.95	54%	0	5
AGD2	Gill score (second infection) January 2013	739	2.14	1.09	51%	0	5
Weight (Tag)	Weight (g) June 2012	1858	111	32	28%	49	318
Weight (Nov)	Weight (g) Nov 2012	1697	483	114	24%	107	1064
CF (Tag)	Condition Factor June 2012	1853	1.38	0.12	8%	0.74	1.79
CF (Nov)	Condition Factor November 2012	1695	1.23	0.09	7%	0.81	1.65

Due to limitations of the swim tank, power supply issues and the operational need to minimise challenge period, our aim was to achieve 50% fatigue in each challenge. In freshwater, 48.4% of fish became fatigued (range 47.3% to 49.7%) and 55.6% in the marine swim (range 46.4% to 62.3%). Time to fatigue could not be fully assessed in these challenges due to power issues, therefore swim times are corrected as a percentage of total test time expressed as 0 to 100%.

All traits assessed were heritable apart from the initial freshwater swim (Swim1) (Table 3.2). The impact of gill score upon swim result in November is illustrated by the moderate negative r_p (-0.20) with higher gill score fish tending to be less successful in swim challenge, though there was no significant genetic correlation. This phenotypic relationship between gill score and fatigue in the swim challenge is further illustrated in Fig. 3.3 which indicates that 50-60% of gill score 0 to 2 fish failed in the challenge, while losses of gill score 3 and 4 were higher (70-80%) and that only 10% of gill score 5 fish were able to withstand the challenge.

Table 3.2 : Variance components and heritability for swim category (0,1), weight and condition factor in freshwater and at first AGD infection (Nov

Trait	Additive genetic σ^2_a	Family σ^2_f	Residual σ^2_r	Heritability h^2
Swim1	40.67 (27.81)	32.56 (14.54)	519.36 (23.68)	0.07 (0.05)
Swim2	80.55 (22.95)	0.00 (0.00)	399.89 (22.75)	0.16 (0.04)
AGD1	0.21 (0.05)	0.00 (0.00)	0.70 (0.04)	0.23 (0.05)
AGD2	0.37 (0.11)	0.00 (0.00)	0.84 (0.09)	0.31 (0.08)
Weight (Tag)	300.86 (61.28)	12.64 (8.84)	673.94 (42.95)	0.30 (0.05)
Weight (Nov)	4896.11 (886.98)	0.00 (0.00)	8125.13 (606.81)	0.38 (0.06)
CF (Tag)	31.61 (7.22)	0.00 (0.00)	110.13 (5.96)	0.22 (0.05)
CF (Nov)	19.91 (5.25)	2.06 (1.52)	56.83 (3.65)	0.25 (0.06)

There was a moderate genetic correlation between the freshwater and marine challenges (r_g = 0.64), despite the phenotypic influence of gill scores at the November swim. The moderate genetic relationship (0.49) between gill score at first infection (AGD1) and reinfection (AGD2) is in line with our previous reported values (Kube et al., 2012).

Good genetic relationships (r_g = 0.88) are observed between the June (freshwater tagging) and November (marine AGD1) weight measures. Similarly, there is a strong r_g (0.76) between the two condition factor measures. There was a positive genetic correlation between fish weight at tagging and freshwater swim (0.55). There were low positive phenotypic correlations between weight and both swim challenges and a low negative phenotypic correlation (-0.07) between condition factor and %swim time in the marine (November) challenge.

Table 3.3: Genetic (below diagonal) and phenotypic (above diagonal) correlations between %swim time, gross gill scores, weight and CF. Standard errors are shown in parentheses. Values in bold are significantly different from zero ($p < 0.05$).5

Trait	Swim1	Swim2	AGD1	AGD2	Weight (Tag)	Weight (Nov))	CF (Tag)	CF (Nov)
Swim1	-	**0.16 (0.03)**	**-0.07 (0.03)**	0.00 (0.04)	**0.14 (0.03)**	**0.08 (0.03)**	0.00 (0.03)	-0.02 (0.03)
Swim2	**0.64 (0.28)**	-	**-0.20 (0.03)**	0.03 (0.05)	**0.18 (0.03)**	**0.16 (0.03)**	0.06 (0.03)	**-0.07 (0.03)**
AGD1	0.07 (0.26)	-0.24 (0.17)	-	**0.23 (0.04)**	**-0.07 (0.03)**	-0.04 (0.03)	-0.01 (0.03)	0.02 (0.03)
AGD2	-0.22 (0.28)	0.14 (0.21)	**0.49 (0.15)**	-	-0.07 (0.04)	**-0.09 (0.04)**	0.01 (0.04)	0.04 (0.04)
Weight (Tag)	**0.55 (0.22)**	0.10 (0.17)	0.04 (0.17)	0.06 (0.19)	-	**0.78 (0.01)**	**0.31 (0.02)**	**0.19 (0.03)**
Weight (Nov)	0.31 (0.21)	0.11 (0.17)	0.20 (0.14)	0.00 (0.18)	**0.88 (0.04)**	-	**0.23 (0.03)**	**0.38 (0.03)**
CF (Tag)	-0.10 (0.26)	0.04 (0.19)	0.22 (0.16)	0.33 (0.18)	0.23 (0.14)	0.26 (0.13)	-	**0.33 (0.02)**
CF (Nov)	-0.45 (0.28)	-0.21 (0.17)	0.19 (0.19)	0.17 (0.21)	0.17 (0.15)	0.26 (0.14)	0.76 (0.09)	-

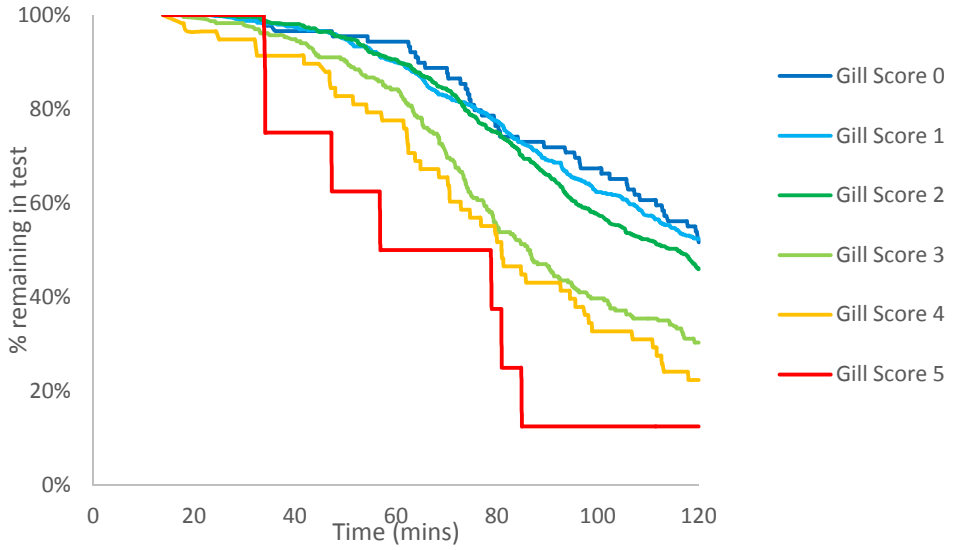

Figure 3.3: Combined result of marine swim trial November 2012 (AGD1) – Kaplan-Meier survival by gill score category across four challenges.

3.4 Discussion

The influence of gross gill pathology upon the fish's ability to withstand handling stress was illustrated by the negative phenotypic correlation between gill score at first infection (AGD1) and the marine swim result (-0.20), meaning that higher gill score fish are more easily fatigued in the crowded swim challenge. Fish with 'zero' to light gill pathology (gill scores 0-2) show a lower response, though some fish appear quite susceptible to handling stress despite apparently low levels of gill pathology (Fig. 3.3). Despite low representation in the high gill score 5 group there is evidence that low numbers (10%) are resilient to crowd stress despite high levels of gross gill

pathology. A similar separation of response by gill score has previously been described for a long term (100 day) AGD survival trial (Taylor et al., 2009b), confirming that gross gill assessment is functionally descriptive. Gill score at the November event (average 1.75, 57% of fish above gill score 1) was higher than normal commercial bathing thresholds (aimed at 30% above gill score 1) but would not be considered an extreme average. The rapid fatigue of higher gill score fish in the challenge illustrates the difficulty that farmers face in handling populations containing high gill score individuals and hence their practice to proactively bathe at lower gill score levels.

Despite issues with power supply in the hatchery and poor survival in anaesthesia following the marine swim challenge, this experiment indicated that handling resilience (measured in a crowded swim challenge) is heritable during marine challenge. The freshwater measure was not heritable but was compromised by unexpected power outages (indeed, when calculated as a binary trait 'failed' and 'not failed' this trait was heritable at 0.15 ± 0.05, data not shown). However, these measures appear to be highly correlated despite the practical operational issues encountered. Unlike the May 2012 result, there was no significant effect of condition factor on fatigue beyond a negative phenotypic correlation (r_p = -0.07) at the November marine challenge. This may reflect the smaller fish size (111g and 483g respectively) compared to the May 2012 marine challenge (15.1°C, 2.3 kg). There was a moderate positive genetic relationship (0.55) between tagging weight and the freshwater swim challenge and positive phenotypic relationships of weight with freshwater and marine swim challenges, suggesting that large fish are more able to withstand crowded swim challenge.

This initial trial using a 48 v electric outboard to generate water current in the swim chamber was successful in creating a more predictable and controllable flow environment in the swim tank, but the overall results were compromised by power supply issues in the freshwater hatchery and seabed cable. Loss of fish following exhaustive exercise and anaesthesia was greater than anticipated and prevented us from running a further swim challenge on this group of fish. A reduction in ventilation rate and amplitude is normally observed during anaesthesia (Basrur et al., 2010). Anaesthesia typically impairs oxygen uptake and may cause an oxygen deficit (Hill, 2004). The slowing of respiratory rate coupled with lactate release and blood acidosis following stressful exercise was undoubtedly the cause of this loss. In consultation with the AEC after this event it was recommended that fish should not be anaesthetised following exhaustive exercise.

4 Refined comparison of freshwater and marine handling resilience.

4.1 Aim

Swim challenge testing of large groups of fish has the potential to rank fitness of individuals within the challenge (AquaGen, 2006). Our initial work demonstrated that the ability to withstand a high density swim challenge is a heritable trait which may be negatively influenced during high AGD expression (Section 3) but is independent of AGD history in recently bathed fish (Section 2). Therefore, there may be opportunity to swim challenge AGD-naive fish in freshwater, allowing potential broodstock to be benchmarked at a young age. Due to issues with power supply at the hatchery and the unexpected loss of over 50% of the marine challenge population following post-handling anaesthesia, the research goal to compare handling resilience in freshwater and marine conditions could not be completed satisfactorily (Section 3).

Our ability to measure variation of fatigue is currently limited to a binary trait (fail, did not fail) or, at best, a 'time to fatigue' which is biased by a large number of individuals that are 'censored' as 'did not fail'. The aim of this study was therefore to further develop the swim challenge to improve discrimination between challenge animals and improve the statistical precision of the measure across a range of AGD expressions. Due to commercial operational limitations, swim challenge needs to be applied to all test animals over a few days. The swim challenge therefore has to be rapid and repeatable.

4.2 Methods

4.2.1 Preparation of fish

Families were produced by 2 x 2 factorial mating design to produce 190 families (98 paternal and 98 maternal half-sib families) at Saltas Wayatinah in May 2012, then combined to a common environment at eyed egg stage for hatching and freshwater nursery. In early June 2013, the fish were weighed (mean = 185.1 g, SD = 57.2 g), PIT tagged and fin clipped for parentage assignment and randomly split to three groups for (i) freshwater ongrowing as potential broodstock (ii) SBP marine test cohort and (iii) a marine swim-challenge cohort which was subjected to freshwater swim challenge in mid-July (Section 4.2.3). Both the SBP and swim cohorts were

subsequently transferred to separate 800 m^3 sea pens at Tassal (Dover) on 7th August 2013. Both pens were fed to satiation on commercial diet and managed to achieve advanced natural AGD, freshwater bathing and reinfection. The marine swim challenge cohort was subjected to multiple high density swim challenges at different levels of AGD expression.

4.2.2 Swim tank

The swim tank design remained largely unchanged from Section 3. Wiring and circuit breakers were upgraded at both hatchery and marine sites. With limitations on the equipment (size and power) the aim was to achieve higher maximum flow rate and to maintain consistent high stocking density by continually crowding and to constantly monitor and control oxygen levels to around 80% saturation to reflect commercial crowding conditions.

4.2.3 Freshwater swim challenge

Fish were swum in three challenges on 2nd and 3rd July 2013 (Table 4.1). For each challenge, approximately720 fish (range 692 – 736) were transferred to the swim tank and allowed to equalise to the challenge environment at low water flow (of approximately 0.8 bl/s) for 10 minutes. Oxygen levels were monitored and controlled to 80 - 90% saturation (mean 84.1% SD 3.4%) at ambient water temperature (mean 7.0°C, SD 0.4°C). The start flow was set at 400 W (approximately 1.5 bl/s) and increased by 300 W every 45 minutes, with the final 1300 W phase (3.5 bl/s) lasting 30 minutes. Fish that fatigued became trapped against the collection screen. In order to ensure a consistent fatigue measure, these animals were turned by hand to face the water flow and categorised as 'failed' if they fell back onto the screen and were unable to swim off. Time to fatigue was recorded for each individual; animals were readily PIT-tag scanned without anaesthesia and returned to an oxygenated recovery tank. To account for the reduction in crowd density as fish were removed from the challenge, the upstream screen was moved down by 10% of the challenge section length for every 10% reduction in fish numbers. The crowding screen was not moved beyond the 30% remaining mark to prevent fish being inadvertently pushed against the collection screen. At the end of each 165 minute challenge, water level was lowered and surviving fish ID's were recorded. Following the freshwater swim trials there was minimal mortality (0.7%).

4.2.4 Marine rearing and swim challenges

Following marine input, the swim cohort was monitored fortnightly for AGD development (Table 4.1) by seine netting a large subsample and dip-netting batches of fish to anaesthetic bin (17 ppm Aqui-S). Fish were batch weighed, with gross gill pathology inspected and scored on 40 individuals. The SBP marine test cohort was treated similarly (data not shown) with the aim of achieving advanced AGD expression for estimation of phenotypic (thus genotypic) variation of AGD gill score. Data from the SBP marine test cohort could thus be utilised to allow comparison of gill score variation in the absence of repeated swim-challenge stress.

By mid- September 2013, AGD was nearing a normal commercial bathing threshold (targeted to 30% of the population at gill score 2 – 5). Therefore, fish were swum in three challenges on 11th and 12th September. Although there were approximately 1950 fish in the population, the requirement was to challenge around 1,300 fish, utilising the half-sib mating design to average 13 fish per parent. The aim was to preserve numbers in case of unexpected losses and also to minimise disruption to commercial operations. For each challenge, the swim-tank was prefilled and allowed to circulate at low velocity (50 W). A small batch of fish was crowded by seine net, dip-netted and counted to the swim chamber. Once the required number of fish (mean 435, range 412 – 450, approximately 171 kg/m^3) had been transferred the seine net was removed to minimise stress effects upon fish remaining in the cage. After 10 minutes of settling at 100 W, power was increased to 400 W (approximately 1.2 bl/s), then increased by 300 W every 45 minutes, with the final phase at 1300 W for 30 minutes (approximately 3 bl/s). The upper crowding screen was moved down 10% of the challenge volume with every 10% reduction in remaining fish numbers. Failed fish were registered without anaesthetic and returned to an oxygenated recovery net. At the end of each challenge the 'winners' were also scanned and returned. Direct mortalities were 0.2% of the swum population.

35 days later (16th October) all individuals in the swim cohort were measured for weight and gill score. AGD was at an advanced level (63% score 2 – 5, index 2.17). The fish were left unbathed to allow approximately 1000 fish (*997*) to be swum on 22nd and 23rd October in four challenges (245 - 252 fish per challenge, approximately 163 kg/m^3). The methodology and power settings were the same as the September swim (400 W -1300 W, approximately 1 bl/s to 2.5 bl/s) with no fish anaesthetised on the day and the screen moved to regulate stocking density. Direct losses from swim were 1.6%. One day later, the entire population was bathed in fresh water.

Fortnightly gill score monitoring continued through to 20th November when an advanced distribution was achieved (index 1.6, 48% at score 2 -5). Approximately

1000 fish (*983*) were swum in five challenges between 26th and 28th November (192 - 200 fish per challenge, approximately 200 kg/m^3) with power settings ranging 600 W (approximately 1.2 bl/s) to 1500 W (approximately 2.2 bl/s). Temperature averaged 16.1°C (SD 0.8°C) and oxygen was maintained at 84.3% (SD 5.1%). Mortalities were 7.7% of the swum fish.

Full AGD score and weight measurement occurred on 5th December 2013. Gross fill pathology had advanced rapidly during the week with 17.7% of fish scored at gill score 5. This rapid increase in AGD was in common with observations of commercial and research cohorts held in the vicinity. All fish were bathed (AGD2).

Figure 4.1: Marine swim challenge March 2014.

Due to fish welfare concerns and commercial operational constraints it was not possible to carry out more swim trials in the height of summer, but regular health monitoring continued (Table 4.1). By late February, gill score was generally low though apart from some overtly mature fish. On 20th February 2014 the entire swim challenge population was gill scored (no weight measures), overtly mature fish were culled and the remaining immature animals were bathed. Between 3rd and 6th March 2014 the final set of swim trials were carried out. Because this was the last challenge, fish were anaesthetised lightly post-swim to allow confirmation of gill score (all scored zero) and weight/length measurement. The challenge regime was similar to previous runs with fish gently crowded and counted (99 – 124 fish per challenge, approximately 201 kg/m^3 across seven challenges) into the tank at low

water velocity (200 W, approximately 0.5 bl/s). The entire population was tested, with the 8th and final swim of 54 fish adjusted to 205 kg/m^3 starting density by starting with upstream screen at 50% of the challenge length. Challenge outboard power settings were from 700 W (1 bl/s) and limited to 1600 W (2 bl/s) at the upper end due to amperage constraints through the underwater cable. Direct mortality was 87 fish (10.7% of the swum number) with a further 140 removed in the next 14 days (16.8% of those swum), totalling 27.2% mortality.

In accordance with normal practice, the marine SBP challenge cohort was measured and bathed on 18th September 2013 (AGD1) at average gill score 1.4. Gill score developed slowly until 28th November (index 1.7, 0% score 5) but rose unexpectedly to reach average gill score 3.1 (24.4% score 5) at second infection (AGD2) on 10th December (Table 4.2). Although not part of our design this cohort provides useful comparisons of sibling fish that were not handled for swim challenges.

4.2.5 Statistical analysis

Freshwater and marine data was analysed with ASReml (Gilmour et al., 2009). Multivariate linear mixed animal models fitted were (a) %Swim time, where time in swim is normalised to 0 to 100%**.** Fish that were still swimming at the end of each challenge were censored at 100%. (b) weight and condition factor (CF = weight/length^3) at tagging in freshwater, at the AGD1 and AGD2 measures and at the final (March 2014) swim trial; and (c) gill score in the swim cohort at three AGD measures and in the SBP cohort at two AGD measures The terms in the fitted model were:

$$Y = \mu + challenge + assess + family + a + \varepsilon$$

where Y is a vector of measured values for all fitted traits, μ is the mean for each trait, *challenge* is the fixed effect of challenge run (1 - 3 in freshwater, 1 – 8 in marine), *assess* is the fixed effect of gill score assessor at each AGD measure, *family* is the random effect of parental interaction, a is the random animal additive genetic effect and ε is the random residual effect.

Heritability was estimated as the proportion of additive genetic variance as a proportion of total phenotypic variance. Genetic and phenotypic correlations were estimated using the components of covariance estimated by the linear model.

Table 4.1: Summary of key events for swim trial cohort. Swim challenges occurred in freshwater (July 2013) prior to marine transfer. Four sets of marine challenges (Sep, Oct, Nov 2013 and Mar 2014) were carried out at different levels of AGD. Regular subsampling allowed growth and gill health to be tracked throughout the trial, figures in parentheses are number of fish sampled for gill score at these 'Health Sample' events. [a]wt based on tagging; [b]wt based on batch average and calculated per individual between tagging and AGD1; [c]wt at AGD1; [d]wt based on batch average and calculated per individual from AGD1. *reduction in population following AGD1 measure was due to culling of 'no tags' and runts.

Date	Event	No. Swim challenges	Total #fish	Mean Wt	Wt (SE)	Start Density kg/m^3	Watts range	Failed%	Mean T°C	Mean O2%	GS0%	GS1%	GS2%	GS3%	GS4%	GS5%
4-12 Jun 2013	Tagging, **Weight1**	-	2209	184	0.63	-	-	-	-	-	-	-	-	-	-	-
2-3 Jul 2013	**Swim1** FW	3	2161	185[a]	1.23	147-154	400-1300	92.4	7.0	84.1	-	-	-	-	-	-
17 Jul 2013	Marine Input	-	2200	-	-	-	-	-	-	-	100.0	0.0	0.0	0.0	0.0	0.0
30 Jul 2013	Health Sample	-	281(40)	189	-	-	-	-	-	-	75.0	25.0	0.0	0.0	0.0	0.0
14 Aug 2013	Health Sample	-	157(40)	226	-	-	-	-	-	-	52.5	45.0	2.5	0.0	0.0	0.0
3 Sep 2013	Health Sample	-	190(40)	326	-	-	-	-	-	-	20.0	67.5	12.5	0.0	0.0	0.0
11 Sep 2013	Health Sample	-	40	-	-	-	-	-	-	-	17.5	62.5	17.5	0.0	0.0	0.0
11-12 Sep 2013	**Swim2** (Low AGD)	3	1305	353[b]	2.94	162-181	400-1300	86.8	12.3	92.3	-	-	-	-	-	-
4 Oct 2013	Health Sample	-	(40)	537	-	-	-	-	-	-	2.5	27.5	57.5	12.5	0.0	0.0
14 Oct 2013	Health Sample	-	161(40)	636	-	-	-	-	-	-	2.5	42.5	32.5	17.5	5.0	0.0
16 Oct 2013	**AGD1** (unbathed) **Weight2, CF2**	-	1898	572	3.62	-	-	-	-	-	6.0	30.6	37.9	18.2	6.3	1.1
22-23 Oct 2013	**Swim3** (Mod AGD)	4	997	591[c]	4.11	162-172	400-1400	87.7	15.4	94.1	-	-	-	-	-	-
24 Oct 2013	Bathed	-	1645*	-	-	-	-	-	-	-	-	-	-	-	-	-
7 Nov 2013	Health Sample	-	138(40)	783	-	-	-	-	-	-	55.0	40.0	5.0	0.0	0.0	0.0
20 Nov 2013	Health Sample	-	216(40)	921	-	-	-	-	-	-	20.0	50.0	10.0	12.5	2.5	5.0
26 Nov 2013	Health Sample	-	192	-	-	-	-	-	-	-	23.4	28.6	23.4	16.7	6.3	1.6
26-28 Nov 2013	**Swim4** (Mod-High AGD)	5	983	918[d]	6.35	195-213	600-1500	77.9	16.0	84.3	-	-	-	-	-	-
5 Dec 2013	**AGD2** (Bathed) **Weight3, CF3**	-	1485	968	5.87	-	-	-	-	-	2.8	7.6	18.9	29.0	24.1	17.7
24 Dec 2013	Health Sample	-	40	1118	-	-	-	-	-	-	55.3	31.6	10.5	2.6	0.0	0.0
8 Jan 2014	Health Sample	-	133(40)	1293	-	-	-	-	-	-	27.5	50.0	20.0	2.5	0.0	0.0
21 Jan 2014	Health Sample	-	110(40)	1300	-	-	-	-	-	-	32.5	57.5	7.5	2.5	0.0	0.0
30 Jan 2014	Health Sample	-	102(40)	1436	-	-	-	-	-	-	27.5	47.5	17.5	2.5	2.5	2.5
6 Feb 2014	Health Sample	-	112(40)	1388	-	-	-	-	-	-	15.0	70.0	7.5	0.0	5.0	2.5
20 Feb 2014	**AGD3** (mature grade and bathed) **Weight4, CF4**	-	1093	1658	-	-	-	-	-	-	20.4	51.8	15.6	6.4	2.8	2.9
3-6 Mar 2014	**Swim5** (Recently bathed)	8	832	1637	11.93	187-217	700-1600	82.5	17.0	87.3	100.0	0.0	0.0	0.0	0.0	0.0
29 Apr 2014	Terminate	-	-	2084	-	-	-	-	-	-	36.7	58.3	3.3	1.7	0.0	0.0

4.3 Results

Following the initial freshwater swim challenge of the entire population in July 2013, four marine challenges were achieved over 8 months, with two (September 2013 and March 2014) at low AGD and two (October and November 2013) at moderate to high AGD (Table 4.1). In order to preserve fish numbers, not all available fish were handled at each marine measure. These were randomly chosen from the main population, so individual fish may not have attended every swim handling. All available individuals were gill scored and measured at AGD assessments (Table 4.2).

Table 4.2: Summary statistics for % swim time for freshwater (July 2013) and marine challenges (September, October and November 2013; March 2014) and weight (June, October and December 2013; March 2014) and gill score at first, second and third infection (AGD1, 2 and 3) of the swim cohort and first and second infection (AGD1 and AGD2) of the SBP marine test cohort.

Trait	Description	N	Mean	SD	CV	Min	Max
Swim1	% Time to fatigue Freshwater, July 2013 (0-100%)	2039	52.91	27.40	52%	0.28	100
Swim2	% Time to fatigue Marine, September 2013 (0-100%)	1214	70.84	19.97	28%	3.80	100
Swim3	% Time to fatigue Marine, October 2013 (0-100%)	975	88.07	13.11	15%	25.27	100
Swim4	% Time to fatigue Marine, November 2013 (0-100%)	957	71.76	20.00	28%	2.35	100
Swim5	% Time to fatigue Marine, March 2014 (0-100%)	818	74.81	24.93	33%	4.90	100
AGD1	Gill score (first infection) October 2013	1812	1.93	1.04	54%	0	5
AGD2	Gill score (second infection) December 2013	1436	3.14	1.29	41%	0	5
AGD3	Gill score (third infection) February 2014	1071	1.28	1.12	87%	0	5
AGD1 (SBP)	Gill score (first infection) SBP cohort September 2013	2714	1.44	0.92	64%	0	4
AGD2 (SBP)	Gill score (second infection) SBP cohort December 2013	2441	3.14	1.51	48%	0	5
Weight1	Weight (g) freshwater tagging	2039	185	57	31%	60	455
Weight2	Weight (g) October 2013	1812	572	154	27%	67	1167
Weight3	Weight (g) December 2013	1384	969	219	23%	320	1845
Weight4	Weight (g) March 2014	814	1638	345	21%	687	2728
CF2	Condition factor (Weight/length3) October 2013	1811	1.11	0.13	12%	0.43	1.63
CF3	Condition factor (Weight/length3) December 2013	1384	1.19	0.11	9%	0.85	1.79
CF4	Condition factor (Weight/length3) March 2015	805	1.21	0.12	10%	0.74	1.63

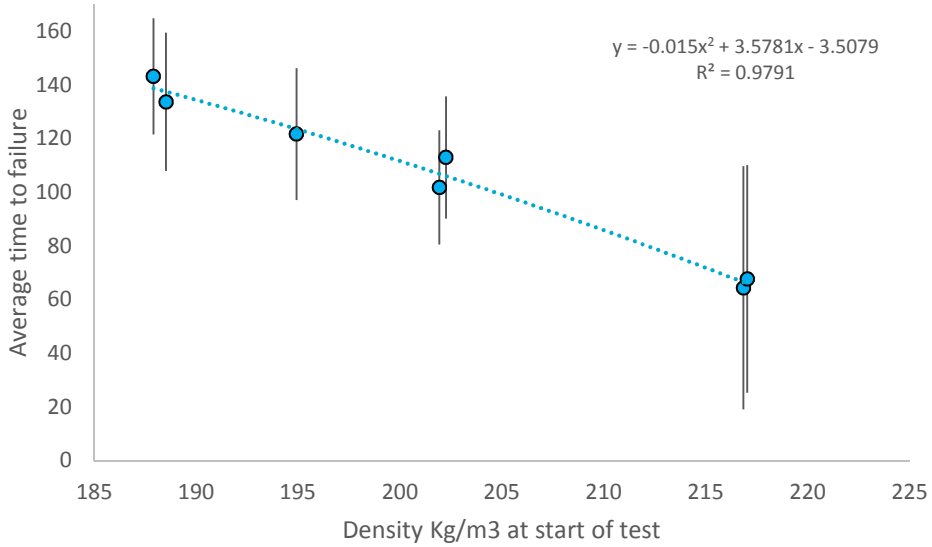

Figure 4.2: Variation in average time to fatigue across marine swim challenges compared to start stocking density in March 2014 (error bars = SD).

%Swim time was variable and a high proportion of fish fatigued, indicating that the flow and crowding technique was able to discriminate differences within the population. At freshwater swim (July 2013) 92.4% of challenged fish fatigued (range 90.5 to 93.5%), while fatigue rates in the marine challenges were 86.8% at September 2013 (85.3 to 89.3%), 87.7% at October 2013 (84.4% to 88.5%), 77.9% at November 2013 (74.4% to 79.7%) and 82.5% in March 2014 (68.5% to 77.9%). Although environmental parameters (water temperature, oxygen control and outboard generated flow) were similar between challenges in a particular month they were undoubtedly responsible for some of the differences in time to fatigue. However, the main driver appears to be stocking density, which had the widest variation in the March 2014 challenges (Fig. 4.2).

%Swim time at all five challenges was of low to moderate heritability (Table 4.3), though this was marginal at the October measure (Swim 3). All gill score measures were heritable, both in the swim challenge and SBP cohorts.

Table 4.3: Heritability and variance components (± standard errors) of swim times and gross gill score at each infection measure

Trait	Additive genetic σ^2_a	Family σ^2_f	Residual σ^2_r	Heritability h^2
Swim1	141.93 (33.01)	0.00 (0.00)	607.38 (28.96)	0.19 (0.04)
Swim2	53.24 (26.23)	9.70 (10.9)	338.14 (20.72)	0.13 (0.06)
Swim3	22.79 (12.66)	2.43 (5.29)	143.71 (9.99)	0.13 (0.07)
Swim4	47.12 (18.12)	0.00 (0.00)	286.56 (19.09)	0.14 (0.05)
Swim5	109.37 (32.36)	0.00 (0.00)	313.34 (27.73)	0.26 (0.07)
AGD1	0.30 (0.08)	0.03 (0.02)	0.74 (0.05)	0.28 (0.07)
AGD2	0.43 (0.14)	0.02 (0.05)	1.22 (0.09)	0.26 (0.08)
AGD3	0.24 (0.07)	0.00 (0.00)	1.03 (0.07)	0.19 (0.05)
AGD1 (SBP)	0.13 (0.04)	0.01 (0.01)	0.71 (0.03)	0.15 (0.04)
AGD2 (SBP)	1.12 (0.19)	0.00 (0.05)	1.20 (0.11)	0.48 (0.07)

Swim challenge results (handling resilience) in freshwater had high genetic correlation to marine challenge results where AGD infection (gill score) was light to moderate (r_g 0.63 to 0.86, r_p 0.15 to 0.28 Table 4.4). There was no significant genetic correlation between freshwater swim and the November marine swim, when AGD infection had increased rapidly over one week since the bath on 24th October, though there was a low r_p of 0.10. The relationship between all marine swim challenges was consistent despite the differing levels of AGD at each swim.

Table 4.4: Correlations (± standard errors) of swim times. Genetic correlation below diagonal, phenotypic correlation above diagonal. *Values in bold are significantly different from zero (p < 0.05).*

Trait	Swim1	Swim2	Swim3	Swim4	Swim5
Swim1	-	**0.28 (0.03**)	**0.22 (0.03)**	**0.10 (0.03)**	**0.15 (0.04)**
Swim2	**0.77 (0.14)**	-	**0.38 (0.04)**	**0.15 (0.04)**	**0.13 (0.03)**
Swim3	**0.86 (0.13)**	**0.94 (0.16)**	-	**0.23 (0.04)**	**0.30 (0.04)**
Swim4	0.03 (0.21)	**0.73 (0.29)**	**0.75 (0.34)**	-	**0.24 (0.04)**
Swim5	**0.63 (0.16)**	**0.69 (0.19)**	**0.72 (0.18)**	**0.71 (0.28)**	-

Genetic correlations between gill score at each AGD infection within the swim cohort were moderate to high. There were also strong genetic correlations between the swim cohort and the SBP cohort despite the additional handling received by the swim cohort. There was a positive (but insignificant) relationship between gill score at third infection (AGD3) in the swim cohort and the first infection (AGD) of the SBP cohort, though both of these measures had been taken at relatively low phenotypic expression 155 days apart at average gill score 1.28 and 1.44 respectively).

Table 4.5: Genetic correlations (± standard errors) of gross gill scores. Genetic correlation below diagonal, phenotypic correlation above diagonal. *Values in bold are significantly different from zero (p < 0.05).*

Trait	AGD1	AGD2	AGD3	AGD1 (SBP)	AGD2 (SBP)
AGD1	-	**0.29 (0.03)**	**0.12 (0.03)**	NA	NA
AGD2	**0.67 (0.12)**	-	**0.23 (0.03)**	NA	NA
AGD3	**0.58 (0.15)**	**0.88 (0.13)**	-	NA	NA
AGD1 (SBP)	**0.83 (0.11)**	**0.47 (0.15)**	0.29 (0.18)	-	**0.12 (0.02)**
AGD2 (SBP)	**0.74 (0.08)**	**0.97 (0.07)**	**0.71 (0.12)**	**0.44 (0.12)**	-

Genetic correlations between the swim challenge test and AGD scores were generally non-significant (Table 4.6). However the September swim result (Swim2) shows a positive genetic correlation with gill score measures at first and second infection in the swim cohort (r_g = 0.58 and 0.47), this is not likely to be an effect of swim handling because the same relationship exists in the unchallenged SBP cohort (r_g = 0.47 and 0.41 respectively). There were no significant genetic relationships between Swim 4 (performed when AGD was rapidly advancing in November 2013) and gill scores, though relationships do appear to be negative. At a phenotypic level (data not shown) Swim 4 showed low phenotypic relationship with AGD1 (rp = -0.11 ± 0.03) and AGD2 (0.09 ± 0.04). Despite being recently bathed, the final swim challenge in May 2014 showed a low phenotypic correlation with gill score at AGD3 (-0.15 ± 0.04, data not shown).

Table 4.6: Genetic correlations (± standard errors) of swim times against gross gill scores. *Values in bold are significantly different from zero (p < 0.05).*

Trait	AGD1	AGD2	AGD3	AGD1 (SBP)	AGD2 (SBP)
Swim1	-0.04 (0.16)	-0.12 (0.17)	-0.08 (0.18)	0.05 (0.16)	-0.01 (0.13)
Swim2	**0.58 (0.19)**	**0.47 (0.20)**	0.22 (0.23)	**0.47 (0.21)**	**0.41 (0.17)**
Swim3	0.34 (0.24)	0.01 (0.26)	-0.13 (0.28)	0.40 (0.22)	0.07 (0.20)
Swim4	0.16 (0.24)	-0.31 (0.26)	-0.50 (0.27)	0.21 (0.25)	-0.31 (0.22)
Swim5	0.10 (0.17)	-0.18 (0.19)	-0.32 (0.19)	-0.02 (0.19)	0.06 (0.16)

Relationships between swim time and fish size parameters (weight and CF) were generally non-significant. However, there were positive genetic correlations between the freshwater swim (Swim1) and subsequent weight measures (r_g = 0.43, 034 and 037 against Weight 2, 3 and 4 respectively, Table 4.7) and a positive but marginal genetic correlation with December condition factor (0.30). Conversely, there were strong negative genetic correlations between the November (Swim4) results and all weight measures (r_g = -0.49, -0.69, -0.78 and -0.62). Swim 4 was also negatively correlated with condition factor in October and December (r_g = -0.38 and -0.51 respectively).

Table 4.7: Heritability, genetic and phenotypic correlations (± standard errors) of weight and CF measures against swim challenges. Genetic correlations (r_g) are shown with grey background, phenotypic correlations (r_p) in italic. *Values in bold are significantly different from zero ($p < 0.05$).*

		Weight1	Weight2	Weight3	Weight4	CF2	CF3	CF4
	h^2	**0.72 (0.07)**	**0.34 (0.08)**	**0.49 (0.09)**	**0.41 (0.12)**	**0.24 (0.05)**	**0.57 (0.07)**	**0.26 (0.11)**
Swim1	r_g	0.20 (0.13)	**0.43 (0.14)**	**0.34 (0.14)**	**0.37 (0.16)**	0.28 (0.15)	**0.30 (0.14)**	0.35 (0.20)
	r_p	***0.19 (0.03)***	***0.16 (0.03)***	***0.17 (0.03)***	***0.19 (0.04)***	*0.04 (0.03)*	***0.08 (0.03)***	*0.07 (0.04)*
Swim2	r_g	0.23 (0.17)	0.30 (0.21)	0.15 (0.20)	0.26 (0.24)	0.10 (0.21)	0.12 (0.19)	0.27 (0.24)
	r_p	***0.09 (0.04)***	***0.17 (0.03)***	*0.06 (0.04)*	*0.09 (0.05)*	***0.12 (0.03)***	*0.02 (0.04)*	*0.03 (0.05)*
Swim3	r_g	0.07 (0.21)	0.07 (0.24)	-0.16 (0.22)	-0.06 (0.25)	0.11 (0.25)	0.00 (0.21)	0.21 (0.30)
	r_p	*0.06 (0.04)*	***0.08 (0.04)***	*-0.03 (0.04)*	*0.03 (0.05)*	*0.04 (0.04)*	*-0.01 (0.04)*	*0.02 (0.05)*
Swim4	r_g	**-0.49 (0.18)**	**-0.69 (0.15)**	**-0.78 (0.13)**	**-0.62 (0.17)**	**-0.38 (0.19)**	**-0.51 (0.17)**	-0.29 (0.22)
	r_p	***-0.13 (0.04)***	***-0.23 (0.04)***	***-0.33 (0.03)***	***-0.30 (0.04)***	***-0.15 (0.04)***	***-0.24 (0.03)***	***-0.16 (0.05)***
Swim5	r_g	-0.05 (0.15)	0.04 (0.18)	-0.10 (0.18)	-0.06 (0.20)	0.02 (0.19)	-0.15 (0.16)	0.15 (0.22)
	r_p	*-0.06 (0.04)*	***-0.10 (0.04)***	***-0.13 (0.04)***	*-0.07 (0.04)*	***-0.13 (0.04)***	***-0.14 (0.04)***	*-0.06 (0.04)*

4.4 Discussion

The resilience tests using a refined swim challenge model have demonstrated the value of the development of a sound challenge model over the previous two years. All traits assessed were found to be under genetic control and therefore have the potential to respond to selection. With further validation trials and in consideration of existing priority traits in the breeding goal (Elliott & Kube, 2009; Kube et al., 2012), the crowded swim challenge provides a potential selection trait to enable Saltas and the Tasmanian Atlantic salmon industry to consider the opportunity to breed for reduced losses during AGD bath crowding events.

For a selection trait to be useful in a breeding program, it should adequately reflect the objective trait. In this case we are presuming that fatigue in a swim challenge with continuous strong water flow at high stocking density is indicative of response to handling stress in a commercial AGD bath handling transaction. The swim challenge is carried out to a point of fatigue from which most fish will recover with adequate oxygenation. During commercial bathing fish are crowded tightly and may be subjected to strong water current (aeration upwelling and fish pump induced), the process of crowding and subsequent sudden exposure to freshwater may cause some fish to die. When high gill score (score 5) fish are in the population, these are invariably over-represented in bath crowding mortalities. However, even at low commercial bathing threshold with few high gill score fish, significant mortality can occur. Once transferred from the crowd, bath variables such as relative oxygen saturation, carbon dioxide build up, temperature, water current and liner pockets which may trap recovering fish and inhibit their recovery undoubtedly influence commercial survival results. Variation in the bathing environment was not modelled

as part of this project. Although the link between the swim challenge fatigue and commercial bath mortality could not be realistically tested within the constraints of this project, it is assumed that resilience to crowding stress is the driving factor in both scenarios.

It is preferable for selection traits to be simple, non-destructive and cost effective to measure. In the case of a freshwater swim challenge it is evident that an entire freshwater cohort can be readily challenged over two to three days with minimal mortality. Handling impacts upon growth were not assessed but are likely to be minimal (although it is not possible to directly compare due to uncontrolled 'cage effect', the swim trial cohort at sea closely mirrored the SBP marine test cohort throughout the marine phase). In our previous study (Section 3), the freshwater swim test was compromised by power failures and was not significantly heritable (due to high test environment variation), but there was a significant genetic correlation (0.64) with a subsequent marine swim challenge of 483g AGD affected fish (mean gill score index 1.75) in November. The results presented in this section indicate that the freshwater swim challenge was heritable ($h^2 = 0.19$) and that there is a moderate genetic correlation between freshwater swim challenge and subsequent marine challenges ($r_g = 0.63$ to 0.86), as long as AGD gill score is at low to moderate levels. At the November swim challenge, gill score was recorded at moderate/high from a subsample on day 1, yet it had progressed to a very high score (18% score 5) within a week (Table 4.1). Similar rapid and unexpected AGD development was also noted in nearby commercial populations, the SBP marine test cohort and in research cages (Maynard et al., 2014). Therefore, apart from cases of extreme AGD development it appears from this single year class set of data that freshwater swim challenge may reflect performance under normal commercial marine handling events. Breeding values obtained from a freshwater challenge swim could therefore be applied to reduce susceptibility to handling in future generations. The opportunity of applying swim challenge directly to potential broodstock in freshwater is that 'within family' selection could be applied to find the best animals within the best families.

The primary components of the SBP breeding objective are improved growth and increased AGD bathing interval (Elliott & Kube, 2009; Kube et al., 2012).The question for the Saltas SBP is whether the addition of AGD handling resilience as a selection trait is worthwhile, given the potential trade-offs with existing commercial traits within the breeding goal?. The selection trait for growth is fish weight at key measures, there were significant positive genetic correlations between swim time in freshwater and fish weight indicating that selection using freshwater swim challenge is unlikely to have a negative impact upon growth selection. Although condition factor was not recorded in freshwater, it invariably shares a moderate to high

genetic correlation with weight (0.62 in October, 0.66 in December and 0.68 in March, data not shown). There was a positive (0.30) but marginal genetic correlation between the freshwater swim and December condition factor and positive (though not significant) relationships with condition factor at the other measures. However, in November (Swim 4) the situation is reversed, with moderate to high negative genetic correlations with condition factor (-0.51 at the December measure) and weight (-0.49 to -0.78 across all measures) indicating that larger and higher conditioned fish fared less well in the test. In this instance, swim challenge selection would negatively impact weight and condition factor. This agrees with our observations from Section 2, where a swim challenge of large fish in May 2012 was negatively correlated with condition factor (r_g = -0.58), though the weight relationship was only significant at the phenotypic level (r_p = -0.15).However, unlike the May 2012 challenge, fish were unbathed at Swim 4 and were experiencing a rapid increase in AGD. There were no significant genetic correlations between gill score and condition factor, though there were low phenotypic correlations between AGD2 and condition factor in October, December and March (-0.11, -0.13 and -0.11 respectively, data not shown). Similarly, there were no genetic correlations of gill score with weight, but phenotypic correlations were low and negative between gill score at AGD1 and weight in freshwater, October, December and March (-0.07, -0.22, -0.21 and -0.10, data not shown) and between gill score at AGD2 and weight in October, December and March (-0.09, -0.15 and -0.21, data not shown). At AGD3 phenotypic correlations of gill score were low and positive with weight (0.12, 0.14, 0.10 in freshwater, October and December respectively). From these results it appears that selection for AGD handling resilience would not usually impact genetic progress in weight or condition factor, though the interplay of fish size and swim time needs to be further examined during high AGD expression. For future work, it may be necessary to separate fish into size groups to more accurately examine size effects on handling tolerance during high AGD expression. The practical application of these observations is that the possible influence of fish size and shape should be taken into account when calculating genetic parameters of handling tolerance, requiring weight and condition measurements close to conducting group swim challenges, the tendency is for larger fish to swim longer and for higher condition (plumper) fish to fatigue more quickly, though these observations can become confused due to the high correlation between fish size and condition.

The selection criterion for AGD bathing interval is gill score (Elliott & Kube, 2009; Taylor et al., 2009a; Kube et al., 2012). Freshwater swim challenge results were not genetically correlated against gill score on any of the measures in either cohort. However, the September swim (which was conducted at a low gill score expression close to commercial bathing threshold) showed low positive genetic correlations with two rounds of subsequent gill score (AGD1 and AGD2). This could suggest that

fish that had lasted longer in the swim became more susceptible to AGD, however the relationship holds true against the SBP cohort which was not swim challenged. Despite a low AGD expression at the time of challenge, this swim challenge is the only one to have shown a genetic correlation with gill score over the three years of our study but was not phenotypically related to gill score at any measure (data not shown). In this case, selection for longer time to fatigue would decrease gains for AGD resistance. Despite the September swim result, the overall findings suggest that breeding for improved resilience will not reduce selection for AGD resistance. During high AGD expression (Swim 4) there was a low phenotypic impact of higher gill score fish tending to fatigue earlier (r_p = -0.11 and -0.09 at AGD1 and AGD2 respectively). There was also a negative phenotypic effect of gill score at AGD3 against Swim 5 (-0.15), which had been recently bathed. Therefore, although AGD resistance and handling tolerance appear to be independent traits, there can be phenotypic effects of advanced gill score upon handling which may still express up to 10 days post bath. Despite this relatively short-lived effect, the opportunity for the SBP is to concurrently select for AGD resistance and AGD handling resilience.

Despite the potential to improve handling resilience through breeding, our data also indicates that improved husbandry will also affect the outcome of bath handling events. When there is a high proportion of gill score 4 – 5 fish in a population losses are likely to be higher. Forward planning of handling protocols related to gill score will help to minimise losses when a threshold of high gill score fish is expected. In addition, reducing crowd density and crowd time is likely to minimise loss at handling.

5 Overall Discussion

The progress of this project is summarised against the three main objectives set out in Section 1.3, as follows:

Determine the genetic variation for AGD handling resilience, and the opportunity for genetic improvement

A significant part of this project has been in the development of a swim-tank to provide a consistent strong water flow to reliably challenge a large number of tagged and genotyped animals by swimming them and collecting fatigued individuals for tag registration. Over three years this project has developed a repeatable challenge model that consistently demonstrates genetic variation of crowded handling tolerance of AGD naive fish in freshwater and subsequently of AGD affected fish in marine conditions. This challenge was designed to mimic the high density environment experienced by fish during commercial bath crowding events, but due to practical and animal welfare limitations we did not attempt to take fatigued animals through subsequent freshwater bathing to intentionally cause handling mortality. Therefore it is not possible to gauge how closely the high density swim challenge will relate to commercial bath loss. Acute blood acidosis is a key feature of AGD affected fish in response to exercise or confinement (Powell & Nowak, 2003; Leef et al., 2005a; Powell et al., 2008), therefore the ability of individual fish to recover from crowding stress may be limited in the highly stocked freshwater bath environment. Commercial bath losses may be seen as fish failing during bath transfer or as moribund or dead fish when the fish are released from the liner after two or more hours in fresh water.

Experimental swimming capacity tests are routinely used to quantify individual fish fitness (Beamish, 1978), the swimming ability of fish is linked to fish size, therefore tests normally compare fish of similar size and shape (Hammer, 1995). When fish are of similar size, group swim tests provide similar estimates of swimming ability and minimises variation (Deslauriers & Kieffer, 2011). A number of authors have developed swim challenges that can be applied to large groups of fish (Castro et al., 2013; Anttila et al., 2014; Veiseth et al., 2006). However, these tests are performed at lower stocking density than commercial fish handling. The stress responses of fish may vary with crowding intensity, time and frequency (Wedemeyer, 1976; Carey & McCormick, 1998; Basrur et al., 2010; Gatica et al., 2010b). Oppedal et al. (2007; 2011) showed that salmon in sea cages may congregate into very tight schools with a local density above 180 kg/m^3 in order to avoid high temperatures but there are no measurements of actual densities achieved in commercial crowding beyond

behavioural welfare guidelines (RSPCA, 2012). Gatica et al. (2010a) modelled commercial well-boat transport at >107 to 244 kg/m^3. Veiseth et al. (2006) crowded fish at >200 kg/m^3. Therefore, we have developed a high density crowd challenge which utilises high stocking density and ramped water velocity as a tool to measure genetic variation of handling resilience.

Our initial study (Section 2) of large (2330 g) fish, which had been through four rounds of AGD and were recently bathed, indicated that the response to high density swim challenge is heritable (0.19) and unrelated to previous AGD history. However there was a high negative genetic correlation (-0.58) with condition factor and phenotypic correlation of fish weight. This indicates that larger/higher conditioned fish fared less well in the challenge. This initial result suggested that genetic selection for handling resilience would not negatively impact AGD resistance but may tend to reduce condition factor. In the second year (Section 3), fish averaging 483 g were swum while AGD affected (first infection, mean gill score 1.75, h^2 = 0.16), this result was not genetically correlated with gill score but was positively correlated with freshwater tagging weight (r_g = 0.55), therefore selection for resilience would not negatively impact AGD resilience and would support weight selection. In the third year (Section 4), marine challenges were carried out across four different AGD expressions and resilience was found to be heritable at each measure (h^2 = 0.13 to 0.26, Table 4.3). These marine measures were well correlated (r_g = 0.69 to 0.94, Table 4.4) and independent of gill score, though Swim 2 (carried out at low AGD expression a month prior to the first AGD measure) was positively correlated with gill score in the swim cohort and reference SBP cohort. These measures were mostly independent of fish size (weight and condition factor) though the November challenge, carried out during a period of rapid AGD development, was negatively correlated with fish weight and condition factor (Table 4.7). Together these three studies confirm that AGD handling resilience is under consistent genetic control across a range of test environments (AGD expression, fish size, water temperature) and has the potential to respond to selection. The trait is largely independent of AGD resistance so unlikely to affect progress to reduce bath frequency, so selection for both traits would reduce the need to bath and provide fish more able to withstand bath handling. However, in one instance (Swim 2, Section 4) there were moderate genetic correlations with gill score, so selection based on this result would reduce progress in improving AGD resistance. Size effects on crowded swim challenge were inconsistent but can be highly correlated; therefore there is a risk that resilience selection may impact progress in important growth or harvest yield traits. Aspects of experimental design, such as minimising size variation in test fish or fitting size effects to the model, need to be further examined to ensure that the swim test is a reliable selection trait for assessment of handling resilience.

Establish the level of genetic variation for cardiovascular traits and the association with AGD handling resilience

Changes in cardiac structure and cardiac failure may follow periods of elevated blood pressure or hypertension (Poppe & Taksdal, 2000; Gamperl & Farrell, 2004; Takle et al., 2006). According to the review by Powell et al. (2008), AGD infection results in increased systemic vascular resistance leading to a compensatory tissue remodelling and change in cardiac shape can occur in chronically infected fishes (Powell et al., 2002a). The combined effect of reduced available respiratory gill surface area and cardiovascular compromise leads to increased routine metabolic rate and a significant reduction in swimming performance. Although cardiorespiratory effects of AGD may not lead to death at low to moderate AGD expression, they are likely to make fish more susceptible to handling stress. Fish that succumb during commercial crowding are typically large animals in good condition, reflecting that their cardiac capacity has been sufficient when unexposed to physical challenge (Poppe et al., 2003). There is a strong correlation between cardiac morphology and function in vertebrates; therefore it is reasonable to assume that optimal heart shape will enable AGD salmon to better withstand the physiological demands of acute handling and AGD. Farmed fish may develop suboptimal heart shape or increased levels of cardiac deformity (Mercier et al., 2000; Dunmall & Schreer, 2003; Poppe et al., 2003; Claireaux et al., 2005; Jonsson & Jonsson, 2006; Olesen et al., 2009) due to inadvertent genetic selection or environmental stressors which can lead to fish kills in routine commercial handling conditions (Brocklebank & Raverty, 2002). A key objective of this project was therefore to examine genetic parameters of heart shape and cardiac deformities in the Tasmanian Atlantic salmon population and their possible relationship to handling resilience (as measured by response to a swim challenge and AGD history).

Measurement of cardiac traits was confined to the initial stage (Section 2) of this project. A central concept of this work was that linking of key cardiac traits with AGD handling resilience could lead to testing of non-destructive Doppler heart imaging (Claireaux et al., 2005) of potential broodstock to inform within-family breeding decisions. During this study water flow was driven by two large water pumps and crowding was not controlled as fish sequentially failed, thus stocking density reduced as each challenge progressed. This is not representative of commercial crowding, where the net is regularly tightened to encourage fish to enter the transfer pump. In these conditions, our study of heart shape against handling resilience failed to show strong correlations, though heart shape and marine swim challenge (one week post bath) were all heritable traits. Heart shape within the measured SBP cohort was generally within healthy (wild) salmon ranges as reported by Poppe et al. (2003) and compared favourably against parameters for larger (4.5 kg) Norwegian Atlantic salmon (Olesen et al., 2009; Shehzad, 2009), though comparison may be biased by

the smaller size of our studied fish. There were no significant correlations between heart shape and previous AGD history, though our fish had been relatively AGD free for up to four months; this suggests that cardiac remodelling resulting from AGD (Powell et al., 2002a) is not persistent.

Cardiorespiratory robustness tested by a swim challenge of freshwater parr is routinely measured as part of a commercial breeding program in Norway (AquaGen, 2006). Recent work in support of this program (Anttila et al., 2014) subsampled 'winners' and losers' from a group challenge and reported phenotypic association of thicker compact layer of the ventricle and longer gill filaments with improved swimming performance. These parameters were not measured in our study, but may warrant future re-examination at a genetic level utilising improved swimming protocols.

Examine cost-effective and non-destructive resilience selection traits that can be applied to freshwater broodstock.

Following initial evidence that cardiac traits could not be readily exploited by the breeding program, the focus was to compare swim challenge results in freshwater against subsequent marine swim challenges of fish that had been subjected to AGD. Having changed the swim tank design to a 48 V outboard generated flow (Fig. 3.2), this question was examined in Sections 3. This first study experienced issues in challenging freshwater fish consistently due to poor 240 V power supply in the hatchery. The freshwater challenge was not heritable when expressed as a continuous trait (% swim time) but was assessed as heritable when expressed as a binary trait ($h^2 = 0.15$). Despite the phenotypic impact of higher gill score fish at the marine challenge ($r_{p`} = -0.20$), marine swim challenge carried out at first AGD infection was highly correlated ($r_g = 0.64$), indicating that selection based upon freshwater swim would lead to improved marine handling resilience. However, due to power issues during the freshwater challenge and following the unexpected post-anaesthesia loss of exercised fish at sea, it was necessary to repeat this work (Section 4). This study gave a clearer measure of freshwater performance ($h^2 = 0.19$) which was well correlated with marine handling resilience across a range of AGD expression and fish age (Table 4.4), though there was no genetic correlation against the November swim which was carried out at advanced AGD level and experienced strong negative effect of fish condition factor. At normal commercial AGD levels (low to moderate gill score) it appears that genetic selection from freshwater swim challenge may positively affect marine handling performance. Although our results will require further testing, the opportunity for the SBP is that freshwater swim testing could be applied to young fish in fresh water with minimal mortality. Annual testing of one year old potential broodstock may support within-family selection for handling resilience when these animals mature. At higher AGD levels the phenotypic

effect of high gill score is likely to cause higher bath handling losses. The farmer can counteract this by bathing at proactive gill score thresholds and by minimising crowding intensity and duration (RSPCA, 2012). The best approach to improving fish welfare and reducing losses is likely to be a combination of breeding and husbandry approaches.

6 Benefits and Adoption

This project has developed a high density swim challenge protocol that can be applied to large numbers of salmon in fresh or saltwater across a range of fish sizes and AGD status. High density swim challenge has the potential to further our understanding of phenotypic and genetic responses of salmon to commercial handling stress but requires further work to validate the challenge against commercial handling losses.

6.1 Recommendations on potential for commercial application of AGD handling resilience

The long-term decision to add resilience selection to the overall breeding objective needs to be considered by its likely impact on key traits and the overall economic impact to the combined breeding objective. Genetic selection for AGD resistance is delivering significant progress in reducing the required freshwater bathing frequency for the Tasmanian Atlantic salmon industry but the need for regular treatment will continue. Although the lowered frequency of handling events is likely to directly reduce the number of handling losses, the continued need for crowding and bathing of AGD affected fish remains a concern for marine farmers. Further reduction in the number of handling events is likely to be driven by genetic selection for improved growth which delivers fish to required harvest size in fewer days at sea, though there is also potential that fish could be grown to a larger weight in the same time (15 to 18 months at sea) thus increasing handling risks. The relationship between the proposed selection trait (response to high density swim challenge) and the objective trait (reduced losses following crowding and bathing of AGD affected fish) is assumed in this study but could not be directly tested in commercial crowding and bath conditions. This study has demonstrated that response to high density swim challenge is a robust trait which is heritable in freshwater and marine conditions across a broad range of fish sizes and AGD expression and that this trait does not generally show adverse correlations with AGD resistance (measured by gill score). Therefore it is feasible that, with further validation, selection for AGD handling resilience could complement AGD resistance. However response to high density swim challenge is not independent of weight or condition factor, suggesting that selection based upon the high density swim challenge will limit genetic progress in weight selection. Further work is required to establish whether size effects in the swim challenge are an artefact of the test environment or reflective of commercial handling losses.

The heritability of swim challenge is generally low, therefore it is unlikely to respond well to selection. Low heritability traits can be included in the overall selection index if their economic weighting is high, but there is no compelling evidence that AGD handling loss is a high value trait when compared with AGD resistance or growth. Currently there are no clear estimates on the value of handling loss to the industry beyond the original '5% during a production cycle' provided by personal communication with David Kiemele (Head of farming, Tassal Group Ltd.). In the absence of industry data on handling losses, it is not possible to consider the impact upon the breeding objective. For the industry to assess the value of improved handling resilience it is necessary to improve data collection and analysis to gauge the economic value of the trait. With growing consumer concern for the welfare of farmed animals and willingness to pay for improved welfare indicators (Grimsrud et al., 2013) it may be necessary to include a higher welfare value to the breeding objective, which would support genetic selection handling resilience or other welfare traits

Although further validation is required, this study has identified risk factors that can be managed by the farmer during commercial bathing. Firstly, the rate of fish becoming fatigued was reduced at lower stocking density indicating that farmers can control losses by crowd management, requiring an understanding of the relative impact of stocking density and crowding time. Secondly, fish with higher gill score are more likely to succumb to handling, requiring proactive bathing at lower AGD thresholds or modification of handling when populations are known to be above threshold. Thirdly, larger and heavier fish appear to be more likely to succumb to prolonged crowding. Genetic selection for improved growth is likely to increase this risk and may bias cardiorespiratory traits in future. Knowledge of population average weight and size variation will allow farmers to optimise handling protocols to manage these risks.

7 Further Development

Despite ongoing genetic progress in reducing the frequency of freshwater bathing, the need to regularly handle and treat fish will continue. It is recommended that in the near-term AGD handling losses will be best minimised through improved husbandry practices. Therefore research efforts are best spent in supporting improved husbandry by understanding physiological responses of AGD affected fish during bath handling (including further swim challenge experiments and the use of in-situ biosensors). This process could be enhanced through collection of industry data of fish health, time and biomass transferred in each crowd and the bathing environment (density, water quality, temperature and dissolved gases) to understand the key drivers of handling loss.

The high density swim challenge protocol developed as part of this project can be a useful tool in testing responses of fish to bath crowding parameters but further work is required to understand the relationship of the test environment to commercial losses. In particular, it is known that fish size effects can be significant in swim flume tests (Brett, 1964, 1967; Plaut, 2001). Despite efforts to provide a uniform test environment during combined tests it is difficult to prevent fish from exploiting preferred sections of the swim flume, though this may reflect behaviours that are exhibited during commercial crowding. The opportunity remains to test physiological parameters of individual ‘winners’ and ‘losers’ from the swim challenge (including use of biosensors) to inform improved husbandry approaches.

Commercial data gathering is required to accurately quantify the economic impact of AGD handling loss. This information will assist the industry in quantifying the value of investment in improved equipment and protocols and will enable the breeding program to accurately assess the likely impact of AGD resilience within the overall breeding objective,

8 Outcomes

We have developed a high density swim challenge protocol that can be applied in freshwater or marine conditions across a range of fish sizes and AGD expression. With further validation this challenge could support further research to minimise AGD handling losses.

This study provides genetic parameters of cardiorespiratory traits from the 2010 yearclass of the SBP. This data set is a reference standard for future studies of the Tasmanian salmon population.

We recommend that the main opportunity to reduce AGD handling loss is through improved husbandry (bathing at lower AGD threshold and improving animal handling protocols and equipment).

9 Conclusion

This study developed a high density swim challenge as a low-cost and repeatable selection trait to examine genetic parameters of AGD handling resilience. Response to swim challenge was heritable across a range of AGD status and fish sizes and was largely independent of the AGD resistance trait (gill score) indicating that selection for AGD handling resilience may not adversely impact genetic progress in reducing bathing frequency. Genetic correlation between freshwater and marine swim challenge suggests that there is potential to select directly based upon swim performance of juvenile potential broodstock. However, adverse correlations between swim challenge and fish size (weight or condition factor) indicate that selection for AGD resilience could reduce breeding progress of fish growth.

Commercial genetic progress through the Saltas SBP is reducing bathing frequency through selection for AGD resistance and is likely to decrease days to harvest through improved growth, therefore the number of bathing events required during a production cycle is likely to decline. The economic value of AGD handling to the Tasmanian salmon industry is unclear but is likely much lower than the cost of AGD and the value of improved growth. Coupled with low heritability of the swim challenge trait and the need for further validation of the test against commercial handling and bath losses, we recommend that it is not prudent to consider the addition of AGD handling resilience to the overall breeding objective at this stage.

Currently the main opportunity to reduce AGD handling loss is through improved husbandry which should be based upon analysis of factors driving commercial losses. The swim challenge protocol can be utilised to support other research efforts to understand handling stressors and their physiological effects upon fish.

This research provides a record of heart morphometry in the early phase of the SBP which should stand as a useful comparison in future. Tasmanian salmon hearts appear to have a healthy shape when compared to overseas examples from the literature. All cardiac traits examined were under genetic control but none were strongly linked to handling resilience.

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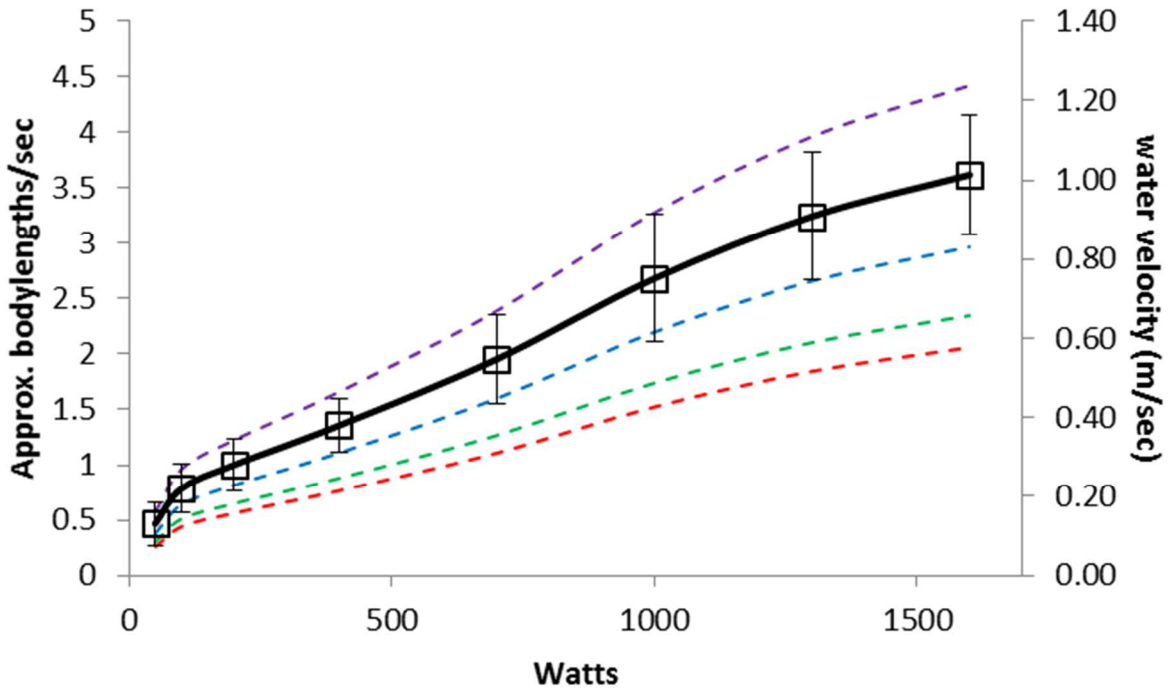
Appendix



Appendix 1: Water velocity (m/sec) related to power (wattage) setting on 48v Torqeedo outboard in swim tank. Error bars are 2 x SD. Approximate body lengths/second are shown for a range of fish sizes of 1.25 Condition Factor (purple = 150g, blue = 500g, green = 1000g, red = 1500g). Water flow was estimated in 10 second intervals using a digital mechanical flow meter with standard rotor (General Oceanics, Miami, Florida) mounted at 250 mm depth in the central radius downstream of the collection screen.

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