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Contents

Genetic variation of handling resilience of Tasmanian Atlantic salmon affected by amoebic gill disease (AGD)

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Abstract

One of the primary breeding goals of the Saltas selective breeding program is resistance to amoebic gill disease (AGD), which is the main health issue affecting production of Atlantic salmon (*Salmo salar*) in Tasmania. Fish farmers regularly assess the intensity and frequency of gross AGD signs ("gill score") in a random subsample of fish from each caged population. Fish are proactively treated at low to moderate infection levels by bathing in fresh water, with each caged population requiring up to 13 treatments in a 15 month marine production cycle. However, the process of densely crowding fish and pumping to the bath can cause up to 5% handling mortality in a transaction or cumulatively over a production cycle. Losses are higher at high gill score, but there is evidence that some high gill score fish are resilient to handling and some low gill score fish can be quite susceptible.

We have assessed genetic variation of handling resilience using a high density crowded non-destructive swim-trial on fish in the freshwater hatchery and later compared this to marine swim-trials at low and advanced levels of AGD. Our results demonstrate that handling resilience is a heritable trait at normal commercial AGD thresholds and measures are mostly repeatable between freshwater and marine conditions. During advanced AGD losses are more closely related to gill score and confirm the need for careful fish handling.

Introduction

Tasmanian Atlantic salmon aquaculture began in the mid1980's and has grown to become Australia's highest value and largest volume fishery product at $513 million (43,989 tonnes) in 20112012 (ABARES, 2013). Since the inception of the industry, amoebic gill disease (AGD) has had significant economic impact upon the marine farming phase, increasing the cost of production by 20% (Kube *et al.*, 2012). The disease is initiated by attachment of the marine ectoparasite *Neoparamoeba perurans* (Adams *et al.*, 2004; Young *et al.*, 2008), the presence of amoebae on the gill causes localised host tissue reactions including hyperplasia, hypertrophy and lamellar fusion that express grossly as raised white spots and patches. Clinical signs include lethargy, respiratory distress, and, if left untreated, death (Munday *et al.*, 1990). Fish

farmers proactively manage the disease by visually inspecting the gills of individual fish for signs of the disease (white lesions). A simple non-destructive "gill score" is used to regularly assess the intensity and frequency of gross AGD signs in a random subsample of fish from each caged population, which is expressed as a six point ordinal scale from "clear" (score 0) to "heavy" (score 5) (Taylor *et al.*, 2009b). The frequency distribution or an average "gill index" are used to assist scheduling of freshwater bathing treatments, with each pen of fish requiring up to 13 baths (Tassal Group Limited, 2009) during a marine production cycle.

While proactive freshwater bathing at low average gill score has ensured that direct losses are minimised, the process of crowding and transferring fish into the freshwater bath invariably causes some animals to die. Handling related mortality may range from a few fish to over 5% of the population in a single transaction. Cumulative mortality due to AGD handling is estimated at 5% over the course of a production cycle (D. Kiemele, pers. comm) and may be impacted by a number of factors such as environmental conditions (temperature, oxygen and algae), crowd dynamics (time and density) and the health status of the fish. Losses are generally of higher gill score fish (Kube *et al.*, 2012) though anecdotal evidence indicates that some fish are resilient to bath handling despite having a high gill score, while others of low gill score can be susceptible to handling events. Fish mortality as a result of health management transactions is both an economic and fish welfare concern that can be minimised by preventative management (Ashley, 2007) such as bathing at low gill index and improved handling procedures. A longer term possibility, next to addressing the disease issue, is to breed for more resilient animals.

The Salmon Enterprises of Tasmania (Saltas) salmon selective breeding program (SBP) commenced in 2004 (Elliott and Kube, 2009). Breeding for 'AGD resistance' is a high priority with the breeding objective being to increase the bathing interval (Kube *et al.*, 2012). Each year, a marine test population is challenged with reiterative rounds of natural AGD infection and bathing with gill score assessed as the selection trait (Kube *et al.*, 2012), thus measurement of AGD resistance is based upon gross gill pathology which may include elements of host *resistance* to *N. perurans* and host *tolerance* in the presence of the parasite. Gill score is a relevant selection trait for the industry and is closely linked to survival in untreated natural field challenge (Taylor *et al.*, 2009a). Although genetic improvement of AGD resistance is predicted to reduce the number of treatments required during a production cycle (Kube *et al.*, 2012), regular freshwater bathing is still needed and there continues to be a need to control handling losses.

The terms 'robustness' and 'resilience' both define coping styles of maintaining equilibrium despite challenges, where robustness is the ability to resist change and resilience is the ability to react to change. In many cases these terms may be used interchangeably, especially when the underlying coping mechanisms are unknown. For example, Knap (2005) defined robustness as 'the ability to combine a high production potential with resilience to stressors". In the case of AGD resilience, we are considering the ability of AGD affected fish to cope with the stress of handling (crowding, pumping, acute environmental change). Crowding stress manifests as lethargy, fish stuck against the net and loss of equilibrium (RSPCA, 2012) leading to mortality in the crowd or subsequently in the freshwater bath. For the SBP to include handling resilience in the breeding goal it is necessary to develop a simple, non-destructive selection trait and es-

timate genetic parameters. The selection trait chosen for this study is a high density crowding test performed in a circulating raceway with strong water current, whereby exhausted fish fall against a collection screen. The benefit of including handling resilience in the overall breeding goal can then be assessed in relation to the effect upon existing breeding objectives and the likely benefit in improved survival and animal welfare. In this paper we describe a high density swim test applied in fresh water and subsequently at sea over a range of fish sizes and AGD infection levels. The aim of this work was to assess whether the fresh water test is related to subsequent marine test, in which case it could be applied directly to potential brood stock at a young age.

Materials and Methods

Preparation of fish

In May 2012, 198 full-sib families were produced by a 2 x 2 factorial mating of 98 sires and 98 dams at the Saltas Wayatinah hatchery and the eggs held in individual family trays. At the eyed egg stage, 250 eggs per family were combined to a common environment for hatching, freshwater nursery and ongrowing. In early June 2013, the fish were weighed (mean = 185.1 g, SD = 57.2 g), passive integrated transponder (PIT) tagged and fin clipped for parentage assignment and randomly split to three groups for (i) freshwater ongrowing as potential broodstock (ii) SBP marine challenge cohort and (iii) a swim-trial cohort which was subjected to freshwater swim test (July 2013) prior to marine input and further swim tests at sea.

Freshwater swim trial

High density swim trials were performed in a 3.6 m x 1.4 m 'D' ended polythene tank (Fig. 1) filled with water to 0.7 m depth (total water volume 3.2 m^3). The tank was divided by a central wall into two straight sections. An 18 v Torqeedo Cruise 4R outboard motor (Starnberg, Germany) was placed in one straight section to provide continuous water flow. Both ends of the tank consisted of three semicircular walls which were evenly spaced to promote consistent water flows. The fish holding test section was 2.2 m in length and 0.7m diameter (1m^3 test volume). Laminar flow was encouraged by a stainless steel screen consisting of 25 x 25 mm stacked cells (100mm horizontal length) which could be moved to alter crowd density. An inclined netting barrier was fitted at the downstream end of the test section to collected failed fish. A wattage throttle control allowed outboard water flow to be set in conjunction with a digital mechanical flowmeter (General Oceanics, USA model 2030R) suspended at 0.3 m in the central radius behind the collection screen. Oxygen levels were controlled using an Oxyguard Atlantic stationary monitor and a ceramic gas diffuser.

Fish were swum in three tests on 2nd and 3rd July 2013 (Table 1). For each test, a batch of ~720 fish (range 692 – 736) was transferred to the swim tank and allowed to equalize to the test environment at low water flow (approximately 0.8 bodylengths per second [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 10.2°C, SD 0.4°C). The start flow was set at 400 W (~1.5 bl/s) and increased by 300 W every 45 minutes, with a final 1300 W phase (~3.5 bl/s) lasting 30 minutes. Fish that failed the test became trapped against the collection screen, to ensure a consistent measure of exhaustion these individuals 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. Failed fish were PIT tag scanned to obtain individual identity and timestamp, then returned to an oxygenated recovery tank. To account for the reduction in crowd density as fish were removed from the system, the steel screen was moved down by 10% of the test section length for every 10% reduction in fish numbers. The crowding screen was not moved beyond the 30% remaining mark to prevent fish being pushed directly onto the collection screen. At the end of each 165 minute test, water level was lowered and surviving fish were scanned and recovered.

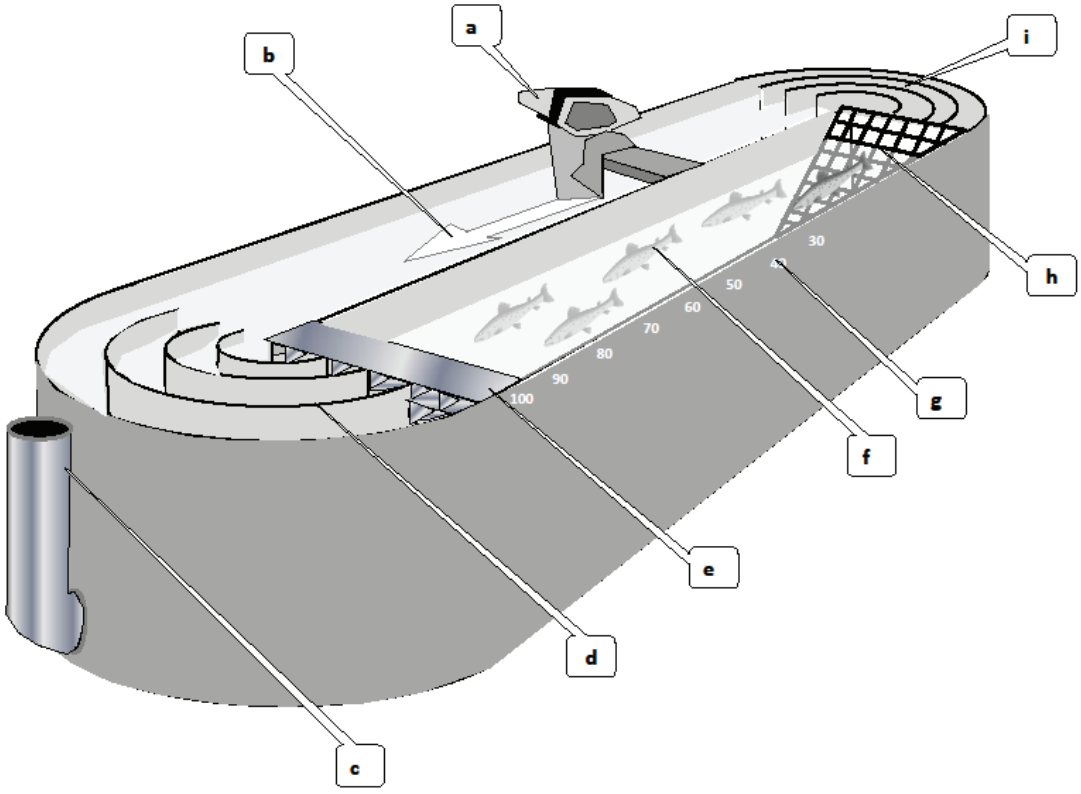

Figure 1. Schematic layout of swim tank showing (a) 48 v/8 hp outboard motor (b) water flow direction (c) standpipe to control water level (d) concentric end walls (e) stacked cell crowding screen (f) fish in test section (g) calibrated scale for crowding screen to regulate fish density (h) failed fish collection screen (i) position of flowmeter and oxygen control systems. Marine rearing and swim trials

The SBP and swim cohorts were transferred to separate 800 m^3 sea pens at Tassal Operations Ltd. (Dover) on 7th August 2013. Both pens were fed to satiation on commercial diet and were managed to achieve reiterative rounds of advanced natural AGD, freshwater bathing and reinfection. Fish were monitored fortnightly for AGD development (Table 1) by collecting a subsample and transferring to anaesthetic (17 ppm AquiS). Fish were batch weighed, with gross gill pathology inspected and scored (0 to 5) on 40 individuals. The SBP cohort was treated similarly (data not shown) with the aim of achieving advanced AGD expression as previously described (Taylor *et al.*, 2009a; Kube *et al.*, 2012).

By mid September 2013, AGD was nearing a normal commercial bathing threshold (normally targeted to 30% of the population at gill score 2 – 5) ready for the first marine swim trial. Our previous (unpublished) experience of the high density swim test at sea is that significant losses can occur. Therefore the aim was to test approximately 1000 fish at each measure to achieve adequate numbers per half-sib family whilst preserving the population numbers (Table 1). Fish were swum in three tests on 11th and 12th September (mean 435 fish per test, range 412 – 450). At each test, the swim-tank was prefilled and allowed to circulate at low velocity (50 W). A small batch of fish was crowded and counted to the test section. After 10 minutes of settling at 100 W, power was increased to 400 W (~1.2 bl/s) and then increased by 300W every 45 minutes, with the final phase at 1300 W (~3 bl/s) for 30 minutes. The crowding screen was moved to account for reduction in remaining fish numbers. Failed fish were registered without anaesthetic and returned to an oxygenated recovery net. At the end of each test the 'winners' were also scanned and returned.

On 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). The fish were left unbathed to allow them to be swum on 22nd and 23rd October in four tests (245 252 fish per test). The methodology and power settings were the same as the September swim (400 W 1300 W, approximately 1bl/s to 2.5 bl/s) with the screen moved to regulate stocking density. One day later, the entire population was freshwater bathed.

Fortnightly gill score monitoring continued through to mid-November when an advanced distribution in gill score was achieved (48% at score 2 5). Fish were swum in five tests (192200 fish per test) with power settings ranging from 600 W (~1.2 bl/s) to 1500 W (~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, weight measurement and freshwater bathing occurred on 5th December 2013. Gross gill 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 cohorts held in the vicinity at the time.

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. By late February 2014, gill score was generally low apart from some overtly mature fish. On 20th February the entire population was gill scored (no weight measures), overtly mature fish were culled and the remaining immatures were freshwater bathed. Between 3rd and 6th March

*Table 1. Summary of key events for swim trial cohort. Swim trials occurred in freshwater (July 2013) prior to marine transfer. Four sets of marine swims (Sep, Oct, Nov 2013 and Mar 2014) were carried out at different levels of AGD. [a]wt based on tagging; [b]wt based on batch average and weighted per individual between tagging and AGD1; [c]wt at AGD1; [d]wt based on batch average and weighted per individual from AGD1. *reduction in population following AGD1 measure was due to culling of 'no tags' and runts.*

Date	Event	No. Swim tests	Total #fish	Av wt (g)	Density kg/m3	Watts	Failed %	Mean T°C	Mean O_2%	Gill score (0,1,2,3,4,5)
4-12 Jun 2013	Tagging	-	2209	184	-	-	-	-	-	
2-3 Jul 2013	Swim FW	3	2161	185[a]	147-154	400-1300	92.4	7.0	84.1	
17 Jul 2013	Marine Input	-	2200	-	-	-	-	-	-	(100, 0,0,0,0)
11-12 Sep 2013	Swim	3	1305	353[b]	162-181	400-1300	86.8	12.3	92.3	(18, 62, 18, 2, 0)
16 Oct 2013	AGD1 (unbathed)	-	1898	572	-	-	-	-	-	(6, 31, 38, 18, 6, 1)
22-23 Oct 2013	Swim	4	997	591[c]	162-172	400-1400	87.7	15.4	94.1	
24 Oct 2013	Bathed	-	1645*	-	-	-	-	-	-	
26-28 Nov 2013	Swim	5	983	918[d]	195-213	600-1500	77.9	16.0	84.3	(23, 29, 23, 17, 6, 2)
5 Dec 2013	AGD2 (Bathed)	-	1485	968	-	-	-	-	-	(3, 8, 19, 29, 24, 18)
20 Feb 2014	AGD3 (bathed)	-	1093	1658	-	-	-	-	-	(20, 52, 16, 6, 3, 3)
3-6 Mar 2014	Swim	8	832	1637	187-217	700-1600	82.5	17.0	87.3	(100, 0, 0, 0, 0)
29 Apr 2014	Terminate	-	-	2084	-	-	-	-	-	(37, 58, 3, 2, 0, 0)

the final set of swim trials was carried out, fish were anaesthetised postswim to allow confirmation of gill score and weight/length measurement. The test regime was similar to previous runs, with fish gently crowded and counted (99–124 fish per test across seven tests) into the tank at low water velocity (200 W, ~0.5 bl/s). The entire population was challenged in 8 tests. Test 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.

In accordance with normal practice, the marine SBP challenge cohort was measured and bathed over reiterative rounds of natural infection. First infection was measured on 18th September 2013 at gill index 1.4. Gill score developed slowly until 28th November (index 1.7, 0% score 5) but rose unexpectedly to reach gill index 3.1 (24.4% score 5) at second infection on 10th December (Table 2). Limited data from this cohort is provided as comparison of sibling fish that were not handled at swim trial.

Table 2. Summary statistics for swim time and gill score of swim trial cohort and gill score in SBP cohort. Swim time is expressed as % (0 – 100%) to account for any differences in overall time per test

Trait	Description	N	Mean	SD	CV (%)	Min	Max
Swim1	% time Freshwater 2013	2039	52.91	27.40	52	0.28	100
Swim2	% time Sept 2013	1214	70.84	19.97	28	3.80	100
Swim3	% time Oct 2013	975	88.07	13.11	15	25.27	100
Swim4	% time Nov 2013	957	71.76	20.00	28	2.35	100
Swim5	% time March 2014	818	74.81	24.93	33	4.90	100
AGD1 (Swim)	Gill score Oct2013	1812	1.93	1.04	54	0	5
AGD2 (Swim)	Gill score Dec 2013	1436	3.14	1.29	41	0	5
AGD3 (Swim)	Gill score Feb 2014	1071	1.28	1.12	87	0	5
AGD1 (SBP)	Gill score SBP Sept 2013	2714	1.44	0.92	64	0	4
AGD2 (SBP)	Gill score SBP Dec 2013	2441	3.14	1.51	48	0	5

Statistical analysis

Freshwater and marine data was analysed with ASReml (Gilmour *et al.*, 2006). Multivariate linear mixed animal models fitted were (a) %Swim time, where time in swim is normalised to 0 to 100% to account for any differences in overall time per test, 'winners' were censored as still swimming at the end of each test (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:

$Y = \mu + test + assess + family + a + \omega + \varepsilon$

where Y is a vector of measured values for all fitted traits, μ is the mean for each trait, test is the fixed effect of test 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, ω is the random effect of weight on Swim time (tagging weight at freshwater swim, AGD1 weight at September and October swims, AGD2 weight at November swim and March weight at March swim) and ε is the random residual effect.

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

Results

Following the initial freshwater swim test of the entire population in July 2013, four swim tests 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. In order to preserve fish numbers, only ~1000 fish were handled at the marine measures. These were randomly chosen from the main population, so some fish were not challenged at every swim handling event. All fish remaining in the population were gill scored and measured at AGD assessments (Table 2).

A high proportion of fish became exhausted (failed) indicating that the swim flume was able to discriminate between the vast majority of the population with the increasing flow and high stocking densities described. At freshwater swim (July 2013) 92.4% of tested fish failed (range 90.5 to 93.5%), 86.8% at the September 2013 test (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%) (Table 1). Environmental parameters (water temperature, oxygen and outboard generated flow) were similar between tests at each swim event (Swim 1 to 5), but were necessarily varied between events as ambient temperature changed. As fish became larger throughout the trial, the upper range of relative water speed (bodylengths/second) was also constrained due to power availability through the submersible electric cable that powered the outboard motor.

Handling resilience (expressed as swim time) at all five tests was of low to moderate heritability (Table 3), though this was marginally significant at the October measure. All gill score measures were heritable, both in the swim trial and SBP cohorts. Handling resilience in freshwater was closely related to handling resilience in marine challenge where AGD was light to moderate (r_g = 0.63 - 0.86, r_p = 0.15 - 0.28, Table 4). There was no significant genetic correlation between freshwater swim and the November swim, when gill score had increased rapidly over

one week, though there was a low r_p of 0.10. The relationship between all marine swims were consistent despite the differing levels of AGD at each swim (r_g = 0.69-0.94).

Table 3. Heritabilities 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	Random σ^2_{wt}	Residual σ^2_r	Heritability h^2
Swim1	141.81 (32.30)	0.00 (0.00)	28.68 (40.98)	579.12 (27.95)	0.19 (0.04)
Swim2	50.30 (24.90)	8.15 (10.59)	8.83 (12.98)	334.32 (20.03)	0.13 (0.06)
Swim3	20.43 (12.93)	3.59 (5.61)	0.76 (1.44)	144.44 (10.07)	0.12 (0.07)
Swim4	43.96 (17.31)	0.00 (0.00)	0.81 (2.32)	289.43 (18.86)	0.13 (0.05)
Swim5	111.16 (32.41)	0.00 (0.00)	0.55 (1.54)	312.04 (27.69)	0.26 (0.07)
AGD1 (Swim)	0.31 (0.08)	0.03 (0.02)	-	0.74 (0.05)	0.29 (0.06)
AGD2 (Swim)	0.41 (0.12)	0.04 (0.04)	-	1.23 (0.08)	0.24 (0.07)
AGD3 (Swim)	0.23 (0.07)	0.00 (0.00)	-	1.02 (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)	1.10 (0.17)	0.00 (0.00)	-	1.21 (0.10)	0.48 (0.06)

Table 4. 2012 YC - Correlations (± standard errors) for swim times. Genetic correlation below diagonal, phenotypic correlation above diagonal

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 AGD gill score within the swim cohort were moderate to high (Table 5). There were also strong correlations between the swim cohort and the SBP cohort. There was a positive, but not significant, relationship between the third infection measure (AGD3) in the swim cohort and first infection (AGD1) in the SBP, though both of these measures had been taken at relatively low phenotypic expression 155 days apart.

Table 5. Genetic correlations (± standard errors) of gross gill scores in swim trial and SBP cohorts. Genetic correlation below diagonal, phenotypic correlation above diagonal

Trait	AGD1 (Swim)	AGD2 (Swim)	AGD3 (Swim)	AGD1 (SBP)	AGD2 (SBP)
AGD1 (Swim)	-	0.29 (0.03)	0.12 (0.03)	NA	NA
AGD2 (Swim)	0.67 (0.12)	-	0.23 (0.03)	NA	NA
AGD3 (Swim)	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 resilience tests and AGD measures were generally non-significant (Table 6). However, the September swim (Swim2) shows a positive genetic correlation with gill score measures at first and second infection (AGD1 and AGD2). This could suggest that handling at the first marine swim has impacted upon later AGD expression, yet the same genetic relationship also exists between Swim2 and the first and second infection measures of the SBP cohort (r_g = 0.47 and 0.41) which were not swim tested. There are no significant relationships between Swim4, performed when AGD was rapidly advancing in November 2013, and gill scores, though relationships do appear to be negative. There was a low phenotypic correlation (r_p = -0.15±0.04, data not shown) between the March swim (Swim5) and AGD3.

Table 6. Genetic correlations (± standard errors) of swim times against gross gill scores

Trait	AGD1 (Swim)	AGD2 (Swim)	AGD3 (Swim)	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)

Discussion

This study demonstrates that handling resilience is under genetic control despite a range of fish size and AGD expression. There is generally a strong genetic correlation between handling events, suggesting a consistent genetic basis to handling resilience. Furthermore, handling resilience is largely unrelated to AGD resistance, although there was a positive genetic correlation between the first marine swim trial (Swim2, September 2013) and gill score at AGD1 and AGD2 (i.e. fish swimming longer in September tended to have higher gill score at AGD1 and

AGD2). This does not appear to be a product of fish handling because the relationship also existed with the SBP cohort.

Selection for AGD resistance is a primary breeding objective for the Tasmanian salmon industry and resistance to AGD is a quantitative trait under genetic control. The low to moderate estimates of AGD gill score heritability and moderate to high genetic correlations between gill score measures presented in this study agree closely with results from previous year classes of the SBP (Taylor *et al.*, 2007; Taylor *et al.*, 2009a; Kube *et al.*, 2012). Gill score selection is therefore expected to provide genetic progress in bathing interval. Although the frequency of treatments is likely to reduce, there remains a need to regularly crowd and bath fish. Therefore selection for handling resilience could help to minimise handling losses.

For a selection trait to be a useful to a breeding program, it should adequately reflect the objective trait. In this case we are presuming that failure 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 test is carried out to a point of exhaustion from which most tested 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 gill score (threshold of 30% score 2 – 5) significant mortalities can occur. The link between the swim test failure and commercial bath mortality could not be realistically tested within the constraints of this project, it is assumed that crowding stress resilience is the driving factor in both scenarios.

It is preferable that a selection trait is simple, non-destructive and cost effective to measure. This study demonstrates that handling resilience can be simply tested in fresh water at early age. The fresh water swim trial was well linked with subsequent marine handling resilience across a range of AGD expression. Due to quarantine constraints, potential brood stock remain in freshwater and breeding values are applied based upon their genetic relationship to the marine tested animals. Therefore, fresh water swim tests could be applied directly to potential brood stock at an early stage, which would support within-family selection for handling resilience. At normal commercial AGD levels (low to moderate gill score) it appears that selection from freshwater breeding values will positively affect marine handling performance. During a rapidly advancing summer AGD there was no genetic relationship, suggesting that at high AGD expression the phenotypic effect of advanced gill score largely overrides inherent handling resilience. Therefore, for farmers to take the best advantage of handling resilience it is important to handle fish at low to moderate average gill score. During heavy AGD outbreaks fish need to be handled carefully by minimising crowd density and crowding times.

Freshwater swim was not genetically correlated with AGD for any of the measures. This suggests that breeding for improved handling resilience will not compromise genetic improvement for AGD resistance. Although they appear to be independent traits, the opportunity is to con-

currently select for AGD resistance and AGD handling resilience. The decision to include more objective traits in the breeding goal needs to be weighed carefully by the Tasmanian industry. There is opportunity to improve fish welfare and survival though selection for handling resilience, but this will reduce selection intensity for key growth and AGD resistance traits.

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