Controlled challenge experiment demonstrates substantial additive genetic variation in resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae*

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ABSTRACT

Streptococcus iniae is an etiologic agent of streptococcal disease in tilapia and is one of several *Streptococcus* spp. that negatively impact worldwide tilapia production. Methods for the prevention and control of *S. iniae* include vaccines, management strategies, and antibiotics. A complimentary preventative approach may include selective breeding for disease resistance, but the potential for this is unknown in tilapia. This study was initiated to challenge Nile tilapia (*Oreochromis niloticus*) families for estimation of genetic parameters for resistance to *S. iniae*. A total of 143 full- and paternal half-sib families (avg. 176 g, sd = 50 g) were divided into two groups with each containing on average 9 fish per family. The challenge was designed with the intent to utilize fish injected with *S. iniae* (Group 1) as shedder fish to transfer the bacterium to cohabitated fish (Group 2). Tilapia from Group 1 were challenged by intraperitoneal injection with a volume containing 1.15×10^8 colony-forming units *S. iniae* per fish, and then cohabitated with tilapia from Group 2 in a single tank. Accumulated mortality at the end of the experiment was 60% for the fish challenged by injection and 6.4% for fish challenged by cohabitation. The results revealed high variation for mean survival of the families injected with *S. iniae* (range from 0% to 100%, CV 69%). The estimated heritability of post-challenge survival in Group 1 was 0.42 ± 0.07 on the observed binary scale and 0.58 ± 0.09 on the underlying liability scale, derived from fitting a linear animal model and a sire-dam threshold model, respectively. In summary, substantial additive genetic variation in resistance to *S. iniae* was observed when fish were challenged by injection, and this suggests promise for genetic improvement of tilapia for resistance to *S. iniae* through selective breeding.

Keywords: *Streptococcus iniae*; Nile tilapia; *Oreochromis niloticus*; selective breeding; disease resistance; heritability

1. Introduction

Tilapia (*Oreochromis* spp.) production from the aquaculture sector was reported to be 3,197,330 t with a value of \$5.2 billion in 2012 (FAO, 2014) and is expanding (Fitzsimmons et al., 2011). Intensification of tilapia production has resulted in disease outbreaks that negatively affect production (Shoemaker et al., 2006). *Streptococcus iniae* is a non-groupable *Streptococcus* sp. (Pier and Madin, 1976) and the economic impact of this pathogen on tilapia aquaculture has been realized for more than a decade (Shoemaker et al., 2001; Agnew and Barnes, 2007). Methods of prevention and control for *S. iniae* include vaccination (Eldar et al., 1997; Locke et al., 2010; Shoemaker et al., 2010; LaFrentz et al., 2011), management strategies such as a reduction of fish density (Shoemaker et al., 2000), and use of antibiotics (Gaunt et al., 2010). Effective control and prevention of streptococcosis in the future will likely require an integrated fish-health management approach consisting of all these methods.

Selective breeding for resistance to disease is a complimentary strategy to improve health and performance in tilapia. Research has demonstrated that resistance to infectious disease can be moderately heritable depending on the host and pathogen (Silverstein et al., 2009; Ødegård et al., 2011; Evenhuis et al., 2015). Therefore, there is potential for genetic improvement and research has illustrated this concept (Ødegård et al., 2011; Wiens et al., 2013). The potential for selectively breeding tilapia for resistance to *S. iniae* is unknown; therefore, this study was initiated to challenge Nile tilapia (*O. niloticus*) families for estimation of genetic parameters for resistance to *S. iniae*.

2. Materials and methods

2.1. Fish and rearing conditions

Fish used for this study were from the third generation of the Spring Genetics Nile tilapia

breeding program with nucleus operations in Homestead, Florida, US. Genetic material used to produce the base population of Spring Genetics had undergone 12 generations of selection and was introduced to Spring Genetics in 2010. The base population at Spring Genetics was derived from a selective breeding program in Nicaragua, with three generations of selection primarily focused on growth rate and fillet yield (Gjerde et al., 2012). The program conducted in Nicaragua was designed and supervised by Akvaforsk Genetics Center, Norway. The material originated from a Vietnamese family-based breeding program started in 1999 that had been genetically improved for growth in freshwater earthen ponds for four cycles of selection. This Vietnamese material originated from the fifth generation of the Genetically Improved Farmed Tilapia (GIFT) breeding project conducted in the Philippines (Bentsen et al., 2012) and local Vietnamese strains. The collaborative GIFT research project was started in the Philippines in 1989 as a complete diallel cross experiment carried out with eight strains of Nile tilapia from Africa and Asia (Bentsen et al., 1998).

A total of 162 full- and paternal half-sib families were produced in 2014 using 93 sires and 162 dams selected for increased harvest weight for two generations in the US. Families were produced during a period of 16 d by natural mating in single pair breeding units. A nested design was used where each male was mated with two different females. Fry or fertilized eggs from each female were collected, placed in individual incubators and after five days, an average of 215 fry from each family were randomly selected and transferred to individual nursery tanks where they were kept for approximately nine days. Then fish from each family were stocked in separate rearing units (144 tanks and 18 hapas) until they reached a size allowing for physical tagging with passive integrated transponder (PIT) tags (Biomark GPT12).

At a mean age of 84 d after egg collection, fish from all families were PIT tagged,

individually weighed (avg. 62 g, sd = 22 g) and representatives of all families were stocked in separate test units for local performance testing, or holding tanks destined for challenge testing. Fish from one of the holding tanks, originating from 143 families, were individually weighed (avg. 176 g, sd = 50 g) and randomly separated into two groups (Group 1 and Group 2) with equal number of fish per group (on average nine fish per family per group). These two groups were transported to the Aquatic Animal Health Research Unit (AAHRU) by truck following protocols previously developed.

2.2. Fish rearing conditions at AAHRU

Upon arrival at the AAHRU, the Group 2 fish were stocked into a single 5,550 L tank, while Group 1 fish were distributed into seven 350 L troughs. The large tank and troughs were supplied with ~ 26 °C dechlorinated municipal water at approximate flow rates of 13 and 2 L min⁻¹, respectively. Fish were acclimated for 12 d prior to bacterial challenge and fed daily (2-3% body weight) with Aquamax Grower feed (PMI Nutrition International, Inc., Brentwood, MO, USA). The PIT tag number and date of death was recorded using an ISO handheld reader (ARE H5; AEG ID, Ulm, Germany) for mortalities that occurred during the acclimation period. The tilapia family information and PIT tag numbers were not provided to AAHRU in order for the experiment to be blinded. All procedures utilizing fish were approved by the USDA-ARS, AAHRU Institutional Animal Care and Use Committee.

2.3. Streptococcus iniae growth conditions and passage through fish

A virulent strain of *S. iniae*, ARS-98-60, was used for the bacterial challenge. This isolate was recovered from hybrid striped bass (*Morone saxatilis* × *M. chrysops*) with natural

streptococcal disease (Shoemaker et al., 2010) and identified by species specific PCR (Mata et al., 2004). The *S. iniae* isolate was resuscitated from a -80 °C glycerol stock and grown for 24 h at 28 °C with shaking at 140 rpm in tryptic soy broth (TSB; BD, Sparks, MD, USA) (Shoemaker et al. 2006). This culture was used to pass the isolate through fish. Briefly, 100 µL of the 24 h culture was injected into the peritoneal cavity (i.p. injection) of five tilapia (mean weight, 54 g). At 24 h post-injection, brain tissues from moribund fish were aseptically plated onto sheep blood agar (SBA; Remel, Lenexa, KS, USA) and incubated at 28 °C for 48 h. A colony of *S. iniae* recovered from fish was passed from the SBA plate into TSB, incubated, and used to inject another group of tilapia as described above. This process was repeated one additional time for a total of three passages through tilapia. After the final passage, a colony was passed into TSB, grown, and then 20% glycerol stocks were prepared and frozen at -80 °C. The isolate was confirmed as *S. iniae* by PCR.

The frozen stock of *S. iniae* generated following passage through tilapia was used for the challenge. Briefly, 100 µL of thawed stock was inoculated into each of four flasks containing 50 ml TSB and then incubated at 28 °C with shaking at 140 rpm. After 24 h of growth, each of the four cultures were aseptically combined into a single flask, the optical density (OD) measured at 540 nm was determined to be 1.35, and this culture was used for the bacterial challenge. The number of viable colony-forming units (cfu) mL⁻¹ was determined by spread plating 100 µL volumes of 10-fold serial dilutions (in duplicate) onto SBA. Plates were incubated for 48 h at 28°C, and colonies were counted and averaged to enumerate the cfu mL⁻¹ using standard procedures.

2.4. *Streptococcus iniae* challenge

The average weight of fish was approximately 176 g (sd = 50 g) and this was determined 12 d prior to bacterial challenge (see section 2.1.). The challenge was designed with the intent to utilize fish injected with *S. iniae* (Group 1) as shedder fish to transfer the bacterium to cohabitated fish (Group 2) and determine the mortality of each family by both injection and cohabitation. To accomplish this, tilapia from Group 1 were injection challenged with *S. iniae*, and then cohabitated with tilapia from Group 2. Groups of 20-40 tilapia from Group 1 were anesthetized with 90 ppm tricaine methane sulfonate (MS-222; Argent Chemicals, Redmond, WA, USA) and injected ip with 100 μ L of the *S. iniae* culture using a 25 gauge 5/8 inch needle. Each fish received 1.15×10^8 cfu, a dose that resulted in ~ 60-70% mortality in preliminary experiments (data not shown). Prior to injection, the PIT tag number of each fish was recorded. Following injection, fish were placed into the 5,550 L tank containing fish from Group 2 and this process was repeated for 3 h until all fish from Group 1 were challenged and stocked into the tank. Following challenge, dead fish were removed twice daily for 45 d and the PIT tag numbers and date of death were recorded. Re-isolation of *S. iniae* was attempted daily from a minimum of 20% of the dead fish by inoculating brain tissue onto SBA. Plates were incubated at 28°C for 48 h and examined for the presence of beta-hemolytic colonies indicative of *S. iniae*. Upon termination of the challenge, all surviving fish were euthanized, examined for clinical signs of disease, and PIT tag numbers were recorded. Water quality parameters were determined over the course of the 45 d challenge. Dissolved oxygen (DO) and temperature were measured with a ProODO meter (Yellow Springs Instruments, Yellow Springs, OH, USA). Nitrate, ammonia, hardness, and alkalinity were determined using a Hach CEL/890 Advanced Portable Laboratory (Loveland, CO, USA) according to manufacturer's directions.

2.5. Data Analysis

Survival at the end of the challenge (scored as 1 for alive fish and 0 for dead fish) was analyzed for both Group 1 and Group 2. Since some mortality occurred during acclimation, families with less than five fish at the onset of the study were not included in the analysis (10 families excluded from Group 1 and six families excluded from Group 2). A total of 1,116 records were used for Group 1 (from 133 families) and 1,226 for Group 2 (from 137 families).

Univariate animal linear (1) and sire-dam threshold (2) models were used to obtain estimates of variance components of random effects for survival by Restricted Maximum Likelihood (REML). Of the two, the threshold models offer the most accurate estimations as they treat binary survival scores as normally distributed observations, while the error of measurement on the binary scale (alive or dead) analyzed with the animal linear model depends on the incidence. Estimates of the fixed effects and predictions of the random effects were obtained using the ASReml software (Gilmour et al., 2009). Pedigree used for analysis traced back four generations.

2.5.1. Univariate linear animal model (Model 1)

Model 1 (Univariate linear animal model): $\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e}$; where $\mathbf{y}$ is the vector of the phenotypic observations for survival; $\mathbf{X}$ is a design matrix for the fixed effects that links individual observations to the fixed effect class; $\mathbf{b}$ is the vector with the estimates of the fixed effects (sex, production week to account for age differences and weight of fish 12 d before challenge as a covariate); $\mathbf{Z}$ is an incidence matrix that links the observations to the animal additive genetic effects; $\mathbf{a} \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2)$ is a vector of random animal additive genetic effect; $\mathbf{W}$ is an incidence matrix that links the observations to the random environmental effects common

to full-sibs; $\mathbf{c} \sim N(\mathbf{0}, \mathbf{I}\sigma_c^2)$ is a vector of random environmental effects common to full-sibs other than additive genetics; and $\mathbf{e}$ is a vector of random residuals. Finally, $\mathbf{A}$ is the numerator relationship matrix that describes the additive genetic relationship among all individuals included in $\mathbf{a}$, and $\mathbf{I}$ is an identity matrix of appropriate size.

For Model 1 the significance of the additive genetic effect and the effect common to full-sibs was assessed with the likelihood-ratio test (Lynch and Walsh, 1998):

$LR = -2(\text{Log}L_R - \text{Log}L_F)$, where $\text{Log}L_R$ is the log of the restricted likelihood of the reduced model (excluding each random effect at a time) and $\text{Log}L_F$ is the log of the restricted likelihood of the full model. The significance of LR was tested with a χ^2 test with $df = 1$ (number of omitted variance components) and $\alpha = 0.05$ for both random effects. For model 1, estimated heritability

for survival was calculated as $h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$, and the relative proportion of the environmental

effects common to full-sibs as $c^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$.

2.5.2. Univariate threshold sire-dam model (Model 2)

Model 2 (Univariate threshold sire-dam model): $\lambda = \mathbf{Xb} + \mathbf{Z}_s\mathbf{a}_s + \mathbf{Z}_d\mathbf{a}_d + \mathbf{Wc} + \mathbf{e}$; where λ_i is the underlying liability which is assumed to be associated to the binary observation of animal i (y_i) such that $\lambda_i \leq 0$ gives $y_i = 0$, and $\lambda_i > 0$ gives $y_i = 1$. The vector λ is a vector of all underlying liabilities. The variance components were estimated on the underlying scale, where residual variance (σ_e^2) was restricted to 1.0, implying that $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I})$; $\mathbf{X}$, $\mathbf{b}$, $\mathbf{W}$, $\mathbf{c}$ and $\mathbf{I}$ are the same as described in Model 1; $\mathbf{Z}_s$ and $\mathbf{Z}_d$ are incidence matrices linking observations to the sire and dam additive genetic effects; $\mathbf{a}_s \sim N(\mathbf{0}, \mathbf{A}\sigma_s^2)$ and $\mathbf{a}_d \sim N(\mathbf{0}, \mathbf{A}\sigma_d^2)$ are vectors of the random

sire and dam additive genetic effects, respectively; sire and dam variance are assumed to be equal $\sigma_s^2 = \sigma_D^2 = \sigma_u^2$. The effect common to full-sibs (c) included the environmental tank/hapa effects caused by separate rearing of the families until tagging and potential dominance and maternal effects. For the individual family, these effects are confounded, and thus fitted as a single random effect. Log-likelihood ratio tests cannot be used to test significance of random effects in the threshold models. For Group1 a Probit link function was used to relate the linear predictor scale to the observation scale. Due to problems with convergence, a Logit link function was used for Group 2.

For model 2, estimated heritability for survival was calculated as $h^2 = \frac{4\hat{\sigma}_u^2}{2\hat{\sigma}_u^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$, and the relative proportion of the environmental effects common to full-sibs as $c^2 = \frac{\hat{\sigma}_c^2}{2\hat{\sigma}_u^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$.

3. Results

3.1. Mortality during acclimation

During the 12 d acclimation period at the AAHRU, a total of 189 fish died, which accounted for approximately 11% of the fish in Group 1 and 4% in Group 2. Most acclimation mortalities occurred during the first three days after transportation (Fig. 1). No mortalities were recorded in the large tank (Group 2) after 6 d post transportation, indicating fish were fully acclimated.

3.2. *Streptococcus iniae* challenge

Accumulated mortality at the end of the challenge experiment was 60% for the fish in

Group 1 that were challenged by injection, and 6.4% for fish in Group 2 challenged by cohabitation (Fig. 2). Most of the fish from Group 1 died on the 1st and 2nd day after injection, and in the remaining 37 d mortalities were low. Brain tissue was cultured from approximately 20% of the fish that died during the trial and 98 % (180/183) of these yielded pure cultures of *S. iniae*. Most of the fish challenged by cohabitation survived, consistent with a pilot study where 0 - 12% mortality was observed in the cohabitants regardless of the bacterial dose and the number of fish injected to serve as shedder animals (data not shown). The water quality parameters during the course of the challenge were determined as follows (mean $\pm$ standard error): DO = 3.58 ± 0.09 mg L⁻¹; temperature = $26.2 \pm 0.07^{\circ}\text{C}$; nitrate = $1.43 \pm$ mg L⁻¹; ammonia = 2.89 ± 0.4 mg L⁻¹; hardness = 140 ± 3.4 mg L⁻¹; and alkalinity = 135 ± 5 mg L⁻¹.

The results revealed high variation between the mean family survival of the fish injected with *S. iniae* (range from 0% to 100%, CV 69%; Fig. 3A). On stark contrast, there was low variation between the mean family survival of the fish challenged by cohabitation (range 60%-100%, CV 10%; Fig. 3B). The Pearson correlation between the mean family survival of Group 1 and Group 2 was positive but of low magnitude ($r = 0.31$).

At the termination of the challenge, all survivors were examined for clinical signs of disease. Overall, eight percent of the surviving fish showed pustules, but a higher percentage of pustules were observed on fish challenged by injection (16.6% for Group 1) when compared with fish that were challenged through cohabitation (4.8% for Group 2). Approximately seventy percent of the surviving fish exhibited cloudy eyes (73.3% for Group 1, 65.7% for Group 2). Distribution of clinical signs of disease observed in survivors from Group 1 and Group 2 are shown in Fig. 4.

3.3. Fixed effects

None of the fixed effects fitted in the models (sex, production week, weight at transport) were found to be statistically significant ($P > 0.05$, Wald F statistics).

3.4. Additive genetic effects

The results showed a strong additive genetic component for survival in fish injected with *S. iniae* (Group 1; Table 1). The heritability estimate on the observed scale was 0.42 ± 0.07 (Model 1), and higher on the underlying scale (0.58 ± 0.09 ; Model 2), as expected. The additive genetic effect was significantly different from zero ($P < 0.05$; Log-likelihood ratio test Model 1).

In contrast, a very low additive genetic component was found for survival in fish challenged by cohabitation (Group 2; Table 1). On the observed scale (Model 1), the heritability estimate was 0.06 ± 0.07 and additive genetic effect for survival was not significantly different from zero ($P > 0.05$; Log-likelihood ratio test Model 1). Heritability for the threshold model (Model 2) was higher (0.54 ± 0.59), however with a standard error of the same magnitude suggesting that the heritability is not statistically different from zero. Results suggest no statistically significant additive genetic variation for survival in fish challenged with *S. iniae* by cohabitation.

3.5. Environmental effects common to full-sibs

The environmental effect common to full-sibs was not significant for either fish challenged with *S. iniae* by injection (Group 1) or for the fish challenged by cohabitation (Group 2) ($P > 0.05$; Log-likelihood ratio test Model 1). The environmental effect common to full-sibs as a proportion of phenotypic variance (c^2) was zero for Group 1 (in both models) and low and

not significant for Group 2 (Table 1).

4. Discussion

Although tilapia production is increasing worldwide, mortality resulting from climatic events and diseases have limited production and the growth of the industry (Thodesen et al., 2013). Streptococcosis caused by *Streptococcus iniae* (Shoemaker et al., 2001) is one important bacterial pathogen impacting tilapia production through disease losses. Resistance against bacterial, viral and parasitic pathogens in fish can be heritable (Ødegård et al., 2011); thus, selective breeding for resistance to *S. iniae* provides a complementary approach for limiting the impact of streptococcosis if there is an additive genetic component to variation in resistance.

Immersion and/or cohabitation disease models are generally preferred for fish disease research, including phenotyping families for resistance to disease. In preliminary studies, attempts were made to develop a cohabitation disease model to mimic natural exposure (data not shown). In these studies, different doses of *S. iniae* were tested in conjunction with different percentages of injected fish (shedders) and naïve fish (cohabitants) in the same tank. Regardless of the number of shedder fish (20 or 50 % of the population) and bacterial doses used, limited transmission of disease to cohabitants was noted. For example, ip injection of shedder fish with 1×10^8 CFU/fish resulted in 60 – 70% mortality in the shedder fish, whereas only 8 – 10% mortality occurred in the naïve fish housed in the same tank. Although these preliminary studies suggested limited mortality in cohabitant fish, a similar experimental design was used for the phenotyping of all families in anticipation of greater disease transmission with larger numbers of fish and higher densities in the 5,500 L tank. The challenge was designed with the intent to utilize fish injected with *S. iniae* (Group 1) as shedder fish to transfer the bacterium to

cohabitated fish (Group 2) and determine the mortality of each family by both injection and cohabitation. However, results of the large tank study largely confirmed the preliminary study results in that 60% mortality was recorded in the fish injected i.p. (Group 1) and only 6.4% mortality observed in the naïve cohabitated fish (Group 2).

Analysis of the mortality data from fish that were ip injected with *S. iniae* (Group 1), demonstrated substantial additive genetic variation in survival of Nile tilapia to *S. iniae* challenge. The estimated heritability of post-challenge survival in Group 1 was 0.42 ± 0.07 on the observed binary scale (Model 1) and 0.58 ± 0.09 on the underlying liability scale (Model 2), derived from fitting a linear animal model and a sire-dam threshold model, respectively. Ødegård et al. (2011) suggested the simplest statistical model for analysis of challenge data are the cross-sectional models if certain assumptions are met (i.e, alive/dead and 50% end point mortality). In this study, the cumulative end of test mortality was 60% for Group 1; therefore, a simple cross sectional model such as Model 1 is considered appropriate since phenotypic variance of a binary trait is maximized at 50% of cumulative mortality and thus difference between results in the observed and underlying scale are consistent. In contrast, analysis of the mortality data obtained from the cohabitant fish (Group 2) suggested no additive genetic variation in survival to *S. iniae* challenge. On the observed binary scale (Model 1), the heritability estimate was 0.06 ± 0.07 and for the threshold model (Model 2) and 0.54 ± 0.59 on the underlying liability scale (Model 2). This finding was not surprising, given the low mortalities in Group 2 and lack of variation between Group 2 mean family survivals. For both groups, the environmental effect common to full-sibs was not significant, indicating that initial separate rearing of individual families prior to tagging, maternal or epistatic effects do not affect the survival to *S. iniae*, as was done in this experiment.

These results suggest good prospect for genetic improvement based on *S. iniae* challenge testing using ip injection. Although injection disease models bypass the natural immune barriers, this route of challenge has demonstrated additive genetic variation for bacterial cold water disease caused by *Flavobacterium psychrophilum* in rainbow trout (*Oncorhynchus mykiss*) (Silverstein et al., 2009; Leeds et al., 2010), cold water vibriosis (*Vibrio salmonicida*) and bacterial kidney disease (*Renibacterium salmoninarum*) in Atlantic salmon *Salmo salar* (Gjedrem and Gjøen, 1995), *Vibrio anguillarum* in Atlantic salmon (Gjøen et al., 1997), *Piscirickettsia salmonis* in Atlantic salmon (Yañez et al., 2013), and infectious salmon anemia virus in Atlantic salmon (Ødegård et al., 2007). Importantly, use of the injection challenge model in rainbow trout for the *F. psychrophilum* selective breeding program has translated to enhanced performance of selectively bred fish in field trials, where the fish were exposed to the pathogen under natural conditions (Wiens et al., 2013). It will be important to validate the relationship between the survival of tilapia in the challenge test for *S. iniae* (via ip injection) and survival to *S. iniae* outbreaks under field conditions. To validate this, fish from the same families (or experimental groups) must be challenged simultaneously under controlled conditions and under commercial field conditions. However, in a practical sense this can be difficult to achieve because you cannot always ensure outbreak of disease in the field.

The results demonstrated substantial additive genetic variation in resistance to *S. iniae* when fish are challenged by injection, and suggest promise in genetic improvement of tilapia for resistance to *S. iniae* by use of selective breeding. The estimates were based on a relatively small family size (on average nine fish per family). Future testing of tilapia from the same population will include a higher number of fish per family to allow for higher accuracy of estimated family breeding values. Additionally, tilapia will be challenged with *S. agalactiae*, another important

tilapia pathogen (Delannoy et al., 2014), to determine if there is a genetic correlation between the resistance of tilapia to both *Streptococcus* species. Evenhuis et al. (2015) recently demonstrated that selection of rainbow trout for resistance to *F. psychrophilum* resulted in a favorable genetic correlation in resistance to another bacterium in the same genus, *F. columnare*. Research is also in progress to determine the potential for marker assisted selection in which selection is based on marker genotypes alone or in combination with phenotypic information on the tilapia families. Such an approach may further improve the accuracy and response to selection for resistance to *S. iniae*. The long term goal of this research is to provide farmers with a more resistant stock of tilapia as an additional management tool for reducing production losses due to *Streptococcus* spp.

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Table 1

Survival variance components, estimated heritability ($h^2 \pm se$) and effect common to full-sibs ($c^2 \pm se$) for the univariate linear animal model (Model 1) and the univariate threshold sire-dam model (Model 2).

<i>Parameter</i>	Model 1 (observed scale)		Model 2 (underlying scale)	
	G1	G2	G1*	G2**
σ^2 sire	-	-	0.207	0.232
σ^2 dam	-	-	0.207	0.232
σ^2 additive	0.100	0.004	0.826	0.927
σ^2 common env.	0.000	0.002	0.000	0.257
$\sigma^2 e$	0.139	0.055	1.000	1.000
$h^2 \pm se$	0.42 ± 0.07	0.06 ± 0.07	0.58 ± 0.09	0.54 ± 0.59
$c^2 \pm se$	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00	0.15 ± 0.29

* PROBIT link function

** LOGIT link function

Figure Legends

Fig. 1. The number of dead Nile tilapia recorded from Group 1 (●) and Group 2 (■) following transportation. Fish were acclimated for 12 d prior to bacterial challenge.

Fig. 2. Cumulative percent mortality in Nile tilapia following challenge with *Streptococcus iniae* by intraperitoneal injection (●; Group 1) or by cohabitation (■; Group 2).

Fig. 3. The number of fish per family (line; right y-axis) and mean survival (bars; left y-axis) of 143 Nile tilapia families following challenge with *Streptococcus iniae* by intraperitoneal injection (A; Group 1) or cohabitation (B; Group 2). Families with less than five fish at the onset of the bacterial challenge were excluded from the data analysis (see section 2.5. Data Analysis).

Fig. 4. Percentage of clinical signs of streptococcosis observed in Nile tilapia surviving challenge with *Streptococcus iniae* by intraperitoneal injection (A; Group 1) or cohabitation (B; Group 2).

Figure 1

Figure 2

Figure 3A

Figure 3B

Figure 4A

Figure 4B