

**QUANTITATIVE GENETICS OF GROWTH, CARCASS-QUALITY TRAITS,  
AND DISEASE RESISTANCE IN HYBRID STRIPED BASS (*Morone chrysops* ♀ x**

***Morone saxatilis* ♂)**

A Dissertation

by

XIAOXUE WANG

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2006

Major Subject: Genetics

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**ABSTRACT**

Quantitative Genetics of Growth, Carcass-Quality Traits, and Disease Resistance in

Hybrid Striped Bass (*Morone chrysops* ♀ × *Morone saxatilis* ♂).

(December 2006)

Xiaoxue Wang, B.E., Ocean University of China

Co-Chairs of Advisory Committee: Dr. John R. Gold  
Dr. Delbert M. Gatlin, III

A 10 x 10 factorial mating design and a ‘common-garden’ rearing approach were employed to examine genetic effects and heritability of growth, carcass-quality traits, and disease resistance, important production traits in the aquaculture of hybrid striped bass (♀ white bass, *Morone chrysops*, crossed with ♂ striped bass, *Morone saxatilis*). Genotypes at four to ten nuclear-encoded microsatellites were used for parentage assignment and a general, linear-mixed model and a Restricted Maximum Likelihood (REML) algorithm were used to estimate variance components associated with dam, sire, and dam x sire interaction effects.

Dam and sire effect on juvenile growth (weight, length and growth rates) were significant, whereas dam by sire interaction effect was not. Estimates of broad-sense heritability for growth, based on family means ( $h^2_f$ ), in dams ranged from  $0.60 \pm 0.20$  to  $0.82 \pm 0.10$  and in sires ranged from  $0.43 \pm 0.20$  to  $0.75 \pm 0.18$ . High correlations were found between growth rates measured at two time intervals. Estimates of general combining ability for growth rates differed significantly among dams and among sires, whereas estimates of specific combining ability for each dam × sire combination did not

differ significantly from zero. These results suggest that additive-effect genes contributed to the differences in juvenile growth.

Dam and sire effect on fillet weight were significant; dam effect on liver weight and sire effect on total viscera weight were also significant. Dam and sire effect on hepatosomatic index and viscerasomatic index were significant, as was dam and sire interaction effect on viscerasomatic index. Phenotypic and genetics correlations between body weight and carcass-quality traits were high (0.85 - 1.00). Phenotypic correlations between body weight and standardized carcass-quality traits were positive but low, ranging from 0.07 to 0.19.

Resistance to *S. iniae* was assessed in a challenge experiment, using the 10 dam x 10 sire factorial mating design. A significant effect of sire on resistance to *S. iniae* was found, and offspring from one sire had a 2.4 times higher probability of dying than offspring from the 'average' sire. Genetic effects on the immune-response parameters and on stress-response parameters assessed were non-significant.

**DEDICATION**

To my mother, Jinying Wang, to my brother, Xiaozhi Wang, and to my husband,  
Peng Li.

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## CHAPTER I

### INTRODUCTION

Hybrid striped bass is the cross between female white bass (*Morone chrysops*) and male striped bass (*Morone saxatilis*). The genus *Morone* belongs to the family Percichthyidae of the order Perciformes. Striped bass are anadromous and native to a variety of habitats, including bays, and estuaries. Striped bass originally were found on the Atlantic Coast of North America, from New Brunswick, Canada, to Florida and along the northern coast of the Gulf of Mexico from Florida to Texas. Striped bass were introduced to the west coast of the United States (U.S.) in the early 1890s and established reproducing populations (Hodson, 1989). Presently, striped bass now range along the western coast of North America from British Columbia, Canada, south to the border between the United States and Mexico. White bass (also genus *Morone*, family Percichthyidae) are found in freshwater in North America and home to spawning sites located on shoals in streams. Originally, white bass were distributed throughout most of the Mississippi basin and along the Gulf Coast. The species has now been widely introduced throughout the U.S., primarily for recreational fishing.

The accepted common name of the hybrid striped bass, when the cross is between female white bass and male striped bass, is sunshine bass. This hybrid is intermediate in appearance between the parental species and possesses improved traits in captivity with

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This dissertation follows the style of *Aquaculture*.

regard to growth, survival, hardiness, and disease resistance. The latter makes the hybrid highly suitable for aquaculture (Bishop, 1968; Myers and Kohler, 2000). Hybrid striped bass survive and thrive in a wide range of environmental conditions. A temperature range of 4 - 33°C is acceptable, but optimum growth occurs within the range 25 - 27°C (Hodson and Hayes, 1989). Hybrid striped bass are generally stocked into freshwater systems, but they do well in salinities of 0 - 25 ppt and can survive in salinities up to full strength seawater (Hodson and Hayes, 1989). Hybrid striped bass can tolerate dissolved oxygen levels as low as 1 mg/L, but optimum dissolved oxygen levels range from 6 to 12 mg/L (Hodson, 1989).

Both female and male hybrid striped bass are fertile and produce eggs and milt, respectively. Males can mature at age one (approximately 250 mm in length and 500 grams in weight), and almost all are mature at age two (Hodson, 1989). A few females mature by age two, and almost all are mature by age three (Hodson, 1989). Second generation individuals (i.e., offspring of female x male hybrid striped bass), however, exhibit morphological abnormalities, variable growth, and reduced viability of eggs, larvae, and juveniles (Harrell, 1997). The backcross of hybrid striped bass females x striped bass males is reported to have low fertilization rate and lower offspring survival and growth rate (Lindell et al., 2004). Consequently, F<sub>1</sub> offspring are the production unit for present-day hybrid striped bass aquaculture (Lindell et al., 2004).

Hybrid striped bass is one of the fastest growing segments of the finfish aquaculture industry in the United States (Kohler et al., 2001). In 2000, hybrid striped bass production was ranked fifth in the U.S. aquaculture production and fourth in value of all

U.S. cultured food fish (Carlberg et al., 2000). Annual production in the U.S. has increased from ~400,000 pounds in 1987 to ~10.6 million pounds in 2001. Other countries, including China, Israel, and Italy also have expanding production of hybrid striped bass.

Expansion of hybrid striped bass production is constrained, however, by high production costs. Efforts had been made to domesticate striped bass and white bass (Kohler et al., 1994); the majority of hybrid striped bass production, however, is still dependent on wild fish as broodstock (Harrell, 1997). The uncontrolled variation in the performances of hybrids derived directly from wild fish thus leads to suboptimal production efficiency of hybrid striped bass (Woods, 2001).

Another important problem in hybrid striped bass aquaculture is infection from the pathogenic bacteria *Streptococcus iniae*. Estimates of the annual economic loss to the U.S. hybrid striped bass industry from *S. iniae* infections are approximately \$2 million (Ostland, 2003). Stressful conditions, essentially unavoidable in aquaculture, make fish such as hybrid striped bass susceptible to infectious agents during normal production, and moreover, may lead to adverse physiological change and mortality (Plumb, 1997). These issues are of even greater concern with increasing intensification of hybrid striped bass production.

For centuries, selective breeding has played an important role in increasing yield and survival and in improving product quality of farmed animals and plants (Dekkers and Hospital, 2002). In finfish aquaculture, however, commercial interest in selective breeding has been overshadowed by efforts to develop optimal husbandry practices

(Gjedrem, 1983). Until recently, only a few fish species have been evaluated in terms of a selective breeding program, the primary species being salmonids and tilapia (Sonesson, 2003).

Studies of the genetic control of production traits in fish are important because of the typically high proportion of genetic variation for such traits and their direct connection to economic value (Perry et al., 2004; Gjedrem, 1983). Phenotypic variation in production traits has been reported in both striped bass and hybrid striped bass (Harrell, 1997; Kohler et al., 2001), as have differences in growth rate within and among different families of striped bass (Woods, 2001). Significant differences in juvenile body weight and in fillet dress-out percentage at market size among hybrid striped bass produced from different geographic stains of white bass also has been documented (Kohler et al., 2001). Improving production efficiency of hybrid striped bass via selective breeding and genetic improvement of broodstock is clearly warranted (Carlberg et al., 2000), and for effective selective-breeding programs to develop, it is essential to have baseline genetic information for commercially important traits in hybrid striped bass.

Growth rate is typically the most important trait for all aquaculture species under selection (Sonesson, 2003). However, selection for faster growth alone may generate correlated responses in other traits that may be unfavourable for either consumers or fish farmers (or both). Negative genetic correlation between or among traits potentially cause offsets in genetic gain from selection on single characters by economic losses in correlated traits (Perry et al., 2004). In the U.S., fillets and gutted fish are two major

process products in the hybrid striped bass market. Consequently, carcass quality traits such as fillet yield and total viscera weight also need to be evaluated in selective breeding programs targeted primarily to improving growth rate. Insights into nutrient and energy utilization and partition between economical valued exports (fillet yield, gutted fish weight) and production wastes (viscera, especially intraperitoneal fat and other inedible organs) associated with various genetic backgrounds is similarly important. It is known that variation in partition of nutrient and energy used for basic metabolite activities and accumulation of muscle tissue and fat among species or individuals of the same species exists (Johnston, 2001).

Selective breeding also is a promising and safe approach to improvement of disease resistance because of its prospects for prolonged and sustainable protection (Wiegertjes et al., 1996). In several fish species significant genetic variation for resistance to various pathogenic diseases has been found (Gjedrem et al., 1991; Beaumont, et al., 2003; Henryon et al., 2005), suggesting that selective breeding for improved disease resistance could be successful.

In this study, a full, factorial design (10 white bass ♀ × 10 striped bass ♂) was employed to generate up to 100 possible full-sib families. Genotypes at ten nuclear-encoded microsatellites were used for parentage assignment. A general, linear-mixed model and a Restricted Maximum Likelihood (REML) algorithm were used to estimate variance components associated with dam, sire, and dam by sire interaction effects on production important traits. In order to predict response to selection of breeders evaluated based on performance of their offspring during controlled crosses, heritability



of the traits was estimated using a family-mean basis. Pairwise genetic and phenotypic correlations among traits were estimated. The expected performance value of an interspecific hybrid is a function of the general and specific combining abilities of the two parental individuals (Falconer and Mackay, 1996). The general combining ability (GCA) of a sire (or dam) is defined as the average performance value of offspring from this sire (dam) when crossed to all other dams (sires) and expressed as a deviation from the mean of all dam by sire crosses (Falconer, 1981). The specific combining ability (SCA) of a cross represents the deviation of the performance of this cross from the expected performance based on the GCA of the dam and the sire involved in the cross; SCA is also a measure of the dam by sire interaction effect(s). Information on combining ability is needed to identify potentially superior parents and help define patterns of gene effects in expression of quantitative traits (Comstock et al., 1949; Goyal and Kumar, 1991).

## CHAPTER II

### QUANTITATIVE GENETICS AND HERITABILITY OF GROWTH-RELATED TRAITS IN HYBRID STRIPED BASS (*Morone chrysops* ♀ x *Morone saxatilis* ♂) \*

#### 1. Introduction

Hybrid striped bass (*Morone chrysops* ♀ × *Morone saxatilis* ♂) is one of the fastest growing segments of the finfish aquaculture industry in the United States (Kohler et al., 2001), ranking fifth in aquaculture production and fourth in value of fish cultured in the year 2000 (Carlberg et al., 2000). The hybrid is similar in appearance to the parental species, but due to heterosis possesses traits such as aggressive feeding behavior and tolerance to a wide range of environmental conditions that make it highly suitable for aquaculture (Bishop, 1968; Myers and Kohler, 2000). A major constraint currently limiting expanded production of hybrid striped bass is suboptimal production efficiency stemming from uncontrolled variation in performance of fish derived from undomesticated broodstock (Woods, 2001). For centuries, selective breeding has played an important role in increasing yield and survival and in improving product quality of farmed animals and plants (Dekkers and Hospital, 2002). In finfish aquaculture, however, commercial interest in selective breeding has been overshadowed by efforts to

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develop optimal husbandry practices (Gjedrem, 1983). Until recently, only a few fish species have been evaluated in terms of a selective breeding program, the primary species being salmonids and tilapia (Sonesson, 2003). Domestication of striped bass (*M. saxatilis*) was initiated in 1983 by spawning fish collected from the wild. In the 1990s, efforts were initiated to domesticate white bass (Kohler et al., 1994).

Studies of genetic control of production traits in fish are important because of the typically high proportion of genetic variation for such traits and their direct connection to economic value (Gjedrem, 1983; Perry et al., 2004). Phenotypic variation in production traits has been reported in both striped bass and hybrid striped bass (Harrell, 1997; Kohler et al., 2001), as have differences in growth rate within and among different families of striped bass (Woods, 2001). Significant differences in juvenile body weight and in fillet dress-out percentage at market size among hybrid striped bass produced from different geographic strains of white bass also has been documented (Kohler et al., 2001). Improving production efficiency of hybrid striped bass via selective breeding and genetic improvement of broodstock is clearly warranted (Carlberg et al., 2000), and for effective selective-breeding programs to develop, it is essential to have baseline genetic information for commercially important traits such as growth and disease resistance.

The objective of this study was to assess genetic parameters of growth-related traits in hybrid striped bass. Heritability of individual traits and pairwise genetic and phenotypic correlations among traits were estimated, as were general and specific combining abilities for dams, sires, and dam  $\times$  sire combinations. Information on combining ability is needed to identify potentially superior parents and help define

patterns of gene effects in expression of quantitative traits (Comstock et al., 1949; Goyal and Kumar, 1991).

## **2. Materials and Methods**

### *2.1. Production of experimental fish*

A classical, factorial design, also known as North Carolina Design II (Roff, 1997), where ten white bass females were crossed *inter se* with ten striped bass males, was employed to produce full-sib, half-sib, and unrelated progeny. Matings were carried out during the spring of 2003 at Keo Fish Farms in Lonoke, Arkansas. White bass females were obtained by personnel at Keo Fish Farms via angling in the Arkansas and Mississippi river drainages. Weight of the ten females ranged from 1.14 - 1.82 kilograms. The striped bass males had been maintained at Keo Fish Farms for several years. The exact origin of each individual male was unknown; two were obtained originally from 'wild' stocks in Maryland, while the remainder were obtained either in the wild from Lake Ouachita, Arkansas, or were provided by Kent SeaTech Corporation in San Diego, California. Weight of the ten males ranged from 2.73-7.95 kilograms.

Both females and males were induced to spawn by injection of human chorionic gonadotropin (hCG) according to established procedures (Hodson and Hayes, 1989). Eggs (200,000 to 400,000/female) were collected by stripping, divided into ten equal aliquots, and placed into ten separate Petri dishes in order to reduce possible bias in family-size due to unequal fertilization and/or unequal hatching success (Fishback et al., 2002). Milt was collected from each male by stripping; 1 ml aliquots of milt were then added to each of the 10 Petri dishes. Spawning and fertilization were achieved within a

6-8 hour period. Following fertilization, the 100 equally sized batches of fertilized eggs were pooled by sire, resulting in ten half-sib families, each representing the eggs from ten dams fertilized by the milt from one sire. Hatching occurred over a 24-hour period. All offspring then were pooled and ~300,000 larvae were placed randomly into each of two 1000-liter indoor production tanks. The tanks were recycled with well water at 18 °C and a 10h/14h of light/dark cycle was maintained. After approximately four days, fry were assigned randomly to two outdoor, earthen ponds (4ha/each). The earthen ponds initially were fertilized with cottonseed meal and inorganic fertilizer to initiate natural production (phytoplankton and zooplankton). After fry begin feeding, they were fed a high-protein meal-type diet. At Phase I (1-4 grams), approximately 3,500 fingerlings from each of the two ponds were selected randomly and transported to the Aquacultural Research and Teaching Facility (ARTF) at Texas A&M University in College Station, Texas. The fingerlings were maintained in 1200-liter tanks connected to a recirculating system and under the same conditions as the growth trial (see below) until tagging.

## *2.2. Growth and maintenance of experimental fish*

Fish were individually marked with PIT (Passive Integrated Transponder) tags when the majority of fish were >20 g. Based on that size criterion, a first group of fish (Group A, 600 fish) were tagged at 152 days post fertilization and allocated to six 1200-liter tanks (100 fish per tank). A second group of fish (Group B, 400 fish) were grown until they reached adequate size (>20 g) for PIT-tagging (201 days post-fertilization) when they were PIT-tagged and allocated to four 1,200-liter tanks (100 fish per tank). Initial weight and length of each fish was recorded when they were PIT-tagged and a small clip

from the dorsal fin was removed and stored in 70% ethanol for subsequent parentage assignment.

The ten 1200-liter tanks were nested within three connected recirculating systems. Water quality was maintained through biological and mechanical filtration; salinity was maintained at 2-3 ppt, using well water and synthetic sea salt (Fritz Industries, Inc., Dallas, TX). Low-pressure electrical blowers provided aeration via air stones; dissolved oxygen levels were maintained at or near saturation. Water temperature was controlled by ambient air and maintained at  $25 \pm 3$  °C throughout the trial. A 12-h light/12-h dark photoperiod was maintained by using fluorescent lights controlled by timers. Fish were fed a commercial striped bass diet (EXTRU 400; 40% crude protein, 10% lipid; Rangen, Inc., Angleton, Texas) to apparent satiation twice daily. Weight and length of fish in Groups A and B was measured at 269 and 318 days post-fertilization, respectively (117 days after the first measurement in both cases). After each measurement, fish from the same tank remained as a unit but were assigned randomly to a different tank within the system. The experiment was terminated when total fish biomass reached the maximum carrying capacity of the recirculating systems (389 days post-fertilization). Final weight and length of all fish in both groups were measured at that time.

### *2.3. Parentage assignment*

Parentage of offspring were determined using four to ten nuclear-encoded genetic markers (microsatellites) developed and/or adapted specifically for this study (Han et al., 2000; Roy et al., 2000; Ross et al., 2004). Polymerase chain reaction (PCR) primer

sequences and reaction conditions for each microsatellite are given in Appendix Table 1. PCR amplification products were screened in 5% denaturing polyacrylamide gels, using an ABI 377 DNA sequencer. Fragment analysis was conducted using GENESCAN® (Applied Biosystem, Foster city, CA, USA) and allele calling was performed with GENOTYPER® (Applied Biosystem, Foster City, CA, USA) software. Multilocus genotypes were used to assign offspring to their parents based on Mendelian principles and using Excel functions incorporated in a macro.

#### 2.4. Trait assessment

Weight and length were assessed prior and subsequent to initiation of the growth trial in both Groups (A & B). Age at initiation of the growth trial was 152 days post fertilization (Group A) and 201 days post fertilization (Group B). Weight and length after initiation of the growth trial were assessed at 269 and 389 days post-fertilization (Group A), and at 318 and 389 days post-fertilization (Group B). Growth rate was estimated as change in weight over time, i.e.,  $G_r = (W_2 - W_1) / (t_2 - t_1)$ , where  $t_1$  and  $t_2$  are ages at the beginning and end of an interval, respectively, and  $W_1$  and  $W_2$  are the weight of fish at those times (Schreck and Moyle, 1990). Growth rates were measured over two time intervals in both groups: *Interval 1* (152 – 269 days, Group A; 201 – 318 days, Group B), and *Interval 2* (152 – 389 days, Group A; 201 - 389 days, Group B).

## 2.5. Statistical analysis

### 2.5.1. Test of significance of genetic effects

Genetic (dam, sire, and dam/sire interaction) and fixed (group) effects on weight and length measured before the growth trail were assessed according to *Model 1*.

*Model 1*:

$$Y_{ijkl} = \mu + dam_i + sire_j + (dam \times sire)_{ij} + G_k + \varepsilon_{ijkl}$$

Genetic (dam, sire, and dam/sire interaction), fixed (group) effects, and the interaction between genetic and non-genetic effects on weight and length measured after initiation the growth trail were assessed according to *Model 2a*. Traits include weight and length measured at the first (269 days post-fertilization, Group A; 318 days post-fertilization, Group B) and second (389 days post-fertilization, Groups A & B) measurement periods, and on growth rates estimated over the two time intervals (see *Section 2.4*). Genetic (dam, sire, and dam  $\times$  sire interaction) effects were considered as random, group effect (fixed) was considered as fixed, and tank (fixed) effect was nested within group.

*Model 2a*:

$$Y_{ijklm} = \mu + dam_i + sire_j + (dam \times sire)_{ij} + G_k + T(G)_{kl} + (dam \times T(G))_{ikl} + (sire \times T(G))_{jkl} + (dam \times sire \times T(G))_{ijkl} + \varepsilon_{ijklm}$$

A simpler model (*Model 2b*) without the interaction between genetic and non-genetic effect also was used to assess the same growth-related traits after initiation of the growth trial.

*Model 2b*:

$$Y_{ijklm} = \mu + dam_i + sire_j + (dam \times sire)_{ij} + G_k + T(G)_{kl} + \varepsilon_{ijklm}$$



In *Model 1*, *Model 2a*, and *Model 2b*,  $\mu$  is the overall mean,  $\text{dam}_i$  represents the effect of the  $i^{\text{th}}$  dam,  $\text{sire}_j$  represents the effect of the  $j^{\text{th}}$  sire,  $(\text{dam} \times \text{sire})_{ij}$  represents the (interaction) effect of the cross between the  $i^{\text{th}}$  dam and  $j^{\text{th}}$  sire,  $G_k$  represents the effect of the  $k^{\text{th}}$  group,  $T(G)_{kl}$  represents the fixed effect of the  $l^{\text{th}}$  tank nested within the  $k^{\text{th}}$  group, and  $\varepsilon$  is the random residual. Significance of random (genetic) and fixed (group /and tank) effects was determined by analysis of variance (ANOVA), with the sum of squares for different effects associated with Type IV estimable functions.

### 2.5.2. *Estimates of variance components and broad-sense heritability*

Variance components and their standard errors were estimated using the REML algorithm implemented in VCE-5 (Groeneveld, 1998), using *Model 1* (for traits measured before the growth trial) and *Models 2a* and *2b* (for traits measured after initiation of the growth trial). Broad-sense estimates of heritability on an individual basis ( $h^2_i$ ) were computed as four times the ratio of the dam or sire component of variance to the total phenotypic variance; standard errors of  $h^2_i$  were computed as four times of the standard error of the ratio of the dam or sire component of variance to the total phenotypic variance, as estimated using VCE-5. The parameter  $h^2_i$  reflects the magnitude of the genetic effect in determining the phenotypic variation of a given trait among individuals (Falconer and Mackay, 1996). In order to predict response to selection of breeders based on performance of their offspring during test crosses, estimates of broad-sense heritability also were generated on a half-sib, family-mean basis as outlined in Holland et al. (2003). Heritability ( $h^2_f$ ) for dam and sire were calculated as the proportion of the variance of dam half-sibs or sire half-sibs relative to

the phenotypic variance of family means for a particular trait. For traits assessed using *Model 1* and *Model 2b*, the corresponding estimates of  $h^2_f$  were estimated according to

$$h^2_{f(dam)} = \frac{\sigma^2_{dam}}{\frac{\sigma^2_{\varepsilon}}{se} + \frac{\sigma^2_{dam \times sire}}{s} + \sigma^2_{dam}}$$

$$h^2_{f(sire)} = \frac{\sigma^2_{sire}}{\frac{\sigma^2_{\varepsilon}}{de} + \frac{\sigma^2_{dam \times sire}}{d} + \sigma^2_{sire}}$$

For traits assessed using *Model 2a*, the corresponding estimates of  $h^2_f$  were estimated according to

$$h^2_{f(dam)} = \frac{\sigma^2_{dam}}{\frac{\sigma^2_{\varepsilon}}{se} + \frac{\sigma^2_{dam \times sire \times T(G)}}{se} + \frac{\sigma^2_{dam \times T(G)}}{e} + \frac{\sigma^2_{dam \times sire}}{s} + \sigma^2_{dam}}$$

$$h^2_{f(sire)} = \frac{\sigma^2_{sire}}{\frac{\sigma^2_{\varepsilon}}{de} + \frac{\sigma^2_{dam \times sire \times T(G)}}{de} + \frac{\sigma^2_{sire \times T(G)}}{e} + \frac{\sigma^2_{dam \times sire}}{d} + \sigma^2_{sire}}$$

In both equations, the values of  $\sigma^2_{dam}$ ,  $\sigma^2_{sire}$ ,  $\sigma^2_{dam \times sire}$ ,  $\sigma^2_{dam \times T(G)}$ ,  $\sigma^2_{sire \times T(G)}$ , and  $\sigma^2_{dam \times sire \times T(G)}$  represent the variance components of dam, sire, and dam by sire, dam by tank, sire by tank, and dam by sire by tank interactions, respectively;  $\sigma^2_{\varepsilon}$  is the residual. The values  $d$ ,  $s$ , and  $e$  are the number of dams, sires, and tanks. Because of the unbalanced experimental design, these numbers were derived as harmonic means (Holland et al., 2003). Approximate standard errors for estimates of  $h^2_f$  were approximated using the ‘delta’ method described in Hohls (1996). REML

estimates of variance components and associated variances and covariances used to compute  $h^2_f$  and their standard errors were obtained from PROC MIXED in SAS.

### **3. Results**

#### *3.1. Parentage assignment of offspring*

A total of 881 offspring were available for parentage assignment; 879 (99.8%) of these were assigned unambiguously to a specific dam, while 841 (95.5%) were assigned unambiguously to a specific sire (Table 1). Paternity of 40 offspring could not be determined using the available microsatellites; 33 of these offspring, however, could be assigned to one of two sires (Table 1). Of the possible 100 full-sib families, 96 were represented with at least one offspring, and all twenty dams and sires contributed to the final total, ranging from 2.0% (Dam 8) to 17.8% (Dam 9) for dams and 5.2 % (Sire 1) to 17.5 % (Sire 10) for sires. Contribution of individual dams and sires to groups A and B was relatively even, varying from 2.0% (Dam 8, Group B) to 19.8% (Dam 9, Group A) for dams and 4.3% (Sire 1, Group B) to 20.4% (Sire 10, Group A) for sires. Similarly, families were relatively evenly distributed among the different tanks (data not shown).

#### *3.2. Weight and length*

Mean and range of weight and length for offspring in Groups A and B measured before (days 152 {A} and 201 {B}) and after (days 269 {A}, 318 {B}, and 389 {A & B}) initiation of the growth trial are shown in Table 2. Overall survival was approximately 86% during the eight-month growth trial. A total of 6% of tagged fish

Table 1. Number of offspring identified for each of 100 full-sib families generated in the 10 x10 factorial cross

Cross	Dam 1	Dam 2	Dam 3	Dam 4	Dam 5	Dam 6	Dam 7	Dam 8	Dam 9	Dam 10	Unknown <sup>b</sup>	Sum
Sire 1	6	4	4	9	3	0	3	2	14	1	0	46
Sire 2	6	12	17	20	6	6	13	1	19	8	1	109
Sire 3	5	6	10	14	9	4	9	4	12	7	0	80
Sire 4	4	2	12	16	9	3	7	1	4	13	0	71
Sire 5	7	13	6	8	7	2	2	3	17	13	0	78
Sire 6	10	8	5	8	9	3	5	1	8	6	0	63
Sire 7	3	6	9	7	6	0	10	0	12	11	1	65
Sire 8	2	7	14	13	6	3	3	0	25	21	0	94
Sire 9	5	13	7	14	13	2	9	1	11	8	0	83
Sire 10	3	18	23	29	22	6	14	3	29	7	0	154
Sire 1/2 <sup>a</sup>	0	5	6	4	5	0	2	2	5	4	0	33
Unknown <sup>b</sup>	0	0	1	0	0	2	0	0	1	1	0	5
Sum	51	94	114	142	95	31	77	18	157	100	2	881

<sup>a</sup> Either Sire 1 or Sire 2<sup>b</sup> Dam or sire could not be determined (see text)

Table 2. Mean (s.d.) of weight and length at different fish ages (in days post-fertilization) for Groups A and B

Post-fertilization	Day 152{A}	Day 201{B}	Day 269{A}	Day 318 {B}	Day 389{A&B}
Weight (g)	23.2 (15.4)	32.5 (17.4)	181.9 (46.8)	182.7 (54.9)	281.9(76.5)
Range (g)	6.0 - 100.5	8.9 - 117.6	46.0 - 360.8	69.8 - 342.0	96.9 - 540.0
Length (mm)	120.5 (22.2)	134.0 (20.0)	226.7 (18.1)	233.7 (21.9)	263.5 (22.6)
Range (mm)	84.0 - 195.0	95.0 - 204.0	159.0 - 278.0	145.0 - 284.0	195.0 - 387.0
Number of fish	608	401	549	369	783

lost their PIT-tags during the growth trials and were re-tagged when weight and length measurements were taken. When the experiment was terminated (389 days post-fertilization), the average weight over all fish (Groups A & B) was 281.9 (range = 96.9 – 540.0 g), while the average length was 270.6 (range = 195.0 – 387.0 mm).

### 3.3. Genetic and non-genetic effects on weight and length

Results from tests of significance of genetic (dam, sire, and dam x sire interaction) effects on weight and length prior to initiation of the growth trials are shown in Table 3. Effects of dam and sire on weight and length were significant ( $P < 0.05$ ), whereas effect of dam  $\times$  sire interaction was not ( $P > 0.05$ ). Effect of group was significant for both traits. The estimate of the dam component of variance was up to two-fold greater than that of sire for both weight and length data. Corresponding estimates of broad-sense heritability (individual basis,  $h^2_i$ ) were between 0.14-0.17 for dams and 0.07-0.08 for sires (Table 3). Estimates of broad-sense heritability based on family means ( $h^2_f$ ) for both dam and sire (both traits) differed significantly ( $>$ two standard errors) from zero. Estimates of  $h^2_f$  for dams were greater than 0.67 for both traits, while estimates for sires (both traits) were greater than 0.43. Genetic correlations between weight and length based on the sire and dam components of variance were unity; the phenotypic correlation was 0.95.

Results of tests of significance of genetic (dam, sire, and dam x sire interaction) and non-genetic (group and tank) effects on weight and length at the two measurement ages subsequent to initiation of the growth trials are shown in Table 4 and Table 5, using *Model 2a* and *Model 2b*, respectively. Nearly identical results were obtained with

Table 3. Probability of significance of genetic and group effects (based on ANOVA) and estimates of heritability ( $h^2 \pm \text{s.e.}$ ), based on *Model 1*, for weight and length measured before initiation of the growth trial.

Effect	Trait (Groups A and B) <sup>b</sup>	
	Weight	Length
Dam	<0.0001	<0.0001
Sire	0.0364	0.0478
Dam × Sire	0.6245	0.6387
Group	<0.0001	<0.0001
Heritability ( $h^2_i$ ) <sup>a</sup>		
Dam	0.14 ± 0.07	0.17 ± 0.08
Sire	0.08 ± 0.04	0.07 ± 0.04
Heritability ( $h^2_f$ ) <sup>a</sup>		
Dam	0.67 ± 0.17	0.71 ± 0.15
Sire	0.46 ± 0.20	0.43 ± 0.20
Number of fish	804	

<sup>a</sup>  $h^2_i$  is heritability estimated on an individual basis;  $h^2_f$  is heritability estimated on a family-mean basis.

<sup>b</sup> Group A measured at 152 days post-fertilization. Group B measured at 201 days post-fertilization.

both models: (i) effects of dam and sire on weight and length were significant ( $P < 0.05$ ) at both measurement ages, whereas effect of dam  $\times$  sire interaction was not ( $P > 0.05$ ); and (ii) effects of group and tank (nested within group) were significant, with one exception, on both weight and length at both measurement ages. The exception was effect of group on length at the first measurement age. All interactions were non-significant ( $P > 0.05$ ) except for the interaction of sire  $\times$  tank on both weight and length at the second measurement age (Table 4). REML analyses implemented in VCE-5, however, revealed only a small and non-significant variance component associated with this interaction effect.

REML analysis (both models) revealed that estimates of variance components of dam were two- to four-fold greater than those of sire for both weight and length at the first measurement age, whereas variance components were similar at the second measurement age. Corresponding estimates of  $h^2_i$ , based on *Model 2b*, at the two measurement ages were between 0.30 - 0.45 for dams and between 0.11 - 0.28 for sires (Table 5). Estimates of  $h^2_i$  and standard errors based on *Model 2a* were similar to estimates based on *Model 2b*. Estimates of  $h^2_f$  (both models) for both traits for both dam and sire exceeded 0.54 and differed significantly from zero (Tables 4 and 5). Estimates of  $h^2_f$  for dam and sire (both models) did not differ significantly from one another. Genetic correlations between weight and length for both dam and sire at the two measurement ages ranged from 0.97 to 0.99; the phenotypic correlation between weight and length ranged from 0.93 to 0.96 at the two measurement ages, respectively.



Table 4. Probability of significance of genetic and environmental effects, based on ANOVA, and estimates of heritability ( $h^2 \pm \text{s.e.}$ ), based on *Model 2a*, for weight and length measured during the growth trial

Effect	Measurement 1 <sup>a</sup>		Measurement 2 <sup>b</sup>	
	Weight	Length	Weight	Length
Dam	<0.0001	<0.0001	0.0027	0.0024
Sire	0.0407	0.0003	<0.0001	<0.0001
Dam × Sire	0.7437	0.7073	0.5824	0.4368
Group	<0.0001	0.5406	<0.0001	<0.0001
Tank (Group)	<0.0001	<0.0001	<0.0001	<0.0001
Dam × Tank (Group)	0.5377	0.5301	0.8312	0.7044
Sire × Tank (Group)	0.3693	0.3451	0.0060	0.0377
Dam × Sire × Tank (Group)	0.7708	0.5189	0.3244	0.4607
Heritability ( $h^2_f$ ) <sup>c</sup>				
Dam	0.76 ± 0.13	0.79 ± 0.16	0.65 ± 0.24	0.60 ± 0.20
Sire	0.54 ± 0.20	0.67 ± 0.13	0.75 ± 0.18	0.69 ± 0.14
Number of fish	733		726	

<sup>a</sup> Measurement 1: Fertilization to Day 269 {A}/Fertilization to Day 318{B}.

<sup>b</sup> Measurement 2: Fertilization to Day 389{A& B}.

<sup>c</sup>  $h^2_f$  is heritability estimated on a family-mean basis.

Table 5. Probability of significance of genetic and environmental effects (without interactions), based on ANOVA, and estimates of heritability ( $h^2 \pm$  s.e.), based on *Model 2b*, for weight and length measured during the growth trial

Effect	Measurement 1 <sup>a</sup>		Measurement 2 <sup>b</sup>	
	Weight	Length	Weight	Length
Dam	<0.0001	<0.0001	<0.0001	<0.0001
Sire	0.0223	0.0039	<0.0001	<0.0001
Dam × Sire	0.6465	0.3497	0.6610	0.5747
Group	<0.0001	0.3769	0.0037	0.0068
Tank (Group)	<0.0001	<0.0001	<0.0001	<0.0001
Heritability ( $h^2_i$ ) <sup>c</sup>				
Dam	0.45 ± 0.16	0.41 ± 0.16	0.30 ± 0.13	0.32 ± 0.13
Sire	0.11 ± 0.05	0.15 ± 0.07	0.28 ± 0.10	0.28 ± 0.10
Heritability ( $h^2_f$ ) <sup>c</sup>				
Dam	0.85 ± 0.07	0.83 ± 0.08	0.74 ± 0.12	0.76 ± 0.11
Sire	0.58 ± 0.16	0.66 ± 0.14	0.77 ± 0.10	0.77 ± 0.10
Number of fish	733		726	

<sup>a</sup> Measurement 1: Fertilization to Day 269 {A}/Fertilization to Day 318 {B}.

<sup>b</sup> Measurement 2: Fertilization to Day 389 {A& B}.

<sup>c</sup>  $h^2_i$  is heritability estimated on an individual basis;  $h^2_f$  is heritability estimated on a family-mean basis.

### 3.4. Genetic and non-genetic effects on growth rates

Growth rate during the first and second intervals averaged  $1.24 \pm 0.32$  g/day and  $1.18 \pm 0.30$  g/day, respectively. Tests of significance of genetic (dam, sire and dam x sire interaction) and non-genetic (group and tank) effects on growth rate at the two time intervals and based on using *Model 2a* and *Model 2b* are shown in Table 6 and 7, respectively. Estimates of genetic effects on growth rates were consistent between two models; effect of dam and sire on growth rate at both intervals differed significantly from zero ( $P < 0.05$ ), while effect of dam x sire interaction did not ( $P > 0.05$ ). Group and tank (nested within group) effects also were significant at both time intervals (Tables 6 and 7) except for group effect, based on *Model 2a*, on growth rate at the second interval (Table 6). None of the interactions (dam x tank {group}, sire x tank {group}, dam x sire x tank {group}) assessed using *Model 2a* were significant (Table 6). Estimates of the variance component due to dam were two times larger than those due to sire during the first interval but similar during the second interval.

Corresponding estimates of  $h^2_i$ , based on *Model 2b*, at the two measurement ages were greater than 0.31 for dams and 0.17 for sires (Table 7). Estimates of  $h^2_i$  and standard errors based on *Model 2a* were similar to estimates based on *Model 2b*. Estimates of  $h^2_f$  at both time intervals for both dam and sire were greater than 0.64 and differed significantly from zero (Tables 6 and 7). Estimates of  $h^2_f$  for dam and sire (both models) did not differ significantly from one another at either time interval. Genetic correlations of growth rate at both time intervals were 0.99 based on the dam

Table 6. Probability of significance of genetic and environmental effects, based on ANOVA, and estimates of heritability ( $h^2 \pm \text{s.e.}$ ), based on *Model 2a*, for growth rate measured at two intervals during the growth trial

Effect	Growth Rate	
	Interval 1 <sup>a</sup>	Interval 2 <sup>b</sup>
Dam	<0.0001	<0.0001
Sire	0.0001	0.0048
Dam × Sire	0.9617	0.5235
Group	<0.0001	0.1135
Tank (Group)	<0.0001	<0.0001
Dam × Tank (Group)	0.5453	0.7389
Sire × Tank (Group)	0.2178	0.1198
Dam × Sire × Tank (Group)	0.8108	0.6183
Heritability ( $h^2_f$ ) <sup>c</sup>		
Dam	0.82 ± 0.10	0.69 ± 0.11
Sire	0.66 ± 0.19	0.64 ± 0.14
Number of fish	782	701

<sup>a</sup> Interval 1: Day 152 – Day 269 {A} and Day 201 – Day 318 {B}

<sup>b</sup> Interval 2: Day 152 – Day 389 {A} and Day 201 – Day 389 {B}

<sup>c</sup>  $h^2_f$  is heritability estimated on a family-mean basis.

Table 7. Probability of significance of genetic and environmental effects (without interactions), based on ANOVA, and estimates of heritability ( $h^2 \pm \text{s.e.}$ ), based on *Model 2b*, for growth rate measured at two intervals during the growth trial

Effect	Growth Rate	
	Interval 1 <sup>a</sup>	Interval 2 <sup>b</sup>
Dam	<0.0001	<0.0001
Sire	0.0015	<0.0001
Dam $\times$ Sire	0.8360	0.7256
Group	<0.0001	0.0154
Tank (Group)	<0.0001	<0.0001
Heritability ( $h^2_i$ ) <sup>c</sup>		
Dam	0.54 $\pm$ 0.18	0.31 $\pm$ 0.13
Sire	0.17 $\pm$ 0.07	0.37 $\pm$ 0.13
Heritability ( $h^2_f$ ) <sup>c</sup>		
Dam	0.69 $\pm$ 0.12	0.82 $\pm$ 0.09
Sire	0.69 $\pm$ 0.13	0.81 $\pm$ 0.08
Number of fish	782	701

<sup>a</sup> Interval 1: Day 152 – Day 269 {A} and Day 201 – Day 318 {B}

<sup>b</sup> Interval 2: Day 152 – Day 389 {A} and Day 201 – Day 389 {B}

<sup>c</sup>  $h^2_i$  is heritability estimated on an individual basis;  $h^2_f$  is heritability estimated on a family-mean basis.

component of variance and 0.98 based on the sire component of variance; the phenotypic correlation was 0.79.

### 3.5. *General and specific combining abilities*

Estimates (and standard errors) of GCA for the ten dams and ten sires on growth rate at Intervals 1 and 2 are shown in Figures 1 and 2, respectively, and revealed considerable differences in general combinability among both dams and sires. Estimated GCA values for individual dams and sires were consistent at both time intervals; GCA values for dams, however, were generally higher at Interval 1, while GCA values for sires were generally higher at Interval 2. Positive GCA values were obtained for dams 4, 6, and 9, and for sires 2, 8, 9, and 10. GCA values at both intervals differed from zero by more than one standard error for dams 6 and 9 (Figure 1) and for sires 2, 9, and 10 (Figure 2). Estimates of SCA for each possible dam  $\times$  sire combination (96 pairwise combinations total) did not differ significantly from zero (data not shown).

## 4. Discussion

### 4.1. *Parentage assignment*

The ten polymorphic microsatellites used in this project yielded unequivocal parentage (sire and dam) assignment for >95% of the 881 progeny assayed and eliminated the need for separate rearing of full- or half-sib families that can confound genetic and environmental effects (Vandeputte et al., 2004). Similar results have been obtained in studies of other farmed fish species including rainbow trout, Atlantic

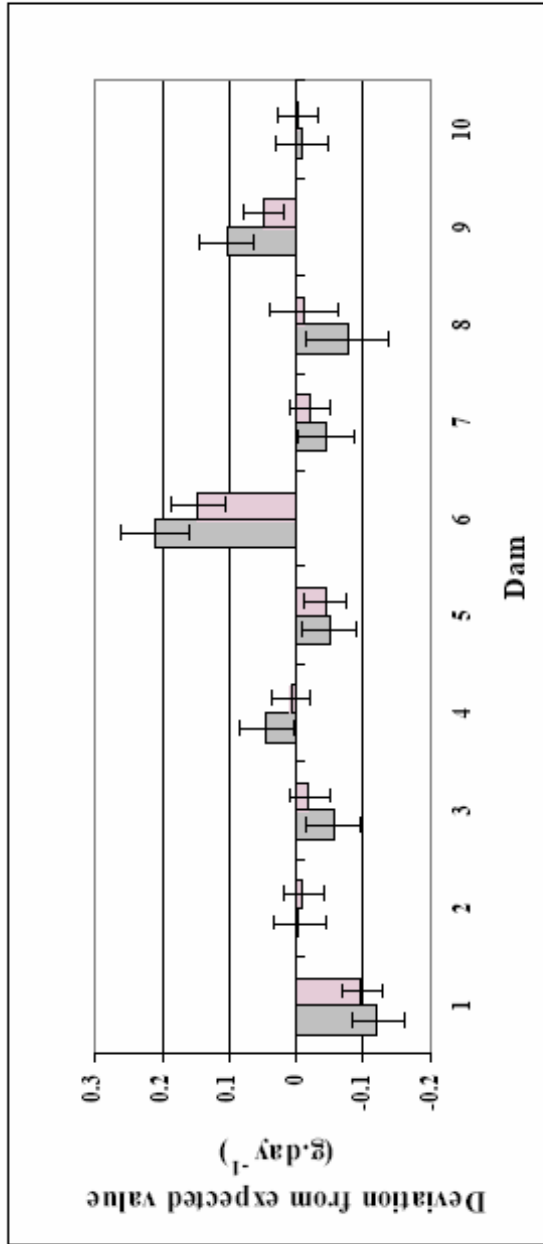


Fig. 1. Estimates of General Combining Ability (GCA) for growth rates for each of ten dams at Intervals 1 & 2 (cf Tables 6 and 7). Interval 1 (solid), Interval 2 (cross-hatched); error bars represent one standard error.

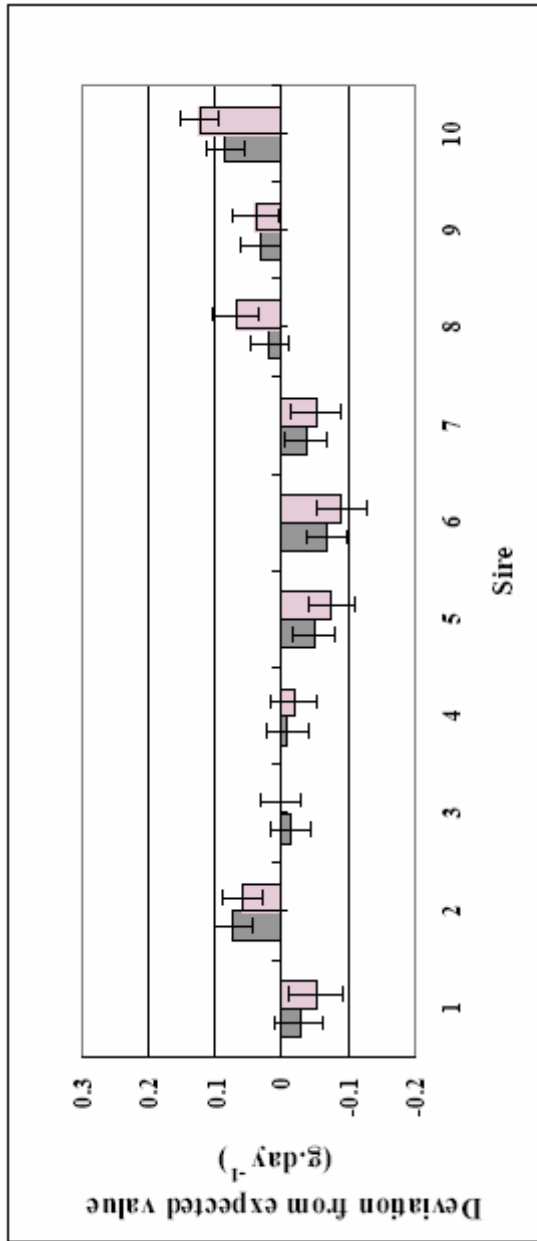


Fig. 2. Estimates of General Combining Ability (GCA) for growth rates for each of ten sires at Intervals 1 & 2 (cf Tables 6 and 7). Interval 1 (solid), Interval 2 (cross-hatched); error bars represent one standard error.



salmon, and common carp (Herbinger et al., 1995; Norris et al., 2000; Vandeputte et al., 2004). In addition, progeny were recovered from 96 of the 100 possible dam x sire combinations, with the number of progeny recovered per dam and per sire in this study being relatively uniform. We attribute the success in recovering representative progeny in part to the mating strategy where equal aliquots of eggs from each dam were separated and fertilized separately with equal aliquots of milt from each male. Such a strategy potentially reduces possible bias caused by unequal fertilization and/or hatching success (Fishback et al., 2002).

#### *4.2. Genetic and non-genetic effects on growth and growth rates*

Significant dam and sire effects on both weight and length were found prior to initiation of the growth trials when fish had reached an average weight and length of ~23g and 120mm (Group A) and ~32g and 134mm (Group B), respectively. For both traits, dam effect was approximately two-fold greater than sire effect, with estimates of broad-sense individual basis heritability ( $h^2_i$ ) ranging from 0.14 to 0.17 for dams and 0.07 to 0.08 for sires; the dam x sire interaction (both traits), however, was non-significant. Significant dam and sire effects for the same growth parameters also were found at two measurement ages subsequent to initiation of the growth trials. Values of  $h^2_i$  for weight and length, respectively, ranged from 0.41 to 0.45 for dams and 0.11 to 0.15 for sires (measurement age 1), and 0.30 to 0.32 for dams and 0.28 (both traits) for sires (measurement age 2). For both traits, dam effect was considerably greater than sire effect at the first measurement age, but not the second; the dam x sire interaction (both traits) was non-significant at both measurement ages. Nearly identical results were

obtained with growth rates estimated at each of two time intervals measured subsequent to initiation of the growth trials; dam and sire effect(s) were significant at both intervals, whereas dam x sire interaction was not.

To our knowledge, this study is the first to report the magnitude of genetic effects on body weight and length and growth rate in hybrid striped bass. Significant dam and sire effects on early growth have been reported for other cultured fish species, including rainbow trout (Gall and Huang, 1988; Herbinger et al., 1995; Wangila and Dick, 1996; Fishback et al., 2002), common carp (Vandeputte et al., 2004), Nile tilapia (Gall and Bakar, 2002) and European sea bass (Garcia de Leon et al., 1998; Saillant et al., 2006). In several of these studies (Herbinger et al., 1995; Wangila and Dick, 1996; Garcia de Leon et al., 1998) dam effects on early growth were more pronounced than sire effects and it was hypothesized that this could be due to a maternal phenotypic effect. In a factorial design, dam effect is expected to include both additive-genetic effects and maternal phenotypic effects (Falconer and Mackay, 1996). It also is commonly observed that maternal effects lessen with age in fish (Resftie, 1980; Herbinger et al., 1995; Garcia de Leon et al., 1998), and this could account for the observation in our study that dam effects were greater than sire effect only during early growth stages. However, methods to estimate maternal effects in factorial designs rely on the assumption that covariances between paternal and maternal half-sibs are equivalent with respect to inherited nuclear genes (Lynch and Walsh, 1998). Such an assumption cannot be made *a priori* in interspecies hybrids such as hybrid striped bass, thus precluding estimation of phenotypic maternal effects (Chevassus, 1983). Further characterization of

maternal phenotypic and genetic effects, using an appropriate experimental design, is clearly warranted.

Effect of group on growth (weight and length) prior to initiation of the growth trial and, with few exceptions, effect of both group and tank on growth and growth rate during the growth trial were highly significant ( $P < 0.005$  in most cases). The exceptions were effect of group on length at Measurement period 1 and on growth rate at Interval 2 when using *Model 2a*. The significant effect of group was likely an indication of age, at least in part, as fish in the two groups (A and B) were measured at different ages except for the final measurement (Measurement period 2). Variation in growth rate with increasing age in hybrid striped bass has been reported previously (Hodson, 1989). The highly significant effect of tank (found in all experiments) was perhaps a function of different rearing densities and the concomitant behavioral interactions among fish maintained in the same tank. This has been shown in other studies of other cultured fishes, including Arctic charr (Jobling and Wandsvik, 1983; Jobling and Reinsnes, 1986) and rainbow trout (Bagley et al., 1994).

#### 4.3. *Genetic and phenotypic correlations {growth and growth rates}*

Genetic and phenotypic correlations between weight and length for both dams and sires over both measurement ages ranged from 93-99%. Genetic correlations between growth rates at the two measurement periods also were high for both dams and sires (0.98-0.99). High genetic and phenotypic correlations between body weight and length have been reported in juveniles of other cultured fishes, including common carp (Vandeputte et al., 2004), Atlantic salmon (Gunnes and Gjedrem, 1978), rainbow trout

(Refstie, 1980; Fishback et al., 2002; Henryon et al., 2002), and chinook salmon (Winkelman and Peterson, 1994). High correlations between growth (body weight) measured at different stages (before sexual maturation) also have been reported in other cultured fish species, including rainbow trout (Su et al., 1996; 2002) and European sea bass (Saillant et al., 2006), and indicated that growth estimated at early stages could be used as a predictor of growth at later stages. Finally, the magnitude of genetic correlations between traits is thought to generally reflect the extent to which the same genes are involved in expression of the traits (Falconer and Mackay, 1996). Taken together, the high genetic and phenotypic correlations found in our study indicate that a selective breeding program for either (or both) traits in hybrid striped bass would result in increased fish size (weight and length) at juvenile stages and potentially larger fish at harvest.

#### *4.4. Heritability, combining ability, and selection strategy*

The significant sire and dam components of phenotypic variance of growth traits in the interspecies F<sub>1</sub> hybrids evaluated in this study indicate that selective breeding could be implemented in either or both parental species. In such a breeding program, however, candidate dams and sires can only be evaluated based on the performance of their crossbred offspring. Combining ability analysis for growth rate revealed significant differences in GCA values among both dams and among sires at both growth intervals measured; SCA values for each possible dam × sire combination, however, did not differ significantly from zero. General Combining Ability (GCA) primarily reflects additive-genetic effects, whereas Specific Combining Ability (SCA) primarily reflects dominance,

additive-genetic x dominance, and dominance x dominance interaction effects (Sprague and Tatum, 1942; Falconer, 1981). Results of the present study indicate that additive-effect genes contributed to most of the genetic differences in juvenile growth of hybrid striped bass.

In order to predict response to selection implemented on the basis of progeny testing, heritability of growth traits were estimated on a family-mean basis for both dams and sires. These estimates of heritability, with one exception, exceeded 0.58 for growth (weight and length) and growth rate for both dams and sires, and differed significantly from zero at developmental times measured both prior and subsequent to initiation of the growth trial. The exception was heritability of sire on weight and length prior to initiation of the growth trial, where estimates of  $h^2_f$  were 0.46 (weight) and 0.43 (length). Estimates of heritability for growth rates at the two time intervals essentially paralleled those for body weight and length, i.e.,  $h^2_f$  estimates for both dams and sires exceeded 0.69 and differed significantly from zero. In addition, estimates of  $h^2_f$  for dams were invariably higher than estimates for sires for all traits at earlier developmental stages, i.e., prior to initiation of the growth trial and at the first time interval after initiation of the trial.

The estimates of heritability ( $h^2_i$  and  $h^2_f$ ) should be interpreted in the context of a specific selection program. Because  $F_1$  rather than  $F_2$  progeny are the production unit in commercial production of hybrid striped bass (Hallerman, 1994), heritability estimated on an individual basis ( $h^2_i$ ) has little meaning in the context of a selection program. Estimates of  $h^2_i$ , however, do reflect the magnitude of genetic effects on the variation in

phenotype among individuals, and in that sense indicate that there is a significant heritable component to growth and growth rate in hybrid striped bass. Heritability based on family-means ( $h^2_f$ ), on the other hand, could be used to predict the fraction of the selection differential to be gained in F<sub>1</sub> hybrids if breeders (white bass females and striped bass males) are selected based on crossbred progeny testing such as conducted in this study (Holland et al., 2003). The estimates of  $h^2_f$  obtained in this study thus indicate that a substantial fraction of the selection differential would be expected to be gained in growth-related traits in offspring of selected parents. Selection response and heritability, however, are dependent on the accuracy of the estimate of the combining ability of breeders. Consequently,  $h^2_f$  for the traits assessed here may differ in a selective breeding program where the number of breeders tested and number of progeny assayed differ from those employed in this study. Further assessment of the expected response to selection as a function of the number of parents tested, number of progeny measured, and magnitude of genetic variance may be warranted in order to design selective breeding programs for hybrid striped bass. Estimating phenotypic variance on a family-mean basis is commonly employed in plant breeding when inbred lines or different species are used to generate F<sub>1</sub> hybrids for production purposes (Betran and Hallauer, 1996; Wolf et al., 2000; Holland et al., 2003). In such situations, estimates of heritability for quantitative traits such as grain yield or plant height generally range from 0.6 to 0.9 (Betran and Hallauer, 1996; Wolf et al., 2000).

The estimates of heritability (both  $h^2_i$  and  $h^2_f$ ) in this study were broad- rather than narrow-sense, and hence include non-additive genetic effects due to dominance and

epistasis as well as additive effects. Narrow-sense heritability generally is of primary interest in classical selective breeding programs, in part because it measures the extent to which phenotypes of progeny can be predicted based on genes transmitted from parents (Falconer, 1981), and in part because dominance and epistatic effects, while typically present in first generation ( $F_1$ ) offspring, are partially lost in future generations (Argue et al., 2003). In hybrid striped bass, however, the  $F_1$  offspring are the production unit, meaning that both additive and dominance effects could be exploited in a selective breeding program (Hallerman, 1994). In this sense, a selective breeding program for production of hybrid striped bass could be to select parents (or parental lines) that produce better quality (faster growing)  $F_1$  offspring regardless of whether genetic effects were additive, dominant, or epistatic. In addition, the genetic disequilibrium generally expected in interspecific hybrids (Gordon, 1999) potentially renders estimates of narrow-sense heritability problematic.

The significant differences in GCA values among both dams and sires and the heritability estimates for growth (both dams and sires) suggest that ‘backward selection’ and reciprocal recurrent selection for increased growth and growth rate would be successful in producing hybrid striped bass. During ‘backward selection’ (Gordon 1999), parents with superior combining ability are identified and then used repeatedly to propagate desired offspring. Reciprocal recurrent selection or RRS (Falconer and Mackay, 1996) requires maintenance of ‘pure’ breeding lines of each parental species, where breeders are selected each generation within each parental line based on their crossbred performance. Backward selection does not require maintaining pure lines in

each species but genetic progress in this approach is lost when selected breeders are culled or die. RRS on another hand would allow accumulation of genetic progress from one generation of selection to the next. However, application of significant selection differential in selected lines while maintaining sufficient effective size in order to prevent inbreeding likely would involve high cost and significant infrastructure. Given the low margin and investment capacity of the current hybrid striped bass industry in the United States (J. Carlberg, personal communication to J. R. Gold), development of a RRS program may not be economically feasible in the short term. Further economic evaluation of both approaches for hybrid striped bass production is clearly warranted.



## CHAPTER III

### QUANTITATIVE GENETICS AND HERITABILITY OF CARCASS-QUALITY TRAITS IN HYBRID STRIPED BASS (*Morone chrysops* ♀ × *Morone saxatilis* ♂)

#### 1. Introduction

In the previous chapter, moderate to high levels of broad-sense heritability (estimated based on family means) for growth rate in hybrid striped bass were documented, suggesting that genetic improvement of this trait would be successful. However, selection of candidate breeders based on their estimated genetic value for growth rate could result in a correlated response in other traits that might be unfavorable for either consumers or fish farmers (Neira et al., 2004; Perry et al., 2004). Knowledge of genetic correlations among traits of economic importance is therefore critical when developing a selective breeding program, if only to prevent offsets in genetic gain from selection on single characters by economic losses in correlated traits (Falconer and Mackay, 1996).

Hybrid striped bass are sold in the U.S. market as whole fish and as processed products (fillet and gutted fish). Therefore, maximizing body weight and fillet yield and minimizing relative viscera weight are potentially important goals for hybrid striped bass aquaculture. Level of fat deposition in fish tissue is another trait of potential interest as it may influence market acceptance (Quinton et al., 2005).

The objective of this aspect of the study was to assess the potential for genetic improvement in various carcass-quality traits such as fillet yield, viscera weight, and visceral fat content. Correlated responses of these traits with growth rate also were examined. Heritability of each carcass-quality trait and genetic correlations between pairs of traits were estimated in full-sib families generated according to the same factorial design (10 white bass ♀ x 10 striped bass ♂) used in the study of heritability of growth-related traits (Chapter II). Carcass-quality traits were measured at 389 days post-fertilization; genotypes at ten nuclear-encoded microsatellites were used for parentage assignment. Variance and covariance components associated with genetic effects were estimated using mixed models and a Restricted Maximum Likelihood (REML) approach. Estimates of broad-sense heritability on an individual basis ( $h^2_i$ ) and on a family-mean basis ( $h^2_f$ ) were generated in order to assess, respectively, the magnitude of genetic effects on the variation in phenotype among individuals and to predict response to selection based on crosses of candidate breeders. Pairwise genetic and phenotypic correlations between quality traits and body weight also were estimated. Finally, estimates of general combining abilities of each trait for individual dams and sires and specific combining abilities for various dam x sire combinations were generated.

## **2. Materials and Methods**

### *2.1. Experimental fish*

Genetic parameters were estimated in offspring from ten white bass females crossed with ten striped bass males according to a full factorial design. Eggs from each white bass female were artificially fertilized with milt from each of ten striped bass males,

thereby generating 100 full-sib families. Fingerlings were incubated separately and then mixed for grow-out as described in Chapter II. At 152 days post-fertilization, 600 fish (Group A) were marked individually with PIT tags and equally divided into six 1200-L tanks connected to a recirculating system. Another group of 400 fish (Group B) were too small to be PIT-tagged at 152 days post-fertilization. They were further grown until 201 days post-fertilization when they were PIT-tagged and stocked into another four tanks connected to the same recirculating system. Fish were grown in the experimental units under the same conditions described in Chapter II. Sampling and measurement of phenotypic traits occurred 389 days post-fertilization. At that time, a small clip from the dorsal fin was removed and stored in 70% ethanol for subsequent parentage assignment. Details regarding parentage assignment are provided in Chapter II (Materials and Methods, *Section 2.2.*).

## *2.2. Trait measurement*

Five hundred fish were selected randomly from nine of the ten tanks at 389 days post-fertilization and euthanized by an overdose of tricaine methane sulfonate (MS-222). Each fish was weighted to the nearest gram (g) and total length was measured to the nearest millimeter (mm). Fillets were obtained manually by cutting along the rib cage and processing to the tail; fillets were weighed to the nearest gram. Fish were eviscerated and the liver and intraperitoneal fat excised and weighed; total viscera from each fish also was weighted. The phenotypic sex of each fish was recorded by visual examination of the gonads. Testes were identified based on their triangular section and high density; ovaries were recognized based on their tubuliform section, pink color,

and occurrence of an ovarian cavity. The following traits were recorded: body weight (BW), body length (BL), fillet weight (FW), viscera weight (VW), intraperitoneal fat weight (IFW), liver weight (LW) and gonad weight (GW). Also recorded were measures of these traits standardized (normalized) to body weight. The reason for the latter is that in a selective breeding program that uses body weight as the selective criterion, the proportion of these carcass-quality traits are the correlated traits of interest. The ‘normalized’ traits were as follows: fillet percentage (FP), estimated as  $FW \times 100/BW$ ; viscerosomatic index (VSI), estimated as  $VW \times 100/BW$ ; intraperitoneal fat percentage (IFP), estimated as  $IF \times 100/BW$ ; hepatosomatic index (HSI), estimated as  $LW \times 100/BW$ ; and gonadosomatic index (GSI), measured as  $GW \times 100/BW$ . Finally, Fulton’s condition factor ( $K$ ) was recorded for each fish.  $K$  was estimated (after Ricker 1975) as  $BW \times 10^5 / BL^3$ .

### 2.3. Statistical analysis

#### 2.3.1. Genetic and non-genetic effects on carcass-quality traits

All carcass-quality traits were assessed according to *Model 3* (below)

$$Y_{ijklmn} = \mu + dam_i + sire_j + (dam \times sire)_{ij} + G_k + T(G)_{kl} + Sex_m + \varepsilon_{ijklmn}$$

where  $Y_{ijklmn}$  is an individual observation,  $\mu$  is the overall mean,  $dam_i$  represents the random effect of the  $i^{\text{th}}$  dam,  $sire_j$  represents the random effect of the  $j^{\text{th}}$  sire,  $(dam \times sire)_{ij}$  represents the random interaction between the  $i^{\text{th}}$  dam and  $j^{\text{th}}$  sire,  $G_k$  represents the fixed effect of the  $k^{\text{th}}$  group,  $T(G)_{kl}$  represents the fixed effect of the  $l^{\text{th}}$  tank nested within the  $k^{\text{th}}$  group,  $sex_m$  represents the fixed effect of sex, and  $\varepsilon_{ijklmn}$  is the random residual.

Significance of random and fixed effects was determined by analysis of variance (ANOVA); sum of squares for the effects tested were estimated using Type IV estimable functions in SAS.

Variance components and their standard errors were estimated based on *Model 3* and using the REML algorithm implemented in VCE-5 (Groeneveld, 1998). Estimates of broad-sense heritability on an individual basis ( $h^2_i$ ) were computed as four times the ratio of the dam or sire component of variance to the total phenotypic variance; standard errors of  $h^2_i$  were computed as four times of the standard error of the ratio of the dam or sire component of variance to the total phenotypic variance, as estimated using VCE-5. Broad-sense heritability based on family means ( $h^2_f$ ) was estimated as the proportion of the variance of dam half-sibs or sire half-sibs relative to the phenotypic variance of family means for a particular trait. Estimates of  $h^2_f$  values followed equations given in Chapter II (Material and Methods, *Section 2.5.2*). Approximate standard errors for estimates of  $h^2_f$  employed the 'delta' method described in Hohls (1996). REML estimates of variance components and associated variances and covariances used to compute  $h^2_f$  and their standard errors were obtained from PROC MIXED in SAS.

To examine the effect of sex on body weight and the carcass-quality traits, the magnitude of the difference between female and male progeny from the same family was estimated employing *Model 3* and using Best Linear Unbiased Estimators (BLUE) analysis in PEST 4.2.3 (Groeneveld and Kovac, 1990).

### 2.3.2. *Phenotypic and genetic correlations*

Pairwise phenotypic correlations among traits were estimated via Pearson's product-moment correlation coefficient, as implemented in the Statistical Package for Social Sciences (SPSS INC., 2001). Two-trait REML analysis in VCE-5 (Groeneveld, 1998) was used to estimate genetic correlations (and their standard errors) between pairs of traits. Genetic correlations were based on dams and sires, respectively, and employed *Model 3*. The dam x sire interaction effect was not included in two-trait analysis when the variance component associated with this interaction effect did not differ significantly from zero in single-trait analysis. Because estimates of dam and/or sire genetic correlations between some of the traits assessed were unity, standard errors for the correlations between the two traits could not be estimated.

### 2.3.3. *Combining abilities*

Best Linear Unbiased Predictors (BLUP) of individual dams, sires, and crosses, and their respective standard errors, were generated in PEST 4.2.3 (Groeneveld and Kovac, 1990) and used as estimates of GCAs of dams and sires and SCAs of crosses. Single-trait analyses were performed to generate BLUPs for each non-standardized and standardized carcass-quality trait, using *Model 3*.

## 3. Results

### 3.1. *Parentage assignment of offspring*

Data were recorded for a total of 474 tagged fish. Of these, 448 were assigned to a single parental pair, based on multilocus genotypes; the remaining 26 fish were

Table 8. Number of offspring identified for each of 100 full-sib families generated in the 10 x10 factorial cross

Cross	Dam 1	Dam 2	Dam 3	Dam 4	Dam 5	Dam 6	Dam 7	Dam 8	Dam 9	Dam 10	Sum
Sire 1	2	2	3	4	2	0	1	0	10	0	24
Sire 2	3	7	10	8	4	6	6	0	10	5	59
Sire 3	3	4	4	8	4	3	3	2	9	4	44
Sire 4	1	0	5	9	5	1	5	1	2	4	33
Sire 5	3	8	3	2	2	1	1	1	6	9	36
Sire 6	5	6	3	6	7	1	2	1	4	2	37
Sire 7	2	3	5	1	4	0	3	0	4	5	27
Sire 8	1	5	11	8	3	3	2	0	12	13	58
Sire 9	2	4	0	7	7	2	5	1	6	2	36
Sire 10	3	11	14	17	9	5	10	3	18	4	94
Unknown sire <sup>a</sup>	0	5	5	5	3	1	0	0	4	3	26
Sum	25	50	58	70	47	22	38	9	81	48	474

<sup>a</sup> Sire could not be determined (see text).

assigned to a single dam but could not be assigned to a single sire. The number of offspring from each full-sib family present in the sample is given in Table 8. A total of 91 of the possible 100 full-sib families were represented with at least one offspring, and all twenty dams and sires contributed to the sample. Contributions ranged from 1.9% (Dam 8) to 17.1% (Dam 9) for dams, and 5.1 % (Sire 1) to 19.8 % (Sire 10) for sires. Contribution of individual dams and sires to groups and tanks was relatively even with respect to tanks. Sex ratio among the progeny was balanced (236 females versus 238 males).

### *3.2. Genetic and non-genetic effects on carcass-quality traits*

Mean and standard deviation of body weight and the various carcass-quality traits measured at 389 days post-fertilization are given in Table 9 for all offspring and for females and males separately. The mean body weight (S.D.) in grams over all fish was 275.33 (75.03); mean body weight (S.D.) for females and males were 279.52(75.80) and 272.48 (69.88), respectively.

Results of tests of significance of genetic effects on the non-standardized carcass-quality traits and estimates of heritability (both  $h^2_i$  and  $h^2_f$ ) for each trait are shown in Table 10. There was a significant effect of dam ( $P < 0.05$ ) on body weight, fillet weight, and viscera weight; the effect of the sire was significant on body weight, fillet weight, and liver weight. The dam x sire interaction was not significant ( $P > 0.05$ ) for any trait. The estimate of the dam component of variance was approximately two-fold greater than that of sire for body weight, fillet weight, and viscera weight. Estimates of broad-sense heritability (individual basis,  $h^2_i$ ) for body weight and fillet weight were 0.44 for dams



Table 9. Mean (s.d.) of body weight and carcass-quality traits of hybrid striped bass measured at 389 days post-fertilization

Trait	Total (n=474)	Females (n=236)	Males (n=238)
Body weight (g)	275.33 (75.03)	279.52 (75.80)	272.48 (69.88)
Fillet weight (g)	98.56 (31.43)	100.33 (33.20)	96.81 (29.54)
Intraperitoneal fat weight (g)	13.61 (5.32)	14.18 (5.76)	13.18 (4.87)
Liver weight (g)	7.35 (2.50)	7.47 (2.68)	7.29 (2.31)
Total viscera weight (g)	29.33 (9.35)	30.80 (9.14)	28.02 (8.35)
Gonad weight (g)	1.02 (0.71)	1.60 (0.55)	0.46 (0.40)
Condition factor	1.39 (0.84)	1.40 (0.08)	1.39 (0.09)
Fillet percentage, FP	35.62 (4.56)	35.70 (4.16)	35.55 (4.92)
Intraperitoneal fat percentage, IFP	4.87 (1.19)	4.98 (1.17)	4.76 (1.20)
Hepatosomatic index, HSI (in %)	2.66 (0.46)	2.66 (0.46)	2.66 (0.47)
Viscerosomatic index, VSI (in %)	10.59 (1.35)	10.95 (1.29)	10.23 (1.30)
Gonadosomatic index, GSI (in %)	0.37 (0.23)	0.58 (0.12)	0.16 (0.11)

and differed significantly ( $>$ two standard errors) from zero; all other  $h^2_i$  estimates for dams were either non-significant or nearly so as were all estimates of  $h^2_i$  for sires (Table 10). Estimates of heritability based on family means ( $h^2_f$ ) for body weight and fillet weight were  $0.72 \pm 0.12$  and  $0.76 \pm 0.11$ , respectively, for dams, and  $0.60 \pm 0.16$  and  $0.54 \pm 0.17$ , respectively, for sires; all other  $h^2_f$  values (dams and sires) were non-significant except for dam effect ( $h^2_f = 0.56 \pm 0.21$ ) on viscera weight (Table 10).

There was an apparent effect of sex on body weight and on all other non-standardized carcass-quality traits (Table 10). However, mean values of these traits did not differ significantly between all female progeny and all male progeny except for gonad weight (Table 9) where average gonad weight for males (0.46 g) was well less than a third of that for females (1.60 g). The effect of tank on all carcass-quality traits was highly significant ( $P < 0.001$ ) except for gonad weight, while effect of group was not significant on any trait (Table 10).

Results of tests of significance of genetic effects on condition factor and standardized carcass-quality traits and estimates of heritability (both  $h^2_i$  and  $h^2_f$ ) for each trait are given in Table 11. There was a significant effect of dam on condition factor ( $K$ ), hepatosomatic index, and viscerosomatic index; effect of the sire was significant only on hepatosomatic index and viscerosomatic index. Dam and sire effects on all other traits, including fillet percentage, were non-significant. The dam x sire interaction was not significant ( $P > 0.05$ ) for any trait except viscerosomatic index ( $P < 0.0001$ ), where the associated variance component accounted for 7.4% of the total phenotypic variance. All estimates (dam and sire) of broad-sense heritability (individual basis,  $h^2_i$ ) for condition

Table 10. Probability of significance of genetic and non-genetic effects, based on ANOVA, and estimates of heritability ( $h^2 \pm$  s.e.), based on *Model 3*, for body weight and non-standardized carcass-quality traits

Effect	Body weight	Fillet weight	Intraperitoneal fat weight			Liver weight	Viscera weight	Gonad weight
Dam	0.0007	0.0011	0.0901	0.4645	0.0181	0.6734		
Sire	0.0201	0.0239	0.2194	0.0050	0.0906	0.1325		
Dam x Sire	0.9839	0.9655	0.6144	0.9591	0.8054	0.3599		
Sex	0.0401	0.0094	0.0121	0.0185	<0.0001	<0.0001		
Group	0.4057	0.8114	0.3029	0.5613	0.4618	0.1724		
Tank (Group)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1541		
Heritability ( $h^2$ ) <sup>a</sup>								
Dam	0.44 $\pm$ 0.12	0.44 $\pm$ 0.16	0.08 $\pm$ 0.08	NA	0.20 $\pm$ 0.08	NA		
Sire	0.20 $\pm$ 0.08	0.16 $\pm$ 0.08	0.00 $\pm$ 0.00	0.32 $\pm$ 0.12	0.08 $\pm$ 0.08	NA		
Heritability ( $h^2$ ) <sup>a</sup>								
Dam	0.72 $\pm$ 0.12	0.76 $\pm$ 0.11	0.37 $\pm$ 0.28	NA	0.56 $\pm$ 0.21	NA		
Sire	0.60 $\pm$ 0.16	0.54 $\pm$ 0.17	0.06 $\pm$ 0.29	0.66 $\pm$ 0.40	0.37 $\pm$ 0.24	NA		
Number of fish	448							

<sup>a</sup>  $h^2_i$  is heritability estimated on an individual basis;  $h^2_f$  is heritability estimated on a family-mean basis.

Table 11. Probability of significance of genetic and non-genetic effects, based on ANOVA, and estimates of heritability ( $h^2 \pm$  s.e.), based on *Model 3*, for condition factor ( $K$ ) and standardized carcass-quality traits

Effect	Condition factor ( $K$ )	Fillet percentage	Intraperitoneal fat percentage	Hepatosomatic Index	Viscero-		Gonado-somatic index
					somatic index	somatic index	
Dam	0.0033	0.3722	0.1563	<0.0001	0.0093	0.3019	
Sire	0.3604	0.7349	0.2890	0.0008	<0.0001	0.6318	
Dam x Sire	0.7088	0.7371	0.8976	0.0507	<0.0001	0.5119	
Sex	0.8783	0.2354	0.0754	0.4303	<0.0001	<0.0001	
Group	<0.0001	0.0011	0.3131	<0.0001	<0.0001	<0.0001	
Tank (Group)	<0.0001	<0.0001	0.0043	<0.0001	<0.0001	0.0003	
Heritability ( $h^2$ ) <sup>a</sup>							
Dam	0.16 $\pm$ 0.08	NA	0.12 $\pm$ 0.08	0.28 $\pm$ 0.12	0.04 $\pm$ 0.04	NA	
Sire	0.08 $\pm$ 0.08	NA	0.20 $\pm$ 0.08	0.24 $\pm$ 0.08	0.12 $\pm$ 0.08	NA	
Heritability ( $h^2$ ) <sup>a</sup>							
Dam	0.51 $\pm$ 0.20	NA	0.30 $\pm$ 0.23	0.64 $\pm$ 0.15	0.14 $\pm$ 0.33	NA	
Sire	0.27 $\pm$ 0.28	NA	0.54 $\pm$ 0.18	0.64 $\pm$ 0.16	0.39 $\pm$ 0.20	NA	
Number of fish	448						

<sup>a</sup>  $h^2$  is heritability estimated on an individual basis;  $h^2_f$  is heritability estimated on a family-mean basis.

factor and standardized carcass-quality traits were either non-significant or nearly so (Table 11). Estimates of heritability based on family means ( $h^2_f$ ) differed significantly (dams and sires) for condition factor ( $0.72 \pm 0.11$ , dams;  $0.60 \pm 0.17$ , sires) and hepatosomatic index ( $0.64 \pm 0.15$ , dams;  $0.64 \pm 0.16$ , sires). All other  $h^2_f$  values were non-significant except for sire effect on intraperitoneal fat percentage ( $0.54 \pm 0.18$ ).

The effect of sex on viscerosomatic index and gonadosomatic index was significant (Table 11). However, mean values of the standardized quality traits did not differ significantly between all female progeny and all male progeny except for gonadosomatic index (Table 9). Effect of tank was significant on all traits, as was effect of group except for intraperitoneal fat percentage (Table 11).

### 3.3. Phenotypic and genetic correlations

Phenotypic ( $r_p$ ) and genetic ( $r_g$ ) correlations between body weight and the various carcass-quality traits (non-standardized and standardized) are shown in Table 12.

Phenotypic correlations between body weight and all non-standardized carcass-quality traits were high (range = 0.80 - 0.92) and significant, while phenotypic correlations between body weight and all of the standardized carcass-quality traits except for hepatosomatic index, although positive and significant, were relatively low (range = 0.14 - 0.19). The phenotypic correlation between body weight and hepatosomatic index ( $r_p = 0.07$ ) was non-significant, whereas the phenotypic correlation between body weight and condition factor ( $r_p = 0.62$ ) differed significantly from zero (Table 12).

Genetic correlations between body weight and non-standardized traits (both dams

Table 12. Phenotypic and genetic correlations ( $\pm$  s.e.) between body weight and carcass-quality traits

Trait	Body weight	
	Phenotypic correlation	Genetic correlation (Sire)
Fillet weight	0.92*	0.99 $\pm$ 0.01
Intraperitoneal fat weight	0.80*	1.00
Liver weight	0.85*	1.00
Total viscera weight	0.92*	0.99 $\pm$ 0.01
Gonad weight	0.48*	0.99 $\pm$ 0.01
Condition factor ( $K$ )	0.62*	0.51 $\pm$ 0.25
Fillet percentage	0.15*	NA
Intraperitoneal fat percentage	0.19*	-0.77
Hepatosomatic index	0.07	-1.00
Viscerosomatic index	0.13*	-0.95 $\pm$ 0.25
Gonadosomatic index	0.08	NA

\*  $P < 0.001$ , NA: not available

and sires) were high (range = 0.87 - 1.00) except for the correlation ( $r_g = 0.37$ ) in sires with intraperitoneal fat weight (Table 12). The genetic correlations between body weight and condition factor were  $0.51 \pm 0.25$  (dam) and  $0.73 \pm 0.20$  (sire) and differed significantly from zero. Genetic correlations between body weight and standardized carcass-quality traits (both dams and sires) were relatively high but negative, ranging from  $-0.70$  to  $-1.00$  (Table 12). The exception was the genetic correlation between body weight and hepatosomatic index in sires ( $r_g = 0.67$ ).

#### *3.4. Combining abilities*

Estimates (and standard errors) of General Combining Ability (GCA) for the ten dams and ten sires for body weight, fillet weight, intraperitoneal fat percentage, hepatosomatic index, and viscerosomatic index are presented in Table 13. Positive and significant (means greater than two standard errors) GCA values for body weight and fillet weight were found for Dam 6 and Sire 10, whereas negative (and significant) values were found for Dams 1 and 5. In addition, a significant and negative GCA value for body weight was found for Sire 6. Significant, negative GCA values were found for intraperitoneal fat percentage (Dam 10 and Sire 10) hepatosomatic index (Dam 10 and Sire 5), and viscerosomatic index (Sire 10), while significant, positive values were found for hepatosomatic index (Dam 1 and 5 and Sires 9) and viscerosomatic index (Sire 6). Of interest is that Sire 10 had a significant, positive GCA values for body weight but significant, negative GCA values for intraperitoneal fat percentage and viscerosomatic index. Estimates of GCA values (for both dams and sires) for the remaining carcass-quality traits (non-standardized and standardized) and

condition factor did not differ significantly from zero (data not shown). With one exception, estimates of Specific Combining Ability (SCA) for each possible dam × sire combination (91 pairwise combinations total) for all carcass-quality traits (standardized and non-standardized ) also did not differ significantly from zero (data not shown). The exception was for viscerosomatic index in the cross of Dam 1 x Sire 4 where the estimated SCA value was  $6.53 \pm 3.04$ .

#### **4. Discussion**

##### *4.1. Genetic and non-genetic effects on carcass-quality traits*

Significant effects of both dam and sire were found for body weight and fillet weight at 389 days post-fertilization. The significance of the genetic effects on fillet weight, however, is due largely to a scale effect given the absence of a significant dam or sire effect on fillet percentage. Significant dam or sire effects also were found for liver weight (sire) and viscera weight (dam). These genetic effects were not due solely to a scale effect, as significant dam and sire effects were found for both hepatosomatic index and viscerosomatic index. No significant genetic effect was found for intraperitoneal fat weight, gonad weight, intraperitoneal fat percentage, and gonadosomatic index; a significant dam x sire interaction effect was found only for viscerasomatic index. Finally, a significant dam effect was found for condition factor. A genetic basis for the carcass-quality traits fillet weight, intraperitoneal fat weight, condition factor, and/or fillet has been reported in other cultured fish, including salmonids (Gjedrem, 1997; Kause et al., 2002; Neira et al., 2004; Quinton et al., 2005),



Table 13. Estimates and standard errors (s.e.) of General Combining Ability (GC.A) for body weight (BW), fillet weight, intraperitoneal fat percentage (IFP), hepatosomatic index (HSI), and viscerosomatic index (VSI) for each of ten dams and ten sires. GC.A values in bold differ significantly from zero ( $P < 0.05$ )

Trait	BW (g)		FW (g)		IFP (%)		HSI (%)		VSI (%)	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Dam 1	<b>-24.18</b>	<b>11.38</b>	<b>-9.56</b>	<b>4.82</b>	0.18	0.16	<b>0.16</b>	<b>0.07</b>	0.48	0.94
Dam 2	5.16	9.86	3.06	4.12	0.04	0.14	-0.03	0.06	0.04	0.91
Dam 3	-3.51	9.58	0.74	4.00	-0.06	0.14	-0.04	0.05	-0.27	0.90
Dam 4	-2.59	9.22	-0.02	3.84	0.20	0.13	0.03	0.05	0.28	0.89
Dam 5	<b>-23.78</b>	<b>9.91</b>	<b>-9.76</b>	<b>4.14</b>	0.07	0.14	<b>0.15</b>	<b>0.06</b>	0.54	0.91
Dam 6	<b>34.74</b>	<b>11.65</b>	<b>16.74</b>	<b>4.16</b>	0.04	0.16	-0.05	0.07	0.04	0.95
Dam 7	-1.23	10.38	-0.54	4.36	-0.01	0.15	-0.04	0.06	0.11	0.92
Dam 8	-1.93	14.21	-6.16	6.10	-0.09	0.18	0.01	0.08	-0.17	0.98
Dam 9	12.31	9.03	4.52	3.76	-0.06	0.13	-0.07	0.05	-0.07	0.88
Dam 10	4.99	9.94	0.98	4.16	<b>-0.30</b>	<b>0.14</b>	<b>-0.11</b>	<b>0.06</b>	-0.97	0.91
Sire 1	-9.91	9.34	-0.92	3.64	0.03	0.19	-0.07	0.06	0.74	1.67
Sire 2	6.93	7.66	1.96	3.02	0.13	0.16	0.08	0.05	1.82	1.47
Sire 3	-6.14	8.13	-3.26	3.20	-0.10	0.17	-0.06	0.05	0.27	0.15
Sire 4	5.83	8.72	1.10	3.42	0.20	0.18	0.03	0.06	0.49	1.60
Sire 5	-12.29	8.56	-3.68	3.36	0.27	0.17	<b>-0.12</b>	<b>0.06</b>	0.09	0.16
Sire 6	<b>-14.96</b>	<b>6.48</b>	-5.78	3.38	0.22	0.17	-0.06	0.06	<b>0.49</b>	<b>0.16</b>
Sire 7	-0.84	9.08	-1.92	3.56	-0.08	0.18	0.06	0.06	-0.75	1.63
Sire 8	8.81	7.70	3.72	3.04	-0.08	0.16	0.05	0.05	0.44	1.49
Sire 9	2.65	8.57	1.38	3.36	-0.16	0.17	<b>0.12</b>	<b>0.06</b>	-0.14	1.58
Sire 10	<b>19.93</b>	<b>6.95</b>	<b>7.40</b>	<b>2.74</b>	<b>-0.45</b>	<b>0.14</b>	-0.04	0.05	<b>-3.42</b>	<b>1.40</b>

tilapia (Rutten et al., 2005) and catfish (Argue et al., 2003).

The effect of sex at 389 days post-fertilization was significant ( $P < 0.05$ ) for all of the non-standardized traits including body weight. The significance of the sex effect on fillet weight, intraperitoneal fat weight, and liver weight, however, also appears to be due in large part to a scale effect as a significant sex effect was found only for viscerosomatic index and gonadosomatic index. The effect of sex on the non-standardized traits appeared to be due to faster growth (leading to larger body size) in females, as females had a 4.36% higher body weight, 5.93% higher fillet weight, 8.89% higher intraperitoneal fat weight, and 18.51% higher viscera weight. The larger female body size, however, did not appear to affect condition factor, as the sex effect on this trait was non-significant. Female progeny also had larger gonads and higher gonadosomatic index than male progeny, and the low gonadosomatic index in males (<1%) indicated that there was hardly any development of male gonads at 389 days post-fertilization. Late sexual differentiation has been observed in European sea bass (*Dicentrarchus labrax*) where gonad differentiation in females occurs earlier than in males (Blázquez et al., 1998).

Differences in early growth between sexes prior to first sexual maturation have been reported in a few fish species (Craig, 2000; Saillant et al., 2001). Potential factors responsible for an early sexual dimorphism in growth include sex-specific genetic factors involved in somatic growth rate and/or early physiological mechanisms associated with sexual development (Saillant et al., 2001). Interestingly, Davis and

Ludwig (2004) reported that male hybrid striped bass grew faster than females during the first year, but that females were heavier at harvest when fish were two years old.

A significant effect of tank was found for all non-standardized and standardized carcass-quality traits assessed except gonad weight. A significant group effect was found on almost all of the standardized carcass quality-traits (intraperitoneal fat percentage excepted), while no significant effect of group was found for the non-standardized traits. Given that many environmental factors (i.e., temperature, dissolved oxygen, salinity, photoperiod, and nutrition) were fairly well controlled during the trial, the significant tank and group effects may have stemmed from other factors such as rearing densities. The latter have been shown to affect growth in other cultured fishes, including Arctic charr (Jobling and Wandsvik, 1983; Jobling and Reinsnes, 1986) and rainbow trout (Bagley et al., 1994).

#### *4.2. Phenotypic and genetic correlations*

Phenotypic and genetic correlations between body weight and all of the non-standardized carcass-quality traits except gonad weight generally were high; whereas correlations between body weight and the standardized traits were either low (phenotypic) or generally negative (genetic). The high correlations (phenotypic and genetic) between body weight and the non-standardized traits likely reflect a genetic basis for differences in growth such as has been reported for fillet weight in other cultured fish species, including rainbow trout (Kause et al., 2002), tilapia (Rutten et al., 2005), channel catfish (Dunham, 1987), and Coho salmon (Neira et al., 2004). Because the hybrid striped bass market in the U.S. consists primarily of whole fish or processed

products (fillet and gutted fish), selection for increased body and/or fillet weight could be realized by improving fillet yield at equal body weight, improving body weight at equal fillet yield, or both (Rutten et al., 2005). However, the high genetic correlation between body weight and fillet weight and the absence of genetic variance for relative fillet yield (fillet percentage) found in this study indicate that improvement of fillet weight in hybrid striped bass would be realized primarily by improvement of body weight at equal fillet yield.

Additional considerations for the hybrid striped bass market would be minimizing relative viscera weight and level of fat deposition. Fish viscera are generally a waste product (Neira et al., 2004), but might be an issue relative to the whole-fish market, while level of fat deposition might either shorten shelf-life (Frankel, 1996) or influence market acceptance (Quinton et al., 2005). The low phenotypic correlations and negative genetic correlations between body weight and viscerosomatic index and intraperitoneal fat percentage might indicate that selection for increased body weight does not necessarily lead to a significant increase in the proportion of either of these two, less desirable traits. Indeed, the negative genetic correlations ( $r_g = -0.95$  {dam} and  $-0.70$  {sire} for viscerosomatic index;  $r_g = -0.77$  {dam} and  $-1.00$  {sire} for intraperitoneal fat percentage) may suggest that selection for increased body weight might generate a decrease in the proportion of these two traits. However, a discrepancy between phenotypic and genetic correlations is unusual (Lynch and Walsh, 1998) and may simply reflect a low genetic variance for the traits (Falconer and McKay, 1996).

Both phenotype and genetic (dam and sire) correlations between body weight and condition factor ( $K$ ) differed significantly from zero:  $r_p = 0.62$ ,  $r_g = 0.51$  {dam} and  $0.73$  {sire}.  $K$  is considered to be an approximate indicator of shape (Ricker, 1975) and to contribute to market acceptance (Ankorion et al., 1992). Longer, less deep-bodied fish (low  $K$  values) are preferred in some markets, while shorter, more deep-bodied (high  $K$  values) are preferred in other markets, e.g., those for salmonids (Barnham and Baxter, 1998). The observed phenotypic and genetic correlations between  $K$  and body weight indicate that selection for increased body weight would lead to a moderate increase in body depth.

#### *4.3. Heritability, combining ability, and selection strategy*

Estimates of family-mean heritability ( $h^2_f$ ) for both dam and sire for body weight and fillet weight were greater than 50 % and differed significantly from zero. Family-mean heritability reflects the fraction of the selection differential (the difference between the mean of a population and the mean of the individuals selected to be parents of the next generation {Falconer and Mackay, 1996}) that can be gained when selection is practiced on the parents (Holland et al., 2003). Thus, the moderate to high estimates of  $h^2_f$  for body weight and fillet weight indicates that genetic improvement of these traits in offspring would be feasible when selection is practiced on the parents. In addition, positive and significant general combining ability (GCA) values for body weight and fillet weight were found for Dam 6 and Sire 10, whereas negative (and significant) values were found for Dam 1, Dam 5, and (for body weight only) Sire 6.

The significant differences in GCA values among both dams and sires suggest that ‘backward selection’ for increased body weight and fillet weight would be successful in either parental species or both. The estimates of individual-basis heritability ( $h^2_i$ ) for body weight and fillet weight ranged from low to moderate (0.44 for dam and 0.16 - 0.20 for sire) and differed significantly from zero, confirming existence of a significant genetic component in these two traits among individuals.

Estimates of  $h^2_f$  greater than 50% also were found for viscera weight (dam only), condition factor (dam only), intraperitoneal fat percentage (sire only) and hepatosomatic index (both dam and sire). Estimates of individual-basis heritability ( $h^2_i$ ) for each of these also differed significantly from zero. Significant and positive GCA values were found for Dam 1, Dam 5, and Sire 9 (hepatosomatic index) and for Sire 6 (viscerosomatic index); whereas significant and negative GCA values were found for Dam 10 and Sire 10 (intraperitoneal fat percentage), for Dam 10 and Sire 5 (hepatosomatic index), and for Sire 10 (viscerosomatic index). While liver weight (and hepatosomatic index) are not traits of general interest to the hybrid striped bass industry, reducing both intraperitoneal fat percentage and viscera weight (and viscerosomatic index) would be of interest. The finding of significant, negative GCA values among dams and sires for these two traits indicates that ‘backward selection’ for reduced intraperitoneal fat and viscerosomatic index (i.e., proportionally reduced viscera weight) could be successful by selecting one parental line or both. The expected gain of selection, however, also would depend on the amount of selection differential that could be practiced in the parental populations available to hybrid striped bass farmers.

Estimates of family-mean heritability for all remaining carcass-quality traits did not differ significantly from zero, indicating that genetic gains for these traits would be very limited. Estimates of individual-basis heritability ( $h^2_i$ ) for all of these remaining traits also did not differ significantly from zero.

The single-trait GCA values of the ten dams and sires used in this study suggest that selection for multiple traits of potential interest could be achieved. Progeny from Sire 10, for example, had a significantly lower intraperitoneal fat percentage, lower viscerasomatic index, and higher body weight. Such breeders potentially could be bred repeatedly (backward selection) to produce progeny that would have desired phenotypes for multiple traits. However, significant GCA values for favorable carcass-quality traits in the same breeder (dam or sire) occurred only in one or two of twenty (dams and sires) used in this study. Thus, genetic improvement in hybrid striped bass for multiple traits simultaneous likely will require further study and identification of appropriate breeder individuals.

## CHAPTER IV

### GENETIC VARIATION IN IMMUNE AND STRESS RESPONSE TRAITS AND RESISTANCE TO INFECTION BY *Streptococcus iniae* IN HYBRID STRIPED BASS (*Morone chrysops* ♀ x *Morone saxatilis* ♂)

#### 1. Introduction

Infection from streptococcal bacteria is a cause of significant economic losses to the aquaculture industry worldwide (Evans et al., 2000). In hybrid striped bass, streptococcal infections caused primarily by the pathogen *Streptococcus iniae* have increased markedly with intensification of aquaculture practices, resulting in an estimated loss in 2002 of ~\$2 million to hybrid striped bass producers (Ostland, 2003).

*Streptococcus iniae* is a gram positive, aerobic bacterium that has been isolated from brain, eye, or kidneys of naturally infected fish (Bowser et al., 1998). Symptoms in hybrid striped bass include orientation loss, corneal opacity, lethargy, and darkened skin (Perera et al., 1994; Evan et al., 2000). The prevalence of *S. iniae* infection among six hybrid-striped-bass farms in the U.S. averaged 2-3% among nursery and market-size fish and ~ 10% among fish at the grow-out stage (Shoemaker et al., 2001).

Improvement in resistance of hybrid striped bass to *S. iniae* infection via dietary supplementation or vaccination has been studied by Li and Gatlin (2003) and Li et al. (2004). The current vaccination procedure relies on injecting each individual fish with a strain of *S. iniae* attenuated via genetic engineering (Buchanan et al., 2005). This



procedure routinely results in successful protection of 90-100% of injected fish, but involves both significant labor and stress induced by handling each fish individually (John Buchanan, pers. comm.). In addition, the ecological consequences of using DNA vaccines on a large scale are still not known (Myhr and Dalmo, 2005).

Selective breeding is a promising and safe approach to improving disease resistance because of its prospects for prolonged and sustainable protection (Wiegertjes et al., 1996). Significant genetic variation for resistance to various pathogenic diseases has been reported in several fish species (Gjedrem et al., 1991; Beaumont, et al., 2003; Henryon et al., 2005), suggesting that selective breeding for improved resistance could be successful. Assessment of the potential for selection to improve resistance to *S. iniae* in hybrid striped bass requires knowledge of the level of genetic variation for this trait among cultured fish. This assessment can be implemented by means of disease-challenge tests where levels of resistance, determined by estimating survival rate after infection, are compared among distinct genetic groups (Wiegertjes et al., 1996). The objective of this study was to use disease-challenge assays to assess genetic variation for resistance to *S. iniae* among hybrid striped bass at the grow-out stage.

Another approach to genetic improvement of disease resistance in fish relies on improvement of immune- and/or stress-response traits. Here, selection for immune- or stress-response traits, assuming they are genetically determined and are correlated genetically with disease resistance, potentially could improve resistance to bacterial infection (Lund et al., 1995). It has been shown recently that blood neutrophil (an immune-response trait) is an important component in the defense to *Streptococcus*

infection in terrestrial animals (Buchanan et al., 2006). Neutrophils are involved in pathogen resistance via several mechanisms. First, they produce oxidative radicals that are highly toxic to invading bacteria (Brinkmann et al., 2004). Second, they produce lysozymes, potent immune enzymes that catalyze hydrolysis of  $\beta$  (1-4) glycosidic bonds of cell walls associated with gram positive bacteria such as *S. iniae*. Finally, granules of neutrophil produce myeloperoxidase, an enzyme that catalyzes conversion of hydrogen peroxide and chloride ions to hypochlorous acid, a potent compound that causes mortality of various microbes including bacteria, viruses, fungi, and both protozoan and helminth parasites. To date, there is no information on genetic variation relative to the levels of oxidative-radical production, lysozyme activity, and myeloperoxidase activity produced by neutrophils in cultured hybrid striped bass. The objective of this study was to assess phenotypic and genetic variation of these immune-response traits.

Stress-response traits also are considered as an indirect selection criterion for disease resistance because of a possible relationship with disease susceptibility (Ellis, 1999). In nature, stress response is viewed as an acute response that has evolved to enable fish to mobilize their energy reserves as they attempt to avoid or overcome an immediate threat (Donaldson 1981). In aquaculture, however, when environmental stress may be of a continuous (chronic) nature, stress response could have damaging effects on a fish's state of health by increasing its susceptibility to disease (Pickering, 1991). Stress-response traits from healthy fish thus may be used as indirect measurements of disease resistance (Fjalestad et al., 1993). Their value as selection marker in a breeding program, however, depends on the occurrence of significant

genetic variation and their correlation with disease resistance. Serum cortisol, a primary stress-related indicator, increases in fish after various types of external disturbance (Donaldson 1981), and a genetic basis for stress-related cortisol release has been demonstrated in salmonids (Fevolden et al., 1993; 1999; Pottinger and Carrick, 1999). Stress-related cortisol release could therefore be a relevant criterion if there is a correlation with disease resistance. Glucose levels in fish serum also appear to increase in response to both acute (Davis and Griffin, 2004) and chronic (Lankford et al., 2005) stress, and thus potentially could be used to characterize stress severity.

In this study, a disease-challenge experiment was carried out on hybrid striped bass generated from a factorial design (10 white bass ♀ x 10 striped bass ♂). Genetic effects on variation in both immune-response (neutrophil oxidative radical production, lysozyme activity, and myeloperoxidase activity) and stress-response (serum cortisol and glucose levels) traits were investigated.

## **2. Materials and Methods**

### *2.1. Physiological parameters*

#### *2.1.1. Experimental fish*

Fish used for analysis of immune-response and stress-response traits were derived from a factorial mating design where eggs from ten white bass females were fertilized with milt from each of ten striped bass males, establishing 100 full-sib families. Progeny were raised together in the same culture system following fertilization. A subset of fish was brought to the Aquaculture Research and Training Facility (ARTF) at Texas A&M University in College Station. A total of 1,000 fish (800 at 152 days post-

fertilization - Group A; and 400 at 201 days post-fertilization - Group B), were sampled, individually PIT-tagged, and transferred to 1200-L tanks for an 8-month growth period. A small clip from the dorsal fin was removed from each fish at tagging and stored in 70% ethanol for subsequent parentage assignment. Details regarding mating procedures, rearing condition, and parentage assignment may be found in Chapter II.

### *2.1.2. Preparation of blood and serum*

At the end of the growth trial, 450 fish from six tanks in Group A were randomly netted and anesthetized in 25 mg/l of tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, Mo) for 15 - 20 minutes. Approximately 1.5 - 2 ml of whole blood was collected from the caudal vein, using a 5-ml heparinized syringe, and transferred into 2-ml Eppendorf tubes. Approximately 100 $\mu$ l of whole blood was sub-sampled and used in analysis of neutrophil oxidative-radical production. The remaining blood sample was centrifuged at 2,750 x g for 5 minutes. The serum was collected following centrifugation and frozen at -80°C.

### *2.1.3. Assays*

Neutrophil oxidative-radical production was assayed by the Nitro Blue Tetrazolium (NBT) test as described by Siwicki et al. (1994). Blood and 0.2% NBT were mixed in equal proportion and incubated for 30 minutes at 25°C. Reduced formazan product was solubilized via addition of dimethyl formamide (SRL, India) at a concentration of 1 $\mu$ l per 50 $\mu$ l incubated product. The supernatant was then separated by centrifugation at 3000  $\times$  g for 5 minutes. The extent of NBT reduced was measured by

spectrophotometry at an optical density of 545 nm. Absorbance was converted to NBT units based on a standard curve of NBT diformazan/ml blood.

A turbidimetric assay, as described by Jorgensen et al. (1993), was used to quantify serum levels of lysozyme. The enzyme assay is based on decrease in absorbance of a cell suspension of *Micrococcus leisodeikticus*, following digestion with lysozyme in the presence of sodium ions. A total of 5µl of serum sample was added to a 3 ml of a suspension of *M. lysodeikticus* (Sigma). The optimum pH for the lysozyme reaction in hybrid stripe bass was determined empirically to be 6.01. Absorbance of the mixture was measured by spectrophotometry at 450nm at 0.5 minutes and 4.5 minutes after addition of the *M. lysodeikticus* suspension. Lysozyme activity was characterized as the decrease in absorbance during the time interval between the two measurements; one activity unit was defined as the amount of enzyme producing a decrease in absorbance of  $0.001 \text{ min}^{-1}$  (Jorgensen et al., 1993).

Myeloperoxidase present in serum was measured according to Rodríguez et al. (2003). A total of 15 ml of serum was diluted in 135 ml of Hanks' Balanced Salt Solution (HBSS), without calcium or magnesium, and mixed with 50 ml of 20 mM 3, 3', 5, 5'-tetramethylbenzidine hydrochloride (TMB, Sigma) and 5 mM hydrogen peroxide. The reaction was stopped after 2 minutes by addition 50 ml of 4 M sulphuric acid. Optical density was read at 450 nm by fluorimetry.

Serum cortisol levels were determined by radioimmunoassay (RIA) using the BioChem ImmunoSystems Cortisol Bridge kit (#14394, Polymedco, Cortlandt Manor, NY) and reported as ng/ml. Details of this method are provided by Davis and Griffin

(2004). Level of glucose in serum was determined by the Nelson-Somogyi method (Nelson, 1944; Somgyi, 1952; Hawk and Bernard, 1954), using a spectrophotometer. A total of 2.0 ml Somogyi reagent was added to 0.2ml of serum sample and placed in a boiling water bath for 15 minutes. After cooling, 2.0 ml of Nelson reagent were added to the above mixture and optical density (at 505 nm) read within 1-2 hour.

## *2.2. Disease challenge experiment*

### *2.2.1. Experimental fish*

Fish used in the disease-challenge experiment were derived from the same factorial mating design as described previously. A total of 450 randomly selected fish were transferred at 440 days post-fertilization to a 3800-L tank for a two-week conditioning period. Water quality was maintained through biological and mechanical filtration; salinity was maintained at 2-3 ppt, using well water and synthetic sea salt (Fritz Industries, Inc., Dallas, TX). Photoperiod was set to 12 h light/12h dark; aeration was supplied through air stones. Fish were fed daily to satiation by hand with a commercial striped bass diet (EXTRU 400; 40% crude protein, 10% lipid; Rangen, Inc., Angelton, TX).

### *2.2.2. Preparation of bacterial suspension and bacterial challenge*

Approximately 2 ml of an isolate of *S. iniae*, obtained originally from tilapia (*Oreochromis* sp.) and maintained by the Texas Veterinary Medical Diagnostic Laboratory (TVMDL), was injected into several sub-adult hybrid striped bass (~ 200 g) by intraperitoneal injection. The pathogen was re-isolated from the brain of infected fish

and positively identified, using DNA analysis, by personnel at TVMDL. The re-isolated pathogen was then grown overnight in brain–heart infusion broth (EM Science, Darmstadt, Germany) in a shaking bath at 27°C as described by Sealey and Gatlin (2002). Concentration of the bacterial suspension was determined by the serial plate count method and used the agar medium specific for *S. iniae* (Nguyen and Kanai, 1999).

At the time of the challenge experiment, the water level in the experimental tank was lowered to reach a volume of approximately 500-L. A bacterial suspension was added to the tank in an amount determined to achieve a concentration of  $9.3 \times 10^5$  colony forming units (CFU)/ml. Fish were exposed to the bacteria under these conditions for four hours at a water temperature of  $26 \pm 2^\circ\text{C}$ . The water level was then returned to 3800-L via addition of 2-3 ppt (salinity) water, resulting in an approximately seven-fold dilution of the bacteria suspension. Subsequent rearing conditions were the same as during the acclimation period. Dead fish were removed twice a day (8:00 am and 8:00 pm) during a 45-day period. A clip from the dorsal fin was removed from each dead fish and stored in 70% ethanol for subsequent parentage assignment. A random sample of dead fish was sent to TVMDL for determination of infection status. The experiment was terminated when mortality reached 45%. All surviving fish were euthanized by a lethal dose of anesthetic (MS 222) and a clip of the dorsal fin removed (and stored) for parentage assignment. Infection status of surviving fish was determined both by visual examination of external symptoms and by assessment of the presence of *S. iniae* in the brain (after Nguyen and Kanai, 1999). External symptoms assessed during visual determination were darkened skin, eye opacity, hemorrhage of the skin, and body

curvature. Brain extracts of each fish were streaked on Petri dishes filled with a modified selective agar medium specific for *S. iniae* (Nguyen and Kanai, 1999). Extracts were cultured at  $26 \pm 1^\circ\text{C}$  for 48 hours in order to assay for presence of colonies of *S. iniae*.

### 2.3. Statistical analysis

#### 2.3.1. Immune- and stress-response traits

Immune- and stress-response traits were assessed according to *Model 4* (below)

$$Y_{ijkl} = \mu + dam_i + sire_j + (dam \times sire)_{ij} + T_k + \varepsilon_{ijkl}$$

where  $Y_{ijkl}$  is an individual observation,  $\mu$  is the overall mean,  $dam_i$  represents the random effect of the  $i^{\text{th}}$  dam,  $sire_j$  represents the random effect of the  $j^{\text{th}}$  sire,  $(dam \times sire)_{ij}$  represents the random interaction between  $i^{\text{th}}$  dam and  $j^{\text{th}}$  sire,  $T_k$  represents the fixed effect of the  $k^{\text{th}}$  tank, and  $\varepsilon_{ijkl}$  is the random residual. Significance of random and fixed effects was determined by analysis of variance (ANOVA), with the sum of squares for different effects associated with Type IV estimable functions based on *Model 4*.

Variance components associated with each random effect and their standard errors were estimated using the REML algorithm implemented in VCE-5 (Groeneveld, 1998).

Phenotypic correlations between pairs of traits were estimated via Pearson's product-moment correlation coefficient, as implemented in the Statistical Package for Social Sciences (SPSS INC., 2001). Significance of the estimated correlation was tested via Student's  $t$  test (Sokal and Rohlf, 1981).



### 2.3.2. *Resistance to S. iniae infection*

Homogeneity of the distribution of dead versus surviving fish and of infected versus non-infected fish among dam and sire half-sib families was tested via G-tests (Sokal and Rohlf, 1981). In the comparison of infected versus non-infected fish, dead fish were classified as ‘infected’ and added to the counts of fish diagnosed as infected at the end of the experiment. Possible genetic effects on disease resistance were examined further by considering survival time data; disease resistance of an individual fish was characterized as the duration of survival during the challenge. The dataset thus included both censored records (survivors at the end of the experiment) and uncensored records (fish that died during the 45 days of survey and for which the duration of survival is therefore known). Survival data were analyzed according to the proportional hazard model

$$h(t, x, z) = h_0(t) \exp\{z' s\}$$

where  $h(t, x, z)$  is the survival probability function of an individual at time  $t$  given that it was alive immediately before  $t$ ,  $h_0(t)$  is the baseline survival function,  $z$  is the vector of additive effects of dams and sires, and  $s$  is the design matrix associated with  $z$ . A Cox model (Cox, 1972) was used to evaluate effects of various factors included in the model on the response variable ( $h(t)$ ). The model allows incorporation of both censored and uncensored data in the estimation and does not require estimation of the parameters of the baseline survival curve. Effects of the factors included in the model are assumed to be multiplicative (proportional) on a log scale, while the baseline hazard function is left arbitrary, as described in Cox (1972). Computations were implemented as in the Survival Kit (Ducrocq and Sölkner, 1998), available at

<http://www.nas.boku.ac.at/1897.html>. Significance of the effect of dam and sire covariates on the estimated survival function was tested via likelihood-ratio tests that compared the likelihood of the full model (including both covariates) to the likelihood of models excluding one covariate at a time. Estimated breeding values (EBVs) for disease resistance were generated in the Survival Kit.

### **3. Results**

#### *3.1. Immune- and stress-response traits*

##### *3.1.1. Parentage assignment of offspring*

A total of 450 fish were bled. PIT-tag identification was unavailable for 32 of the sampled fish, preventing determination of their parentage. Consequently, analysis of genetic effects on immunological and stress parameters was carried out on the remaining 418 fish. Representation of offspring from different families is given in Table 14. A total of 89 of the possible 100 full-sib families were represented with at least one offspring, and all ten dams and ten sires made some contribution, ranging from 2.0 % (Dam 8) to 18.0 % (Dam 9) for dams and 6.0 % (Sire 1) to 21.8 % (Sire 10) for sires.

##### *3.1.2. Genetic and non-genetic effects*

Mean, standard deviation, and range of observed values of immune- and stress-response traits are given in Table 15. Average values ( $\pm$ SD) for oxidative-radical production, serum lysozyme, and myeloperoxidase activities were  $1.54 \pm 0.40$  mg/ml,  $1729.3 \pm 722.8$  units/ml and  $0.88 \pm 0.42$ , respectively. Serum-cortisol level averaged

Table 14. Number of offspring identified for each of 100 full-sib families generated in the  $10 \times 10$  factorial cross and used in the assay of immune- and stress-response parameters

	Dam 1	Dam 2	Dam 3	Dam 4	Dam 5	Dam 6	Dam 7	Dam 8	Dam 9	Dam 10	Sum
Sire 1	2	2	3	4	1	0	3	0	9	1	25
Sire 2	2	7	8	7	3	4	6	1	7	5	50
Sire 3	4	4	7	5	1	2	4	2	6	4	39
Sire 4	0	1	7	9	2	2	3	1	2	5	32
Sire 5	3	6	2	3	2	2	1	0	3	5	27
Sire 6	3	5	3	5	6	1	2	0	1	4	30
Sire 7	2	1	5	2	3	0	5	0	6	6	30
Sire 8	1	3	6	8	4	2	2	0	13	7	46
Sire 9	0	4	2	5	9	1	5	1	7	0	34
Sire 10	0	10	15	20	10	5	6	3	18	4	91
Sire 1/2 <sup>a</sup>	0	0	2	3	3	0	1	1	3	1	14
Sum	17	43	60	71	44	19	38	9	75	42	418

<sup>a</sup> Either Sire 1 or Sire 2

Table 15. Mean, standard deviation (s.d.), and range of immune-response and stress-response values obtained

Trait	Mean $\pm$ s.d.	Range	# fish
Neutrophil-oxidative radical production			
(NBT diformazan mg/ml)	1.54 $\pm$ 0.40	0.04 ~ 2.60	410
Lysozyme (units/ml)	1729.3 $\pm$ 722.8	100.0 ~ 3183.3	406
Myeloperoxidase (absorbance at 450 nm)	0.88 $\pm$ 0.42	0.08 ~ 2.21	330
Cortisol (ng/ml) <sup>a</sup>	183.3 $\pm$ 74.4	25.1 ~ 413.5	176
Glucose (mg/dl) <sup>b</sup>	276.4 $\pm$ 78.2	115.7 ~ 584.1	377

\* mg/dl = milligrams per deciliter

\* ng/ml = nanograms per milliliter

Table 16. Probability of significance of genetic and non-genetic effects, based on ANOVA, on immune-response and stress-response parameters

Effect	Neutrophil			
	oxidative radical production	Lysozyme activity	Myeloperoxidase level	Cortisol level
Dam	0.0142	0.2348	0.5969	0.6631
Sire	0.5225	0.0430	0.5913	0.7987
Dam × Sire	0.8107	0.8438	0.8239	0.6386
Tank	<0.0001	<0.0001	0.3835	0.5863
				Glucose level
				0.6551
				0.2373
				0.9654
				<0.0001

183 ± 74.4 ng/ml and serum-glucose level averaged 276.4 ± 74.4 mg/dl. Results of ANOVA are given in Table 16. There was a significant dam effect on neutrophil oxidative-radical production and a significant sire effect on lysozyme activity. None of the genetic effects, including dam x sire interaction, on any other immune- or stress-response trait was significant. REML estimates of the proportion of dam, sire, and dam x sire interaction variance components to the total phenotypic variance did not differ significantly from zero for any of the traits. Because estimates of genetic variance for all traits did not differ significantly from zero, genetic correlations between traits were not estimated. Effect of tank on oxidative-radical production, lysozyme activity, and serum glucose level was significant; whereas effect of tank on total myeloperoxidase and serum cortisol was not.

There was a positive and significant ( $P < 0.001$ ) correlation between oxidative-radical production and lysozyme activity ( $r_p = 0.26$ ), between serum glucose level and myeloperoxidase activity ( $r_p = 0.15$ ), and between body weight and oxidative-radical production ( $r_p = 0.22$ ). All other estimates of pairwise phenotypic correlation did not differ significantly from zero.

### 3.2. Disease resistance

A total of 394 fish were used in the disease-challenge experiment. Representation of offspring from different families is given in Table 17. All ten dams and ten sires contributed to offspring, ranging from 1.2 % (Dam 6) to 19.3 % (Dam 2) for dams and from 3.0 % (Sire 9) to 19.3 % (Sire 10) for sires. A total of 79 full-sib families were represented with at least one offspring. Only three mating pairs involving Dam 6 were

Table 17. Number of offspring identified for each of 100 full-sib families generated in the 10 x10 factorial cross and used in the disease challenge experiment

Cross	Dam 1	Dam 2	Dam 3	Dam 4	Dam 5	Dam 6	Dam 7	Dam 8	Dam 9	Dam 10	Sum
Sire 1	0	4	2	3	0	0	5	0	4	1	19
Sire 2	6	4	4	6	2	0	2	2	2	5	33
Sire 3	2	6	1	2	1	1	3	0	3	1	20
Sire 4	9	14	5	12	3	0	4	4	4	10	65
Sire 5	5	5	3	2	4	0	1	0	6	5	31
Sire 6	5	7	0	0	0	0	1	2	3	1	19
Sire 7	2	4	2	3	2	0	6	0	3	5	27
Sire 8	5	10	6	9	0	1	7	1	11	10	60
Sire 9	2	2	1	1	0	1	4	0	1	0	12
Sire 10	7	16	8	10	7	0	14	0	10	4	76
Sire1/2 <sup>a</sup>	4	4	3	5	1	2	5	2	0	6	32
Sum	47	76	35	53	20	5	52	11	47	48	394

<sup>a</sup> Either Sire 1 or Sire 2

represented among the offspring (Dam 6 × Sires 3, 8, and 9), while only four mating pairs involving Dam 8 (Dam 8 × Sires 2, 4, 6, and 8) were represented.

A total of 361 fish were unambiguously assigned to parents and were used in analysis of genetic effects. A total of 161 fish (44.6%) died during the 45-day experimental period. The cumulative mortality curve is shown in Figure 3. The proportion of offspring from each of the ten dams and ten sires among the survivor and dead ‘groups’ is given in Figure 4. The proportion of offspring among the ten dams in the survivor group ranged from 1.3% (Dam 6) to 18.0% (Dam 2) and among the ten sires from 2.5% (Sire 1) to 23.5% (Sire 10). The proportion of offspring from Dam 1 and Dam 10 in the survivor group was higher than in the dead group (% offspring in the survivor group - % offspring in the dead group = +5.80% for Dam 1 and +4.18% for Dam 10). Conversely, the proportion of offspring from Dam 4 in the survivor group was lower (-5.15%) than in the dead group. The proportion of offspring from Sire 2, Sire 5, and Sire 10 was higher in the survivors group (+5.29% for Sire 2, +4.29% for Sire 5, and +5.49% for Sire 10); whereas the proportion of offspring from Sire 1 and Sire 3 was lower in the survivor group (-5.07% for sire 1 and -6.82% for Sire 3) than in the dead group. The remaining half-sib families had similar proportions of survivor and dead fish (less than 4% difference).

Of the 200 surviving fish at the end of the experiment, 39 were diagnosed as ‘infected’ based on external symptoms and detection of *S. iniae* in brain extracts. The total percentage of fish infected during the challenge period was therefore 61.2%. The proportion of offspring from each of the ten dams and ten sires among non-infected and



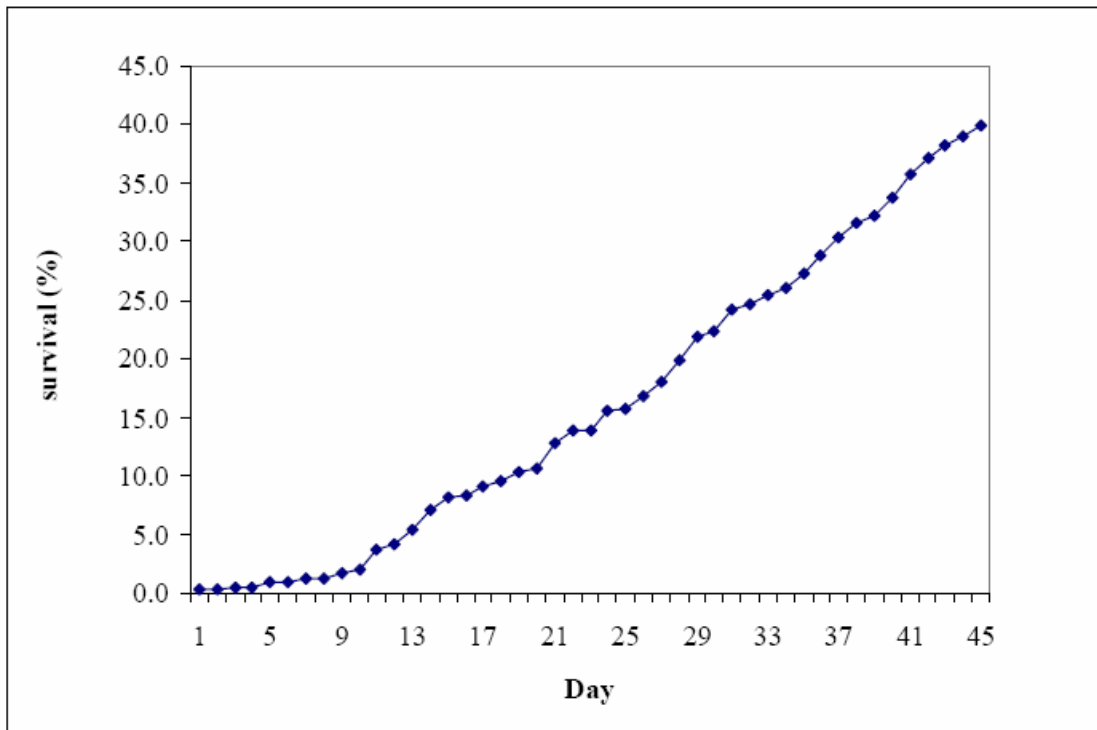


Fig. 3. Cumulative mortality following exposure to *Streptococcus iniae*.

infected 'groups' are given in Figure 5. Only offspring from nine dams were found in the non-infected group; the proportion of offspring per dam in the non-infected group ranged from 1.4% (Dam 8) to 18.6% (Dam 2). The proportion of offspring (in the non-infected group) from the ten sires ranged from 2.1% (Sires 3 and 9) to 22.8% (Sire 10) from each of the ten sires in the non-infected group. The proportion of offspring from Dam 1 was higher in the non-infected group (+9.71%), whereas the proportion of offspring from Dam 9 was higher (+4.93%) in the non-infected group. The proportion of offspring from Sire 2 and Sire 5 were higher in the non-infected group (+8.40% for Sire 2 and +4.64% for Sire 5), whereas the proportion of offspring from Sire 3 was higher (+5.55%) in the infected group. The remaining half sib families had similar proportions of non-infected and infected fish (less than 4% difference).

Results of G-tests indicated that the distribution of offspring in survivor versus dead groups differed significantly among sires ( $G_{[9]} = 19.22, P < 0.05$ ) but not among dams ( $G_{[9]} = 8.42, P > 0.05$ ). Similar results were obtained when comparing the distribution of offspring in infected versus non-infected groups ( $G_{[9]} = 19.52, P < 0.05$  for sires;  $G_{[9]} = 14.81, P > 0.05$  for dams). Likelihood-ratio tests indicated that effect of the sire on the survival probability function was of borderline significance ( $P = 0.054$ ), while effect of the dam was non-significant ( $P > 0.05$ ). Estimated breeding values (EBVs) for all ten dams did not differ significantly from zero, whereas the EBV of Sire 3 was positive with an associated probability of significance was 0.056. The corresponding risk ratio for offspring from this sire was 2.4 (i.e., the probability of dying was 2.4 times higher than

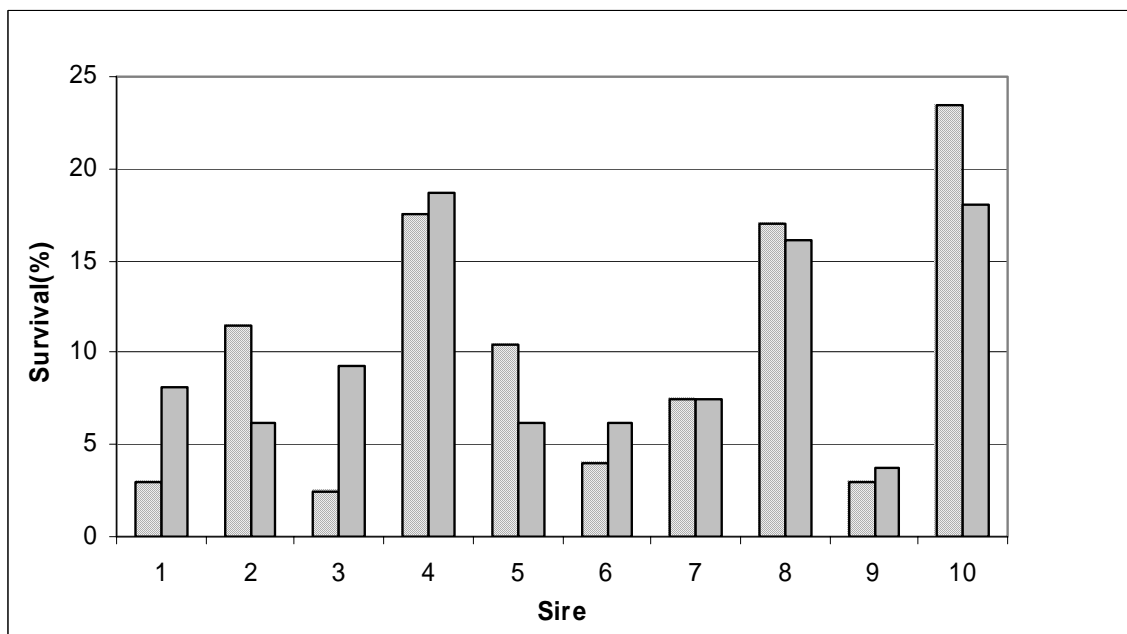
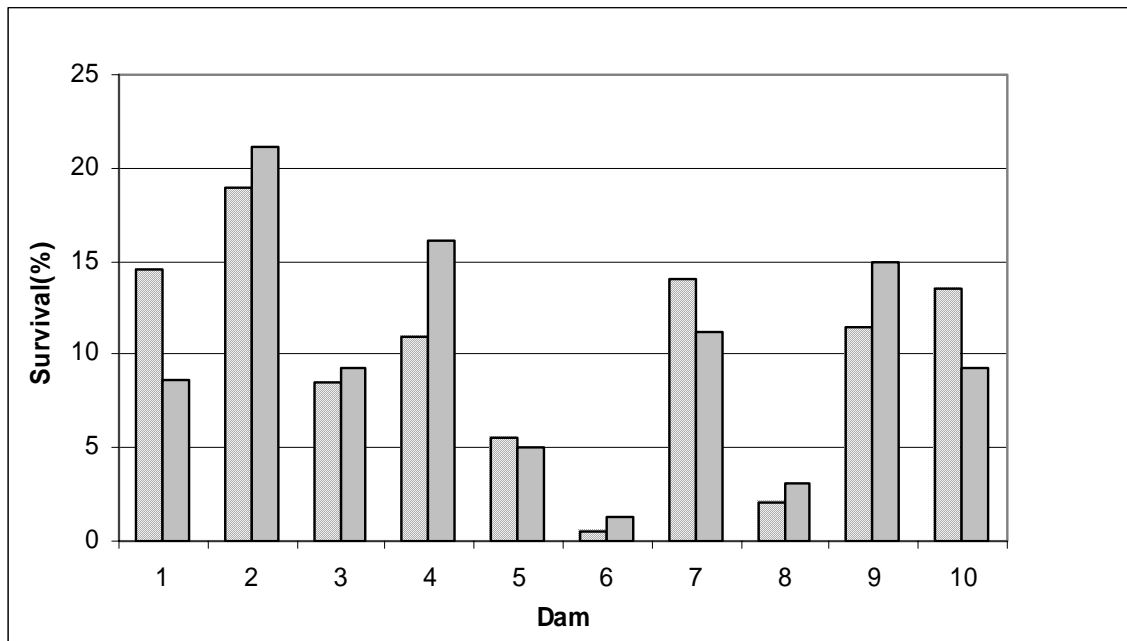


Fig. 4. The proportion of offspring from each of the ten dams and ten sires in the survivor (cross-hatched) and dead groups (solid).

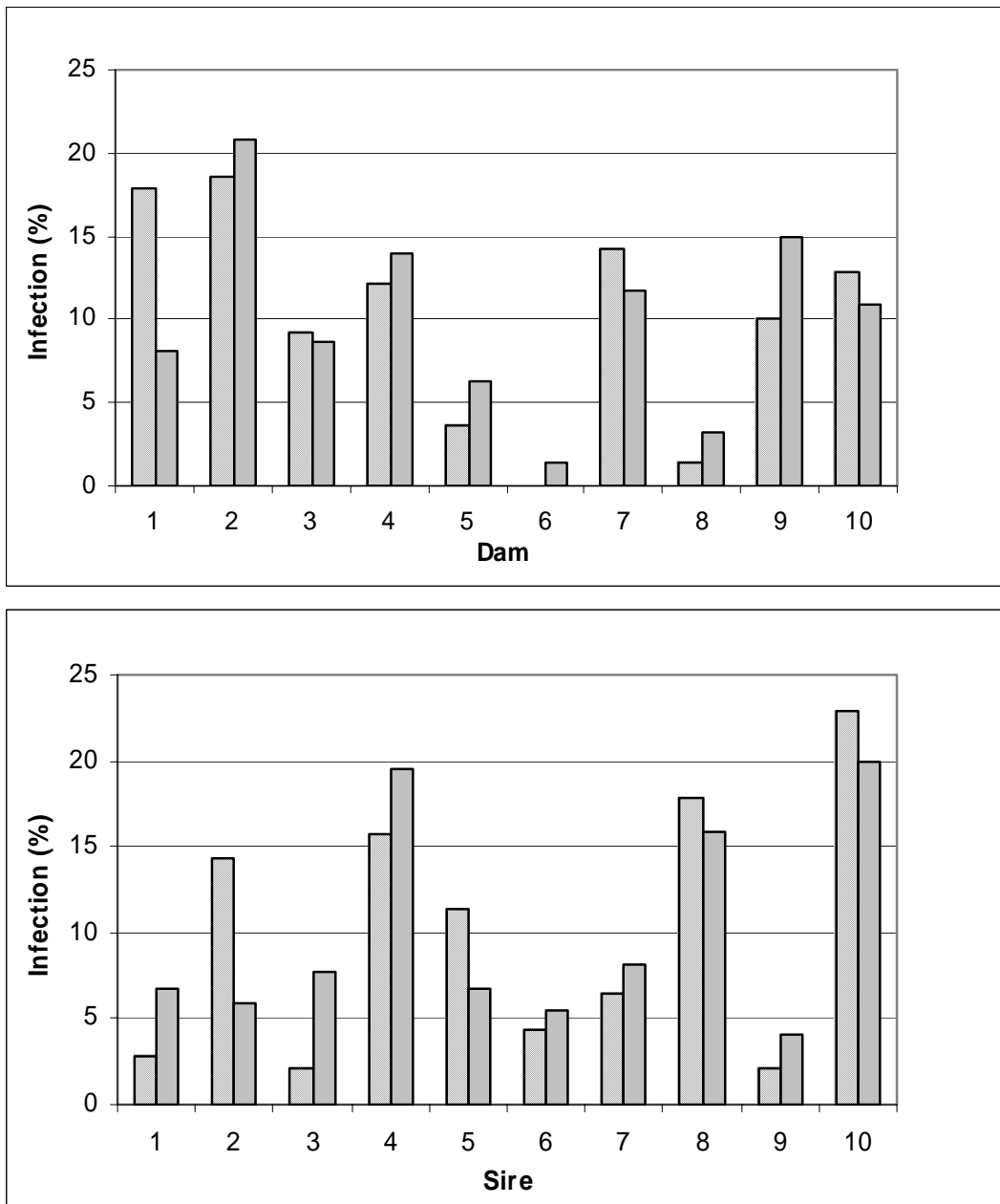


Fig. 5. The proportion of offspring from each of the ten dams and ten sires in non-infected (cross-hatched) and infected groups (solid).

average for offspring from this sire). EBVs of all other sires did not differ significantly from zero.

#### **4. Discussion**

##### *4.1. Genetic and non-genetic effects on immune-and stress-response traits*

A significant effect of dam on neutrophil oxidative-radical production and a significant effect of sire on lysozyme activity were found. However, the proportion of dam and sire variance components to the total phenotypic variance did not differ significantly from zero for both traits. All other genetic effects on immune-response traits were non-significant. Effect of tank on oxidative-radical production and lysozyme activity was significant; whereas effect of tank on total myeloperoxidase was not. While a significant genetic effect on lysozyme activity has been reported in rainbow trout (Fevolden et al., 1991; Røed et al., 1993a) and Atlantic salmon (Røed et al., 1993b; Lund et al., 1995), immune-response traits in general are often sensitive to variation in stress and temperature (Pickering et al., 1982; Pickering, 1989). The occurrence of a significant tank effect on neutrophil oxidative-radical production and lysozyme activity in these experiments may suggest there was differential stress on hybrid striped bass cultured at different tanks, and it is possible that variable stress obscured any significant genetic effects on the immune-response traits.

No genetic effect (dam, sire, or dam x sire interaction) was found for the two stress-response traits (serum cortisol and glucose levels). A significant effect of tank was found for serum-glucose level but not for serum-cortisol level. Results of other studies that explored the genetic basis for stress-related cortisol and glucose levels in various

salmonid species were inconsistent and varied depending on the type and duration of the stress (Fevolden et al., 1993; 1999; Pottinger and Carrick, 1999). Overall, there appears to be little potential of using these immune- and stress-response traits as indirect markers for disease resistance in hybrid striped bass.

#### 4.2. Genetic effects on disease resistance

G-tests revealed a significant effect of sire on resistance to *S. iniae* and offspring from Sire 3 had a 2.4 times higher probability of dying, on average, than offspring from other sires. No such effect was found for dams. In other cultured fish, including channel catfish (Plumb et al. 1975; Wolters and Johnson, 1995), Atlantic salmon (Gjedrem et al., 1991; Gjøen et al., 1997), rainbow trout (Henryon et al., 2005) and Atlantic cod (Kettunen and Fjalestad, 2006), significant genetic variation in mortality after infection with specific pathogens has been reported. Collectively, these results indicate that selection for resistance of hybrid striped bass to infection by *S. iniae* might be feasible.

#### 4.3. Disease resistance in $F_1$ hybrids

It has been suggested that transmission of disease-resistance traits in  $F_1$  hybrids may not be equivalent with respect to paternally and maternally inherited nuclear genes, and that hybrids appear to inherit, at least partly, the specific resistance of one of the parental species (Chevassus and Dorson, 1990). One study showed that hybrids between common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) were particularly susceptible to dropsy (Pojoga and Negriu, 1981), while in another study, hybrids between rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*)

displayed variation between the sexes in resistance to viral hemorrhagic septicemia (Ord et al., 1976). However, unequal genetic effects observed between dam and sire also may be due to unequal genetic variation for specific traits within each parental species (or line) used in a study. An example is resistance to the hemoflagellate *Cryptobia salmositica* in coho salmon where Bower et al. (1995) reported that the genetic variation for survival detected in the intraspecific hybrids between susceptible and resistant strains was attributable to the susceptible strain only. Evaluation of genetic variation within each parental species, as well as the genetic variation in resulting F<sub>1</sub> hybrids, would help to elucidate underlying mechanisms in inheritance of resistance to pathogens in hybrid striped bass.

Heterosis in resistance to pathogen infection, stemming ostensibly from dominance, overdominance, and epistatic effects, has been studied in both intra- and inter-species fish hybrids (Fjalestad et al., 1993; Lutz, 2001). These studies were designed to examine heterotic effects by comparing survival (after infection to pathogen) in parental species with that of in their F<sub>1</sub> hybrids; the occurrence and levels of heterosis, however, have been variable. Non-additive genetic effects on disease resistance have been identified in inter-strain hybrids of salmonid fish (Ibarra et al., 1994), as has susceptibility of different strains of coho salmon and sockeye salmon, and their interspecies hybrids, to the hemoflagellate *Cryptobia salmositica* (Bower et al. 1995). In coho salmon, survival was 95% in the resistant strain, 3% in the susceptible strain, and intermediate (30%) in intraspecific hybrid strains. For sockeye salmon, survival was 90% in the resistant strain, 40% in the susceptible strain, and 70% in the hybrid strains. Schisler et al. (2006) found

increased resistance to whirling disease (caused by *Myxobolus cerebralis*) in F<sub>1</sub> progeny of a cross between a resistant strain and a susceptible strain of rainbow trout. However, Wolters et al. (1996) reported that F<sub>1</sub> interspecies hybrids between channel catfish females and blue catfish males showed intermediate survival after challenge with *Edwardsiella ictaluri* as compared to their parents. No previous study has been conducted to study the genetic effects on disease resistance to *S. iniae* in either parental species of hybrid striped bass. Consequently, the question of whether heterosis occurs in resistance of F<sub>1</sub> hybrids between striped bass and white bass remains open.



## CHAPTER V

### CONCLUSIONS

This study focused on quantitative-genetic aspects of production traits in hybrid striped bass. A  $10 \times 10$  factorial mating design was employed to examine genetic effects and heritability based on dam half-sib and sire half-sib families. Genotypes at ten polymorphic microsatellites yielded unequivocal parentage (sire and dam) assignment for >95% of progeny assayed. Traits assessed were as follows: (i) growth-related traits, including body weight, total length, and growth rate; (ii) carcass-quality traits, including fillet weight, total viscera weight, intraperitoneal fat weight, liver weight, and gonad weight; (iii) standardized (to body weight) measures of the carcass-quality traits; (iv) conditional factor ( $K$ ); and (iv) traits associated with resistance to infection by *Streptococcus iniae* and with immune and stress response.

Dam and sire effect(s) on juvenile growth and growth rate were significant, whereas dam by sire interaction effect was not. Estimates of broad-sense heritability, based on family means ( $h^2_f$ ), in dams ranged from  $0.65 \pm 0.24$  to  $0.76 \pm 0.13$  for weight, and  $0.60 \pm 0.20$  to  $0.79 \pm 0.16$  for length; estimates in sires ranged from  $0.46 \pm 0.20$  to  $0.75 \pm 0.18$  for weight, and  $0.43 \pm 0.20$  to  $0.69 \pm 0.14$  for length. Estimates of  $h^2_f$  for growth rate ranged from  $0.69 \pm 0.11$  to  $0.82 \pm 0.10$  for dams, and from  $0.64 \pm 0.14$  to  $0.66 \pm 0.19$  for sires.

Dam and sire effect(s) on fillet weight were significant; estimates of heritability ( $h^2_f$ ) for this trait were  $0.76 \pm 0.11$  (dams) and  $0.54 \pm 0.17$  (sires). Dam effect on liver weight was significant ( $h^2_f = 0.60 \pm 0.40$ ), whereas sire effect was not. Alternatively, sire effect on total viscera weight was significant ( $h^2_f = 0.56 \pm 0.21$ ), whereas dam effect was not. Phenotypic correlations ( $r_p$ ) between body weight and carcass-quality traits were high (0.85 - 0.92) and differed significantly from zero. Genetic correlations ( $r_g$ ) between body weight and most of the carcass-quality traits also were high (0.87-1.00).

Dam and sire effect(s) on standardized (by body weight) values of the carcass-quality traits hepatosomatic index (HSI) and viscerasomatic index (VSI) were significant, as was dam and sire interaction effect on VSI. Phenotypic correlations between body weight and standardized carcass-quality traits were positive but low, ranging from 0.07 to 0.19. Genetic correlations between body weight and standardized carcass-quality traits were relatively high and negative for both dams and sires, ranging from  $-0.70$  to  $-1.00$ . The exception was the genetic correlation between body weight and HSI for sires ( $r_g = 0.67$ ). Dam and sire effect on condition factor ( $K$ ) were significant; estimates of  $h^2_f$  were  $0.72 \pm 0.11$  (dams) and  $0.60 \pm 0.17$  (sires). The phenotypic correlation between body weight and condition factor was 0.62, while the genetic correlations were 0.51 (dam) and 0.73 (sire); all three correlations differed significantly from zero. The observed genetic correlation between body weight and fillet weight and the absence of a genetic effect on relative fillet yield (standardized fillet weight) indicate that improvement of fillet weight may be best realized by improvement of body weight.

In this study, ten white bass and ten striped bass were crossed and genetic effects and heritability of traits that are important in aquaculture production were evaluated based on the mean values of half-sibs and full-sibs. Dam and sire effects were significant for all the growth-related traits in F<sub>1</sub> hybrids, while dam x sire interaction effects were not significant for any of the traits. These results indicate that most of the variation in growth of F<sub>1</sub> progeny was due to additive genetic effects rather than dominance effects. The additive genetic variance thus could be exploited within pure lines of each species (striped bass and white bass). A point to note, however, is that maternal effects also may be present. In addition, the conclusions reached in this study are valid only for the genotypes of the breeders employed. As pointed out by Gordon (1999), however, the level of heterozygosity in the breeders could affect genetic variance in the F<sub>1</sub> hybrids, meaning that the conclusions reached here should be interpreted with caution. Finally, the additive genetic variance within populations of white bass and striped bass has not been adequately documented, so the expected or potential gain from selection for any white bass or striped bass combinations can not be estimated at this time. Partitioning of the additive variance for production traits of parental lines could be obtained by methods such as parent-offspring regression and/or half-sib breeding designs (Falconer and Mackay 1996; Lynch and Walsh 1998), but would require larger numbers of parental lines and higher maintenance costs of rearing in different environments.

The estimates of the genetic parameters of production traits important in hybrid striped bass aquaculture will help in designing effective selective-breeding programs to

improve production efficiency. Currently, there is no consensus as to which method of selection will provide the highest sustainable returns over the shortest period of time for production of hybrid striped bass (Hallerman, 1994). The possible selection approaches in hybrid striped bass include reciprocal recurrent selection and backward selection.

Reciprocal recurrent selection of striped bass and white bass for production of hybrid striped bass would involve testing many inter-specific crosses among various lines of each parental species. Parents that produce superior hybrids would be retained as candidate breeders within pure lines to produce parents for the next hybrid generation (Hallerman, 1994). Reciprocal recurrent selection involves the exploitation of both additive genetic variance ( $V_A$ ) and dominance genetic variance ( $V_D$ ) and is preferably used between two populations that are known to give heterotic effects when crossed (Falconer and Mackay, 1996). Overall, although reciprocal recurrent selection approach could allow accumulation of genetic progress from one generation of selection to the next, application of significant selection differential in selected lines while maintaining sufficient effective size in order to prevent inbreeding likely would involve high cost and significant infrastructure.

Backward selection, on the other hand, does not need to maintain the 'pure' lines of parental species for the production of next generation of breeders. Parents with superior combining ability are identified and then used repeatedly to propagate desired offspring (Gordon, 1999). This approach suffers, however, from the drawback of a long generation interval as selection of parents cannot be carried out until offspring have been evaluated (Falconer and Mackay, 1996). The results in this study indicate that backward

selection could be implemented to improve production efficiency (mainly growth) in hybrid striped bass. Because dam x sire interaction effects in this study were not a significant source of variation in growth-related traits, selection could be implemented by selecting individual dams and sires with significant general combining ability. This approach does not require maintenance of parental lines, as superior dams could be used for multiple spawns. Given considerable research on short-term storage (He and Woods 2003a; He et al., 2004) and long-term cryopreservation of striped bass sperm (Jenkins-Keeran and Woods 2002; He and Woods, 2003b), sperm from sires with superior general combining abilities could be collected and sent to various hybrid striped bass facilities that hold white bass stocks for producing hybrid striped bass offspring.

In the long term, collection and evaluation of large numbers of potential breeders (both striped bass and white bass) from various geographic locations would be necessary to select the 'best breeders' that could generate hybrid striped bass with best performance. The recent progress in the development of molecular markers for species of *Morone* (Couch et al., 2006) will help in identifying quantitative-trait loci for important production traits and ultimately for use in marker-assisted selection.

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## APPENDIX I



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**APPENDIX II**

**PRIMER SEQUENCES, ANNEALING TEMPERATURE (T<sub>a</sub>), AND  
REFERENCE FOR MICROSATELLITES USED IN PARENTAGE  
ASSIGNMENT**

Microsatellite	Primer sequences (5'---3')	T <sub>a</sub> (°C)	Reference
<i>Hsb1B</i>	F: GCAGCAGAAGTTGGGACTGGT R: GGCACCAAACAAGACATATAGTGA F: GTACGGTGTTCCTGCCTCA	54	Ross et al., 2004
<i>Hsb1C</i>	R: GGAGTGTCATAGATACAGTAAGTG F: CAGAAACACTCGCTTCGCATCA	47	Ross et al., 2004
<i>Hsb6C</i>	R: GGAGCGTTCTTCAATGTTCTCTCAA F: GGCTGAGGGCAGTAGTCAGA	57	Ross et al., 2004
<i>Hsb7C</i>	R: GGTGATACTGGTGGGTTTCAA	52	Ross et al., 2004
<i>SB6</i>	F: ACAGCAAAGATAAACATCTG R: TTCATGATGTTTCACCAGG	46	Han et al., 2000
<i>SB83</i>	F: TGGGCCTGATTGGAATCAAAA R: GATAGGTTGTATCAATGTTGC	50	Han et al., 2000
<i>SB13</i>	F: TGCTGAGCCGGTAATTCAAG R: CACACATATGCATGGATGCA	51	Han et al., 2000
<i>SB 91</i>	F: AGACACCAGATAAGGAGA R: AAATAGATTCACACAAGG	58	Roy et al., 2000
<i>SB 113</i>	F: GATCGCGTTATTACAGT R: GACTATCTCCCCTGAAAT	50	Roy et al., 2000
<i>SB 117</i>	F: TTAAAGTTTTCCAGTCAT R: CTTTTTAGAGCCAGTGTT	52	Roy et al., 2000



### APPENDIX III

#### METHODOLOGY FOR ANALYSIS OF PHYSIOLOGICAL RESPONSES

##### Neutrophil Oxidative Radical Production

###### A. Reagents:

1. Hank's Balance Salt Solution (HBSS, 1×)  
Weigh 4.5 g HBSS (Sigma), dissolve in 500 ml DI water in volumetric flask filter and store at room temperature.
2. Nitro Blue Tetrazolium (NBT, 0.2%)  
Weigh 0.020 g NBT, dissolve in 10 ml HBSS solution prepared as above. Store in dark bottle or aluminum foil wrapped bottle at 2-8°C. 10 ml NBT solution is sufficient for 96 samples in a 96- well plate.
3. *N, N*-dimethyl formamide  
Ordered from VWR.
4. Moisture Chamber  
Put 2 pieces of wet paper towel into the bottom of a 1-ml tip container. This is important to transport samples.

###### B. Procedure:

1. Add 0.1 ml of NBT solution to each well of a 96-well plate with multi-channel pipettman.
2. Add 0.1 ml of fresh blood to each individual well.
3. Incubate blood sample with NBT solution at least 30 min. Shake gently and periodically. Transport plates in moisture chamber if needed.
4. Transfer 50  $\mu$ l of sample mixture to 1 ml of *N, N*-dimethyl formamide in eppendoff tubes in fume hood.
5. Centrifuge the samples at  $3000 \times g$  for 5 min to separate blood cell debris.
6. Read supernatant in a spectrophotometer at 545 nm. Be sure to use glass cuvette.
7.  $\text{mg NBT diformazan/ml blood} = 40 * (\text{absorbance} - 0.0245) / 5.8564$

###### C. Notes:

1. *N, N*-dimethyl formamide is highly flammable and cancer potentiating. Wearing mask is recommended. It also reacts with disposable cuvette and plastic weigh pan.
2. This whole procedure should be completed within 10 hours.
3. NBT is light sensitive, so avoid light as much as possible during the procedure.

###### D. References:

- Siwicki, A. K., Anderson, D. P., Rumsey, G. L. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Vet. Immunol. Immunopathol.* 41, 125-139.

### Serum Lysozyme Analysis (Turbidimetric method)

#### A. Reagents:

1. Phosphate Buffer Solution (0.04 M, pH 6.1; 1 liter)  
Weigh 5.0286 g  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  and 2.5791 g  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ , dissolve in 1 liter of DI water, make sure pH is 6.01 by pH meter.
2. *Micrococcus lysodeikticus* suspension (0.2 g/l; 1 liter)  
Weigh 0.2 g *Micrococcus lysodeikticus* (Sigma), suspend in 1 liter of PBS prepared above.
3. Lysozyme standard (Optional)  
Weigh 0.01 g hens egg lysozyme (Sigma) dissolve in 100 ml DI water. And serially diluted to prepare standard

#### B. Procedure:

1. Add 3 ml of *M. lysodeikticus* suspension into disposable cuvette.
2. Add 25  $\mu\text{l}$  - serum sample into the same cuvette. Note down the time immediately.
3. Read the absorbance 1 at 540 nm after 30 seconds.
4. Read the absorbance 2 at 540 nm after 4 minutes and 30 seconds.
5. Serum lysozyme (units/l) = (absorbance 1 – absorbance 2)  $\times 10^7$
6. (Optional) Standardize to  $\mu\text{g}$  lysozyme by serial dilutions of lysozyme standard and measure decrease in absorbance and establish a standard curve corresponding absorbance to actual quantities.

#### C. Notes:

1. Buffer pH dramatically influences lysozyme activity. This may be species specific. This procedure is based on hybrid striped bass. For other fish species, determination of optimum pH before assay is recommended.
2. Because pH for chicken egg lysozyme activity is significantly different from fish serum lysozyme activity, unit standardization is not recommended in our protocol.
3. Although it rarely occurs, *M. lysodeikticus* may cause the reading to increase. If so, it is suggested to purchase another batch.
4. Maintaining the same time of sampling is of obvious importance. Do not try to process too many samples at one time.

#### D. References:

- Jørgensen, J. B., Sharp, J. E., Secombes, C. J., Robertsen, B., 1993. Effect of a yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. *Fish Shellfish Immunol.* 3, 267-277.
- Ellis, A. E., 1990. Lysozyme assays. In: Stolen, J. S., Fletcher, T. C., Anderson, D. P., Roberson, B. S., van Muiswinkel, W. B. (Eds.), *Techniques in Fish Immunology*. SOS Publications, Fair Haven, NJ, USA. pp. 101-103.

## Serum Peroxidase

### A. Reagents:

1. Hank's Balance Salt Solution (HBSS, 1×, without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ )  
Weigh 4.5 g HBSS (Sigma), dissolve in 500 ml DI water in volumetric flask and filter it, store at room temperature.
2. TMB- $\text{H}_2\text{O}_2$  solution (for 100 samples)
  - 1) TMB-solution (40 mM, 5ml): Weigh 0.0313 g 3, 3', 5, 5'-tetramethylbenzidine hydrochloride (TMB, Sigma T8768, M.W. 313.3), dissolve in 2.5 ml DI water.
  - 2)  $\text{H}_2\text{O}_2$  solution (10 mM, 1 L): Measure 0.5825 ml 30%  $\text{H}_2\text{O}_2$  solution and dilute to 500 ml with DI water in a volumetric flask.
  - 3) Mix 2.5 ml TMB-solution and 2.5 ml  $\text{H}_2\text{O}_2$  solution.
3. Sulfuric acid (4 M)  
Measure 22.4 ml  $\text{H}_2\text{SO}_4$  and dissolve in 60 ml DI water in 100 ml volumetric flask and dilute to 100 ml with DI water.

### B. Procedure:

1. Add 15  $\mu\text{l}$  serum with 135  $\mu\text{l}$  HBSS in flat-bottomed 96-well plates.
2. Add 50  $\mu\text{l}$  TMB-  $\text{H}_2\text{O}_2$  solution to each well using multichannel pipettor.
3. Incubate samples for 2 min.
4. Add 50  $\mu\text{l}$   $\text{H}_2\text{SO}_4$  to stop reaction using multichannel pipettor.
5. Read the optical density at 450 nm in a 96-well plate reader.
6. Serum peroxidase content can be expressed as absorbance at 450 nm.

### C. Notes:

1. TMB- $\text{H}_2\text{O}_2$  solution must be prepared immediately before adding to plates. TMB can be oxidized by air rapidly.

### D. References:

- Rodriguez, A., Cuesta, A., Ortuno, J., Esteban, M. A., Meseguer, J. 2003.  
Immunostimulant properties of a cell wall-modified whole *Saccharomyces cerevisiae* strain administered by diet to seabream (*Sparus aurata* L.). Vet. Immunol. Immunopathol. 96, 183-192.

## Serum Glucose

### A. Reagents:

1. Nelson chromogen reagent (1 L)  
Add 50 g ammonium molybdate  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  in 900 ml DI water. Add 42 ml concentrated  $\text{H}_2\text{SO}_4$ . Then dissolve 6.0 g  $\text{Na}_2\text{HAsO}_4\cdot 7\text{H}_2\text{O}$  in 50 ml DI water and mix with the solution prepared above. Incubate at  $37^\circ\text{C}$  for 24-48 h. Store at room temperature in a glass-stopped dark bottle.
2. Somogyi oxidizing reagent (2 L)  
Add 48 g  $\text{Na}_2\text{CO}_3$  and 24 g potassium sodium tartrate (Rochele salt,  $\text{C}_4\text{H}_4\text{KNaO}_6$ ) in 1 L hot DI water. Then add 32 g  $\text{NaHCO}_3$  and allow some time to dissolve. Then dissolve 8 g  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$  in 100 ml water and add to the solution prepared above. Then dissolve 36 g  $\text{Na}_2\text{SO}_4$  in 500 ml boiling water to the solution prepared above. Allow the solution cool down and dilute to 2 L. Filter through glass wool to remove insoluble materials if necessary. Store the prepared solution in a dark bottle at room temperature.
3. Glucose standard solutions (0.1%)  
Weigh 0.1 g glucose and dissolve in 100 ml DI water to prepare the glucose stock solution (1000  $\mu\text{g}/\text{ml}$ ). Make the standard by

	Glucose standard solutions (ppm)									
	40	80	120	160	200	240	280	320	360	400
Glucose stock (ml)	1	2	3	4	5	6	7	8	9	10
DI water (ml)	24	23	22	21	20	19	18	17	16	15

### B. Procedure:

#### Sugar Determination

1. Pipette 0.2 ml of sample prepared in Step A in a test tube.
2. Add 2.0 ml Somogyi reagent.
3. Place in boiling water bath for 15 min and then allow it cool.
4. Add 2.0 ml Nelson reagent.
5. Add 5.0 ml DI water and mix thoroughly.
6. Read O. D. at 505 nm within 1-2 h.
7. Use a conversion factor of 0.93 to calculate glycogen from glucose.

### C. Notes:

1. Color density develops almost immediately.
2. Stable for many hours.
3. Mixing should be complete to release  $\text{CO}_2$ .

### D. References:

Hassid, W.Z., Abraham, S., 1957. Chemical procedure for analysis of polysaccharides. In: Colowick, S.P., Kaplan, N.O. (Eds.), *Methods of Enzymology*, Vol. 3. Academic Press, New York, p. 37.

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- Li P., Wang X. & Gatlin D. M. III., 2006. Evaluation of levamisole as a feed additive for growth and health management of hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture* 251, 201-209.
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