EFFECTS OF PARENTAL SIZE AND AGE ON LARVAL GROWTH AND DEVELOPMENT: IMPLICATIONS FOR IMPROVED INTENSIVE LARVAL YELLOW PERCH (*Perca flavescens*) CULTURE TECHNIQUES

By

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ABSTRACT

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Yellow perch aquaculture has been constrained by inadequate larval culture techniques due, in part, to the small size of first feeding larvae. This study was designed to: 1) identify mouth gapes for first feeding larvae, 2) estimate heritabilities, and 3) correlate *Artemia* cyst diameter to nauplius hatching size. Mouth gapes for larval yellow perch less than 10 mm total length (TL) were described by linear regression models: $width = 0.081599 + 0.006664\ (TL)^2 + e$ and $height = 0.060535 + 0.007155\ (TL)^2 + e$. Heritabilities ($h^2$), based on spawner TL and age, were estimated for larval yellow perch TL ($h^2 = 0.1393$), mouth gape width ($h^2 = 0$), and mouth gape height ($h^2 = 0.2289$). Separating *Artemia* cysts into discrete size categories facilitated the significant, positive correlation of cyst diameter to nauplius size. These results can be used to help design breeding programs and develop intensive larval yellow perch culture feeding strategies.

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INTRODUCTION

In the North Central Region (NCR) of the United States (U.S.), yellow perch (perch, *Perca flavescens*) is an excellent candidate for commercial aquaculture. It is recognized for its value as a food fish and for certain biological characteristics that benefit aquaculture (Calbert 1975). With the inception of the North Central Regional Aquaculture Center (NCRAC) in 1988, perch became a high-priority species for aquaculture production research and has been funded every year (Batterson, personal communication, 1997). Throughout the NCR, researchers and fish-culturists believe that perch is an important species for aquaculture development (Garling 1991; Starr 1991; Hushak et al. 1992) based on market presence and buyer demand (Gleckler et al. 1991).

Perch is one of the more valuable sport, commercial, and forage fish species in the U.S. (Hokanson and Kleiner 1974; Jobes 1952; Ney and Smith 1975; Smith 1977; Tharratt 1959; Thorpe 1977; Tsai and Gibson 1971; Whiteside et al. 1985) with commercial harvests from the Great Lakes comprising the major supply of perch fillets in the U.S. However, recent declines in perch populations throughout Lake Michigan have had significant impacts on the industry. In January 1995, the Perch Task Group (YPTG) was asked to investigate the reasons for the decline in Lake Michigan perch populations (Hess 1996). In all states surrounding Lake Michigan, commercial fishing for perch has

been closed for the 1998 fishing season. In addition, recreational fishing harvests have been reduced.

Larval perch are small at the time of hatching and first feeding, and their size may limit their ability to utilize a large portion of potential food supplies. Wong and Ward (1972) have speculated that larval perch are restricted to limited amounts of prey due to the maximum mouth gape width. Mouth gape limitations have occurred when the size of the entire prey exceeded the size of the mouth gape (Hansen and Wahl 1981; Wong 1972) or the diameter of the esophagus (Kestemont et al. 1996; Raisanen and Applegate 1983). This appears to be the most limiting factor affecting the survival of larval perch.

Two approaches could be used to help increase survival rates of larval perch. The first approach could concentrate on developing a method that would provide the smallest possible *Artemia* nauplii at first feeding. Variations observed in cyst diameters and nauplii length within a single strain may indicate a possible correlation between cyst diameter and nauplii length. The smaller diameter cysts may potentially result in smaller *Artemia* nauplii lengths. If a correlation exists between nauplii size and cyst diameter, then a process could be developed to initially hatch the smallest diameter cysts. The smallest nauplii could be fed first, since the small size of certain first feeding larval fish and crustacean limits the ability to utilize a large portion of available *Artemia* nauplii.

A second approach that could be used to help increase survival rates of larval perch would focus on the selection of broodstock. Identifying the effects of paternal size and age on their offspring could help lead to selecting broodstock which might favor increased survival rates in intensive culture conditions. Mansueti (1964) observed that perch egg diameters varied in size, which suggests that eggs from the same female or

from different females varied in size. The size of eggs can influence development and growth of larvae (Kamler 1992). However, no correlation has been reported between the size of the female perch spawner and the average size of eggs and larvae. Larger sized females could potentially produce larger eggs and larvae.

An alternative to this approach is genetic improvement through selective breeding and genetic analysis. Perch broodstock has not been genetically improved through selective breeding programs nor has any significant genetic analyses been completed. A basic approach in comparing genetic differences was completed on perch. The study compared survival and growth of larval perch, which were collected from various geographical sources of wild broodstock and raised in intensive culture conditions (NCRAC 1997). The results of the study indicated there was not a single source of perch broodstock that produced superior larvae. However, genetic selection for larval perch could help improve survival and other desirable characteristics.

This thesis is divided into three chapters and explores several strategies for improving intensive culture of larval perch including, new uses of *Artemia* nauplii, identifying the effects of parental size and age on offspring, and developing an intensive culture system and feeding strategy.

OBJECTIVES

The goal of this research project was to develop and enhance current intensive larval perch culture techniques. The specific objectives for this study were:

1) to evaluate strains of *Artemia* for variations in cyst diameter

2) to evaluate the effect of different cyst diameters on nauplii size (length and width) at the Instar I stage.

3) to determine the relationships of maternal size to total fecundity, egg size, larval total length (TL) at the time of hatch, and mouth gape size at first feeding for larval perch;

4) to define the range of mouth gape sizes for larval perch less than 10 mm TL;

5) to determine the relationships of parental size and age to mouth gape size and TL of larval perch at the time of hatch;

6) to determine the significance of gape size on growth, survival, and feed acceptance during intensive larval culture conditions.

The first chapter includes Objective 1 and 2 and details the variation observed in diameters of *Artemia* cysts, along with the potential use of size variation for larval perch culture. The second chapter includes Objectives 3, 4, and 5, which attempt to identify the reasons for size differences observed in larval perch at hatch based on parental influence. The third chapter includes Objective 6, which describes the diet strategies and culture methods used in this study.

HYPOTHESIS

The specific hypotheses tested for each objective in this study are:

Objective 1:

Null Hypothesis 1_1: All *Artemia* cysts are equal in size based on the diameter.

Prediction 1_1: The cysts can be separated into groups using the diameter of the chorion shell. If a large variation exists in the diameter of the cysts, then the groups will be statistically different from each other based on One-way ANOVA testing.

Large variations in cyst diameters have been reported, but correlating the size of newly hatched nauplii has not been studied. Objective 1 is designed to separate cysts into size categories based on the diameter and test the significance between cyst size categories. Two strains of brine shrimp, San Francisco Bay and Great Salt Lake, were evaluated.

Objective 2:

Null Hypothesis 2_1: All newly hatched Artemia nauplii are the same size (length, width, and appendage length).

Prediction 2_1: Nauplii size (length, width, and appendage length) can be correlated to cyst diameter. If a significant, positive correlation exists, larger cyst diameters will result in a statistically significant larger nauplii.

Large variations in nauplii length have been reported, but correlating the size of newly hatched nauplii has not been studied previously. Objective 2 is designed to correlate cyst diameter size categories to the length, width, and appendage length of newly hatched nauplii.

Objective 3:

Null Hypothesis 3_1: All perch larvae are equal in total length (TL) at the time of hatching.

Prediction 3_1: The predictor variable, larval TL, can be correlated to female spawner TL. If a significant correlation exists, the female spawner size groups will be significantly different from each other. Analysis of Variance (ANOVA) procedures can test the differences between female spawner size groups.

Null Hypothesis 3_2: All spawned and fertilized perch eggs are of equal diameter.

Prediction 3_2: The predictor variable, egg yolk diameter and chorion shell diameter, can be correlated to female spawner TL. If a significant correlation exists, egg diameters from different sized female spawner groups will be significantly different. ANOVA procedures can test egg diameter differences between female spawner size groups.

Null Hypothesis 3_3: Total fecundity, total number of eggs, will be equal for all female spawners.

Prediction 3_3: The predictor variable, total fecundity, can be correlated to female spawner TL. If a significant correlation exists, the female spawner size groups will be significantly different from each other. ANOVA procedures can test the differences between female spawner size groups.

The study for objective 3 was designed to describe and predict the effects of female spawner TL on total fecundity, egg yolk diameter, egg chorion shell diameter, and larval TL. The design allowed for testing the variation that might exist in larvae and eggs from the same female spawner as well as between different female spawners. The study for objective 3 was also designed to describe and predict the effects of female spawner TL on various egg and larval parameters.

Objective 4:

Null Hypothesis 4_1: Larval mouth gape size, width and height, increases with an increase in larval TL.

Prediction 4_1: The predictor variable, mouth gape width and height, can be correlated to the larval TL. If a significant correlation exists, the slope of the line will be significantly different from zero.

Objective 4 was designed to describe and predict the effects of larval TL on mouth gape width and height. The design allowed for testing the variation that might exist in larvae from the same female spawner as well as between different spawners.

Objective 5:

Null Hypothesis 5_1: All perch larvae have an equal TL at the time of hatching.

Prediction 5_1: The predictor variable, larval TL , can be correlated to female and male TL and age. If a significant correlation exists, the slope of the line will be significantly different from zero.

Objective 5 was designed to test the significance of the paternal contribution observed in Objective 3 and 4. The study was also designed to estimate heritability for larval TL, mouth gape width and height, based on the sire and dam components of the

broodstock used. A true estimation of heritability was not calculated, since the design of this study includes a fixed assignment of parental stock. However, the estimation of non-additive genetic variance, dominance, was calculated.

Objective 6:

Null Hypothesis 6_1: All perch larvae have an equal survival rate.

Prediction 6_1: If all perch larvae have an equal chance to survive, then other factors can be tested. The type of diet fed to first feeding larval perch can be correlated to survival over time. If a significant correlation exists, the slope of the regression line will be significantly different from zero. ANCOVA procedures can test the difference between regression lines for each diet.

Null Hypothesis 6_2: All perch larvae have an equal growth rate.

Prediction 6_2: If all perch larvae have an equal chance to grow at the same rate, then other factors can be tested. The type of diet fed to first feeding larval perch can be correlated to growth over time. If a correlation exists, the slope of the regression line will be significantly different from zero. ANCOVA procedures can test the difference between regression lines for each diet.

Objective 6 was designed using the results from Objective 3. The present study failed to result in rejecting the null hypothesis 3_1 (review Chapter 2, this thesis). This indicated that there were no significant differences between larvae from different sizes of female spawners. Hence, all larvae were pooled for further studies. Objective 6 was designed to test the effects of different diets on the survival and growth of larvae.

CHAPTER 1

EFFECTS OF *ARTEMIA* CYST DIAMETER ON NAUPLII SIZE

INTRODUCTION

Since 1933, many species of larval fish and crustacea have been intensively cultured using *Artemia* nauplii as first food. In some cases, *Artemia* nauplii were the only available source of live food for the youngest stages of most cultured species of larval fish and crustacea (Bardach et al. 1972; Kinne and Rosenthal 1977). High survival rates are associated with feeding *Artemia* nauplii, which has resulted in a large aquaculture demand.

In the commercial *Artemia* industry, *Artemia* cysts are graded and sold based on various parameters such as nutritional value, hatching rate, cysts per gram, and naupliar size. Depending on the quality of the *Artemia* cysts being sold, the variation of reported values for these parameters could be quite large within each graded batch. The variations for each of these parameters have been investigated and compared. However, the correlation between cyst diameter and nauplii length has not been studied previously.

When a large variation in cyst diameter occurs, another grading could be used to separate cysts into more uniform diameter size categories. This would facilitate an evaluation of the effect of cyst size on nauplii size at hatch. Smaller diameter cysts may potentially result in smaller *Artemia* nauplii lengths. The smaller nauplii could be fed to first feeding larval fish and crustaceans, which are limited by mouth gape size. Larger diameter cysts could be used when the larvae have grown large enough to eat larger nauplii.

By making more *Artemia* nauplii available to larvae limited by mouth gape, less *Artemia* would have to be cultured and fed. Less waste from uneaten *Artemia* nauplii would collect in the tanks and reduce the amount of *Artemia* needed. This process could potentially make the feeding process more efficient and could reduce feed costs.

This study was designed to explore the variation in cyst diameters, the impact of cyst diameter on nauplii size, and the potential application of cyst grading to larval fish and crustacean culture. San Francisco Bay (SFB) and Great Salt Lake (GSL) strains of *Artemia* cysts were compared.

OBJECTIVES

1) to evaluate strains of *Artemia* for variations in cyst diameter

2) to evaluate the effect of different cyst diameters on nauplii size (length and width) at the Instar I stage.

LITERATURE REVIEW

Many species of larval fish and crustacea have been successfully raised on newly hatched *Artemia* nauplii, including: Eurasian perch (Vlavonou et al. 1995), perch (Hinshaw 1985; Starr, personal communication, 1996; Dabrowski 1998), striped bass (Webster and Lovell 1990a; Lemm and Lemarie 1991), bluegill (Mischke 1995), smallmouth bass (Ehrlich et al. 1989), shrimp and prawn (Sorgeloos et al. 1983), as well as many others. *Artemia* comprises approximately 85% of the total live food used for aquaculture worldwide (Lovell 1990).

The aquaculture demand for *Artemia* cysts was met by the harvest of natural areas until the 1960's, when a severe shortage of cysts stimulated new research on the optimal use of *Artemia* (review Sorgeloos et al. 1986). Methods for processing cysts and increased hatching success were two examples of the results from the research. From these advances, uses of *Artemia* for aquaculture operations were made more efficient. In 1993, another shortage in the harvest of *Artemia* cysts occurred. After *Artemia* cyst reserves were depleted in 1995, prices soared to 500% above 1994 costs (Sawtell, personal communication, 1996). Problems continue to plague the supply of *Artemia* cysts. During the 1997 collection season, the Utah Division of Wildlife Resources (Perschon, personal communication, 1997) enacted an emergency closure of the Great

Salt Lake *Artemia* strain harvest on October 27, when the season quota of 4.5 million pounds of *Artemia* cysts had been collected.

The Great Salt Lake is an important source of *Artemia* cysts and has represented up to 90% of the world's market supply (Sawtell, personal communication, 1996), but approximately 90% of the harvest is exported to prawn farms in Asia (Jensen, personal communication, 1997). The high price of *Artemia* and the uncertainties of supply have renewed interests in the development of more efficient uses of *Artemia* in aquaculture (Starr, personal communication, 1996).

The demand for *Artemia* cysts has grown with the growth of intensive larval fish and crustacean culture. Contributing to the increased demand for *Artemia* has been the low survival of larvae fed formulated feeds and the high survival of larvae fed *Artemia* nauplii. Many larvae are unable to survive on formulated feeds alone, and in some cases, newly hatched *Artemia* nauplii were the only available source of live food for the youngest stages of most cultured species of larval fish and crustacea (Bardach et al. 1972; Kinne and Rosenthal 1977). This is partly due to a rudimentarily developed alimentary tract of larval fish that is often unable to effectively use formulated diets at first feeding (Lovell 1990). Short, poorly developed alimentary tracts, rapid evacuation rates, and low production of digestive enzymes are considered to be some of the constraints of larval food digestion (Kamler 1992). Enzymes derived from live food have been identified as providing a substantial contribution to the total enzymatic activity in the alimentary tract (Lauff and Hofer 1984) which Dabrowski (1979) suggested were needed for digestion of food by larvae. However, survival of certain larvae raised in intensive culture conditions and fed *Artemia* nauplii is still low. To help increase survival rates, researchers and

culturists have been searching for the smallest possible prey or formulated diets that still provide the necessary nutritional requirements (Brown et al. 1996).

While *Artemia* nauplii have been used to successfully raise many types of larval fish and crustacea, a search continues to identify smaller strains of *Artemia*. Feeding smaller *Artemia* could help to increase the survival of intensively cultured larvae. However, a different approach could be explored to help reduce production costs and increase larval survival rates. Instead of searching for smaller *Artemia* strains, developing ways to reduce the variation in cyst diameter and nauplii length could be explored.

Several authors have reported large statistical variations for various *Artemia* parameters (Vanhaecke and Sorgeloos 1980; Beck and Bengtson 1982; Quynh et al. 1988; Webster and Lovell 1990b; Webster and Lovell 1991). In addition, different strains from various geographical locations were compared and the values reported. Vanhaecke and Sorgeloos (1980) described variations in cyst diameters from different geographical locations. Mean cyst diameter values reported ranged from 224 to 285 μm and standard deviations ranged from 9.7 to 19.8. The authors also reported nauplii length for strains from different geographical locations. Mean nauplii length values ranged from 429 to 517 μm with standard deviations that ranged from 16.5 to 37.2. The cysts/gram ratio for the GSL brand was reported to be 280,000 dehydrated cysts/gram, while one Argent brand reported 330,000 dehydrated cysts/gram. Other parameters have also varied depending on grades and brands. Some strains of *Artemia* provided high levels of essential highly unsaturated fatty acids (20:5ω3 and 18:ω3), while other strains did not have significant amounts (Sorgeloos et al. 1983; Quynh et al. 1988; Webster and Lovell 1990a). In the commercial *Artemia* industry, cysts are graded and sold based on various

parameters such as nutritional value, hatching rate, cysts/gram ratio, and naupliar size. Higher values of these parameters result in higher prices (Sawtell, personal communication, 1996). Depending on the quality of the *Artemia* cysts being sold, the variation of reported values for these parameters could be quite large within each graded batch.

The variations observed in cyst diameters and nauplii length within a single strain may indicate a possible correlation between cyst diameter and nauplii length. A more detailed analysis of these variations might lead to specific applications in aquaculture. For example, if the cysts can be separated into different size categories according to diameter, then it might be possible to evaluate nauplii size at hatch for each category. Another grading could be used to separate cysts into more uniform diameter size categories. By grading cysts into more uniform diameter size categories, the effect of cyst size on nauplii size at hatch would be facilitated for evaluation. Smaller diameter cysts may potentially result in smaller *Artemia* nauplii lengths.

If a significant, positive correlation exists between nauplii size and cyst diameter, then a process could be developed to initially hatch the smallest diameter cysts. The smallest nauplii could be fed first, since the small size of certain first feeding larval fish and crustacea limits the ability to utilize a large portion of available *Artemia* nauplii. To be potential food, an organism must be small enough for larvae to ingest (Siefert 1972). Mouth gape limitations of first feeding larvae occurs when the size of the prey exceeds the size of the mouth gape (Hansen and Wahl 1981; Wong 1972) or the diameter of the esophagus (Kestemont et al. 1996; Raisanen and Applegate 1983). Mouth gape size has been observed to limit the optimal utilization of *Artemia* nauplii for first feeding Eurasian

perch (*Perca fluviatilis*) larvae (Kestemont et al. 1996). Hyatt (1979) observed that stickleback larvae could consume invertebrates with a maximum body width almost equal to the mouth size. By providing the smallest *Artemia* nauplii, a greater percentage of nauplii could be available as food, since the larvae are not restricted by the size of nauplii.

As larval size increases, the size of *Artemia* nauplii could be increased by culturing larger diameter cysts. The morphological capability to consume larger prey increases with growth (Wong and Ward 1972; Werner 1974). Larger diameter cysts could be saved for later uses when the larvae have grown large enough to eat larger *Artemia* nauplii. Larger nauplii could also be saved for later in the feeding process when nauplii "packing" could be used. Packing *Artemia* refers to a method of increasing fatty acid profiles, which are often absent in nauplii (see review in Sorgeloos et al. 1986). After approximately 12 hours, newly hatched nauplii molt and begin feeding. Typically, a food source that is high in essential fatty acids is provided. The nauplii retain the newly acquired fatty acids and are fed to larval fish and crustacean as a complete diet.

This strategy could potentially make the feeding process more efficient. By making more *Artemia* nauplii available to larvae limited by mouth gape, less *Artemia* would have to be cultured and fed. Less waste from uneaten *Artemia* nauplii would collect in the tanks and reduce the amount of *Artemia* needed. Feed costs would be reduced and water quality improved.

METHODS

San Francisco Bay[1] and Great Salt Lake[2] *Artemia* cysts were compared for the variations in cyst diameter and effects on nauplii size. Cyst diameters were separated into groups using micro-sieves constructed from nitex plankton screen stretched over PVC pipe. The nitex mesh sizes used were labeled with the prefix MS (Table 1) and were separated into 200, 220, 236, 243, 253, 265, 280 and 300 μm sizes. The pipe diameter was 4 inches and made of schedule 40 PVC. Nitex plankton screen was held in place using 4-inch slip to slip couplings made of schedule 40 PVC. The screens were placed in descending order beginning with the 300 μm screen.

Artemia cysts were separated by diameter using the micro-sieves. Six grams of cysts were weighed and added to tap water at room temperature for hydration. After one hour, the cysts were placed into the micro-sieves and washed through with tap water. Triplicate trials of each strain were run with samples of 100 collected from each screen. Filter containers and micro-sieves were washed thoroughly between sample trials.

Cysts from each screen were rinsed with tap water into 250 ml Millipore plastic filter funnels. Excess water was filtered off using a six-place kit Millipore filter system with 47 mm, #1 glass filters. After removing excess water, the concentrated cysts were

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[1] San Francisco Bay (Platinum grade Argentemia, Argent Chemical Laboratories, Inc., Seattle, WA; lot BP0103K)

[2] Great Salt Lake (Bonneville Artemia International, Inc., Salt Lake City, UT; no lot number)

Table 1. Label designation for each micro-sieve based on the mesh size.

Micro-sieve	Mesh size
MS200	200 μm
MS220	220
MS236	236
MS243	243
MS256	256
MS265	265
MS280	280
MS300	300

placed under a dissecting microscope. The dissecting microscope was connected to a camera, which digitized the video image. Diameters were measured using digital analysis software, OPTIMAS Imaging System, BioScan™.

After measuring the diameter of the cysts, the percent of cysts collected on each micro-sieve was calculated. Cysts were rinsed from the filter into aluminum pans and allowed to dry in an oven at 38 °C for 48 hours (modified from Sorgeloos et al. 1986). The dry cysts were weighed and calculated as a percentage of the total.

Nauplii size at hatch for the 200 and 280 µm size categories were evaluated. The separated cysts were hatched separately and samples collected to measure nauplii length according to diameter. Three *Artemia* cyst samples weighing 2.5 grams each were placed into room temperature tap water. After one hour, the hydrated cysts were placed into the top of the stacked micro-sieves and rinsed through with tap water. The cysts in the 200 and 280 µm micro-sieves were collected and placed into a 1.5 L beaker with 1 L of hatching medium. Procedures for hatching *Artemia* and making the hatching medium (Appendix A) follow the guidelines reviewed by Sorgeloos et al. (1986). After approximately 21 hours of incubation at 28 °C, first Instar I nauplii were randomly collected from the triplicate samples. Procedures for random nauplii collection are outlined in Sorgeloos et al. (1986). The nauplii were preserved in Lugol's solution (Edmondson 1959). Sample sizes of 20 were selected after conducting power analysis tests (Snedecor and Cochran 1989). *Artemia* nauplii size was determined by measuring the 20 Instar I nauplii along the long axis, at the widest point, and the length of the appendages. The nauplii were placed under a dissecting microscope and measured using

digital analysis software, OPTIMAS Imaging System, BioScan™. Methods for determining hatching percentage (Appendix B) are outlined in Sorgeloos et al. (1986).

STATISTICAL ANALYSIS

Once separated into the different size categories, cyst diameters were measured and statistically analyzed. The success of separating the cysts into the different size categories was determined by calculating the mean cyst diameter for each micro-sieve and comparing the means. Significance was based on an alpha level of 0.05%.

The means were tested first for normal distribution and equal variances. If the sample size was greater than 2,000, the test for normal distribution was based on the *Kolmogorov D* statistic (Neter et al. 1996). If the sample size was less than 2,000, the test for normal distribution was based on the *Shapiro-Wilk statistic W* (Neter et al. 1996). The assumption of equal variances was tested using Hartley's F-maximum test (Sokal and Rohlf 1995). If the assumption of equal variances was violated, the nonparametric Kruskal-Wallis test was used (Miller 1985). One-way Analysis of Variance (ANOVA) was used to test differences between means from each micro-sieve. If the ANOVA test indicated an overall significance, contrast statements were used to determine which micro-sieves were significantly different from each other.

The mean nauplii size for the 200 and 280 μm micro-sieves were tested for normal distribution and equal variances. Two sample t-tests were used to test significant differences between means from each screen and significant differences between strains.

RESULTS

The first objective of this study was to evaluate the variation in cyst diameters for from each sample used. Using the stacked micro-sieves, *Artemia* cysts were separated into different diameter size categories. The means and percentage of the total cyst biomass used were calculated (Table 2). The majority of cysts were collected in micro-sieves MS236, MS265, and MS280. These micro-sieves represented 67.9% of the total for GSL and 67.3% for SFB. Very few cysts were collected from the micro-sieves MS200 and MS220. These micro-sieves represented 6.0% of the total for GSL and 9.2% of the total for SFB. Many of these cysts in these two micro-sieves were not hydrated and were not used for measurements. The reasons for the failure to hydrate were not investigated.

A sample of cyst diameters from each micro-sieve was measured, and the means and standard deviations were calculated (Table 3). A general increase in variance was observed as screen size increased. The data were not normally distributed ($p = 0.0001$) over all treatments, and the assumption of equal variances across treatments was violated ($p > 0.05$; Appendix C). The non-parametric test indicated that were significant differences (Appendix C) which are summed in Figures 1 and 2.

Table 2. Averages and percentages of San Francisco Bay (SFB) and Great Salt Lake (GSL) *Artemia* cyst dry weight for each micro-sieve. Values are means ± standard deviations (sd). All other values are reported as percent.

	GSL		SFB	
Micro-sieve	Mean ± sd	Percent	Mean ± sd	Percent
MS200	0.156 ± 0.017 g	2.6 %	0.221 ± 0.028 g	3.8 %
MS220	0.202 ± 0.012	3.4	0.313 ± 0.019	5.4
MS236	0.914 ± 0.063	15.4	1.043 ± 0.054	17.9
MS243	0.485 ± 0.047	8.2	0.493 ± 0.038	8.5
MS256	0.451 ± 0.043	7.6	0.416 ± 0.055	7.2
MS265	1.397 ± 0.049	23.6	1.367 ± 0.061	23.5
MS280	1.712 ± 0.125	28.9	1.508 ± 0.033	25.9
MS300	0.607 ± 0.072	10.2	0.454 ± 0.019	7.8
Total	5.924	100	5.815	100

Table 3. Averages of San Francisco Bay (SFB) and Great Salt Lake (GSL) *Artemia* cyst diameters for each micro-sieve. Values are reported as means ± standard deviations (sd).

GSL				
Micro-sieve	N	Mean ± sd	Minimum	Maximum
MS200	309	213 ± 6.7 µm	194 µm	234 µm
MS220	267	229 ± 7.5	207	247
MS236	309	228 ± 6.4	202	245
MS243	309	235 ± 4.5	225	253
MS256	309	242 ± 7.6	223	260
MS265	309	252 ± 10.5	230	282
MS280	309	273 ± 9.1	240	296
MS300	309	292 ± 26.0	223	358

SFB				
Micro-sieve	N	Mean ± sd	Minimum	Maximum
MS200	309	216 ± 10.1 µm	191 µm	261 µm
MS220	309	225 ± 11.3	203	274
MS236	309	231 ± 10.1	198	277
MS243	309	232 ± 8.5	205	265
MS256	309	235 ± 7.9	200	271
MS265	309	251 ± 10.3	223	280
MS280	309	262 ± 14.0	216	329
MS300	296	284 ± 23.3	208	356

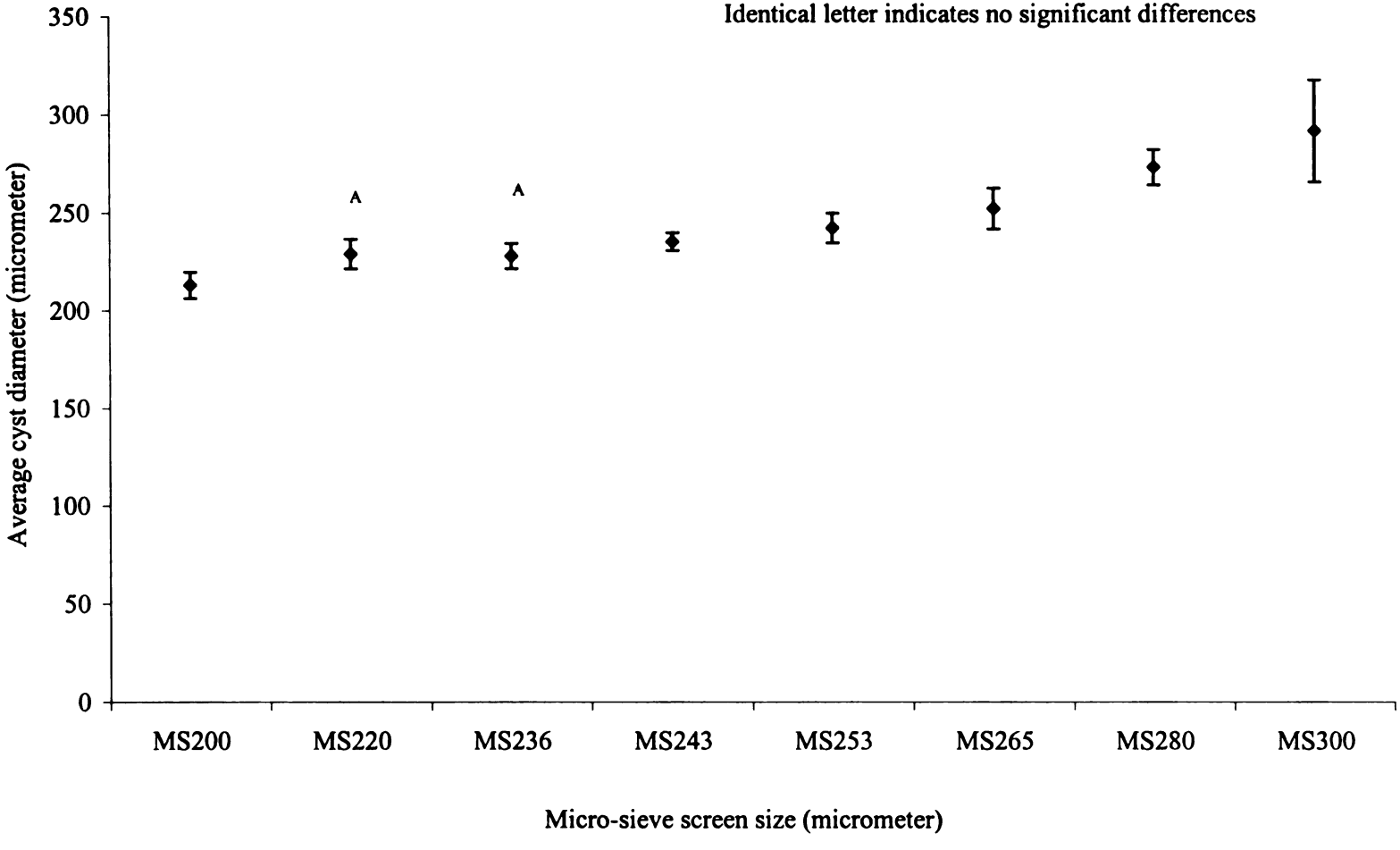

Figure 1. Average cyst diameter (micrometer) for commercially available Great Salt Lake *Artemia* strain from each micro-sieve (mesh size in micrometer).

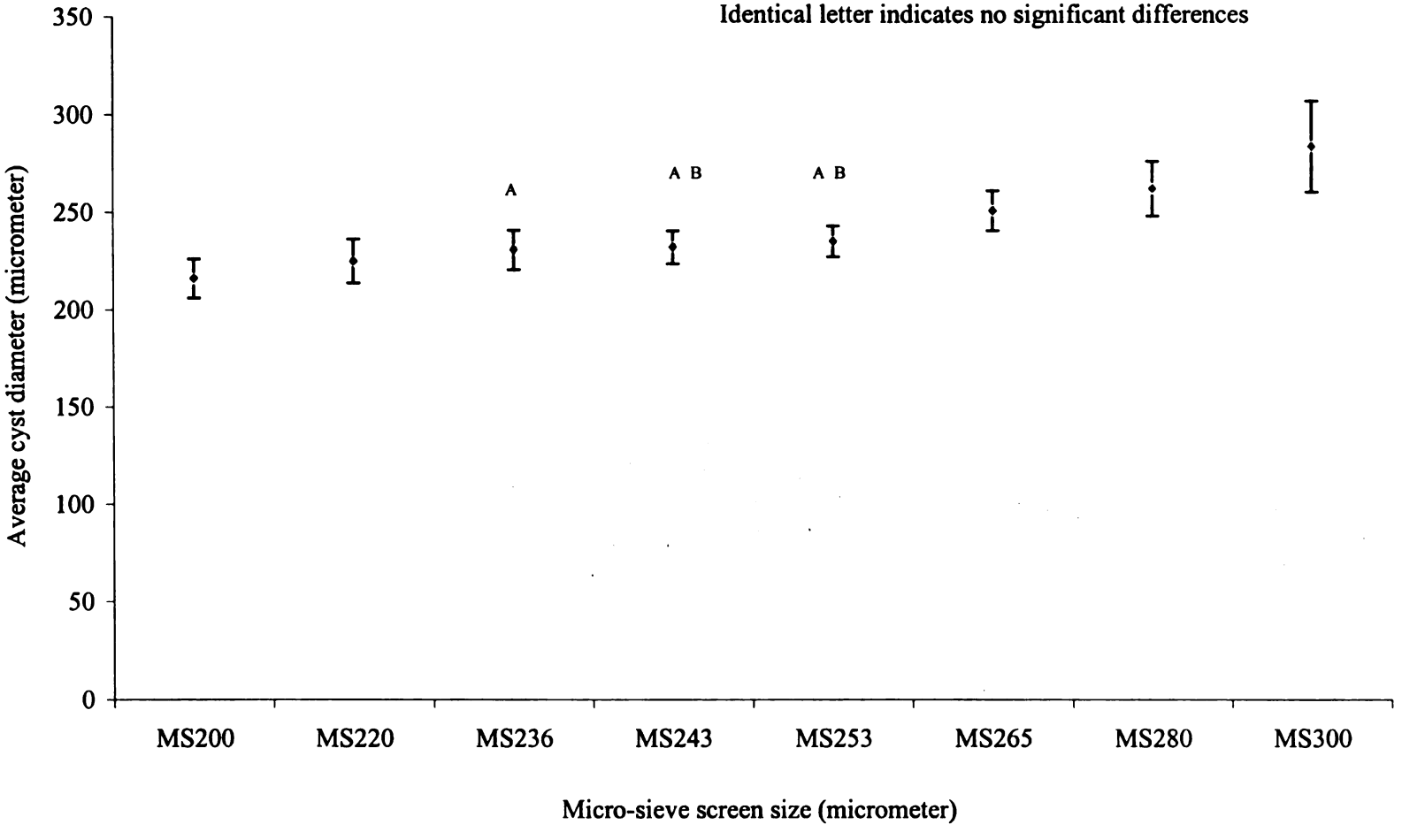

Figure 2. Average cyst diameter (micrometer) for commercially available San Francisco Bay *Artemia* strain from each micro-sieve (mesh size in micrometer).

The second objective of this study was to determine if the Instar I nauplii size was correlated to cyst diameter. Cysts from micro-sieves MS200 and MS280 were chosen because the cyst diameters were significantly different (Figure 1; Figure 2) and were at the opposite ends of the stacked micro-sieve (Table 1). The means for nauplii length, widths, and appendage length were calculated and compared (Table 4). The data were normally distributed for all parameters, and the variances were homogeneous across treatments (Appendix C). One-way ANOVA indicated that there were statistically significant differences between nauplii from the different micro-sieves for both strains (Appendix C). Contrast statements indicated significant differences between micro-sieves (Figure 3; Figure 4).

The parameters measured for both strains of *Artemia* were compared (Table 4; Figure 5). The t-tests indicated that there were significant differences between strains for nauplii width and appendage length in micro-sieve MS200. The SFB strain was significantly larger for both parameters. The t-tests also indicated that there were significant differences between strains for nauplii length and appendage length in micro-sieve MS280. The GSL strain had a longer length than the SFB strain, but the SFB strain had a larger appendage length than the GSL strain. Hatching percentage for both sizes and both strains are summed in Table 5.

Table 4. Averages of San Francisco Bay (SFB) and Great Salt Lake (GSL) *Artemia* nauplii length, width, and appendage length for the MS200 and MS280 micro-sieves. Values are reported as means ± standard deviations (sd).

GSL					
Variable	Micro-sieve	N	Mean ± sd	Minimum	Maximum
Nauplii length	MS200	60	416 ± 28.7 µm	345 µm	519 µm
Nauplii width	MS200	60	148 ± 12.1	126	183
Appendage length	MS200	60	603 ± 52.9	484	749
Nauplii length	MS280	60	487 ± 27.4	429	576
Nauplii width	MS280	60	170 ± 12.2	142	207
Appendage length	MS280	60	685 ± 40.0	561	764

SFB					
Variable	Micro-sieve	N	Mean ± sd	Minimum	Maximum
Nauplii length	MS200	60	427 ± 25.3 µm	387 µm	495 µm
Nauplii width	MS200	60	154 ± 14.6	124	187
Appendage length	MS200	60	635 ± 36.6	570	730
Nauplii length	MS280	60	467 ± 21.5	396	515
Nauplii width	MS280	60	171 ± 14.6	144	216
Appendage length	MS280	60	706 ± 45.7	593	837

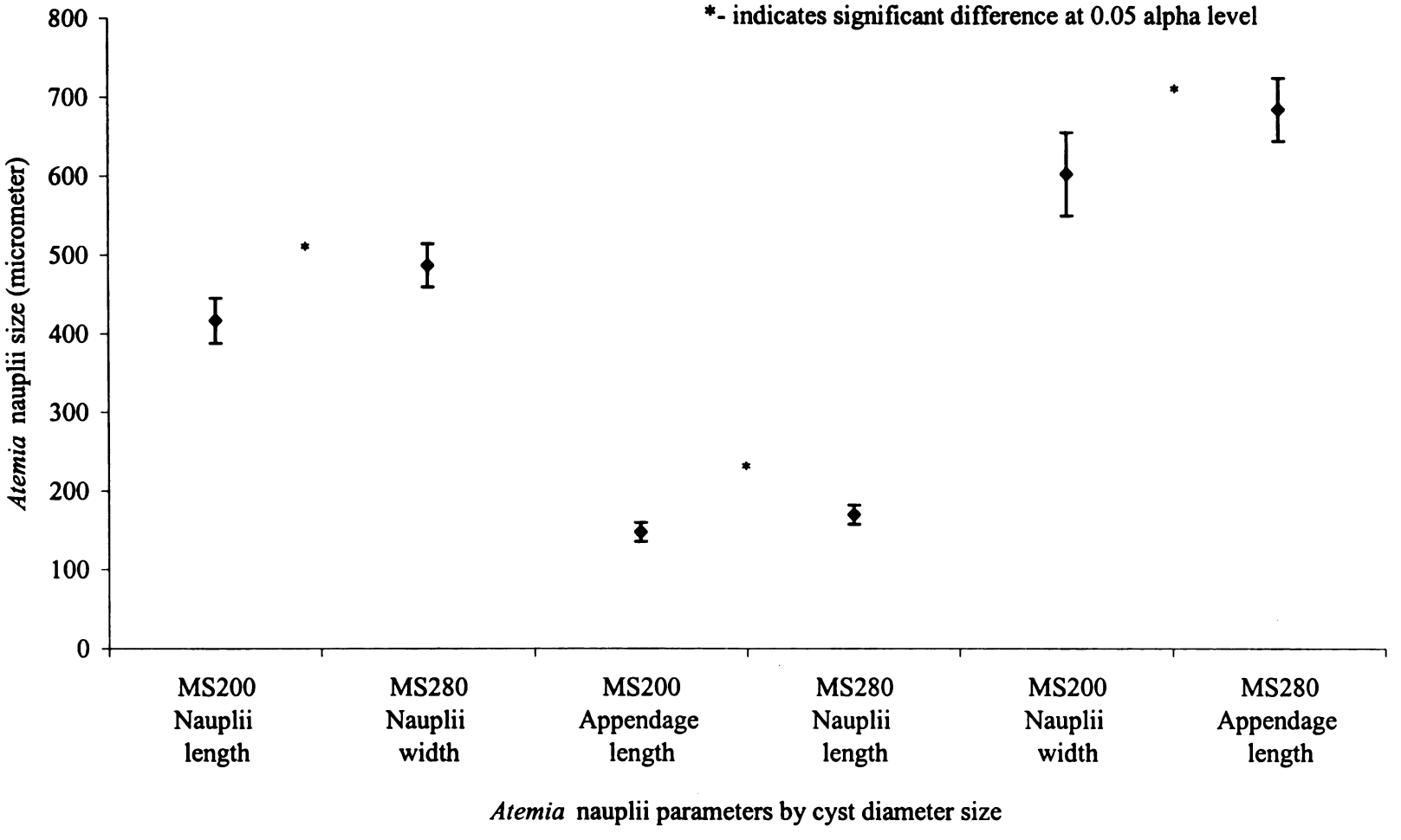

Figure 3. Comparison of Great Salt Lake *Artemia* strain instar I nauplii length, width, and appendage length for cysts collected from MS200 and MS280 micro-sieves (mesh size in micrometer).

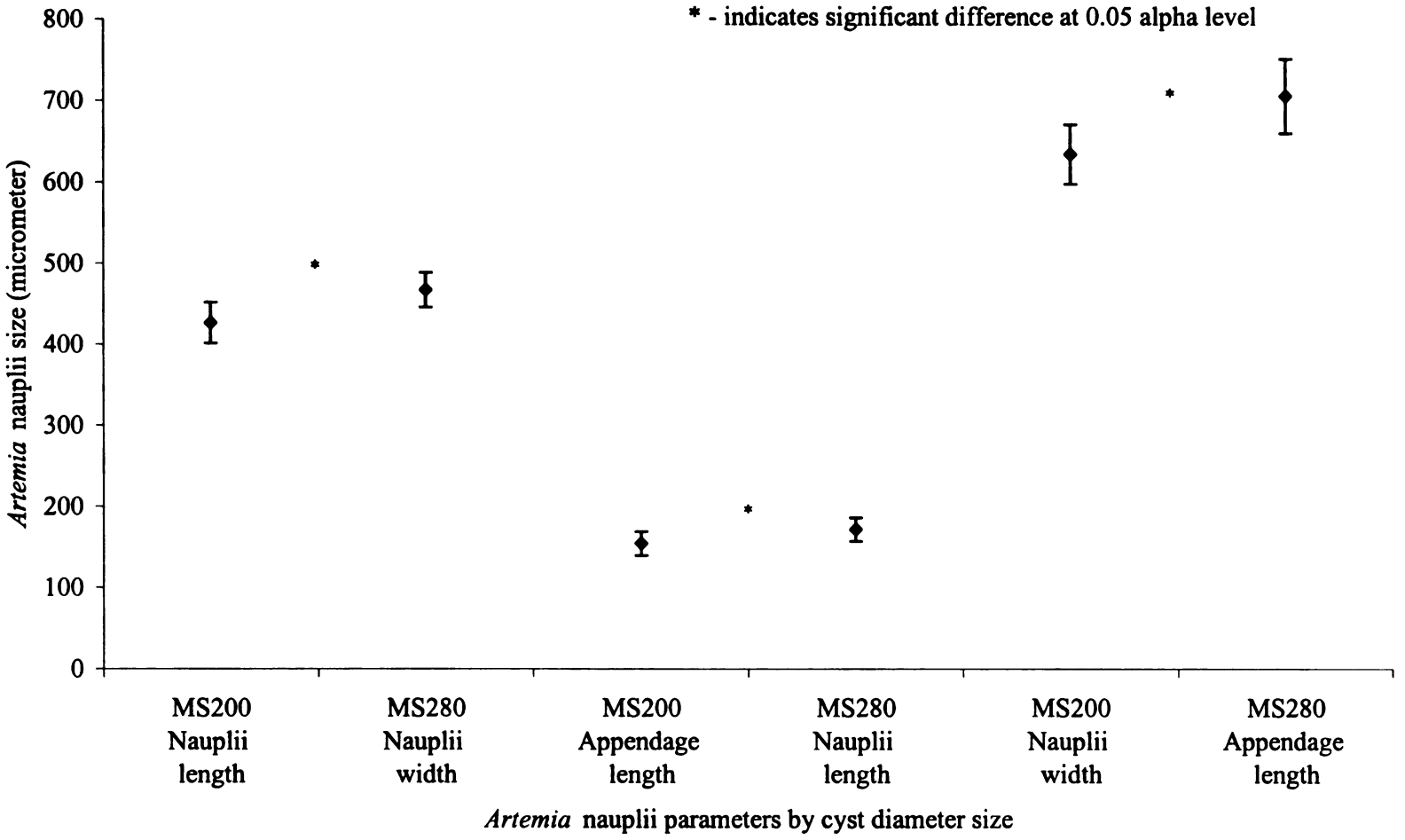

Figure 4. Comparison of San Francisco Bay *Artemia* strain instar I nauplii length, width, and appendage length for cysts collected from MS200 and MS280 micro-sieves (mesh size in micrometer).

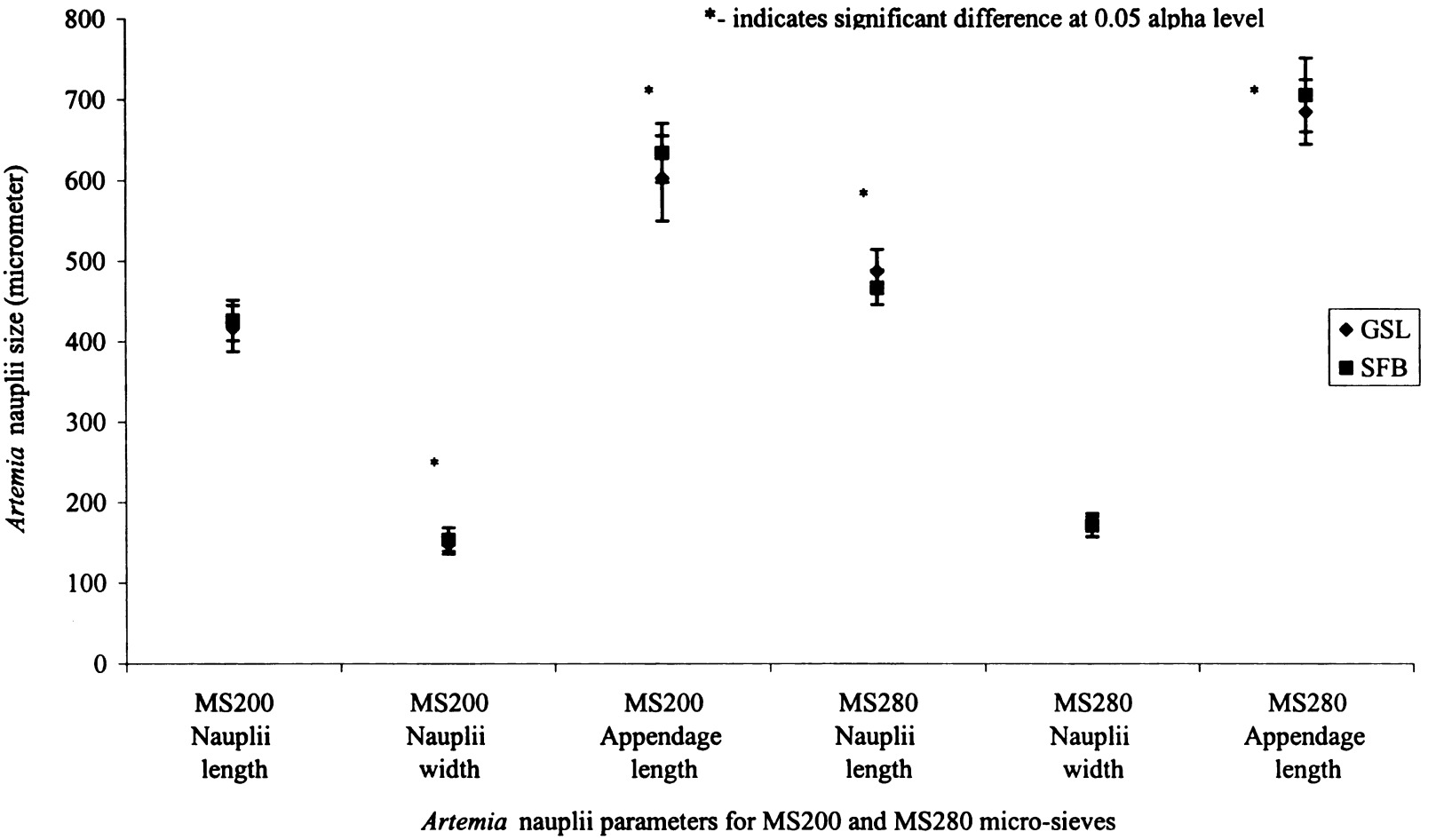

Figure 5. Comparison of first instar I nauplii for the commercialy available Great Salt Lake and San Francisco Bay *Artemia* strains from micro-sieve MS200 and MS280 (mesh size in micrometer).

Table 5. Hatching percentage of San Francisco Bay (SFB) and Great Salt Lake (GSL) *Artemia* strains in micro-sieves MS200 and MS280. All values are reported as percent.

GSL		
	Micro-sieve	Percent hatched
	MS200	76.0 %
	MS280	60.2
SFB		
	Micro-sieve	Percent hatched
	MS200	72.0
	MS280	83.8

DISCUSSION

Variations in cyst diameters from different geographical locations have been described by Vanhaecke and Sorgeloos (1980). Their study revealed large statistical variation in *Artemia* cyst diameters for the SFB and GSL strains ($\bar{x}_{SFB}$ = 224.7μm, σ^2 = 153.76; $\bar{x}_{GSL}$ = 252.5 μm, σ^2 = 169). Similar values were found in the current study ($\bar{x}_{SFB}$ = 241.8μm, σ^2 = 0.589; $\bar{x}_{GSL}$ = 246.1 μm, σ^2 = 0.736). The large variation observed indicated that the cysts could be separated into size categories.

Once the *Artemia* cysts were separated into different size categories according to diameter, a more detailed analysis of these variations revealed the distribution of cyst diameters. For both strains, the majority of *Artemia* cysts were found in micro-sieves MS236 (GSL – 15.4%; SFB – 17.9%), MS265 (GSL – 23.6%; SFB – 23.5%), and MS280 (GSL – 28.9%; SFB – 25.9%). Less *Artemia* cysts were found in the remaining micro-sieves. Although the number of *Artemia* cysts collected in each micro-sieve was significantly different, the distribution helped reduce the number of micro-sieves needed. By reducing the number of micro-sieves, the process of separating *Artemia* cysts was improved.

The stacked micro-sieves were further improved by analyzing the cysts collected in each micro-sieve. The cyst diameters were measured and statistically analyzed. For the SFB *Artemia* strain, there were no significant differences between micro-sieves

MS236 vs. MS243 and MS243 vs. MS256, and the MS243 micro-sieve was removed. For the GSL *Artemia* strain, there were no significant differences between micro-sieves MS220 vs. MS236 and MS280 vs. MS300, and the micro-sieves, MS236 and MS300 were removed. The inability to achieve significant differences between some of the micro-sieve groups may have been the result of three possible factors. First, the mesh sizes may have been too similar. For example, some of the micro-sieves had a maximum difference of 20 μm, while the minimum difference was 7 μm (Table 1). The difference between micro-sieves MS236 and MS243 was 7 μm. The mesh sizes represented the Nitex screen availability in the commercial industry. The second reason for the inability to achieve significant differences may have resulted from contamination. In all trials, it was noted that there were leaks between several of the micro-sieves. The Nitex screen, which was stretched over the PVC pipe, bunched up and prevented the PVC coupling from fitting completely. As a result, some water and *Artemia* cysts escaped through the side of the stacked micro-sieves and may have contaminated other micro-sieves. A third reason for the lack of significant differences between some micro-sieves was the large percentage of cysts collected in micro-sieves MS265 and MS280. The cysts clogged the Nitex screen and made it difficult to process all cysts through the stacked micro-sieves. Dehydrated cysts could not be used for this experiment, since the cysts have a convex shape.

Statistically significant differences between micro-sieves and a more uniform diameter size made it possible to evaluate nauplii size at hatch. Vanhaecke and Sorgeloos (1980) reported variations in newly hatched *Artemia* nauplii length from the GSL and SFB strains ($\bar{x}_{SFB}$ = 429.5 μm, σ^2 = 689; $\bar{x}_{GSL}$ = 487.5 μm, σ^2 = 894). Similar nauplii

length values were found in the current study ($\bar{x}_{SFB}$ = 447μm, σ^2 = 961; $\bar{x}_{GSL}$ = 452 μm, σ^2 = 1225).

Nauplii size at hatch was evaluated by taking *Artemia* cysts from the micro-sieves MS200 and MS280. Cysts from these two micro-sieves were chosen because the cyst diameters were significantly different (Figure 1; Figure 2) and were at the opposite ends of the stacked micro-sieves (Table 1). For both strains in the current study, nauplii had statistically significant differences in length, width, and appendage length. This indicated that there was an increase in nauplii size as *Artemia* cysts increased in size. Smaller diameter cysts produced smaller *Artemia* nauplii sizes. If cysts were separated into more uniform sizes, the smaller cysts could be cultured first, and the nauplii could be fed to larval fish and crustaceans with the appropriate mouth gape and esophagus. As larvae increase in size, the size of *Artemia* nauplii could be increased by culturing progressively larger diameter cysts.

In the final part of this study, the differences between these two *Artemia* strains were compared. It is generally accepted that the SFB strain is one of the smallest nauplii overall (Sorgeloos et al. 1986). However, comparisons of the same size cyst diameter from both strains indicated the opposite. GSL cysts collected from the micro-sieve MS200 resulted in smaller newly hatched nauplii than SFB. This indicates that GSL nauplii hatched from the smallest cysts can be fed to larval fish and crustaceans. This trend was not repeated for larger diameter cysts.

Reducing size variation in cysts and, consequently nauplii size, has the potential to make the feeding process more efficient when using newly hatched *Artemia* nauplii. This process would help to provide a greater percentage of small *Artemia* nauplii to first

feeding larvae that are limited by mouth gape size. Less *Artemia* would have to be cultured and fed, which reduces the amount of *Artemia* needed. Less waste from uneaten *Artemia* nauplii would collect in the tanks and feed costs could potentially be reduced.

SUMMARY AND CONCLUSIONS

1) The high prices and decreased supply of *Artemia* have renewed interest in developing more efficient use of *Artemia* for aquaculture.
2) Newly hatched *Artemia* nauplii from the GSL strain were as small or smaller than nauplii from the SFB strain. Either strain could be fed to first feeding larval fish and crustaceans. However, the SFB strain is marketed as the smallest nauplii and should be fed to small larvae. The demand and price for the SFB strain is higher, reaching as much as $100 per lb, while the GSL strain is priced less than $35 per lb.
3) Large statistical variations in cyst diameters were observed for the SFB and GSL *Artemia* strains.
4) Nauplii hatched from cysts collected in the micro-sieves MS200 and MS280 had statistically significant differences in length, width, and appendage length for both strains, SFB and GSL. These results indicate that there was a significant, positive correlation between *Artemia* cyst diameter and nauplii length, width, and appendage length.
5) Although separating *Artemia* cysts by size would enhance their use, the process used in this study would not be efficient for small-scale aquaculture operations. After the hydration and separation processes, the *Artemia* cysts would have to be dehydrated for storage (review Sorgeloos et al. 1986).

6) After harvesting, separating cysts into smaller size categories could be done during cyst processing, but prior to dehydrating the cysts. The cysts could be graded, dehydrated, and sold according to diameter.

CHAPTER 2

EFFECTS OF PARENTAL SIZE AND AGE ON DIFFERENCES OBSERVED IN EGG SIZE, MOUTH GAPE SIZE, AND TOTAL LENGTH OF LARVAL YELLOW PERCH AT HATCH

INTRODUCTION

Successful intensive larval culture of perch is limited by high mortality rates. The small size of larval perch limits their ability to search and feed on certain prey. In addition, Wong and Ward (1972) suggested that limited prey selection was due to a restriction of gape width.

Variations in the size of eggs and larval fish from the same female or among females could influence development and growth. Larger sized females and males could potentially produce larger larvae with larger mouths. Several species of larval fish with larger mouths grew faster than larvae with smaller mouths (Shirota 1970).

This study was designed to identify the effects of parental size and age on differences in eggs and larvae of perch. In the first year of this study, the relationships between maternal size and age to total fecundity, egg size, larval TL at the time of hatch, and mouth gape size at first feeding were identified. The range of mouth gape sizes for larval perch under 10 mm TL were defined. In the second year, the relationships of parental (both male and female) size and age to larval perch mouth gape size and TL at

the time of hatch were identified. Estimates of heritability were calculated for potential selection of desirable characteristics.

OBJECTIVES

3) to determine the relationships of maternal size to total fecundity, egg size, larval TL at the time of hatch, and mouth gape size at first feeding for larval perch;

4) to define the range of mouth gape sizes for larval perch under 10 mm TL;

5) to determine the relationships of paternal size and age on larval mouth gape size and TL of perch at the time of hatch.

LITERATURE REVIEW

Intensive culture of larval perch must be refined to achieve commercial production success (NCRAC 1997). However, larvae of perch are difficult to rear intensively due to their small size (Mansueti 1964; Nickum 1978; Best 1981). Perch larvae have been observed to hatch within the range of 4.7 to 6.6 mm TL (Mansueti 1964; Hokanson and Kleiner 1974; Ney 1978). After hatching, the larvae begin absorbing the yolk sac and developing a more complete and functional mouth (for review, see Mansueti 1964). While larvae have not been observed feeding immediately after hatching, exogenous feeding has been observed to begin before the yolk sac has been fully absorbed. First feeding larvae were as small as 6.0 mm TL (Siefert 1972). By the time the larvae had reached approximately 7.0 mm TL, the yolk sac had been completely absorbed (Mansueti 1964; Siefert 1972).

The transition period from yolk absorption or endogenous feeding to exogenous feeding characterizes the transition from prolarvae to postlarvae (Hubbs 1943). This transition period has also been described as the "critical period" for larval fish because of the increased mortality associated with the metamorphosis of larvae (Hjort 1914; 1926). In general, failure to start exogenous feeding when the yolk sac is completely absorbed results in mortality due to starvation (Wang and Eckmann 1994). This period of high

mortality appears to be the most limiting factor for developing larval perch intensive culture at commercial levels.

Since larval perch are small at the time of hatch and first feeding, their size may limit their ability to utilize a large portion of potential food supply. To be potential food, an organism must be small enough to be ingested and slow enough to be captured by the larvae (Siefert 1972). Wong and Ward (1972) have speculated that larval perch are restricted to limited amounts of prey due to the maximum mouth gape width. Mouth gape limitations have occurred when the size of the prey exceeded the size of the mouth gape (Hansen and Wahl 1981; Wong 1972) or the diameter of the esophagus (Kestemont et al. 1996; Raisanen and Applegate 1983). Mouth gape size has limited the prey selection for other planktivorous fish (Wankowski 1979) and has limited the optimal utilization of *Artemia* nauplii for first feeding Eurasian perch (*Perca fluviatilis*) larvae (Kestemont et al. 1996).

Researchers and culturists have been searching for the smallest possible prey or formulated diet that provides their nutritional requirements to help increase survival of larval perch raised in intensive culture conditions (Brown et al. 1996). Identifying the mouth gape size of first feeding perch larvae could help researchers and culturists identify the appropriate sized prey or formulated diet.

Attempts have been previously made to determine the maximum mouth gape size for various larval fish. Using a modified equation developed by Shirota (1970), Guma'a (1978) determined the maximum mouth gape height for larval Eurasian perch using the following equation:

(2.1) maximum mouth gape height = $\sqrt{U^2 + L^2}$

where U and L are the lengths of the upper and lower jaws respectively, when opened at 90°. The calculated mouth gape height was used with larval TL to develop a linear regression model. By using the linear regression model, the maximum mouth gape height for natural stocks of Eurasian perch could be determined for first feeding larvae. The maximum size of prey or formulated food for first feeding Eurasian perch larvae was estimated to be 684 μm.

However, it is not clear from the available data or methods, if Guma'a (1978) measured mouth gapes for larvae under 10 mm TL. If mouth gape heights were not measured for larvae smaller than 10 mm TL, statistical predictions on mouth gape sizes cannot be made for smaller lengths (Tempelman, personal communication, 1997). The relationship between the mouth gape size and length of larvae less than 10 mm TL might not be linear. The rate of increase may be much higher or lower than predicted by a straight-line regression. For example, Henderson and Ward (1978) concluded that the body weight of perch increased exponentially after hatch. If the rate of growth or increase in mouth gape sizes is exponential for Eurasian perch or perch larvae under 10 mm TL, then the linear regression model cannot be applied.

Wong and Ward (1972) measured the mouth gape widths of larval perch that were greater than 10 mm TL. Their results indicated that the mouth size of perch larvae initially increased more rapidly than body length. The measurements were made using a calibrated, tapered brass cone with increments of 0.2 mm. Arts and Evans (1987) measured the mouth gape width of larval perch using a modification of the brass cone technique. Larval fish smaller than 10 mm TL could not be measured effectively using

either technique. The maximum mouth gape size for larval perch less than 10 mm TL, first feeding larvae, has not been identified.

While some researchers have studied the theoretical maximum prey size that larval fish can consume, other researchers have studied the size preference that larval fish exhibit. Stickleback larvae consumed invertebrates with a maximum body width almost equal to the mouth gape size (Hyatt 1979). However, Dabrowski and Bardega (1984) concluded that cyprinid larvae consumed zooplankton prey that were only 40 - 60 % of the total mouth gape.

Various approaches can be taken to help increase the survival of larval perch raised in intensive culture conditions. Researchers have concentrated on developing feeding strategies using small prey, such as small zooplankters and newly hatched *Artemia* nauplii, or by providing a small, nutritionally complete diet. Some researchers have attempted to raise larval perch on a commercial scale using both types of live food (NCRAC 1995; Starr, personal communication, 1996; Dabrowski 1998) with limited success. Other researchers that have attempted to use formulated diets exclusively to raise larval perch have not been successful (Brown et al. 1996).

Melard and Kestemont (1994: in Kestemont et al. 1996) have illustrated the importance of mouth size. While approximately 60-70 % of newly hatched Eurasian perch larvae consumed a small strain *Artemia* nauplii, the remaining 30-40 % disappeared due to starvation or lack of vigor throughout the first week of experiments. The authors speculated that the inability to consume *Artemia* nauplii was potentially due to differences in mouth size between those that survived and those that died.

Another method for increasing the survival of larval perch raised in intensive culture conditions could concentrate on selecting larvae with desirable characteristics or selecting broodstock that produce larval offspring with desirable characteristics. Shirota (1970) concluded that several species of larval fish with larger mouths grew faster compared to larvae with smaller mouths. Selection for larger larvae or larvae with larger mouths could potentially increase the survival of larval fish past the critical period. Positive correlations between larval TL and mouth gape size have been established for perch larvae greater than 10 mm TL (Wong and Ward 1972; Art and Evans 1987). Longer larvae would have larger mouth gapes. By selecting for larvae with longer TL, the potential for survival increases. However, researchers have not developed methods for selecting longer larvae.

Wang (1994), Wang and Eckmann (1994), and Kestemont et al. (1996) have suggested that eggs from the Eurasian perch have varied in size from the same female or from different females, which could influence development and growth of larvae. Similarly, Mansueti (1964) observed that perch egg diameters varied in size. The egg diameters ranged from 1.6 to 2.1 mm before water hardening and expanded to 1.7 to 4.5 after water hardening. The variations observed in egg diameter might account for variations observed in larval TL. Perch larvae hatch at variable lengths. Researchers have reported TL values ranging 4.7 to 6.6 mm TL (Mansueti 1964; Hokanson and Kleiner 1974; Ney 1978). However, no correlation has been reported between the size of the female perch spawner and the average size of eggs and larvae. The size of eggs and larvae from some marine fish increase with the size of female spawners within the same strain of fish (Kamler 1992).

Genetic improvement through selective breeding and genetic analysis can improve desirable characteristics. European carp and freshwater salmonids were some of the first fish to be genetically improved through carefully controlled experiments (Moav 1979). As a result, fish have been observed with genetically increased fecundity rates, egg size, hatching percent, growth, and height/length ratio. Perch broodstocks have not been genetically improved through selective breeding programs nor has any significant genetic analyses been completed. A basic approach in comparing genetic differences among perch compared survival and growth of intensively cultured larval perch, which were collected from various geographical sources (NCRAC 1997). The results indicated that no single source of perch broodstock produced superior larval offspring.

Genetic analysis of desirable characters is required to design efficient selection programs and predict expected gains (Moav 1979). In particular, the additive genetic variance and its proportionate amount for each phenotype can be used to predict whether selection will be effective (Tave 1993). Estimates of heritability are calculated using the additive genetic variance, which ranges from 0 to 1 (for review, see Falconer 1989). In cases where heritabilities are closer to 0, selection for that particular trait is not expected to improve the existing mean genetic value (Purdom 1993). By establishing a breeding program with carefully designed experiments, the gains observed in European carp and freshwater salmonids potentially could be observed for perch. Genetic selection could help improve survival and other desirable characteristics in larval perch.

METHODS

Year 1: Using maternal size and age as indicators for egg and larval size of perch

Perch broodstock was collected from the outer Saginaw Bay, Lake Huron for a two week period beginning on April 19, 1996 and transported to Bay Port Aquaculture in West Olive, Michigan (Figure 6). Eggs were collected over a two week period from April 22 to May 4. All experiments were conducted at Bay Port Aquaculture's hatchery facilities.

Female Spawner and Egg Analysis

The broodstock was held in a flow through system supplied with 11 °C aerated well water. Females were separated into six female spawner size groups by length (Table 6). Attempts were made to replicate each spawner size group five times. Data collected for the female spawners included TL, weight and age. The TL to the nearest 1 mm of the spawners (from the tip of the head to the tip of the tail, with lobes compressed) was determined using a measuring board. Weights were recorded to the nearest 1 g using a top loading, electronic balance. Scale samples were taken from below the lateral line on

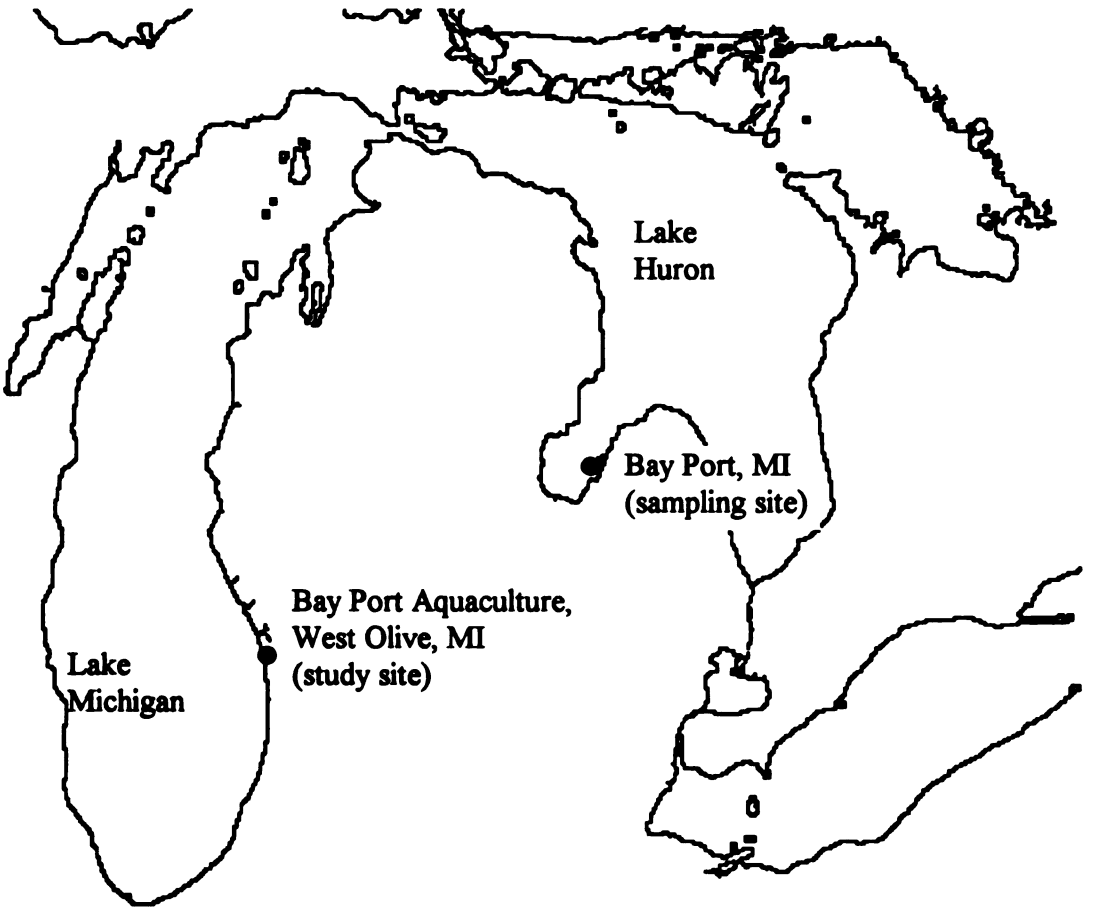

Figure 6. Map showing sampling location of yellow perch broodstock in Lake Huron and study site at Bay Port Aquaculture research facilities near Lake Michigan.

Table 6. Separation of female yellow perch broodstock into six spawner size groups (SG) based on total length.

Female spawner size group	Total length
SG 201	201-225 mm
SG 226	226-250
SG 251	251-275
SG 276	276-300
SG 301	301-325
SG 326	326-350

the left side (DeVries and Frie 1996). Age was determined after the spawning season by counting the scale annuli and reporting completed years of life. Age analysis was checked and verified by a second independent analysis of every third scale.

Egg ribbons were collected from each of the female spawner size groups (Table 6). Data collected for each egg ribbon included weight, number of eggs per gram of eggs, total fecundity, chorion shell diameter, and yolk diameter. For all measurements, a video recorder connected to an Olympus dissecting microscope was used to record all egg and larval images. The video images were converted into a digital image using the OPTIMAS Imaging System, BioScan™. For each female, the eggs were manually stripped into a dry bowl. Two egg samples (approximately 5g and 1g) were collected from the posterior (i.e. situated nearest to the urogenital vent) portion of the egg ribbon, weighed, and recorded to the nearest 0.1 g using a top loading, electronic balance. The remaining egg ribbon was weighed for total fecundity rate calculations.

After weighing the 1g egg sample, the eggs were mixed with well water and allowed to water harden in a separate container. After one hour, the egg sub-samples were fixed in Stockard's solution (Galat 1972). After 48 hours, the Stockard's solution was replaced with a 10% formalin solution. The eggs were counted in August 1996, and the number of eggs per gram of eggs was determined and the diameters of the chorion shell and yolk were measured.

After weighing the 5g sample, the eggs were fertilized using milt from one or more males. The dry method was used because it enhances fertilization rates for eggs (Piper et al. 1982). In a dry container, milt was mixed with the eggs before water was added. The fertilized eggs were allowed to water harden for 20 min before being placed

into an incubating unit. After the egg samples were fertilized and water hardened, they were randomly placed in specially designed incubator chambers (Westmaas 1992). The incubator chambers were randomly placed in an eight tray vertical incubator unit and supplied with 11.5 °C +/- 0.5 °C well water with a flow rate of 12 L/min (Leitritz and Lewis 1980).

In addition to the samples collected from the six female spawner size groups, egg samples were collected from a sub-sample of the female spawner size groups. The sub-samples were collected from three fish in SG 226 and three fish in SG 301 (Table 6). These two female spawner size groups were chosen because adequate numbers of gravid females were available and provided a large enough size difference for contrasts. Two egg samples (approximately 5g and 1g) were collected from the anterior, median, and posterior positions of each egg ribbon. The posterior portion of the egg ribbon was the region of the egg ribbon that was closest to the urogenital vent. The median portion was the region of the egg ribbon that was the mid-point between the two ends. The 5g and 1g egg samples were weighed, fertilized, and fixed using the same methods described above. The remaining egg ribbon was weighed to calculate the total fecundity rate. The parameters for number of eggs per gram of eggs, diameters of the chorion shell and yolk were determined using the OPTIMAS Imaging System, BioScan™.

Larval Offspring Analysis

Beginning with the first day of the hatch, five prolarvae were randomly selected from each incubator chamber. The larvae were mildly agitated first and then selected with a Pasteur pipette. The opening of the pipette was large enough to aspirate larvae without causing damage. Larvae were anesthetized using CO_2 saturated tonic water. After the larvae were anesthetized, they were placed in a 90 mm diameter Petri dish containing 10 ml of distilled water. The larvae were placed on the left ventral side for TL measurements. Methods for measuring the mouth gape were modified from Arts and Evans (1987). While on the ventral side, a needle was inserted into the mouth to separate the jaws. Care was taken to insure that the mouth was not stretched or the larvae damaged. The distance from the upper to the lower jaw was measured as the maximum gape height. The larvae were placed on the dorsal side to measure the mouth gape width. The needle was carefully inserted without stretching the mouth or causing damage to the larvae. The width of the jaw was measured at the point where the two jaws connected. Sampling continued daily in the same manner until all larvae were removed from the chambers.

STATISTICAL ANALYSIS

Variations within groups of female spawners and differences between groups of female spawners were tested using One-way analysis of variance (ANOVA) (Snedecor and Cochran 1989). Variation was also tested for eggs taken at different areas of the same egg ribbon and compared to another spawner size group using Two factor ANOVA (Neter et al. 1996). Relationships were analyzed using simple linear regression analysis. All values, unless otherwise stated, were based on a significant alpha level = 0.05%. The test for normal distribution was based on the *Shapiro-Wilk statistic W* (Neter et al. 1996). If the data were not normally distributed, they were transformed to create a normal distribution. The appropriate transformation was determined using the *Box-Cox* procedure (Tempelman, personal communication, 1997). The assumption of equal variances was tested using a *Modified Levene's* test (Neter et al. 1996). If the assumption of equal variances were violated, differences between the means were determined using the non-parametric *Kuskal-Wallis* test instead of One-way ANOVA (Miller 1985). If the variances were not homogeneous for the two factor ANOVA, the data were ranked transformed (Conover and Iman 1981). All linear regression models were based on the following basic model (Neter et al. 1996):

(2.2) $y = \beta_o + \beta_1 (x) + e$

where β_o was the regression coefficient for the intercept;

β_1 was the regression coefficient for the slope of the line;

x was the explanatory variable;

y was the response variable;

e was the residual error term.

Analysis of covariance (ANCOVA) was used to test for statistically significant differences between regression coefficients in the linear regression models (Neter et al. 1996). In cases where a single explanatory variable did not explain the relationship well, multiple linear regression models were explored using a stepwise regression selection procedure (Neter et al. 1996).

Female Spawner Analysis

Data collected on the female spawner groups included TL, weight, and age. Means for each of the female spawner group parameters were calculated and tested for normal distribution and homogeneity of variances. One-way Analysis of Variance (ANOVA) tests were used to determine overall statistical significance between groups of females. ANOVA contrast statements were used to determine statistical significance between group comparisons. Simple linear regression analysis was used to describe and predict the relationship between female spawner TL and weight. The linear regression model was based on the power function:

(2.3) $W = a\,L^b$,

where W was weight;

L was length;

a and *b* were the regression parameters (Carlander 1969; Anderson and Neumann 1996).

The simple linear regression model can also be written as:

(2.4) $\log_{10}(W) = a' + b * \log_{10}(L) + e$,

where a' was $\log_{10}(a)$ and the y-intercept parameter;

b was the slope regression parameter;

e was the residual error term.

Egg Analysis

Data collected on the egg ribbons for two spawner groups included number of eggs per gram of eggs, chorion shell diameter, and yolk diameter, sampled from three different areas of the egg ribbon: anterior, median, and posterior. A 2 x 3 factorial design was used to test significance between locations of the egg ribbon. Means and standard deviations for each female spawner group parameter were calculated and tested for normal distribution and homogeneity of variances. Factorial analysis was used to determine overall statistically significant differences between sampling locations in the egg ribbon. Contrast statements were used to estimate statistically significant differences between the locations in the ribbon within the two spawner size groups.

Data collected on the egg ribbons for each spawner group included total fecundity, egg ribbon weight, number of eggs per gram of eggs, chorion shell diameter, and yolk diameter. Means and standard deviations for each female spawner group parameter were calculated and tested for normal distribution and homogeneity of

variances. One-way ANOVA tests were used to determine overall statistical significance between groups of females. ANOVA contrast statements were used to determine statistical significance between group comparisons. Simple linear regression analysis was used to describe and predict the relationship between female spawner TL or weight with total fecundity, egg ribbon weight, number of eggs per gram of eggs, chorion shell diameter, and yolk diameter. The linear regression models were based on the basic model (2.1).

Larval Offspring Analysis

Data collected on the larval offspring for each spawner group included total length, mouth gape width and height. Larvae were separated into six groups based on the female spawner size classification.

Means and standard deviations for each larval group parameter were calculated and tested for normal distribution and homogeneity of variances. One-way ANOVA tests were used to determine overall statistical significance between the six different groups of perch larvae. ANOVA contrast statements were used to determine statistical significance between group comparisons. Simple linear regression analysis was used to describe and predict the relationship between female spawner TL or spawner weight to larval TL, larval mouth gape width, and larval mouth gape height. The linear regression models were based on the basic model (2.1). Simple linear regression models were developed that described and predicted the relationship between larval TL and larval mouth gape width and height. Individual linear regression models were developed for each larval

group. ANCOVA was used to test for statistically significant differences between the regression coefficients in the different larval group linear relationships. The initial mouth gape size and rate of mouth gape increase were tested. To standardize the hatching period of larval perch, the time of hatching was standardized from day 0, the day when the eggs were fertilized.

METHODS

Year 2: Effects of perch parental size and age on larval total length and mouth gape width and height

The additive genetic variance and non-additive genetic variance (dominance) for TL, mouth gape width and height were estimated using a cross-classification design (Dickerson 1969; Falconer 1989; Tave 1993). Two slow growing and two fast growing male perch were crossed with each of the four females, two slow growing and two fast growing (Figure 7). This mating produced eight families sired by slow growing males and eight families sired by fast growing males.

Male and female spawners were selected from the pool of spawning stock collected from the outer Saginaw Bay, Lake Huron during a two week period starting April 15, 1997 and were transported to Bay Port Aquaculture. The spawning stock was held in a flow through system supplied with 11 °C well water.

Age of the spawners was determined before selection and analyzed at a later time for verification. Spawners were selected based on age-length frequencies for native perch in Lake Huron based on data provided by the Michigan Department of Natural Resources, Fisheries Division (Fielder, personal communication, 1997). The TL's were compared to age and a sampling frequency was calculated. Based on a normal distribution (Central Limit Theorem) for the age selected, an 80% two tailed t-based distribution was

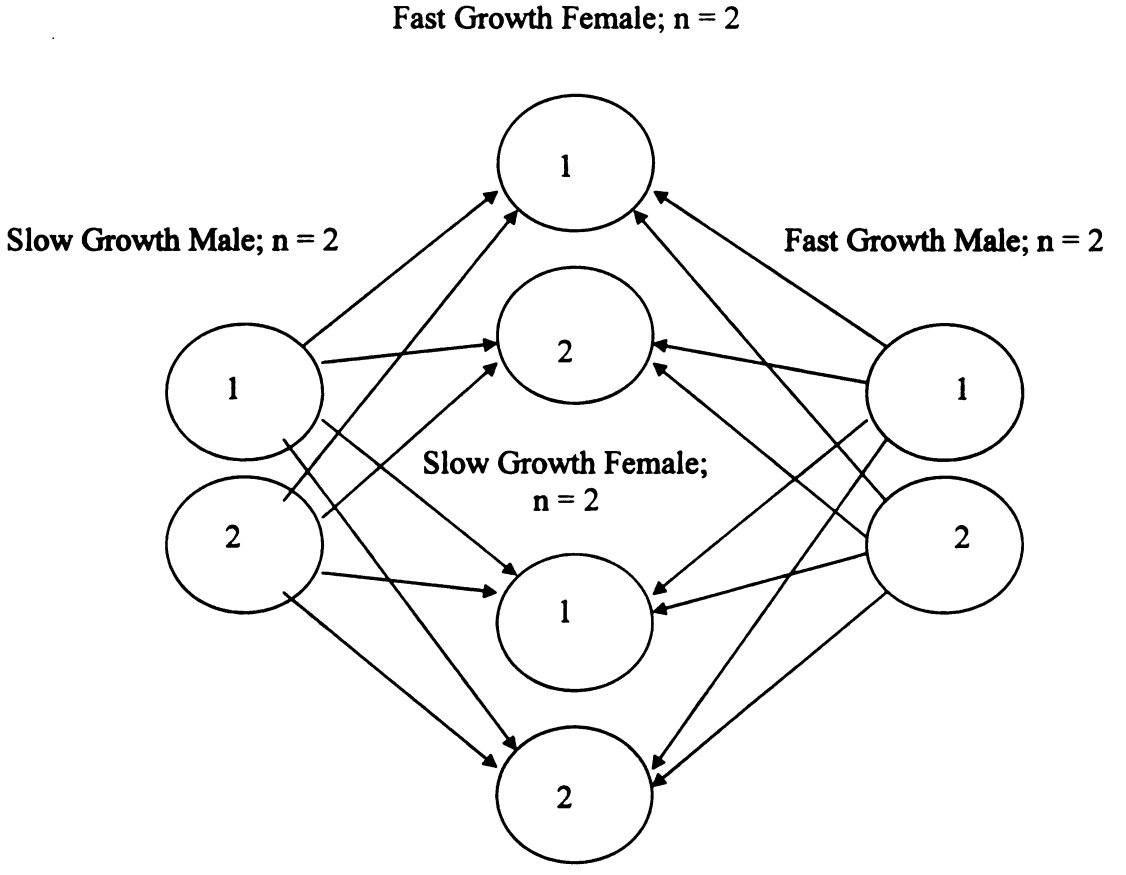

Figure 7. Experimental design for Objective 5, paternal influence on the larval TL and mouth gape size of larval yellow perch.

calculated for TL (Snedecor and Cochran 1989). The test was used to select slow and fast growth rates based on TL for the age (Figure 8). TL's in the upper limit of the 10% tail represented a fast growth rate. TL's in the lower limit of the 10% tail represented a slow growth rate.

For each female, the eggs were manually stripped into a dry bowl. Eight samples of 5g were initially cut from the egg ribbon and weighed. The egg samples were randomly placed into separate, dry containers. The milt from each of the four male spawners randomly fertilized two of the egg samples from each female spawner before water was added. Each cross was replicated twice. The fertilized eggs were allowed to water harden for 30 min before being placed in a 55 gallon cooler. The eggs were transported back to the MSU aquaculture laboratory within three hours of fertilization.

After arriving at the lab, the fertilized egg samples were placed in 11.5 °C $\pm$ 0.5 °C well water bath. After the eggs had tempered, they were placed in specially designed incubator chambers (Westmaas 1992). The incubator chambers were randomly placed in an eight tray vertical incubator unit and supplied with 11.5 °C $\pm$ 0.5 °C well water with a flow rate of 12 L/min (Leitritz and Lewis 1980).

Beginning on the second day of the hatch, the larvae were randomly selected in each incubator chamber for measurements of TL. On day 5 of the hatch, 10 larvae were randomly selected for measurements of mouth gape width and height. While still in the incubating chamber, the larvae were randomly sampled by mildly agitating and selecting with a Pasteur pipette. The opening of the pipette was large enough to aspirate larvae without causing damage. Larvae were anesthetized using CO_2 saturated tonic water. Sampling continued daily in the same manner until all larvae were sampled.

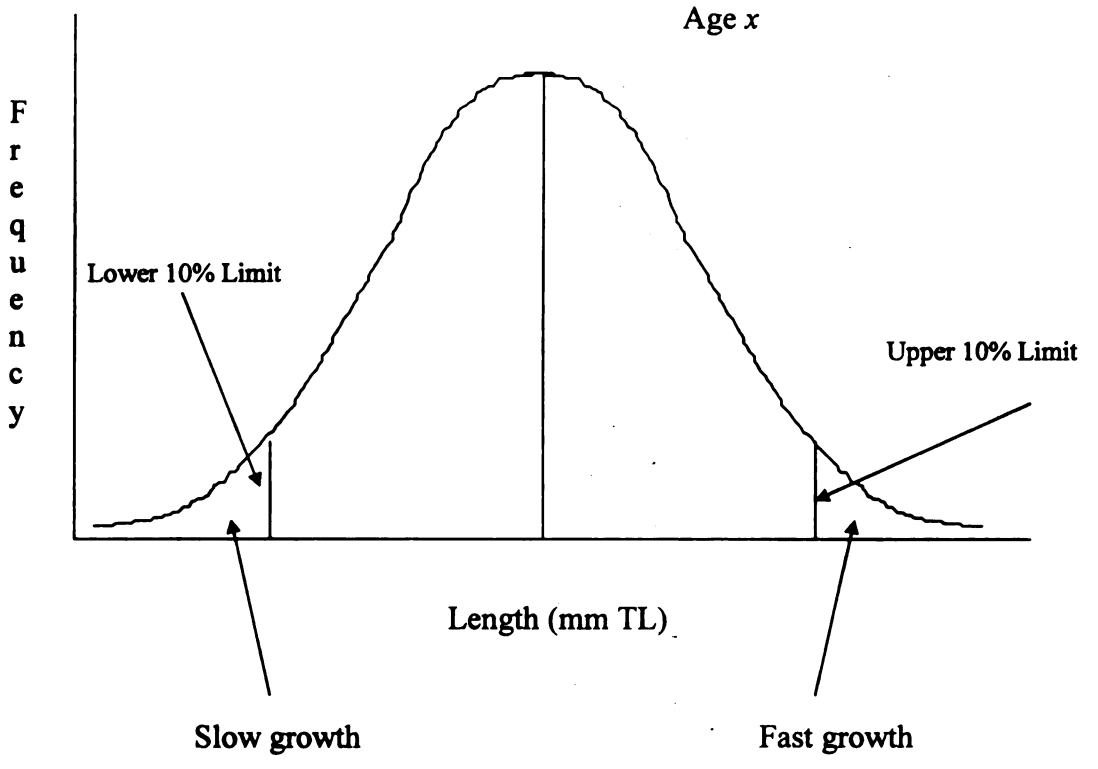

Figure 8. Experimental illustration of two-tailed t-based distribution for selection of growth rates (slow vs. fast) based on age-length frequency distributions in Saginaw Bay, Lake Huron (data provided by the MI DNR; Dave Fielder 1997).

STATISTICAL ANALYSIS

The average additive and non-additive genetic strain effects for perch growth rates, fast and slow, were estimated. The average non-additive strain effect was separated into the two reciprocal crosses and estimated. The variations around the average additive and non-additive genetic strain effects were estimated as the additive and non-additive genetic individual effects. The non-additive genetic strain effect was partitioned into two heterotic effects based on mating fast growth dams with slow growth sires (hd) and slow growth dams with fast growth sires (hs).

Detection of significant treatment and interaction (specific combining ability) effects were performed on coded data. Variance components were estimated and used to determine the relative contributions of maternally and paternally derived sources of variation to the total variation. The specific combining ability was also partitioned out (Falconer 1989). Contrast statements were used to determine significance between fast and slow genotypes and between the average non-additive genetic strain effects of sire and dam (hs and hd).

The ANOVA model used to test for significance was based on a partially nested factorial design (Winer 1971). The model was:

(2.5) $Y_{ijklm} = \mu + c_f(\alpha_f)_{ik} + c_s(\alpha_s)_{ik} + sire_j(sire\ group_i) + dam_l(dam\ group_k) +$

$sire_j * dam_l(sire\ group_i * dam\ group_k) + hs(Recip)_{12} + hd(Recip)_{21} + e_{ijklm}$

where, μ was the overall mean;

c_f was the covariate for fast growth;

$c_f = 1$, when $sire_f$ x dam_f cross;

$= 0.5$, when $sire_f$ x dam_s cross or $sire_s$ x dam_f cross;

$= 0$, when $sire_s$ x dam_s cross;

c_s was the covariate for slow growth;

$c_s = 0$, when $sire_f$ x dam_f cross;

$= 0.5$, when $sire_f$ x dam_s cross or $sire_s$ x dam_f cross;

$= 1$, when $sire_s$ x dam_s cross;

$(\alpha_f)_{ik}$ was the additive effect of genotype for fast growth;

$(\alpha_s)_{ik}$ was the additive effect of genotype for slow growth;

$sire_j$ (sire $group_i$) was the effect of the *j*th male within the *i*th male group;

$i = 1$, when sire was genotype fast growth;

$= 2$, when sire was genotype slow growth;

dam_l (dam $group_k$) was the effect of the *l*th female within the *k*th female

group;

j = 1, when dam was genotype slow growth;

= 2, when dam was genotype fast growth;

$sire_j$ * dam_l ($sire\ group_i$ * $dam\ group_k$) was the effect of the *j*th male by the *l*th female within the *i*th male and *k*th female group;

hs was the covariate for male fast growth (*i*=1) by female slow growth (*k*=2) heterotic component;

hs = 1, when $sire_f$ x dam_s cross;

= 0, otherwise;

hd was the covariate for male slow growth (*i*=2) by female fast growth (*k*=1) heterotic component;

hd = 1, when $sire_s$ x dam_f cross;

= 0, otherwise;

$(Recip)_{12}$ was the heterotic effect of male fast growth (*i*=1) and female slow growth (*k*=2), and;

$(Recip)_{21}$ was the heterotic effect of male slow growth (*i*=2) and female fast growth (*k*=1).

For the determination of heritability and dominance, the variance component was partitioned for the sire and dam combinations based on Becker (1975). Models described in Falconer (1989) were used to estimate heritability and dominance for TL, mouth gape width, and mouth gape height for each day (Appendix D and E, respectively).

RESULTS

Female Spawner Analysis

Weight and TL for each female was measured, and the age determined from the scales. The means and standard deviations for each female spawner size group were calculated (Table 7). The means for spawner TL, weight, and age increased from the smallest to the largest female spawner size group. Means and standard deviations for spawner TL and weight at each completed age were also calculated (Table 8). The means for spawner TL and weight increased from the smallest to the largest female spawner age group.

The data were normally distributed for TL ($p = 0.0980$) and age ($p = 0.0509$) but were not normally distributed for weight ($p = 0.0074$, Appendix F). The weight data were log transformed to bring the data back to a normal distribution ($p = 0.0874$). The test for the assumption of equal variances across female spawner size groups indicated a violation ($p < 0.05$) of equal variances for all three parameters. Since hypothesis testing was not conducted on these parameters, nonparametric tests were not used. Two factor ANOVA tests indicated an estimated overall statistical significance ($p = 0.0001$) for the age-group effect in spawner TL and weight (Appendix G). However, the age effect was not significant. The age effect was eliminated and One-way ANOVA tests were

Table 7. Average spawner total length (TL), weight, and age for each female spawner size group (SG). Values are reported as means ± standard deviations (sd).

Parameter	Group	N	Mean ± sd	Minimum	Maximum
Female	SG 201	6	221.50 ± 3.50 mm	216 mm	225 mm
Spawner	SG 226	5	242.60 ± 5.46	237	250
Total	SG 251	7	259.14 ± 7.40	252	274
Length	SG 276	7	280.71 ± 4.92	276	287
	SG 301	6	312.83 ± 8.06	301	322
	SG 326	4	340.50 ± 11.24	328	350
Female	SG 201	6	119.17 ± 7.28 g	109 g	129 g
Spawner	SG 226	5	150.60 ± 13.13	139	171
Weight	SG 251	7	212.71 ± 24.63	181	260
	SG 276	7	269.86 ± 20.03	248	301
	SG 301	6	395.33 ± 26.61	365	428
	SG 326	4	497.75 ± 39.87	460	541
Female	SG 201	6	4.5 ± 0.84	4	6
Spawner	SG 226	5	5.4 ± 0.55	5	6
Age	SG 251	7	6.3 ± 1.13	5	8
	SG 276	7	6.6 ± 1.27	5	8
	SG 301	6	7.3 ± 1.03	6	9
	SG 326	4	8.5 ± 0.58	8	9

Table 8. Averages for spawner total length (TL) and weight for each female spawner completed age. Values are reported as means ± standard deviations (sd).

Parameter	Age	N	Mean ± sd	Minimum	Maximum
Female	4	4	220.0 ± 3.37 mm	216	224
Spawner	5	8	254.9 ± 21.15	224	287
Total length	6	7	259.7 ± 25.95	225	301
	7	7	288.3 ± 30.18	252	322
	8	6	298.0 ± 29.83	258	334
	9	3	339.0 ± 19.05	317	350
Female	4	4	118.0 ± 8.87 g	109	129
Spawner	5	8	191.6 ± 62.28	119	301
Weight	6	7	213.7 ± 89.31	124	380
	7	7	312.1 ± 96.73	200	428
	8	6	343.8 ± 104.37	223	468
	9	3	486.7 ± 78.23	397	541

conducted on group effects, which were statistically significant (Appendix F). The tests indicated an estimated overall statistical significance ($p = 0.0001$) for both parameters, female spawner TL and weight (Appendix F). Spawner TL and weight contrast statements indicated statistical significance between all group means (Table 9). Age contrast statements indicated statistical significance between the following groups: SG 201 vs. SG 251, SG 201 vs. SG 276, SG 201 vs. SG 301, SG 201 vs. SG 326, SG 226 vs. SG 301, SG 226 vs. SG 326, SG 256 vs. SG 326, and SG 301 vs. SG 326 (Table 9).

Linear regression analysis was used to describe the relationship between female spawner weight and TL. The response variable, log(weight) parameter, was highly correlated to the predictor variable, log(spawner TL) parameter ($r^2 = 0.9820$) and the slope of the line was statistically significant from zero ($p = 0.0001$, Appendix H).

Factorial Analysis of positions within the egg ribbon

The means and standard deviations for number of eggs per gram of eggs (Table 10), egg yolk diameter (Table 11), and chorion shell diameter (Table 12) were calculated for each position of the egg ribbon. The mean values demonstrated an increase from the anterior position to the median position of the egg ribbon. The mean values for number of eggs per gram of eggs decreased from the median position to the posterior position for both female spawner size groups (Table 10). This trend was also observed for egg yolk diameter (Table 11) and chorion shell diameter (Table 12), in SG 226. However, the mean values for both of these parameters in SG 301 continued to increase from the median position to the posterior position.

Table 9. ANOVA contrast statements for spawner total length, weight, and age. Mean values for spawner total length, weight, and age are compared between female spawners groups (SG). Significance determined on the 0.05 % alpha level.

		TL		Weight		Age	
Contrast	df	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
SG 201 vs SG 226	1	25.91	0.0001	5.10	0.0315	2.27	0.1424
SG 201 vs SG 251	1	97.70	0.0001	53.56	0.0001	10.60	0.0029
SG 201 vs SG 276	1	241.77	0.0001	138.98	0.0001	14.27	0.0007
SG 201 vs SG 301	1	534.09	0.0001	433.44	0.0001	24.79	0.0001
SG 201 vs SG 326	1	725.34	0.0001	651.63	0.0001	39.52	0.0001
SG 226 vs SG 251	1	17.04	0.0003	21.32	0.0001	2.35	0.1357
SG 226 vs SG 276	1	90.43	0.0001	78.58	0.0001	4.12	0.0517
SG 226 vs SG 301	1	287.11	0.0001	309.44	0.0001	10.49	0.0030
SG 226 vs SG 326	1	454.56	0.0001	507.33	0.0001	21.98	0.0001
SG 251 vs SG 276	1	34.76	0.0001	21.65	0.0001	0.29	0.5918
SG 251 vs SG 301	1	198.76	0.0001	204.11	0.0001	3.65	0.0660
SG 251 vs SG 326	1	359.58	0.0001	391.77	0.0001	12.85	0.0012
SG 276 vs SG 301	1	71.13	0.0001	96.36	0.0001	1.93	0.1753
SG 276 vs SG 326	1	194.18	0.0001	250.43	0.0001	9.74	0.0041
SG 301 vs SG 326	1	39.21	0.0001	47.69	0.0001	3.36	0.0770

Table 10. Averages for number of eggs per gram in each position of the egg ribbon (anterior, median, and posterior) for female spawner size groups (SG), SG 226 and SG 301. Values are reported as means ± standard deviations (sd).

Group	Position	N	Mean ± sd	Minimum	Maximum
SG 226	Anterior	3	634.61 ± 181.84 μm	477.18	833.65
SG 226	Median	3	662.60 ± 166.89	530.00	850.00
SG 226	Posterior	3	653.75 ± 143.20	516.13	801.95
SG 301	Anterior	3	635.15 ± 32.54	600.00	664.23
SG 301	Median	3	669.78 ± 15.58	653.04	683.87
SG 301	Posterior	3	659.35 ± 37.78	618.60	693.22

Table 11. Averages for egg yolk diameter in each position of the egg ribbon (anterior, median, and posterior) for female spawner size groups (SG), SG 226 and SG 301. Values are reported as means ± standard deviations (sd).

Group	Position	N	Mean ± sd	Minimum	Maximum
SG 226	Anterior	75	0.3160 ± 0.0468 μm	0.1954	0.3946
SG 226	Median	75	0.3355 ± 0.0489	0.2391	0.4435
SG 226	Posterior	75	0.3274 ± 0.0809	0.1323	0.4548
SG 301	Anterior	75	0.2879 ± 0.0330	0.2097	0.3405
SG 301	Median	75	0.3514 ± 0.0451	0.2463	0.4493
SG 301	Posterior	75	0.3926 ± 0.0423	0.3091	0.5025

Table 12. Averages for chorion shell diameter in each position of the egg ribbon (anterior, median, and posterior) for female spawner size groups (SG), SG 226 and SG 301. Values are reported as means ± standard deviations (sd).

Group	Position	N	Mean ± sd	Minimum	Maximum
SG 226	Anterior	75	1.3731 ± 0.0632 μm	1.2158	1.4838
SG 226	Median	75	1.4003 ± 0.0685	1.2702	1.5581
SG 226	Posterior	75	1.3918 ± 0.1107	1.1415	1.5758
SG 301	Anterior	75	1.3343 ± 0.0436	1.2333	1.4057
SG 301	Median	75	1.4225 ± 0.0636	1.2793	1.5672
SG 301	Posterior	75	1.4822 ± 0.0630	1.3622	1.6528

The data were normally distributed for all of the parameters (Appendix I). The assumption of homogeneous variances across positions for the chorion shell and egg yolk diameters was violated ($p < 0.05$) but was not violated ($p > 0.05$) for the parameter number of eggs per gram of eggs (Appendix I).

Since the assumption of homogeneous variances was violated for the chorion shell and egg yolk diameter, the data were ranked transformed. Multi-factorial analysis was completed using the ranked transformed data which demonstrated an overall statistically significant difference ($p = 0.0001$, Appendix I). Contrast statements for both parameters indicated statistically significant differences ($p < 0.05$) between the three positions of the egg ribbon in SG 301 (Table 13). There were no statistically significant differences ($p > 0.05$) between positions of the egg ribbon in SG 226 (Table 13).

The number of eggs per gram of eggs data were not ranked transformed, and multi-factorial analysis was completed which demonstrated that there were no statistically significant differences ($p = 0.9984$) between positions of the egg ribbon (Appendix I). The female spawner size group, SG 226, was more variable than SG 301, but there were no significant differences between the positions within the two groups.

Egg Analysis

Data for egg ribbon weight, total fecundity, and number of eggs per gram of eggs were collected. The means and standard deviations were calculated for the parameters by female spawner size groups (Table 14) and by completed age (Table15). The means calculated for egg ribbon weight and total fecundity data demonstrated an

Table 13. ANOVA contrast statements for chorion shell and egg yolk diameters. Mean values for chorion shell and egg yolk diameters are compared between positions within the egg ribbon (anterior, median, and posterior) for female spawner size groups (SG) SG 226 and SG 301. Significance determined on the 0.05 % alpha level.

Chorion shell diameter

Contrast	Group	Estimate	Std error	p-value
Anterior vs. Median	SG 226	-0.72	0.0117	0.4689
Anterior vs. Posterior	SG 226	1.60	0.0117	0.1112
Median vs. Posterior	SG 226	5.03	0.0117	0.0589
Anterior vs. Median	SG 301	5.10	0.0117	0.0001
Anterior vs. Posterior	SG 301	12.62	0.0117	0.0001
Median vs. Posterior	SG 301	7.53	0.0117	0.0001

Egg yolk diameter

Contrast	Group	Estimate	Std error	p-value
Anterior vs. Median	SG 226	-0.96	0.0084	0.3382
Anterior vs. Posterior	SG 226	1.35	0.0084	0.1778
Median vs. Posterior	SG 226	6.11	0.0084	0.0513
Anterior vs. Median	SG 301	4.88	0.0084	0.0001
Anterior vs. Posterior	SG 301	12.40	0.0084	0.0001
Median vs. Posterior	SG 301	7.52	0.0084	0.0001

Table 14. Averages for egg ribbon weight, number of eggs per gram, and total fecundity for each female spawner size group (SG). Values are reported as means ± standard deviations (sd).

Parameter	Group	N	Mean ± sd	Minimum	Maximum
Egg ribbon Weight	SG 201	6	37.24 ± 5.75 g	31.78 g	47.64 g
	SG 226	5	53.23 ± 5.04	49.29	62.02
	SG 251	7	65.21 ± 12.63	49.39	83.76
	SG 276	7	80.19 ± 8.62	72.83	97.13
	SG 301	6	121.94 ± 18.14	101.14	143.15
	SG 326	4	176.79 ± 19.36	154.07	200.38
Number of eggs per g	SG 201	6	689.80 ± 63.42	573.04	753.75
	SG 226	14	671.50 ± 126.84	479.18	850.00
	SG 251	7	662.41 ± 72.67	572.78	784.21
	SG 276	7	644.80 ± 114.75	492.38	784.36
	SG 301	15	632.00 ± 39.68	583.33	674.86
	SG 326	4	591.11 ± 66.27	524.10	671.43
Total Fecundity	SG 201	6	25910.34 ± 5965.33	18211.32	35908.65
	SG 226	5	37934.64 ± 7291.07	30597.69	49089.03
	SG 251	7	42934.97 ± 7991.43	32073.01	56178.42
	SG 276	8	52416.78 ± 6263.50	46586.38	62487.27
	SG 301	5	79823.62 ± 12659.95	62154.17	94436.27
	SG 326	4	107802.80 ± 17953.27	81004.90	119173.40

Table 15. Averages for number of egg per gram, egg ribbon weight, and total fecundity parameters for each completed age of female spawners. Values are reported as means ± standard deviations (sd).

Parameter	Age	N	Mean ± sd	Minimum	Maximum
Number of	4	4	672.50 ± 70.23	573.04	733.33
Eggs per gram	5	8	738.67 ± 46.98	670.71	796.55
	6	7	648.53 ± 89.38	547.75	784.21
	7	7	632.03 ± 67.29	579.45	771.95
	8	6	582.93 ± 60.72	492.38	649.38
	9	3	661.94 ± 19.48	639.53	674.86
Egg ribbon	4	4	35.90 ± 2.85 g	31.78	38.36
Weight	5	8	60.47 ± 16.36	32.20	83.76
	6	7	71.28 ± 32.11	47.64	137.99
	7	7	96.99 ± 31.37	59.94	143.15
	8	6	113.08 ± 54.96	49.39	200.38
	9	3	154.18 ± 38.75	109.82	181.44
Total	4	4	24293 ± 4283	18211	28131
Fecundity	5	8	44629 ± 11771	22381	57125
	6	7	45553 ± 19952	30598	88915
	7	7	61057 ± 19955	36982	94436
	8	6	64953 ± 31288	32073	119173
	9	3	101715 ± 23910	74113	116037

increase from the smallest to largest female spawner size groups (Table 14). These two parameters also increased from age 4 to age 9 (Table 15). The means calculated for number of eggs per gram of eggs data demonstrated a decrease from the smallest to largest female spawner size groups (Table 14). The mean number of eggs per gram of eggs increased from age 4 to age 5, decreased from age 5 to age 8, and increased from age 8 to age 9 (Table 15).

The data for number of eggs per gram of eggs were normally distributed ($p = 0.4726$) but were not normally distributed for egg ribbon weight ($p = 0.0013$) and total fecundity ($p = 0.0028$, Appendix H). The egg ribbon and total fecundity data were log transformed which converted the data into a normal distribution ($p = 0.3603$ and $p = 0.7372$, respectively).

The assumption for homogeneous variances across female spawner size groups was tested and did not indicate a violation ($p > 0.05$) for egg ribbon weight and number of eggs per gram of eggs (Appendix F). Two factor ANOVA tests indicated an estimated overall statistical significance ($p = 0.0001$) for the age-group effect in log(egg ribbon weight) and log(total fecundity), but was not statistically significant overall ($p = 0.0834$) for number of eggs per gram of eggs (Appendix G). The age effect was not statistically significant for log(egg ribbon weight) and log(total fecundity) parameters. The age effect was eliminated and One-way ANOVA tests were conducted on size group effects, which were statistically significant. The tests indicated an overall statistical significance ($p = 0.0001$) for the log(egg ribbon weight) data (Appendix F). Contrast statements demonstrated statistical significance between all groups except for the group contrast SG 226 vs. SG 251 ($p = 0.1091$, Table 16). Statistical significance was found

Table 16. ANOVA contrast statements for egg ribbon weight. Mean values for egg ribbon weight are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	df	Estimate	p-value
SG 201 vs SG 226	1	4.55	0.0414
SG 201 vs SG 251	1	16.51	0.0003
SG 201 vs SG 276	1	38.92	0.0001
SG 201 vs SG 301	1	140.58	0.0001
SG 201 vs SG 326	1	305.28	0.0001
SG 226 vs SG 251	1	2.73	0.1091
SG 226 vs SG 276	1	13.84	0.0008
SG 226 vs SG 301	1	84.10	0.0001
SG 226 vs SG 326	1	221.60	0.0001
SG 251 vs SG 276	1	5.13	0.0312
SG 251 vs SG 301	1	67.92	0.0001
SG 251 vs SG 326	1	207.01	0.0001
SG 276 vs SG 301	1	36.79	0.0001
SG 276 vs SG 326	1	155.16	0.0001
SG 301 vs SG 326	1	47.16	0.0001

between groups SG 201 vs. SG 226 and SG 251 vs. SG 276, although the p-values were marginal ($p = 0.0414$ and $p = 0.0312$, respectively). One-way ANOVA test of the number of eggs per gram of eggs data demonstrated that there were no overall statistical significance ($p = 0.4816$, Appendix F). No contrasts were conducted.

The test for homogeneity of variances indicated a violation for the total fecundity data. Nonparametric testing indicated overall statistically significant differences between groups (overall chi-square value, $p = 0.0001$, Appendix F). The group contrasts that were statistically significant were SG 201 vs. SG 301 and SG 201 vs. SG 326 (Table 17).

Linear regression analysis was used to describe the relationship between female spawner TL or spawner weight to log(egg ribbon), log(total fecundity), and number of eggs per gram of eggs. The response variables, log(egg ribbon) and log(total fecundity), were highly correlated to the predictor variables, spawner TL and spawner weight (Appendix H). The analysis indicated that the r^2 values ranged from 0.8567 to 0.9089 and that the slope of each line was statistically significant from zero ($p = 0.0001$). The response variable, number of eggs per gram of eggs, was not highly correlated to the predictor variables, spawner TL and spawner weight (Appendix H). The analysis indicated that the r^2 values were 0.0983 to 0.1000, respectively, and that the slope of both lines were not statistically significant from zero ($p = 0.0667$ and $p = 0.0643$, respectively).

Data for the chorion shell and egg yolk diameters were collected. The means and standard deviations calculated for the two parameters did not indicate a trend across female spawner size groups (Table 18). For both parameters, the data were normally distributed ($p = 0.8979$ and $p = 0.9915$, respectively, Appendix F). The test for

Table 17. Contrast statements for total fecundity based on the Kruskal-Wallis non-parametric test. Mean values for total fecundity are compared between female spawner size groups (SG). Significance determined if D is greater than R.

Contrast	D	R	D>R
SG 201 vs SG 226	7.23	21.25	no
SG 201 vs SG 251	10.40	19.53	no
SG 201 vs SG 276	16.83	18.96	no
SG 201 vs SG 301	25.43	21.25	yes
SG 201 vs SG 326	29.03	21.25	yes
SG 226 vs SG 251	-3.17	20.55	no
SG 226 vs SG 276	-9.60	20.01	no
SG 226 vs SG 301	-18.20	22.20	no
SG 226 vs SG 326	-21.80	22.20	no
SG 251 vs SG 276	-6.43	18.17	no
SG 251 vs SG 301	-15.03	20.55	no
SG 251 vs SG 326	-18.63	20.55	no
SG 276 vs SG 301	-8.60	20.01	no
SG 276 vs SG 326	-12.20	20.01	no
SG 301 vs SG 326	-3.60	22.20	no

Table 18. Average for egg yolk and chorion shell diameters in each female spawner size group (SG). Values are reported as means ± standard deviations (sd).

Variable	Group	N	Mean ± sd	Minimum	Maximum
Egg yolk	SG 201	150	1.38 ± 0.07 μm	1.20	1.56
Diameter	SG 226	350	1.37 ± 0.09	1.14	1.58
	SG 251	175	1.39 ± 0.07	1.23	1.58
	SG 276	422	1.38 ± 0.10	0.89	1.65
	SG 301	125	1.39 ± 0.06	1.25	1.50
	SG 326	125	1.38 ± 0.06	1.23	1.50
Chorion	SG 201	150	0.32 ± 0.05	0.19	0.44
Shell	SG 226	350	0.31 ± 0.06	0.13	0.45
Diameter	SG 251	175	0.33 ± 0.05	0.20	0.46
	SG 276	422	0.32 ± 0.07	0.01	0.50
	SG 301	125	0.33 ± 0.05	0.22	0.40
	SG 326	125	0.32 ± 0.04	0.21	0.41

homogeneity of variances across female spawner size groups did not indicate a violation for either parameter. Although statistically significant, the One-way ANOVA tests indicated overall statistical significant differences between groups for the chorion shell diameter ($p = 0.0381$, Appendix F). Group contrasts for the chorion shell diameter demonstrated statistical significance between SG 226 vs. SG 251, SG 226 vs. SG 276, and SG 226 vs. SG 301 (Table 19). One-way ANOVA tests did not indicate overall statistically significant differences between groups for the egg yolk diameter ($p = 0.0661$, Appendix F).

Simple linear regression analysis was used to describe the relationship between female spawner TL or spawner weight to chorion shell and egg yolk diameters. The response variables, chorion shell diameter and egg yolk diameter, were not correlated to the predictor variables, TL and weight (Appendix J). The analysis indicated that the r^2 values ranged from 0.0003 to 0.0005 and that the slope of each line was not statistically significant from zero ($p > 0.05$).

Larval Offspring Analysis - *Maternal Contribution*

In general, all means increased over time with the exception of the means for larval TL, which indicated a decreasing trend over time for SG 301 (Table 20). Larval mouth gape width and height increased over time (Table 21 and 22, respectively). The data were normally distributed ($p = 0.3876$ and $p = 0.0942$, respectively) for the larval mouth gape width and height parameters but were not normally distributed ($p = 0.0345$) for larval TL (Appendix F). The larval TL data were squared which transformed the data

Table 19. ANOVA contrast statements for chorion shell diameter. Mean values for chorion shell diameter are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	2.99	0.0839
SG 201 vs SG 251	1	0.67	0.4147
SG 201 vs SG 276	1	0.03	0.8721
SG 201 vs SG 301	1	0.44	0.5090
SG 201 vs SG 326	1	0.16	0.6928
SG 226 vs SG 251	1	7.86	0.0051
SG 226 vs SG 276	1	6.49	0.0110
SG 226 vs SG 301	1	5.70	0.0171
SG 226 vs SG 326	1	1.35	0.2459
SG 251 vs SG 276	1	0.70	0.4013
SG 251 vs SG 301	1	0.01	0.9266
SG 251 vs SG 326	1	1.40	0.2367
SG 276 vs SG 301	1	0.40	0.5253
SG 276 vs SG 326	1	0.38	0.5352
SG 301 vs SG 326	1	1.02	0.3123

Table 20. Average for larval TL in each female spawner size group (SG) sampled through days 15 to 19 post-fertilization. Values are reported as means ± standard deviations (sd).

	Day	N	Mean ± sd	Minimum	Maximum
SG 201	15	6	5.5267 ± 0.1903 mm	5.3695	5.8819
	16	11	5.3173 ± 0.3617	4.7749	5.7604
	17	14	5.2473 ± 0.5155	4.5120	6.0746
	18	17	5.4784 ± 0.3945	4.8205	6.2897
	19	11	5.5822 ± 0.5948	4.3713	6.3593
SG 226	15	3	5.3813 ± 0.6013	4.9773	6.0723
	16	13	5.4232 ± 0.4138	4.6354	6.0025
	17	13	5.4091 ± 0.5611	4.5888	6.2455
	18	28	5.4560 ± 0.5427	4.3724	6.3445
	19	33	5.0480 ± 0.5672	4.1058	6.1159
SG 251	15	2	5.0358 ± 0.0826	4.9773	5.0942
	16	11	5.2085 ± 0.3324	4.7168	5.8087
	17	12	5.3289 ± 0.5793	4.1597	6.5572
	18	9	5.3478 ± 0.5019	4.3811	6.0457
	19	10	5.5124 ± 0.5246	4.7065	6.4660
SG 276	15	6	5.2323 ± 0.1632	4.9544	5.3973
	16	13	5.3462 ± 0.2631	4.8837	5.8384
	17	14	5.5768 ± 0.2925	5.0599	6.1604
	18	14	5.2107 ± 0.5443	4.5067	6.0326
	19	13	5.6695 ± 0.3796	5.1452	6.3152
SG 301	15	14	5.4054 ± 0.3611	4.7272	5.9896
	16	28	5.3018 ± 0.5079	4.4626	6.2793
	17	32	5.1814 ± 0.5486	4.1672	5.9049
	18	39	5.0413 ± 0.5280	4.3002	6.1363
	19	8	4.9037 ± 0.2768	4.5084	5.1884
SG 326	15	2	5.1778 ± 0.2883	4.9740	5.3816
	16	4	5.1235 ± 0.3198	4.7366	5.5074
	17	2	5.3795 ± 0.0890	5.3165	5.4424
	18	4	5.5479 ± 0.2232	5.3509	5.8357
	19	3	5.0420 ± 0.2362	4.7702	5.1972

Table 21. Average for larval mouth gape width in each female spawner size group (SG) sampled through days 15 to 19 post-fertilization. Values are reported as means ± standard deviations (sd).

	Day	N	Mean ± sd	Minimum	Maximum
SG 201	15	6	0.2435 ± 0.0586 mm	0.1545	0.3232
	16	11	0.2772 ± 0.0469	0.1878	0.3292
	17	14	0.2892 ± 0.0508	0.1844	0.3782
	18	17	0.2881 ± 0.0583	0.1815	0.4444
	19	11	0.3103 ± 0.0406	0.2537	0.3909
SG 226	15	3	0.2388 ± 0.0663	0.1920	0.3147
	16	13	0.2663 ± 0.0348	0.2180	0.3549
	17	13	0.2395 ± 0.0669	0.1373	0.3190
	18	28	0.2922 ± 0.0477	0.1915	0.3751
	19	33	0.2632 ± 0.0502	0.1601	0.3623
SG 251	15	2	0.2009 ± 0.0126	0.1920	0.2098
	16	11	0.2278 ± 0.0378	0.1801	0.3102
	17	12	0.2635 ± 0.0445	0.1836	0.3248
	18	9	0.3101 ± 0.0954	0.1949	0.4727
	19	10	0.2863 ± 0.0551	0.1955	0.3450
SG 276	15	6	0.2785 ± 0.0419	0.2142	0.3327
	16	13	0.2622 ± 0.0545	0.1754	0.3597
	17	14	0.2909 ± 0.0502	0.2120	0.3519
	18	14	0.2686 ± 0.0524	0.2087	0.3741
	19	13	0.2972 ± 0.0299	0.2435	0.3479
SG 301	15	14	0.2696 ± 0.0332	0.2068	0.3229
	16	28	0.2632 ± 0.0519	0.1857	0.3558
	17	32	0.2497 ± 0.0572	0.1308	0.3443
	18	39	0.2590 ± 0.0526	0.1564	0.3664
	19	8	0.2674 ± 0.0485	0.1902	0.3398
SG 326	15	2	0.2962 ± 0.0228	0.2801	0.3124
	16	4	0.2636 ± 0.0616	0.1719	0.3023
	17	2	0.2599 ± 0.0486	0.2255	0.2942
	18	4	0.3046 ± 0.0122	0.2934	0.3179
	19	3	0.2840 ± 0.0296	0.2572	0.3157

Table 22. Average for larval mouth gape height in each female spawner size group (SG) sampled through days 15 to 19 post-fertilization. Values are reported as means ± standard deviations (sd).

	Day	N	Mean ± sd	Minimum	Maximum
SG 201	15	2	0.2593 ± 0.0163 mm	0.2478	0.2708
	16	11	0.2745 ± 0.0602	0.1778	0.3626
	17	14	0.2668 ± 0.0493	0.1851	0.3597
	18	17	0.2979 ± 0.0667	0.1886	0.4185
	19	11	0.3125 ± 0.0680	0.2338	0.4167
SG 226	15	3	0.2310 ± 0.0332	0.1981	0.2644
	16	10	0.2705 ± 0.0326	0.2125	0.3333
	17	13	0.2395 ± 0.0661	0.1434	0.3371
	18	28	0.2730 ± 0.0617	0.1477	0.3774
	19	33	0.2561 ± 0.0571	0.1145	0.3583
SG 251	15	2	0.2143 ± 0.0230	0.1981	0.2305
	16	6	0.2186 ± 0.0234	0.1959	0.2598
	17	12	0.2454 ± 0.0392	0.1739	0.2961
	18	9	0.3101 ± 0.0985	0.2145	0.4601
	19	10	0.2744 ± 0.0595	0.1542	0.3513
SG 276	15	6	0.2559 ± 0.0261	0.2125	0.2867
	16	13	0.2549 ± 0.0645	0.1586	0.3729
	17	14	0.2763 ± 0.0548	0.1667	0.4021
	18	14	0.2671 ± 0.0594	0.1848	0.3825
	19	13	0.2880 ± 0.0381	0.2452	0.3860
SG 301	15	14	0.2569 ± 0.0367	0.1717	0.3143
	16	28	0.2427 ± 0.0479	0.1697	0.3664
	17	32	0.2450 ± 0.0568	0.1342	0.3474
	18	39	0.2540 ± 0.0494	0.1624	0.3638
	19	8	0.2645 ± 0.0417	0.2028	0.3225
SG 326	15	2	0.2797 ± 0.0176	0.2673	0.2921
	16	4	0.2570 ± 0.0297	0.2139	0.2803
	17	2	0.2964 ± 0.0409	0.2675	0.3254
	18	4	0.3033 ± 0.0349	0.2608	0.3385
	19	3	0.2648 ± 0.0386	0.2213	0.2948

to a normal distribution ($p = 0.0679$). The assumption of homogeneous variances across female spawner size groups was tested and demonstrated no violations of this assumption.

In general, all means increased over time with the exception of the means for larval TL, which indicated a decreasing trend over time for SG 301. One-way ANOVA tests were used to demonstrate statistically significant differences between offspring within female spawner size groups across all days sampled. The data for larval TL^2 were statistically significant overall ($p = 0.0127$ and $p = 0.0004$) for days 18 and 19 post-fertilization, respectively (Appendix K). Contrast statements for larval TL^2 on day 18 indicated statistically significant differences between groups SG 201 vs. SG 301 and SG 226 vs. SG 301 (Table 23). Contrast statements for larval TL^2 on day 19 indicated statistically significant differences between groups SG 201 vs. SG 226, SG 201 vs. SG 301, SG 226 vs. SG 251, SG 226 vs. SG 276, SG 251 vs. SG 301 and SG 276 vs. SG 301 (Table 24). The data for larval mouth gape width were statistically significant overall ($p = 0.0497$) on day 19 post-fertilization (Appendix K). Contrast statements for larval mouth gape width on day 19 indicated statistically significant differences between groups SG 201 vs. SG 226, SG 201 vs. SG 301, and SG 226 vs. SG 276 (Table 25). The data for larval mouth gape height were not statistically significant for any days post-fertilization.

Since there were no significant differences between egg size from the different spawner size groups, chorion shell diameter and egg yolk diameter were not used as predictor variables to describe any relationship between these maternal characteristics and any offspring characteristic. Instead, spawner TL and spawner weight were used to describe the linear relationships with the response variables, larval TL, larval mouth gape

Table 23. ANOVA contrast statements for larval TL on day 18 post-fertilization. Mean values for larval TL on day 18 post-fertilization are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	0	0.9510
SG 201 vs SG 251	1	0.36	0.5496
SG 201 vs SG 276	1	1.97	0.1638
SG 201 vs SG 301	1	8.12	0.0053
SG 201 vs SG 326	1	0.05	0.8271
SG 226 vs SG 251	1	0.36	0.5523
SG 226 vs SG 276	1	2.21	0.1398
SG 226 vs SG 301	1	10.67	0.0015
SG 226 vs SG 326	1	0.07	0.7930
SG 251 vs SG 276	1	0.37	0.5464
SG 251 vs SG 301	1	2.47	0.1193
SG 251 vs SG 326	1	0.38	0.5404
SG 276 vs SG 301	1	1.07	0.3035
SG 276 vs SG 326	1	1.23	0.2708
SG 301 vs SG 326	1	3.27	0.0733

Table 24. ANOVA contrast statements for larval TL on day 19 post-fertilization. Mean values for larval TL on day 19 post-fertilization are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	9.35	0.0031
SG 201 vs SG 251	1	0.13	0.7176
SG 201 vs SG 276	1	0.13	0.7179
SG 201 vs SG 301	1	8.81	0.0041
SG 201 vs SG 326	1	3.00	0.0878
SG 226 vs SG 251	1	6.30	0.0143
SG 226 vs SG 276	1	13.72	0.0004
SG 226 vs SG 301	1	0.64	0.4274
SG 226 vs SG 326	1	0.01	0.9174
SG 251 vs SG 276	1	0.53	0.4676
SG 251 vs SG 301	1	6.62	0.0121
SG 251 vs SG 326	1	2.17	0.1455
SG 276 vs SG 301	1	11.56	0.0011
SG 276 vs SG 326	1	3.97	0.0502
SG 301 vs SG 326	1	0.14	0.7110

Table 25. ANOVA contrast statements for larval mouth gape width on day 19 post-fertilization. Mean values for larval mouth gape width on day 19 post-fertilization are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	8.60	0.0045
SG 201 vs SG 251	1	1.42	0.2373
SG 201 vs SG 276	1	0.48	0.4895
SG 201 vs SG 301	1	4.00	0.0492
SG 201 vs SG 326	1	0.77	0.3837
SG 226 vs SG 251	1	1.92	0.1699
SG 226 vs SG 276	1	5.06	0.0275
SG 226 vs SG 301	1	0.05	0.8172
SG 226 vs SG 326	1	0.56	0.4576
SG 251 vs SG 276	1	0.32	0.5764
SG 251 vs SG 301	1	0.74	0.3913
SG 251 vs SG 326	1	0.01	0.9395
SG 276 vs SG 301	1	2.06	0.1554
SG 276 vs SG 326	1	0.20	0.6563
SG 301 vs SG 326	1	0.28	0.5976

width, and larval mouth gape height. The simple linear regression analysis indicated that the maternal characteristics were not highly correlated to the offspring characteristics. While the slopes for all of the lines were statistically significant from zero (p values ranged from 0.0170 to 0.0294), the r^2 values ranged from 0.0007 to 0.0100 (Appendix L).

Simple linear regression analysis was used to describe the relationship between larval TL and larval mouth gape width and height. The response variables, larval mouth gape width and height, were linear related to the predictor variable, larval TL ($r^2 = 0.4330$ and $r^2 = 0.4577$, respectively, Appendix M). The slopes for both lines were statistically significantly different from zero ($p = 0.0001$). These relationships were reduced into the six different spawner size groups and analyzed. The analysis indicated linear relationships between the response and predictor variables. The r^2 values ranged from 0.3487 to 0.6100 and the p values ranged from 0.0001 to 0.0100 (Appendix N).

ANCOVA was used to determine if the rate of increase for larval mouth gapes was different for the six spawner size groups. The analysis indicated that there were significant differences between spawner size groups ($p = 0.0001$, Appendix O). Contrast statements indicated that there were no significant differences overall between the rates of growth of the mouth gape width (Table 26) and between initial mouth gape widths (Table 27). The contrast statements indicated that there were significant differences between the rates of growth of the mouth gape height (Table 28) and between initial mouth gape heights (Table 29). The contrast statements that were significant for the rate of growth are SG 201 vs. SG 226, SG 201 vs. SG 301, SG 226 vs. SG 276, and SG 276 vs. SG 301. The contrast statements that were significant between intercepts are SG 201 vs. SG 226,

Table 26. ANCOVA contrast statements for larval mouth gape width regression coefficients. Mean values for larval mouth gape width regression coefficients are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	0.58	0.4470
SG 201 vs SG 251	1	0.18	0.6758
SG 201 vs SG 276	1	0.41	0.5231
SG 201 vs SG 301	1	0.96	0.3274
SG 201 vs SG 326	1	0.06	0.8103
SG 226 vs SG 251	1	1.44	0.2315
SG 226 vs SG 276	1	2.10	0.1483
SG 226 vs SG 301	1	0.07	0.7906
SG 226 vs SG 326	1	0.28	0.5975
SG 251 vs SG 276	1	0.05	0.8313
SG 251 vs SG 301	1	1.99	0.1595
SG 251 vs SG 326	1	0.00	0.9570
SG 276 vs SG 301	1	2.76	0.0973
SG 276 vs SG 326	1	0.00	0.9647
SG 301 vs SG 326	1	0.37	0.5452

Table 27. ANCOVA contrast statements for larval mouth gape width regression intercepts. Mean values for larval mouth gape width regression intercepts are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	1.93	0.1656
SG 201 vs SG 251	1	2.86	0.0917
SG 201 vs SG 276	1	0.84	0.3609
SG 201 vs SG 301	1	2.32	0.1283
SG 201 vs SG 326	1	0.51	0.4769
SG 226 vs SG 251	1	0.32	0.5727
SG 226 vs SG 276	1	0.15	0.6951
SG 226 vs SG 301	1	0.01	0.9331
SG 226 vs SG 326	1	2.48	0.1159
SG 251 vs SG 276	1	0.73	0.3943
SG 251 vs SG 301	1	0.27	0.6025
SG 251 vs SG 326	1	3.30	0.0700
SG 276 vs SG 301	1	0.23	0.6291
SG 276 vs SG 326	1	1.67	0.1968
SG 301 vs SG 326	1	2.72	0.1002

Table 28. ANCOVA contrast statements for larval mouth gape height regression coefficients. Mean values for larval mouth gape height regression coefficients are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	5.57	0.0188
SG 201 vs SG 251	1	1.52	0.2190
SG 201 vs SG 276	1	0.03	0.8711
SG 201 vs SG 301	1	7.86	0.0053
SG 201 vs SG 326	1	0.29	0.5894
SG 226 vs SG 251	1	0.56	0.4561
SG 226 vs SG 276	1	4.08	0.0440
SG 226 vs SG 301	1	0.27	0.6041
SG 226 vs SG 326	1	0.10	0.7478
SG 251 vs SG 276	1	1.05	0.3054
SG 251 vs SG 301	1	1.27	0.2608
SG 251 vs SG 326	1	0.00	0.9854
SG 276 vs SG 301	1	5.90	0.0157
SG 276 vs SG 326	1	0.21	0.6433
SG 301 vs SG 326	1	0.22	0.6379

Table 29. ANCOVA contrast statements for larval mouth gape height intercepts. Mean values for larval mouth gape height regression intercepts are compared between female spawner size groups (SG). Significance determined on the 0.05 % alpha level.

Contrast	DF	Estimate	p-value
SG 201 vs SG 226	1	7.16	0.0078
SG 201 vs SG 251	1	5.45	0.0201
SG 201 vs SG 276	1	5.17	0.0235
SG 201 vs SG 301	1	7.93	0.0051
SG 201 vs SG 326	1	0.16	0.6908
SG 226 vs SG 251	1	0.02	0.8883
SG 226 vs SG 276	1	0.05	0.8251
SG 226 vs SG 301	1	0.00	0.9917
SG 226 vs SG 326	1	4.27	0.0394
SG 251 vs SG 276	1	0.10	0.7549
SG 251 vs SG 301	1	0.02	0.8899
SG 251 vs SG 326	1	3.96	0.0473
SG 276 vs SG 301	1	0.06	0.8091
SG 276 vs SG 326	1	3.50	0.0623
SG 301 vs SG 326	1	4.48	0.0350

SG 201 vs. SG 251, SG 201 vs. SG 276, SG 201 vs. SG 301, SG 251 vs. SG 326, and SG 301 vs. SG 326.

Larval Offspring Analysis - *Parental Contribution*

Analysis was completed over a period of six days (days 13 to 18 post-fertilization) for the measurement of larval TL. Measurements of larval mouth gape width were completed on day 14 post-fertilization. From the ANOVA model, the maternally and paternally derived sources of variation were estimated for larval TL, mouth gape width, and mouth gape height (Table 30). Larval TL estimates ranged from 0 to 0.1278 (maternal) and 0 to 0.0427 (paternal) over the six sampling days. The maternal and paternal variation estimates for mouth gape width and height were 0.00005 and 0.0001, respectively. The specific combining ability of the two strains (fast and slow growth rates) was estimated for larval TL, mouth gape width, and mouth gape height (Table 30). The estimates for larval TL ranged from 0 to 0.1751. The estimates for mouth gape width and height were 0.00007 and 0.0002, respectively. Differences between the average additive genetic strain effects (fast and slow growth rates) were compared. A statistically significant difference was observed on day 13 post-fertilization ($p = 0.0072$, Table 31).

Means and standard deviations for larval TL (Table 32) and mouth gape width and height (Table 33) were calculated for each genotype combination over all days sampled. Estimates of heritability and dominance were calculated for larval TL, mouth gape width, and mouth gape height (Table 34). Heritability estimates for larval TL ranged from 0 to 0.5863. The heritability estimates for mouth gape width was 0 and was

Table 30. Estimation of maternally and paternally derived sources of variation from the average additive genetic strain effects, including the specific combining ability. Estimates include larval total length (TL) for days 13 to 18 post-fertilization and larval mouth gape width and height on day 14 post-fertilization.

	Days post-fertilization	Maternal	Paternal	Specific combining ability	Residual
Larval TL					
	13	0	0	0.0184	0.0484
	14	0.0071	0	0.0655	0.0946
	15	0.0033	0	0.0129	0.1190
	16	0	0	0.0888	0.1599
	17	0.0326	0.0426	0.1751	0.2104
	18	0.1278	0.0106	-[3]	0.1739
Mouth gape width	14	0	0	0.0002	0.0011
Mouth gape height	14	0.0001	0.00005	0.00007	0.0012

[3] Unable to estimate due to missing data.

Table 31. Factorial contrast statements for average additive genetic strain differences between fast growth and slow growth. Estimates include larval total length (TL) for days 13 to 18 post-fertilization and larval mouth gape width and height on day 14 post-fertilization. Significance determined on the 0.05 % alpha level.

Parameter	Days Post-fertilization	DF	Estimate	Std error	p-value
Larval TL					
	13	119	0.3191	0.1166	0.0072
	14	304	0.0214	0.2054	0.9169
	15	144	0.0155	0.1255	0.9017
	16	128	0.1740	0.2475	0.4834
	17	103	0.1391	0.4281	0.7459
	18	73	0.1369	0.3986	0.7323
Mouth gape width	14	126	0.0279	0.0141	0.0505
Mouth gape height	14	126	0.0233	0.0163	0.1557

Table 32. Averages for larval total length (TL) by genotype combinations for days 13 to 18 post-fertilization. Values are reported as means ± standard deviations (sd).

Sire fast growth x Dam fast growth		
Days post-fertilization	N	Mean ± sd
13	40	5.3632 ± 0.2093 mm
14	80	4.9212 ± 0.4294
15	40	5.2210 ± 0.3451
16	30	5.4021 ± 0.4146
17	24	5.5929 ± 0.5852
18	19	5.6430 ± 0.5654
Sire fast growth x Dam slow growth		
Days post-fertilization	N	Mean ± sd
13	40	4.9878 ± 0.2826 mm
14	80	4.9092 ± 0.4690
15	40	5.1148 ± 0.3964
16	36	5.4853 ± 0.4269
17	38	5.5545 ± 0.5565
18	23	5.7696 ± 0.5334
Sire slow growth x Dam fast growth		
Days post-fertilization	N	Mean ± sd
13	40	5.1336 ± 0.1896 mm
14	80	5.2428 ± 0.3036
15	40	5.3103 ± 0.3168
16	38	5.4159 ± 0.5838
17	16	5.3437 ± 0.7601
18	10	6.0379 ± 0.4016
Sire slow growth x Dam slow growth		
Days post-fertilization	N	Mean ± sd
13	40	5.0483 ± 0.3034 mm
14	80	4.8997 ± 0.3179
15	40	5.2365 ± 0.3825
16	39	5.5892 ± 0.4474
17	40	5.5590 ± 0.3996
18	30	5.8534 ± 0.4627

Table 33. Average for larval mouth gape width and larval mouth gape height by genotype combinations on day 14 post-fertilization. Values are reported as means ± standard deviations (sd).

Genotype combination	N	Mean ± sd
Sire fast growth x Dam fast growth		
Mouth gape width	40	0.2744 ± 0.0395 μm
Mouth gape height	40	0.2521 ± 0.0331
Sire fast growth x Dam slow growth		
Mouth gape width	40	0.2566 ± 0.0339
Mouth gape height	40	0.2481 ± 0.0383
Sire slow growth x Dam fast growth		
Mouth gape width	30	0.2569 ± 0.0371
Mouth gape height	30	0.2470 ± 0.0355
Sire slow growth x Dam slow growth		
Mouth gape width	30	0.3023 ± 0.0275
Mouth gape height	30	0.2738 ± 0.0361

Table 34. Estimates of heritability and dominance. Estimates include larval total length (TL) for days 13 to 18 post-fertilization and larval mouth gape width and height on day 14 post-fertilization.

Parameter	Days Post-fertilization	Heritability	Dominance
Larval TL			
	13	0	1.1006
	14	0.1393	1.5668
	15	0.0544	0.3816
	16	0	1.4284
	17	0.5269	1.5201
	18	0.5863	0
Mouth gape width	14	0	0.7303
Mouth gape height	14	0.2289	0.2087

0.2289 for mouth gape height. The dominance estimates for larval TL ranged from 0 to 1.5668. The dominance estimates for mouth gape width was 0.7303 and was 0.2087 for mouth gape height. The dominance estimates were broken down into two heterotic effects and estimated (Table 35). Only days 13 and 14 post-fertilization showed a statistically significant average dominance effect. On day 13 post-fertilization, a significant heterotic effect for male fast growth and female slow growth was observed (hs, $p = 0.0188$). On day 14 post-fertilization, another heterotic effect, male slow growth and female fast growth, was significant (hd, $p = 0.0484$). However, the contrast statement comparing these two heterotic effects were not significantly different from each other ($p = 0.1058$ and $p = 0.1055$, respectively, Table 36).

Table 35. Estimates of average heterotic effects based on mating fast growth sires with slow growth dams (hs) and mating slow growth sires with fast growth dams (hd). Estimates include larval total length (TL) for days 13 to 18 post-fertilization and larval mouth gape width and height on day 14 post-fertilization.

Parameter	Days Post-fertilization	hs	hd
Larval TL			
	13	0.0188	0.5701
	14	0.9938	0.0484
	15	0.2593	0.4195
	16	0.9681	0.7499
	17	0.8622	0.6067
	18	0.5231	0.2694
Mouth gape width	14	0.0072	0.0154
Mouth gape height	14	0.1434	0.0742

Table 36. Factorial contrast statements for differences between average non-additive genetic strain effect of sire and dam. Estimates include larval total length (TL) for days 13 to 18 post-fertilization and larval mouth gape width and height on day 14 post-fertilization. Significance determined on the 0.05 % alpha level.

Parameter	Days Post-fertilization	DF	p-value
Larval TL			
	13	1	0.1058
	14	1	0.1055
	15	1	0.1213
	16	1	0.8051
	17	1	0.6001
	18	1	0.7128
Mouth gape width	14	1	0.7468
Mouth gape height	14	1	0.9816

DISCUSSION

Female Spawner Analysis

Caution needs to be taken when interpreting the values calculated for the female spawning data. Although, the females were randomly selected, the spawning stock was not entirely reflective of the perch population in Saginaw Bay, Lake Huron. The sample size was too small to make specific population comparisons, however, general comparisons can be made.

The ANOVA contrast statements indicated that the TL means for each female spawner size group were significantly different from the other spawner size groups. This complete separation of the size groups allowed for the comparison of maternal and larval offspring characteristics in this study. The mean spawner TL values calculated for each completed age (Table 8; Figure 9) were similar to the values calculated by Hile and Jobes (1941)[3] and were comparable to the unweighted means reported for the strain of perch in Saginaw Bay, Lake Huron (review Carlander 1969). Values calculated by Diana and Salz (1990)[3] were less than the values reported in this study.

In the simple linear regression analysis, spawner weight was log transformed because it was not normally distributed. Although spawner TL was normally distributed,

[3] Values were estimated from figures.

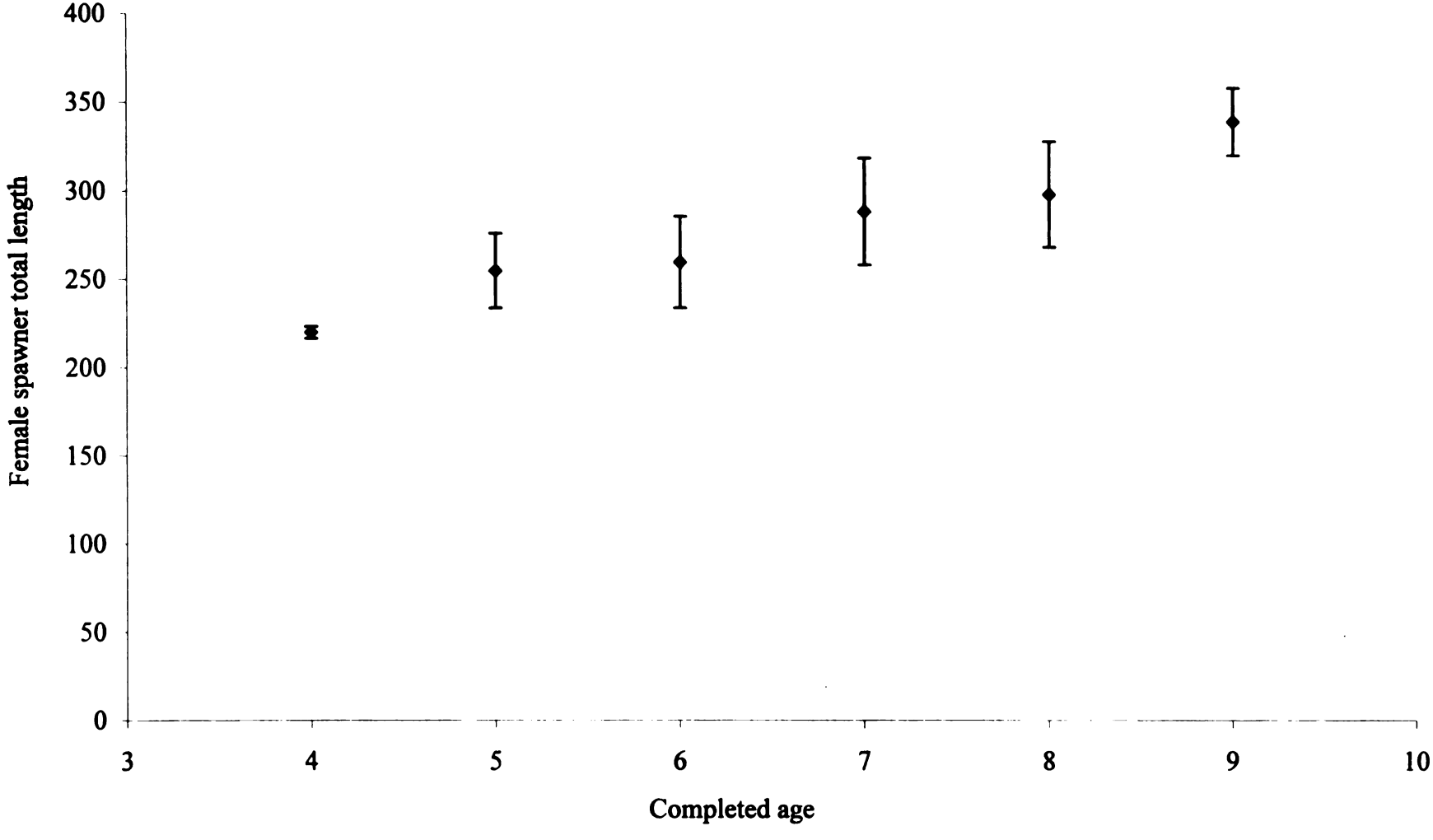

Figure 9. Averages (± standard deviations) of female spawner total length by each completed

it was log transformed to describe the weight-length relationship (Anderson and Neumann 1996). The transformations of the data provided a linear relationship and resulted in the following weight-length linear regression equation (Figure 10):

$$(2.6) \quad \log_{10}(W) = -13.675 + 3.417 * \log_{10}(L) + e.$$

The y-intercept estimate was lower than the natural log values reported by Parker (1958, in Carlander 1969) for perch in Flora Lake, WI. The value of the y-intercept after transforming from the natural log is -2.6156. This value compares to –2.3982 reported for perch in Saginaw Bay, Lake Huron (El-Zarka 1959). The slope regression estimate falls within the range (2.65 to 3.54) of reported values (review Carlander 1969). While the sample size of this study was considerably less than the studies reviewed by Carlander (1969), the estimates were comparable. The sample of females did not produce any females that could be considered "out of the ordinary."

Factorial Analysis of positions within the egg ribbon

Number of eggs per gram of eggs, chorion shell diameter, and egg yolk diameter for each region of the egg ribbon were compared. There were no significant differences between the positions within the egg ribbons for SG 226. There were, however, significant differences between the positions within the egg ribbons for SG 301. The difference observed in the different positions of the egg ribbon is potentially due to the location within the ovary. Toetz (1966; in Kamler 1996) compared differences between eggs from the anterior and posterior positions within the ovary of bluegill

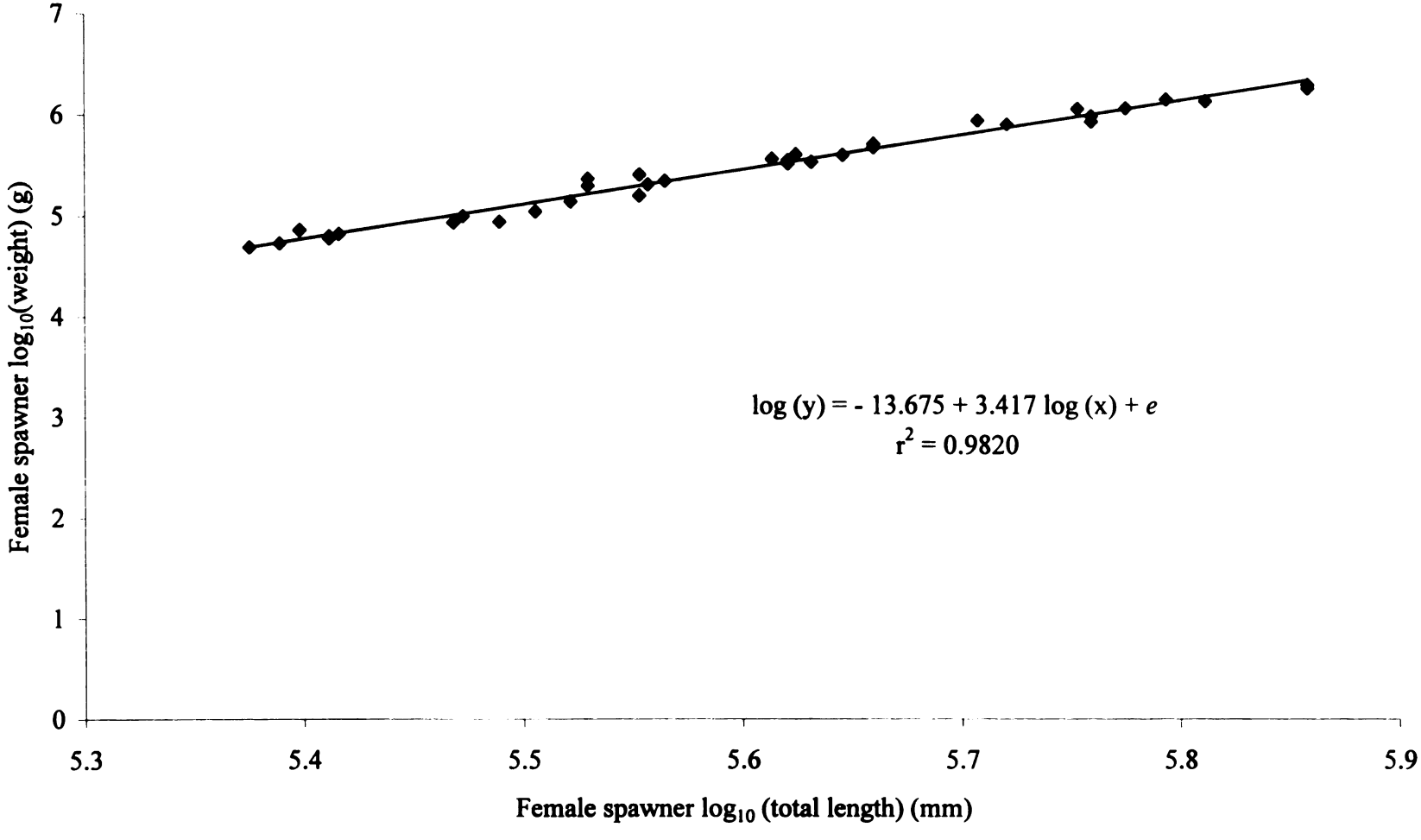

Figure 10. Simple linear regression model for the natural log of female spawner total length to weight relationship.

(*Lepomis macrochirus*). The dry weight, energy content, nitrogen content, and fertilizability were not significantly different between the two positions.

The diameters of the chorion shell and egg yolk for the eggs in SG 301 were smallest in the anterior portion and largest in the posterior portion of the egg ribbon. The ratio between the smallest and largest chorion shell diameter was 1.34 and 2.40 for the egg yolk diameter. The chorion shell diameter ratio is lower than the ratio reported for herring (*Clupea harengus*, Blaxter and Hempel 1963), which indicates that the variability in egg size observed in this study was comparable to reported values in other fish species.

The variability observed within the egg ribbon could be explained by food availability. Mejen (1940; in Kamler 1996) described the mechanism of regulation for this process. When food is adequate, all oocytes near large or small blood vessels receive sufficient nutrients for development. If there is a food shortage during egg development, the nutrient flow is restricted in the oocytes near small blood vessels. This can result in an increase in variability and a decrease in average egg size. In the cases of extreme food shortages, female spawners can utilize lipids present in the gonads for metabolic purposes (Nikolskij 1974; in Kamler 1996). While egg size can be influenced by the amount of food available, Springate et al. (1985) did not find any differences in egg size for rainbow trout (*Onchorhynchus mykiss*) eggs developed during an induced period of starvation. The researchers also did not find significant differences in protein, lipid, and ash percentages of the egg dry matter.

While there were significant differences observed between the positions of the egg ribbon, these differences have been observed in other studies. However, the effect of food availability may not have a large effect on egg size, chemical composition, and

offspring viability. Any effect of food limitation on egg quality can be compensated by the reduction of total fecundity (Kamler 1996). The analysis continued with the assumption that the posterior portion was representative of the entire egg ribbon.

Egg Analysis

Mean fecundity values were higher than the values reported in Carlander (1969). However, all sample sizes, including this study, were small. The total fecundity regression models developed in this study were comparable to reported values in Carlander (1969). The total fecundity-spawner TL relationship resulted in the following regression model (Figure 11):

(2.7) $\log(y) = -5.939 + 3.417 \log(x) + e.$

The y-intercept estimate is lower, but the regression slope estimate almost identical to the values reported for Lake Michigan (Brazo et al. 1975) and Lake Erie (Hartman et al. 1980). The total fecundity-spawner weight relationship resulted in the following regression model (Figure 12):

(2.8) $\log(y) = 2.550 + 0.900 \log(x) + e.$

The y-intercept estimate is higher and regression slope estimate is lower than the values reported for Lake Erie (Sztramo and Teleki 1977). The observed differences may be the result of differences in sample sizes or geographic locations. Sample sizes from earlier studies were much larger than the sample size of this study.

In addition to total fecundity, egg ribbon weight or egg biomass per female can be used to express reproductive effort (Calow 1979). However, the relationship between egg

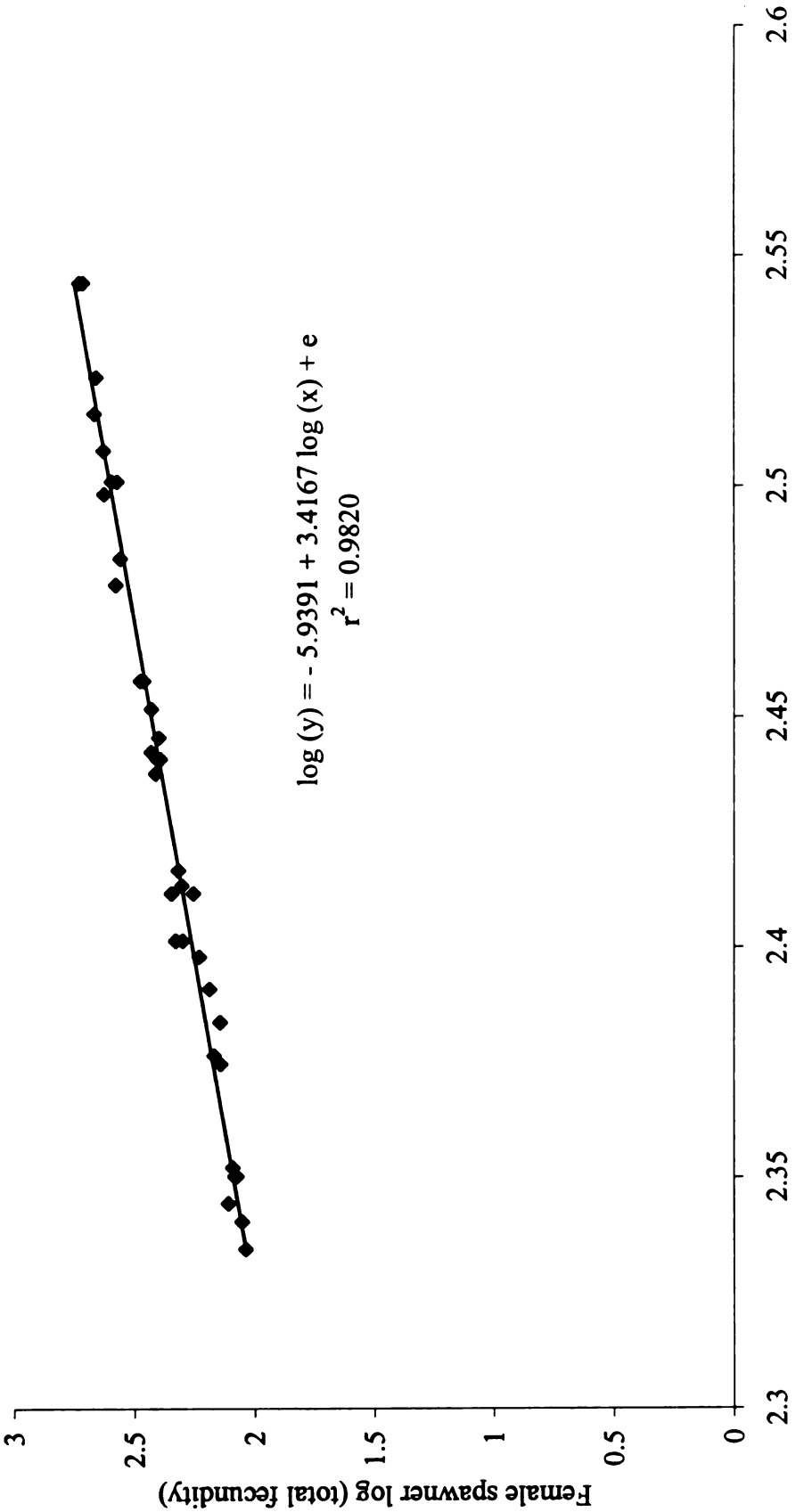

Figure 11. Simple linear regression analysis for female spawner total length to total fecundity relationship.

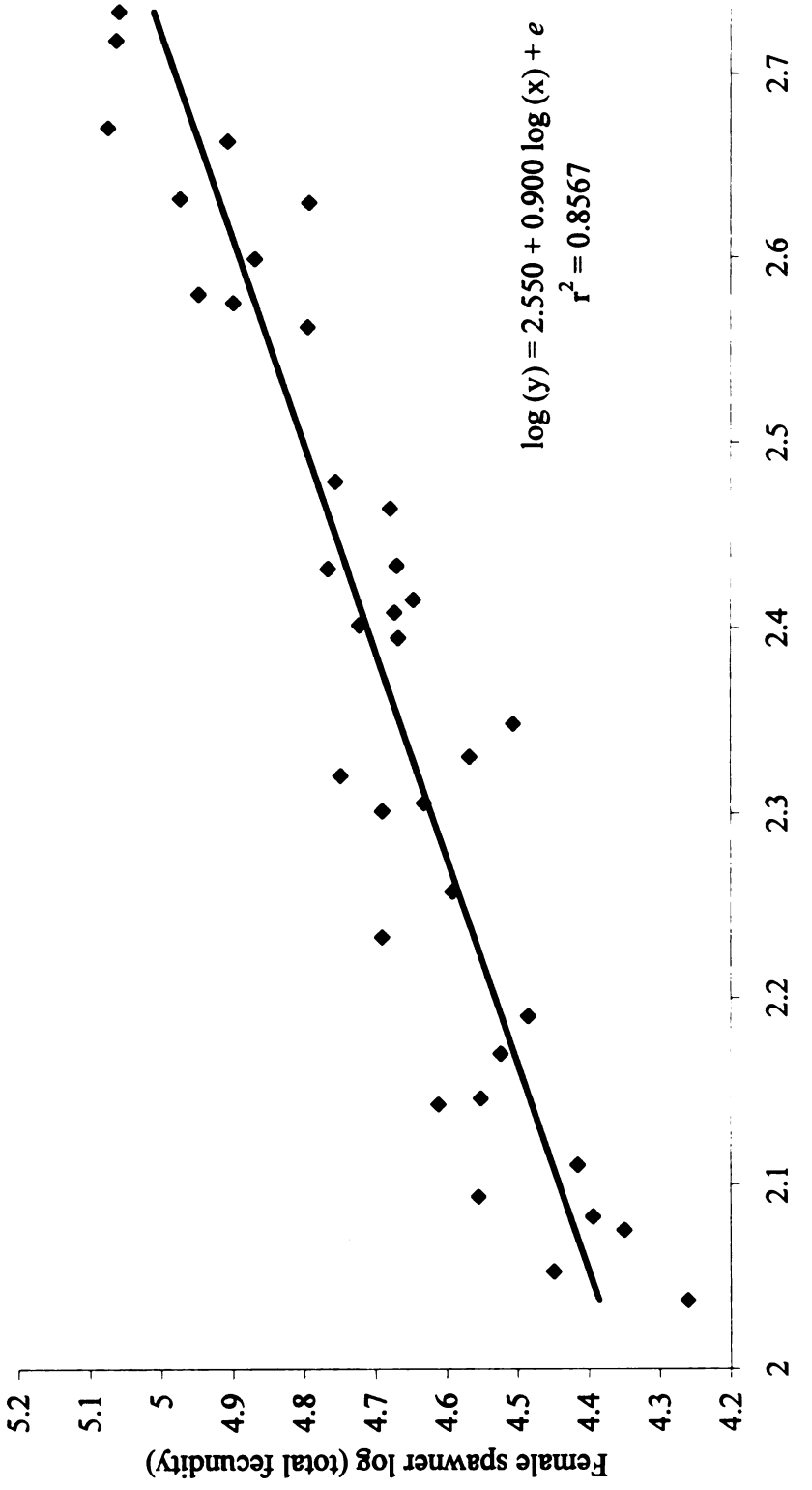

Figure 12. Simple linear regression analysis for female spawner weight to total fecundity relationship.

ribbon weight or number of eggs per gram of eggs to spawner TL or weight have not been previously reported for perch. No comparisons could be made between the relationships. Since the total fecundity follows the same trends reported by other researchers, it might be assumed that egg ribbon weight would follow similar trends.

While this study indicated that there was a linear association for the total fecundity–spawner size and egg ribbon weight-spawner size relationships, the number of eggs per gram of eggs-spawner size relationship was not linear. The trend of the calculated means indicated that this parameter decreased as spawner size increased. This trend is possibly related to egg size. However, the results of this study indicated that there were no significant differences found between chorion shell diameter or egg yolk diameter for the different spawner size groups. The linear regression models constructed poorly represented the egg-spawner size relationship. Schneider (1972) was unable to establish any relationship between perch spawners (97 – 343 mm TL) and egg size, egg yolk size, fry length, or hatching success.

Larval Offspring Analysis - *Maternal Contribution*

Linear regression models were constructed to describe the larval offspring characteristics-spawner size relationship. Like Schneider (1972), a relationship could not be maintained between the larval offspring characteristics and spawner size. The resulting r-square values in this study were less than 0.0300.

A different approach was attempted to establish a relationship between larval offspring and spawner size. First, linear regression models were constructed to describe

the mouth gape width and height-larval TL relationships. Guma'a (1978) constructed a linear regression model for the mouth gape height-larval TL relationship. Although the data were derived differently, the linear model described the relationship well (r = 0.99). The following linear regression model was constructed by Guma'a (1978):

(2.9) $G_{max} = 0.2152 + 0.0781\ (L) + e$,

where G_{max} is the maximum gape height;

L is the total length of the larvae;

e is the residual error term.

This linear regression model compares to the following model constructed in this study (Figure 13):

(2.10) $y = 0.060535 + 0.007155\ (x) + e$,

where y is the mouth gape height;

x is the larval TL^2;

e is the residual error term.

Although the model (2.8) was constructed using data collected from the Eurasian perch, the two species are closely related and are considered biologically similar (Thorpe 1977). A comparison of models (2.8) and (2.9), results in an observed difference for maximum mouth gape height. For example, 6.0 mm TL was the smallest TL that the larvae were observed to begin exogenous feeding (Siefert 1972). Using this length, model (2.8) predicted a maximum mouth gape height of 684 μm and model (2.9) predicted in a maximum mouth gape height of 318 μm. The difference observed between these two models may be due to several factors. First, the difference in data collection techniques may have resulted in the difference observed. Guma'a (1978) measured the jaws at a 90°

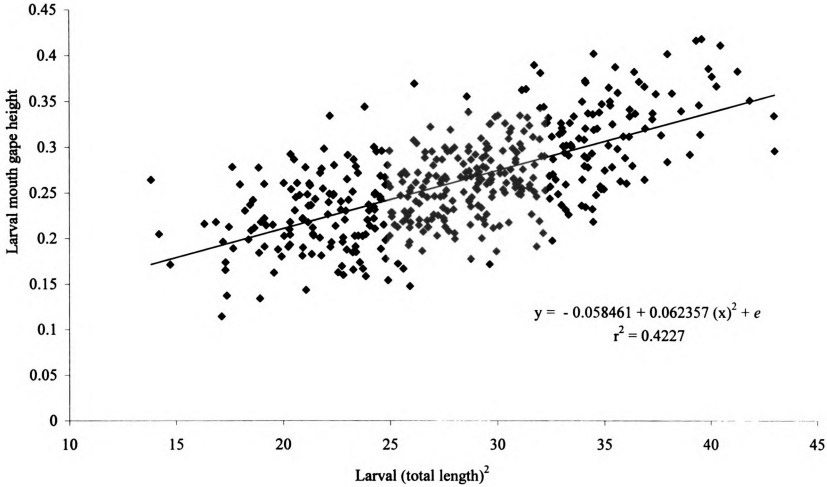

Figure 13. Simple linear regression analysis for larval (total length)^2 to mouth gape height relationship.

angle. In this study, care was taken to minimize any damage to the larvae. The mouth was only opened less than 45°. Secondly, it is not clear from the available data or methods, if Guma'a (1978) measured the mouth gape height of Eurasian perch larvae less than 10 mm TL. If larvae less than 10 mm TL were not used, this could account for the observed differences. Statistical predictions on the mouth gape cannot be made for values outside of the range investigated (Tempelman, personal communication, 1997). Third, the difference could potentially be due to different broodstock used. Researchers in the NCR have observed differences in larval offspring from different sources of broodstock (NCRAC 1997). While this study was unable to establish a relationship between the size of the broodstock and the larval offspring, differences were observed in larval offspring from different sources of spawning stock. All stocks showed the greatest amount of growth at 22 °C. For temperatures higher than 22 °C, growth rates were higher in stocks originating from the southern U.S., but growth rates were higher for stocks originating from the northern U.S. when temperatures were lower than 22 °C.

A second linear regression model was constructed for the larval TL-mouth gape relationship. Data collected for the mouth gape width were used to construct the following model (Figure 14):

(2.11) $y = 0.0585 + 0.0624\,(x) + e$,

where y is the mouth gape width;

x is the larval TL^2;

e is the residual error term.

Using the example of 6.0 mm TL for first feeding larval perch, the mouth gape width can be estimated to be approximately 322 μm. Since the mouth gape width of larval perch

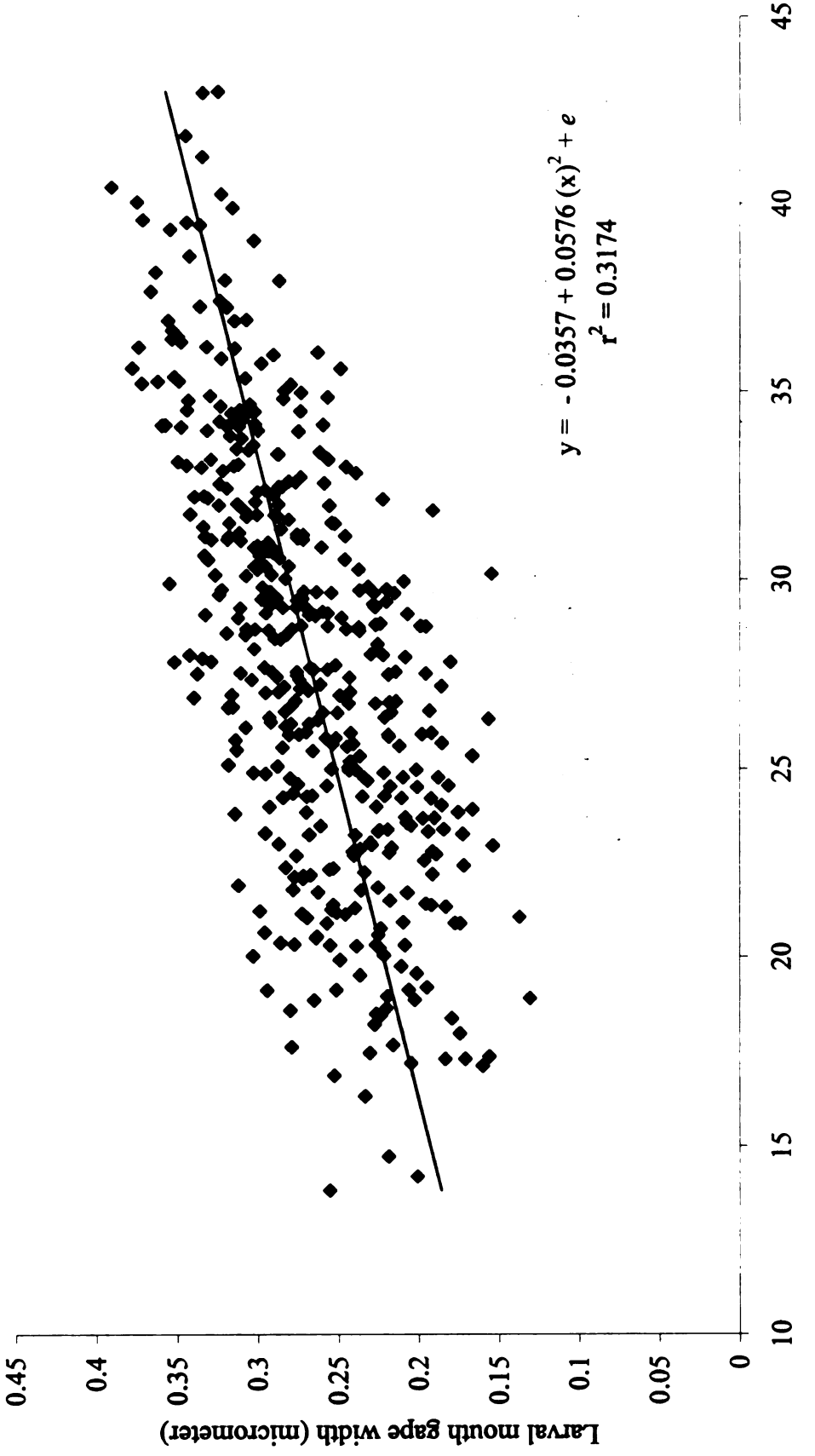

Figure 14. Simple linear regression analysis for larval (total length)^2 to mouth gape width relationship.

less than 10 mm TL has never been reported in the literature, comparisons cannot be made.

Linear regression models were constructed for larval TL-mouth gape relationships in each female spawner size group (Appendix N). This division of models allowed the comparisons between female spawner size groups for initial larval mouth gape size and rate of increase. In this experiment, no statistically significant differences for mouth gape width were observed between female spawner size groups (Table 26 and 27). Statistically significant differences for larval mouth gape height were observed between female spawner size groups. The rate of increase for mouth gape height was significantly different for SG 201 vs. SG 226, SG 226 vs. SG 276, and SG 276 vs. SG 301 (Table 28). The rate of increase for the contrast SG 201 and SG 276 was 0.009 and 0.006 for SG 226 and SG 301 (Appendix N). The rates of increase for the other female spawner size groups fell in between 0.006 and 0.009. This may have indicated that the rates of increase fell over a range of values, and the two opposite extremes of the range were significantly different. Overall, the rates of increase may not be too different. Statistical differences for initial mouth gape height were observed between a range of female spawner size groups. A consistent pattern did not emerge from the statistical analysis and may be a result of large variations.

The variations observed in this study might be the result of the fertilization methods used. For this study, eggs from a single female spawner were fertilized with one or more males. In addition, egg samples were taken over a period of two weeks. During this time, researchers were unable to keep male spawners used in this study separate from new broodstock. Male spawners were chosen at random. Consequently, the same male

spawners were not used to fertilize all of the eggs in this study and several males were often used at one time to ensure that enough milt was available for fertilization. The results of using several males may have contributed to the large variation observed in this portion of the study.

Larval Offspring Analysis - *Parental contribution*

The estimates of heritability were very low for larval TL, mouth gape width, and mouth gape height. All estimated values were less than 0.5. The value of heritability can range from 0 to 1, but values less than 0.5 indicate that selective improvement for these values is unlikely. Moav and Wohlfarth (1976) estimated the heritability for increased growth rate in European carp to be approximately 0. While an estimate for heritability is only a prediction on the effectiveness of selection (Tave 1993), Moav and Wohlfarth (1976) investigated the selection for improved growth rates over five generations. The result was negative and the conclusion was that the additive genetic variance for this trait had reached a plateau due to natural selection and past, uncontrolled, artificial selection. Purdom (1993) offered another explanation for the lack of response to increased growth rates. This trait may depend heavily on environmental conditions. Examples of variables that can result in environmental variation are food availability, food quality, water temperature, and predation. Variance due to environmental conditions, especially if not controlled, can confound with the additive genetic variance, and eliminate any possibilities to quantify improvement due to breeding by interacting with the genome (Tave 1993).

The low heritability values in this study are a reflection of the estimates for additive genetic variance (review Falconer 1989; Appendix D). The additive genetic variance was partitioned into maternally and paternally derived sources of variation. Estimates for both sources of variation were low, which suggests large non-additive genetic and environmental effects. Non-additive genetic variations result from the interaction between alleles at each locus. In this study, two breeding combinations resulted in significantly larger larvae (larval TL). On day 14 post-fertilization, a non-additive genetic or dominance effect was observed for sire fast growth and dam slow growth, but on day 15 post-fertilization, the non-additive genetic effect was observed for sire slow growth and dam fast growth. However, this is not significant since non-additive genetic variance cannot be inherited (Tave 1993). This form of genetic variance depends on interactions and is disrupted during meiosis, which eliminates the possibility of transfer from parent to offspring.

In this study, variation due to the environment was not partitioned out and was included in the error term. This was the result of unknown factors influencing the broodstock. Otherwise, the environmental condition was similar for all offspring.

The results of this study indicate that the low heritability for larval TL, mouth gape width, and mouth gape height do not currently warrant a selection or breeding program with current available broodstock. As Moav and Wohlfarth (1976) suggested, these traits may have reached a plateau due to natural selection. However, if a captive broodstock is developed, then another heritability study should be completed. There is still potential for improvement if environmental conditions for the broodstock are controlled.

SUMMARY AND CONCLUSIONS

1) The width and height of the mouth gapes for first feeding larval perch larvae were identified. For a 6.0 mm TL larvae, the mouth gape width was approximately 322 μm, and the mouth gape height was approximately 318 μm. With the mouth gape identified, researchers can focus on providing suitable diets that are small enough for the larvae to consume.
2) Positive linear relationships were significant between female spawner size (TL and weight) and egg ribbon weight and total fecundity. However, linear relationships were not significant between female spawner size and number of eggs per gram of eggs, chorion shell diameter, and egg yolk diameter. The inability to establish a correlation between female spawner size and these egg characteristics may be due to large environmental variation. Examples of variables that can result in environmental variation are food availability, food quality, water temperature, and predation.
3) Linear relationships could not be established between female spawner size and larval TL and size of the mouth gape, which could have been the result of large paternal influences or environmental variation. The genetics study attempted to identify the amount of influence from both maternal and paternal sources and from the environment.

4) The genetics study was designed to partition out the influence of the maternal and paternal sources of variation on larval TL, mouth gape width and height. Overall, the paternal contribution to the total variation was small (0 – 0.1278). The overall residual term, including any environmental variance, was larger (0.0484 - 0.2104), which indicates the large environmental influence. Another genetics experiment should be conducted, but only after some of the environmental variables can be controlled (i.e. – a captive broodstock).
5) Estimates of heritability were calculated for larval TL, mouth gape width, and mouth gape height. All values of heritability were less than 0.5, which indicates that selection for improvement of these characteristics will be unlikely. Since this study included a fixed assignment of the parental stock, a true estimation of heritability could not be calculated. However, the estimates of heritability provide a valuable insight to the possibility of starting breeding programs, which could select for other desirable characteristics.
6) The number of broodstock used and the number of larvae sampled were large enough to estimate the genetic variance components (review Falconer 1989). However, by increasing the number of broodstock used, the amount of variation in the population would be better represented.
7) The large dominance values (0.2087 - 1.5668) indicate that the variations in larval TL and mouth gape sizes occurred by chance. Larger values for larval TL and the size of the mouth gape resulting from a breeding program could not be duplicated, since this is a result of cell division.

8) The results of this study indicate that, through natural selection, larval TL and the size of the mouth gape for perch may have reached a plateau and cannot be increased through selective improvement. If artificial selection for these traits operates in the same direction as natural selection (i.e. - larger mouths and longer lengths), then it may be difficult to improve on natural selection. However, other traits that are important for culture, such as survival could be investigated

CHAPTER 3

EXPERIMENTAL TANK CULTURE DESIGN AND DIET STRATEGY EVALUATION FOR FEEDING LARVAL YELLOW PERCH

INTRODUCTION

Researchers and fish culturists have been trying to develop successful feeding strategies and culture systems for larval perch. However, current intensive larval culture techniques remain inadequate and cannot supply enough advanced fry and fingerlings for research, stocking, and food production requirements. With the development of out-of-season spawning techniques, successful intensive culture techniques become essential.

One reason for the slow development of intensive culture techniques has been the small size of perch larvae. This morphological characteristic makes it difficult to develop culture systems and to provide a suitable diet or feeding strategy for the small larvae.

This study was designed to develop feeding strategies and culture methods for intensive culture systems. Diet strategies were based on methods developed in other larval perch and walleye culture research (Barrows et al. 1993; Moore et al. 1994; Starr, personal communication, 1996; Summerfelt 1996). Culture methods were based on a combination of successful larval culture methods used for walleye and mahi mahi (Kim et al. 1993; Peterson et al. 1997).

Findings from the first two chapters of this thesis were incorporated into the design of this study. Based on the results of chapters 1 and 2, all larvae were pooled and different diet strategies were compared for growth and survival.

OBJECTIVE

6) to determine the significance of mouth gape size on growth, survival, and feed acceptance during intensive larval culture conditions.

LITERATURE REVIEW

Researchers and fish culturists in the North Central Region have been trying to develop successful feeding strategies and culture systems for larval perch using both, pond and tank rearing approaches to produce advanced perch larvae. However, intensive larval culture techniques remain inadequate and fail to meet commercial production demands.

In the pond rearing approach, eggs have been collected from wild or captive broodstock, which have been stocked as fertilized eggs or hatched larvae into intensively managed, fertilized ponds (Manci et al. 1983; Malison and Held 1992). The fertilized ponds provided a natural forage base for the larvae to survive and grow. At a later time, the larvae could be harvested and habituated to formulated feeds and intensive culture conditions. However, pond production of larval perch has been associated with highly variable survival, growth, and condition of the fish (NCRAC 1995). Results can vary annually as well as by location. In addition, perch stocked into ponds can be subjected to stress from zooplankton forage base depletion and excessive handling during harvest (Malison and Held 1995). These types of stress have been observed to result in higher rates of disease and to increase the amount of time to habituate to feed, resulting in significant mortality rates (Hussain and Summerfelt 1991).

In tank rearing of larval perch, larvae could be monitored during all stages of development, and the environmental and nutritional variables could be controlled by manipulation for the long-term goal of maximum growth and survival (Calbert and Huh 1976; Hale and Carlson 1972; Hinshaw 1985). This culture method is essential if out-of-season spawning for perch is to be developed (Dabrowski 1998). Together, these methods could potentially supply perch year round for research, stocking, and food production requirements.

Despite advancements, development of intensive culture techniques has been slow (NCRAC 1997). Larval perch are difficult to rear intensively due to their small size (Mansueti 1964; Nickum 1978; Best 1981), which has been reported to range from 4.7 - 7.0 mm total length (TL) at hatch (Mansueti 1964; Ney 1978; Whiteside et al. 1985; Heidinger and Kayes 1986). In addition to this morphological limitation, perch larvae have been observed to have small mouth gapes, which suggests that the larvae are restricted to certain prey due to the restriction of mouth gape width (Wong and Ward 1972). Larval perch are limited by the mouth gape when the size of their prey exceeds their mouth gape (Hansen and Wahl 1981; Wong 1972) and possibly the diameter of the esophagus (Kestemont et al. 1996; Raisanen and Applegate 1983). The size of the mouth gape, width and height, has been identified for larval perch less than 10 mm TL (review Chapter 2, this thesis). By identifying the mouth gape size for first feeding larval perch, the appropriate sized prey or pellet can be provided. For example, if the mouth width of first feeding larval perch is approximately 322 μm, then the larvae are capable of consuming prey or pellets that are less than this size. Care should be taken to avoid

providing prey or formulated diets that exceed the mouth gape or diameter of the esophagus.

Several researchers have been exploring the use of formulated diets as the initial food for first feeding larval perch (Starr, personal communication, 1996; NCRAC 1997). Formulated diets would be ideal for intensive culture of perch larvae if the diets were accepted by the larvae and were nutritionally complete. However, first feeding perch larvae do not accept and survive readily on formulated diets (Best 1981; Starr, personal communication, 1996). The development of intensive larval walleye culture also went through the similar problems of providing an appropriately sized and nutritionally balanced diet (Barrows et al. 1988).

Currently, larval perch intensive culture feeding strategies have employed captured or cultured species of zooplankton and phytoplankton (Hale and Carlson 1972; Noble 1973; Raisanen and Applegate 1983; Confer and Lake 1987; Ansari and Qadri 1989; NCRAC 1995) or feeding newly hatched *Artemia* nauplii (Hinshaw 1985; Mansueti 1964; McCormick 1976). The use of captured zooplankton presents several problems for any early season or out-of-season spawning and is often an unreliable food source. Problems may arise if zooplankton blooms do not occur at the same time as the first feeding larval stage. For example, the necessary food requirements of larval fish are sometimes not met due to a difference in timing between first feeding and zooplankton availability. It can also be difficult to find appropriate sizes or abundance of zooplankton.

At different times of the year, certain zooplankton species might not be able to supply the necessary nutritional requirements of the larval fish. Zooplankton population structure and size varies considerably during a growing season and between years due to

different environmental conditions (Houde 1967; Mills et al. 1986). In addition to the size of the zooplankton biomass, the caloric content of zooplankton may vary among species and seasonally within a species (Schindler et al. 1971).

Since larval fish depended on the chemical properties of their food (Watanabe et al. 1983), large numbers of zooplankton are needed to fulfill the fishes nutritional requirements. The National Research Council (1993) reported docosahexaenoic acid (DHA, 22:6ω3), eicosapentaenoic acid (EPA, 20:5ω3), and linolenic acid (18:ω3) are essential fatty acids for several species of fish. Levels of DHA and EPA were higher in young perch than in their major prey item, *Daphnia* (review Dabrowski et al. 1993). In addition, a single species of zooplankton is often incapable of supplying the long-chain fatty acids needed for growth and survival of larval fish. The lipid content of *Daphnia* varied throughout egg development and the molt cycle (Tessier at al. 1983).

Researchers that have focused on collecting zooplankton from rivers and lakes for feeding larval fish have discovered that this has not been a reliable source of food for commercial scale operations (Kamler 1992). The collection process can be labor intensive and problems can be magnified during periods of reduced zooplankton abundance. In addition, zooplankton have been identified as potential carriers of parasites and feeding wild zooplankton has been observed to increase the risk of pathogen introduction (Spotte 1970).

The problems associated with capturing zooplankton can be avoided by culturing zooplankton. Mass production of live feeds has been extensively used throughout Japan with success (Kamler 1992). However, zooplankton culture has been associated with an inability to provide adequate varieties of species as well as an adequate abundance for

commercial operations. Plankton and zooplankton monocultures require large amounts of space and skilled labor.

Binkowski (see review in NCRAC 1995) has developed a "green tank water" (GTW) method for culturing several zooplankton species in single, indoor tanks. The GTW culture is added regularly as a food source for first feeding larval perch. After four to six days, the perch larvae can be fed with newly hatched *Artemia* nauplii. While the need for monoculture has been eliminated, this method has gained limited acceptance for commercial applications, in part due to an inability to duplicate the research results.

An alternative to zooplankton culture could be *Artemia* culture. While the cost of *Artemia* cysts can be high ($25-100 lb^{-1}), the newly hatched *Artemia* nauplii provide a readily available diet with high nutritional quality in the form of fatty acids (Lovell 1990; Webster and Lovell 1990b). In addition, *Artemia* can be "packed" which can increase levels of highly unsaturated and essential fatty acids (Lemm and Lemarie 1991). Approximately 12 hours after hatching, *Artemia* nauplii molt into the Instar II stage when feeding begins (Sorgeloos et al. 1986). Typically, the nauplii can be fed a food source that has concentrated levels of fatty acids which helps to increase the nutritional value.

Feeding *Artemia* nauplii is a viable diet strategy for intensive commercial culture for first feeding larval perch. Survival rates have been reported as high as 85.3% after 14 days for European perch fed *Artemia* nauplii at first feeding (Vlavonou et al. 1995). Perch larvae can also be raised on *Artemia* nauplii as first food (Mansueti 1964; Hale and Carlson 1972; Hinshaw 1985; Starr, personal communication, 1996).

Although newly hatched *Artemia* nauplii are an excellent source of food for first feeding larval fish, there are two drawbacks. First, *Artemia* cysts can be expensive,

especially for certain characteristic traits such as a high hatching rate. Second, the daily production of *Artemia* nauplii can be labor intensive and usually requires additional equipment and space. One alternative to the use of *Artemia* nauplii is decapsulated *Artemia* cysts. The cysts have a non-digestible chorion shell, which can be removed using a chemical method (Bruggeman et al. 1979). The decapsulated cysts have a biochemical composition comparable to newly hatched nauplii and have a 30 to 40 % higher energy content (Schauer et al. 1980; Seidal et al. 1982). Verreth et al. (1987) and Vanhaecke et al. (1990) have reported using decapsulated *Artemia* cysts as a food source for first feeding larvae of carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*).

Intensive culture techniques need to be improved, if a continuous supply of perch can be provided to meet the needs of research, food production, and stocking. By developing intensive larval perch culture techniques, the options available to the hatchery manager increases (West and Leonard 1978). When out-of-season spawning techniques are developed, intensive larval culture techniques will be necessary to help supply perch year round for research, stocking, and food production requirements.

METHODS

Larval feeding strategy experiments were designed to compare survival and growth rates during the first 45 days using different larval diets. During the first experimental trial (year 1) for feeding strategies, there was no attempt to separate female spawners into separate size groups. Data analyzed from year one were used to determine strategies for feed trials in year two. Based on the results from year one (review Chapter 2 in this thesis), all larvae from the different adult female spawners were pooled in the second experimental trial (year 2).

Culture System Design

Cylindrical, 60 L polyethylene black plastic tanks with conical bottoms were used to culture the perch larvae. The conical bottom design was similar to culture tanks designed by Kim et al. (1993). The culture tanks had a 42 cm diameter with a 76 cm depth. In the center of each culture tank, an erect tubular screen was used to keep the fish larvae from entering the drain, which was located at the bottom of each tank. Since a large amount of diet was provided, a screen was constructed, which provided a large surface area and reduced the amount of clogging. The screen was constructed out of a 3.8 cm inside diameter (ID) PVC pipe with approximately 75% removed and covered with

150 μm Nitex mesh. The screen ran the length of the pipe, which was 78 cm long and extended 6.5 cm above the water surface.

The culture tanks were initially supplied with 11 °C aerated well water. Gradually, the well water supply was mixed with heated water from the Bay Port facility. The temperature of the water was increased over the next seven days and held constant at 17 ± 3 °C. The water depth was controlled using an external standpipe with the water volume set to 50 L. Water was supplied to the culture tanks using a similar design that has been used for intensive culture tank systems for walleye larvae (Barrows et al. 1993; Moore et al. 1994). The water was supplied below the surface, along the tank wall through a vertical manifold. The manifold was constructed using a 13 mm ID PVC pipe with 10 - 1.6 mm diameter holes. The manifold was pointed in the direction to deliver a clockwise flow. A water spray was used on the surface and pointed in the direction of the flow to minimize any disruption of flow patterns. The flow rate was regulated to 0.5 maximum exchanges per hour (0.42 liters per min) for the prolarvae stage and increased to 0.75 exchanges per hour (0.625 liters per min) at the onset of exogenous feeding (Summerfelt 1996). The culture tanks were illuminated using overhead, incandescent lights with less than an estimated 100 lux. The culture tank walls and screens were cleaned every two days using a siphon.

Egg and Larval Collection Procedures

Perch spawning stock was collected from the outer Saginaw Bay, Lake Huron during a two week period beginning on April 19, 1996 and on April 15, 1997. The

spawning stock was transported to Bay Port Aquaculture in West Olive, Michigan (Figure 6) and held in a flow through system supplied with 11 °C aerated well water.

The egg ribbon was manually stripped from each ripe female into a dry bowl to enhance fertilization rates (Piper et. al. 1985). Milt was mixed with the eggs before water was added. The fertilized eggs were allowed to water harden for 10 min before being placed into an upwelling incubation unit. Eggs were incubated using techniques developed by Bay Port Aquaculture researchers (Starr, personal communication, 1996).

After hatching, approximately 11-12 days post fertilization, larvae from several incubating units were pooled into one collection tank. The water within the collection tank was mildly agitated to achieve an even mixture of larvae. All culture tanks were randomly stocked, on day 0, with perch larvae at a density of 160 larvae per liter (8,000 larvae per tank) using a Jensorter Electronic Fry Counter, Model FC (Bend, Oregon, U.S.).

Sub-samples of five larvae were collected every three days post stocking to determine percent gas bladder inflation, verify diet consumption and measure TL. Larvae were euthanized using CO_2 saturated tonic water. After the larvae were euthanized, they were placed in a 90 mm diameter Petri dish containing 10 ml of distilled water. Larvae were observed under a dissecting microscope with light provided from the bottom to determine gas bladder inflation (GBI). Inflated gas bladders were readily detected by observation (Barrows et al. 1993). After the initial two week sampling period, larvae were sampled once per week until completion of the study. At each cleaning, moribund larvae were collected and counted to estimate mortality and survival.

Feeding Procedures

Four different larval diet strategies were compared (3 replicates per treatment) in 12 culture tanks (Table 37). Two *Artemia* strains, Great Salt Lake[4] (GSL) and San Francisco Bay[5] (SFB), were compared. *Artemia* cysts and nauplii from these two strains were used as initial food for first feeding larval perch. Incubation of cysts followed standard methods (Hoff and Snell 1993) and decapsulation of cysts followed procedures discussed by Sorgeloos et al. (1977) and Bruggeman et al. (1979).

The control group was initially fed newly hatched *Artemia* nauplii from the GSL strain. This control group designation was based on previous successful research conducted by researchers at the Bay Port research facility (Starr, personal communication, 1996). For the other three feeding strategies, decapsulated *Artemia* cysts from both strains were used as the initial food for first feeding larval perch. The feeding strategies were randomly assigned to culture tanks (SAS, 1988).

Feeding began on day 2 with an initial *Artemia* cyst or nauplii density of 3 per ml for each feeding. The *Artemia* density was increased to 5 per ml for each feeding beginning on day 4 and was increased to 7.5 per ml on day 9. On day 13, the first diet transition began. The initial diet amount was reduced by 25% each day and replaced with the first transition diet (Table 38). This continued until the initial diet was entirely replaced. On day 27, the second diet transition began. The transition occurred in the

[4] Great Salt Lake (Bonneville Artemia International, Inc., Salt Lake City, UT; no lot number)
[5] San Francisco Bay (Platinum grade Argentemia, Argent Chemical Laboratories, Inc., Seattle, WA; lot BP0103K)

Table 37. Larval yellow perch stocking levels for each tank during trial one. Feeding strategies are labeled as decapsulated Great Salt Lake strain *Artemia* cyst group 1 (DG 1), decapsulated Great Salt Lake strain *Artemia* cyst group 2 (DG 2), decapsulated San Francisco Bay strain *Artemia* cyst group (DS 1), and the control group.

Feeding Strategy	Culture tank	Initial stocking	Initial stocking density
Control 1	1	8005 larvae per tank	160 larvae per liter
Control 2	5	8033	161
Control 3	7	8009	160
DG 1_0	2	7990	160
DG 1_1	8	7996	160
DG 1_2	9	8020	160
DG 2_0	3	8000	160
DG 2_1	4	8003	160
DG 2_2	6	8016	160
DS 1_0	10	8016	160
DS 1_1	11	7980	160
DS 1_2	12	7992	160

Table 38. Description of feeding strategies for larval yellow perch during trial one. Feeding strategies are labeled as decapsulated Great Salt Lake (GSL) strain *Artemia* cyst group 1 (DG 1), decapsulated Great Salt Lake (GSL) strain *Artemia* cyst group 2 (DG 2), decapsulated San Francisco Bay (SFB) strain *Artemia* cyst group (DS 1), and the control group.

Feeding strategy	Initial starting food	First transition	Second transition
Control	Instar I GSL nauplii	"packed" *Artemia* nauplii	BioKyowa A and B
DG 1	Decapsulated GSL cysts	Instar I *Artemia* nauplii	"packed" *Artemia* nauplii
DG 2	Decapsulated GSL cysts	BioKyowa A and B	BioKyowa A and B
DS 1	Decapsulated SFB cysts	Instar I *Artemia* nauplii	"packed" *Artemia* nauplii

same manner as described above. The larval starter diet consisted of BioKyowa A and B with a pellet size of 50 – 200 μm.

Changes to Equipment and Feeding Strategies for Trial Two

Perch larvae were pooled and collected using the same methods as described for year one. All tanks were stocked volumetrically with perch larvae at a density of approximately 160 larvae per liter (8,000 larvae per tank). For transportation, larvae were placed into 12 - 40 L bags with 10 L of 11 °C aerated well water. The bags were filled with pure oxygen and sealed. The larvae were transported to Michigan State University's Aquaculture Laboratory in East Lansing, Michigan where larval feeding experiments were conducted. The larvae were tempered to 16 °C before being randomly stocked into the culture tanks. The culture tanks were supplied with heated well water. The temperature of the water was increased to 20 °C over the next seven days and held constant to ± 1 °C. The culture tanks were illuminated using overhead, fluorescent lights with an estimated 400 lux. The culture tanks were cleaned every two days.

Tank design was modified based on findings from the first trial. Initially, a false, flat bottom was provided to increase the bottom surface area. A similar modification in the tank design of Kim et al. (1993) had been used for benthic marine fish larvae at the Oceanic Institute, University of Hawaii (Garling, personal communication, 1996).

Four different larval diet strategies were compared (3 replicates per treatment) in twelve culture tanks (Table 39). The diets consisted of newly hatched *Artemia* nauplii from two different strains, SFB and GSL. Feeding began on day 2 with an initial *Artemia*

Table 39. Larval yellow perch stocking levels for each tank during trial two. Feeding strategies are labeled as Great Salt Lake strain *Artemia* nauplii group (G 1), San Francisco Bay strain *Artemia* nauplii group 1 (S 1), San Francisco Bay strain *Artemia* nauplii group 2 (S 2), and the control group.

Feeding Strategy	Culture tank	Initial stocking	Initial stocking density
Control 1	4	8827 larvae per tank	177 larvae per liter
Control 2	5	7280	146
Control 3	9	8190	164
G 1_0	2	8008	160
G 1_1	6	7462	149
G 1_2	8	8463	169
S 1_0	7	8645	173
S 1_1	10	7280	146
S 1_2	12	8190	164
S 2_0	1	8372	167
S 2_1	3	7735	155
S 2_2	11	8736	175

nauplii density of 3 per ml for each feeding. The *Artemia* density was increased to 5 per ml for each feeding beginning on day 4 and was increased to 7.5 per ml on day 9. On day 13, the first diet transition began. The initial diet amount was reduced by 25% each day and replaced with the first transition diet (Table 40). This continued until the initial diet was replaced entirely. On day 27, the second diet transition began. The transition occurred in the same manner as described above.

Table 40. Description of feeding strategies for larval yellow perch during trial two. Feeding strategies are labeled as Great Salt Lake (GSL) strain *Artemia* nauplii group (G 1), San Francisco Bay (SFB) strain *Artemia* nauplii group 1 (S 1), San Francisco Bay (SFB) strain *Artemia* nauplii group 2 (S 2), and the control group.

Feeding strategy	Initial starting food	First transition	Second transition
Control	Instar I GSL nauplii	"packed" *Artemia* nauplii	BioKyowa A and B
G 1	Instar I GSL nauplii	BioKyowa A and B	BioKyowa A and B
S 1	Instar I SFB nauplii	"packed" *Artemia* nauplii	BioKyowa A and B
S 2	Instar I SFB nauplii	BioKyowa A and B	BioKyowa A and B

STATISTICAL ANALYSIS

Variation in larvae TL means for each diet strategy were calculated and tested for normal distribution and homogeneity of variances. The test for normal distribution was based on the *Shapiro-Wilk statistic W* (Neter et al. 1996). The assumption of equal variances was tested using a *Modified Levene's* test (Neter et al. 1996). One-way Analysis of Variance (ANOVA) tests were used to determine overall statistical significance between TL from the different diet strategies. Variation in mean TL was compared over time for the different diet strategies. The percentage of GBI and feed acceptance were calculated and compared over time.

Growth and mortality were represented as instantaneous growth rates (IGR) and instantaneous mortality rates (IMR). IGR and IMR were calculated using methods from Ricker (1975). Means for larval TL were used to calculate IGR:

$$\mu = \frac{\ln (L_{x+i}) - \ln (L_x)}{t_{x+i}} \tag{3.1}$$

where, μ is the IGR;

L_x, L_{x+i} are the lengths at time x and x + i;

t_{x+i} is the time at x + i represented as days.

In addition, a survivorship curve was used to show the corresponding larval survival to any point in time (Deevey 1947):

(3.2) $$\delta_x = \frac{N_x}{N_0}$$

where, δ_x is the change in population size;

N_0 is the initial population size;

N_x is the population size at any point in the future.

Larval mortalities were used to calculate IMR:

(3.3) $$Z = \frac{\ln (N_x) - \ln (N_{x+i})}{i}$$

where, Z is the IMR;

N_x is the initial population size;

N_{x+i} is the population size time at x + i;

i is the time represented as days.

RESULTS

Trial One

Since each tank was treated as an experimental unit, the observations from each replicate were combined into means for each of the four different feeding strategies. The means and standard deviations for larval TL were calculated every three days during the first feeding trial (Table 41). The data were normally distributed ($p = 0.7839$), and the test for the assumption of equal variances across feeding strategies was not violated ($p > 0.05$). One-way ANOVA tests did not indicate any statistical differences between the mean larval TL for each feeding strategy or for each day measured.

Feed acceptance and GBI were measured at the same time. The first observation of GBI occurred on day 6 in groups DG 2 and DS 1 (Table 42). By day 9, GBI rates for the experiment groups ranged from 0 % to 87 % (Table 42). GBI was not observed in the control group before day 10. The group DS 1 indicated 100 % GBI by day 12 (Table 42). Feed acceptance was low throughout the study. The first observation of feed acceptance occurred on day 9 (Table 42). Approximately 7 % of the larvae in the DS 1 feeding strategy had accepted the diet. In contrast, none of the larvae sampled in this feeding strategy had accepted the diet on day 12. However, 20 % of the larvae from the DG 2

Table 41. Average larval yellow perch TL over time for each diet strategy during the first feed trial. All values are reported as means ± standard deviations. Feeding strategies are labeled as decapsulated Great Salt Lake strain *Artemia* cyst group 1 (DG 1), decapsulated Great Salt Lake strain *Artemia* cyst group 2 (DG 2), decapsulated San Francisco Bay strain *Artemia* cyst group (DS 1), and the control group.

Diet strategy	day 0	day 3	day 6	day 9	day 12	day 15
Control	5.2178 ± 0.4308	5.4675 ± 0.4635	5.9470 ± 0.3174	6.232 ± 0.3634	-	-
DG 1	5.1098 ± 0.3724	5.3764 ± 0.3966	5.9872 ± 0.5115	6.328 ± 0.4483	6.4432 ± 0.3097	6.5978 ± 0.4141
DG 2	5.1782 ± 0.4450	5.4071 ± 0.5021	5.8934 ± 0.4250	6.341 ± 0.3898	6.5671 ± 0.4092	6.6311 ± 0.5048
DS 1	5.2010 ± 0.4004	5.4302 ± 0.6057	5.9797 ± 0.4481	6.307 ± 0.5352	6.5309 ± 0.4410	6.5694 ± 0.4475

Table 42. Gas bladder inflation and feed acceptance rates over time for each diet strategy during the first feed trial. All values are reported as percents. Feeding strategies are labeled as decapsulated Great Salt Lake strain *Artemia* cyst group 1 (DG 1), decapsulated Great Salt Lake strain *Artemia* cyst group 2 (DG 2), decapsulated San Francisco Bay strain *Artemia* cyst group (DS 1), and the control group.

Gas bladder inflation

Diet strategy	day 0	day 3	day 6	day 9	day 12	day 15
Control	0	0	0	0	-	-
DG 1	0	0	0	73.33	93.33	100
DG 2	0	0	13.33	66.67	86.67	93.33
DS 1	0	0	13.33	86.67	100	100

Feed aceptance

Diet strategy	day 0	day 3	day 6	day 9	day 12	day 15
Control	0	0	0	0	-	-
DG 1	0	0	0	0	0	20
DG 2	0	0	0	0	20	13.33
DS 1	0	0	0	6.67	0	13.33

feeding strategy had accepted the diet by day 12. By day 15, 13 % to 20 % of larvae remaining in the three feeding strategies had accepted the diet.

Survival for all diet strategies was above 99 % for the initial six days after stocking (Table 43; Figure 15). Beginning on day 8, survival rates decreased to 65 % for the control group. The survival rates for the rest of the diet strategies were lower than reported for the previous six days but ranged from 89 % to 91 % (Table 43). By day 10, 0.5 % of the 8,000 larvae stocked in each of the control group culture tanks were remaining. The survival rates for the rest of the diet strategies ranged from 71 % to 74 %. By day 10, almost 100 % mortality was observed in the control group, and all sampling was discontinued. By day 12, the experiment for the control group was terminated. For the remaining culture tanks, survival rates ranged from 0.52 % to 1.65 %. By day 16, there were no larvae surviving in the culture tanks, except for 0.15 % in the DS 1 culture tanks.

The growth of perch larvae was measured as larval TL and calculated as IGR. While the TL of the larvae increased over time (Table 41), the IGR initially increased until day 6 and began to decrease (Figure 16).

The number of mortalities in the control group occurred sooner than the other feeding strategies. The number of mortalities peaked by day 10 (Table 44). For the other three feeding strategies, the number of mortalities peaked on day 12. These observations are reflected in the IMR calculations. The IMR peaked at 2.42 on day 10 for the control group (Figure 17). The IMR for the other three feeding strategies peaked on day 14 but was less, ranging from 1.22 to 1.77 (Figure 17).

Table 43. Average survival rates over time for each diet strategy during the first feed trial. All values are reported as percents. Feeding strategies are labeled as decapsulated Great Salt Lake strain *Artemia* cyst group 1 (DG 1), decapsulated Great Salt Lake strain *Artemia* cyst group 2 (DG 2), decapsulated San Francisco Bay strain *Artemia* cyst group (DS 1), and the control group.

Diet strategy	day 0	Day 2	day 4	day 6	day 8	day 10	Day 12	day 14	Day 16
Control	100	99.92	99.84	99.60	64.57	0.51	0	0	0
DG 1	100	99.92	99.81	99.55	89.10	71.26	17.86	0.52	0
DG 2	100	99.88	99.78	99.60	91.38	72.96	12.52	0.81	0
DS 1	100	99.88	99.80	99.48	90.14	73.95	19.05	1.65	0.15

Table 44. Average mortality rates over time for each diet strategy during the first feed trial. All values are reported as means for each feeding strategy. Feeding strategies are labeled as decapsulated Great Salt Lake strain *Artemia* cyst group 1 (DG 1), decapsulated Great Salt Lake strain *Artemia* cyst group 2 (DG 2), decapsulated San Francisco Bay strain *Artemia* cyst group (DS 1), and the control group.

Diet strategy	day 0	day 2	day 4	day 6	day 8	day 10	day 12	day 14	day 16
Control	0	6.33	6.33	19.00	2804.67	5129.00	50.33	0	0
DG 1	0	6.33	8.67	21.00	836.33	1427.67	4273.00	1387.67	41.33
DG 2	0	9.67	8.33	14.33	657.67	1475.00	4838.33	937.00	66.00
DS 1	0	9.33	6.67	25.33	748.00	1297.00	4396.00	1393.00	120.67

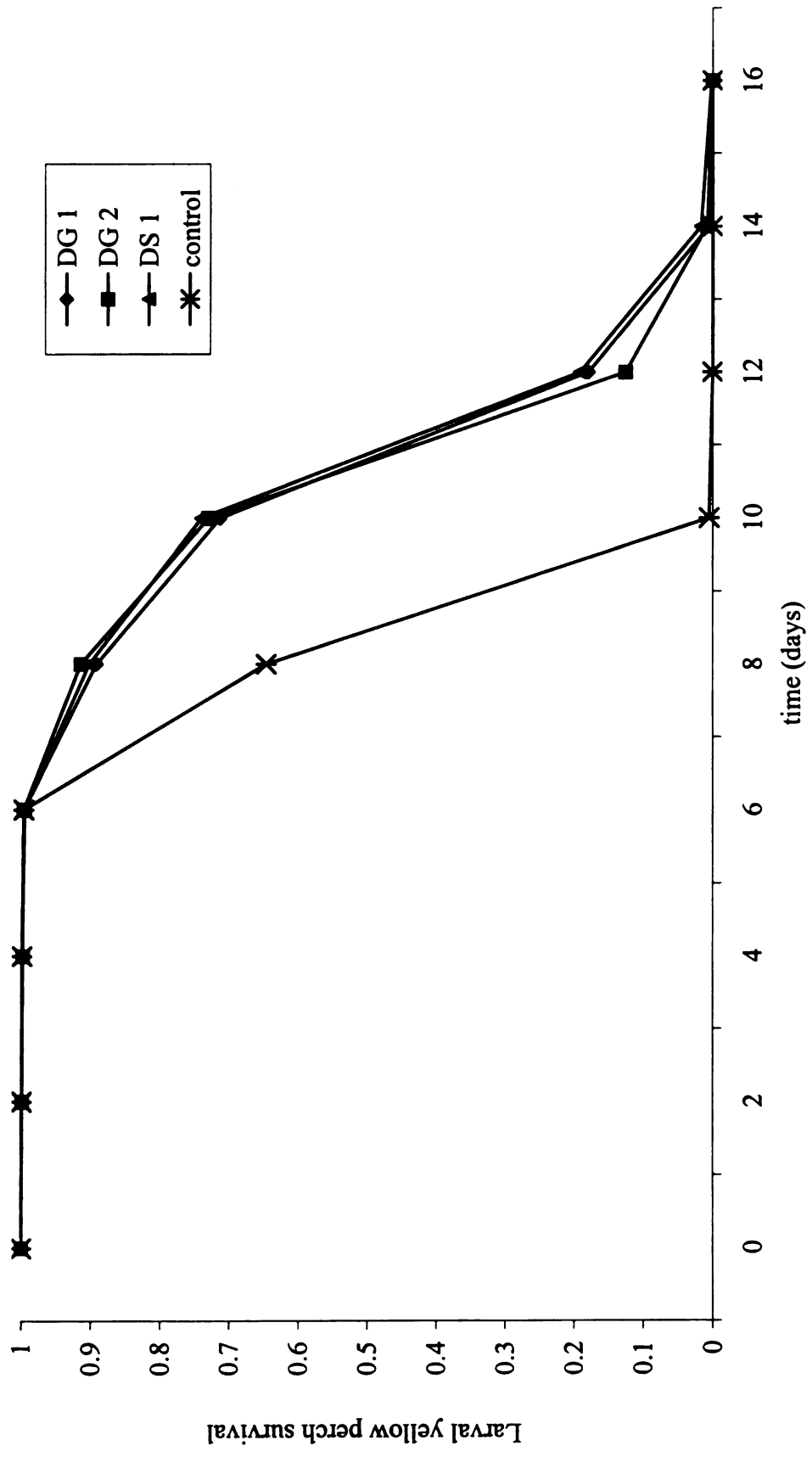

Figure 15. Survivorship curve for larval yellow perch during the first feed trial. Feeding strategies include an *Artemia* control group, Great Salt Lake strain decapsulated *Artemia* cyst groups 1 and 2 (DG 1; DG 2) and San Francisco Bay strain decapsulated *Artemia* cyst group 1 (DS 1).

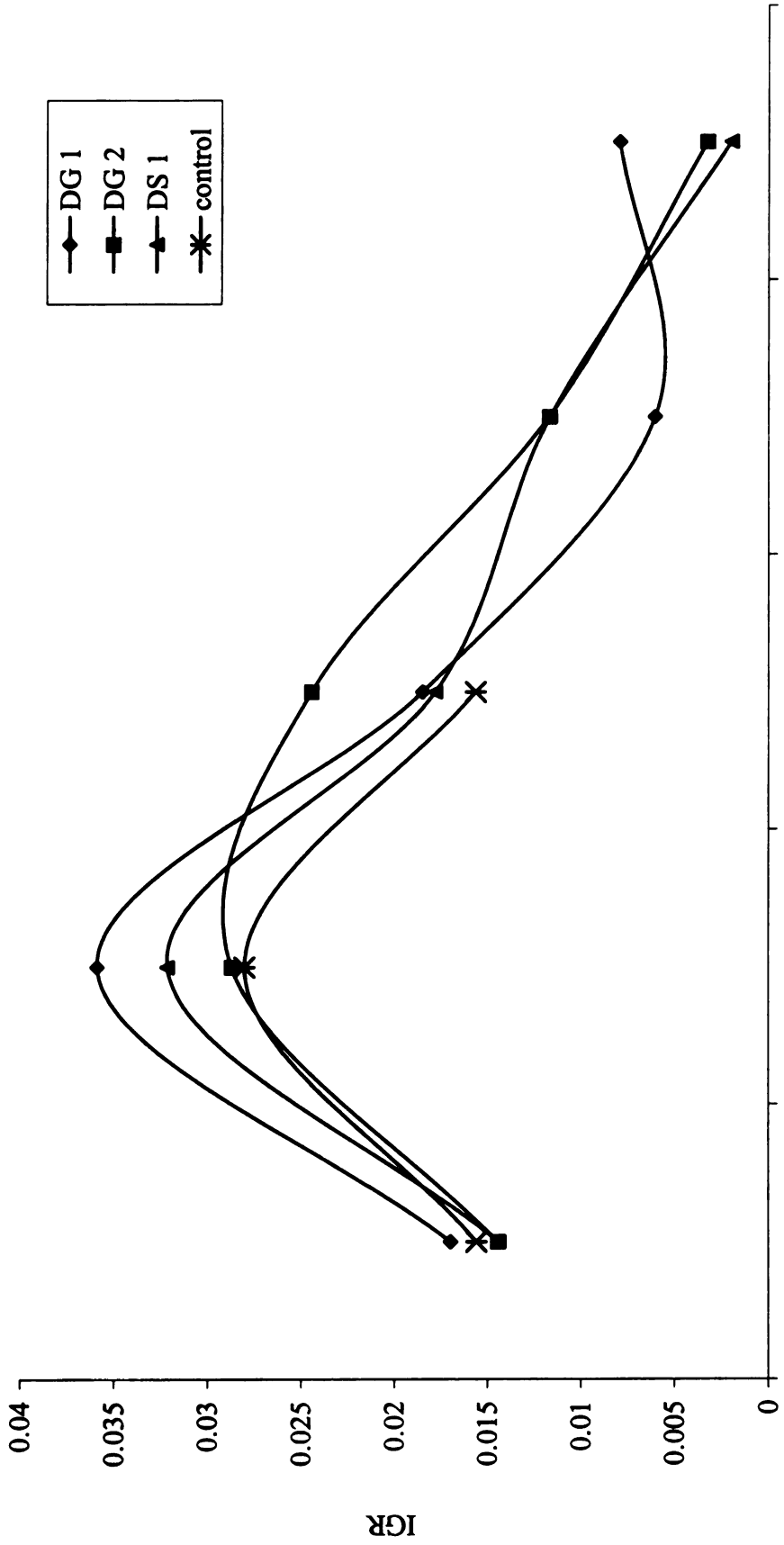

Figure 16. Instantaneous Growth Rates (IGR) for larval yellow perch during the first feed trial. Feeding strategies include an Artemia control group, Great Salt Lake strain decapsulated Artemia cyst groups 1 and 2 (DG 1; DG 2) and San Francisco Bay strain decapsulated Artemia cyst group 1 (DS 1).

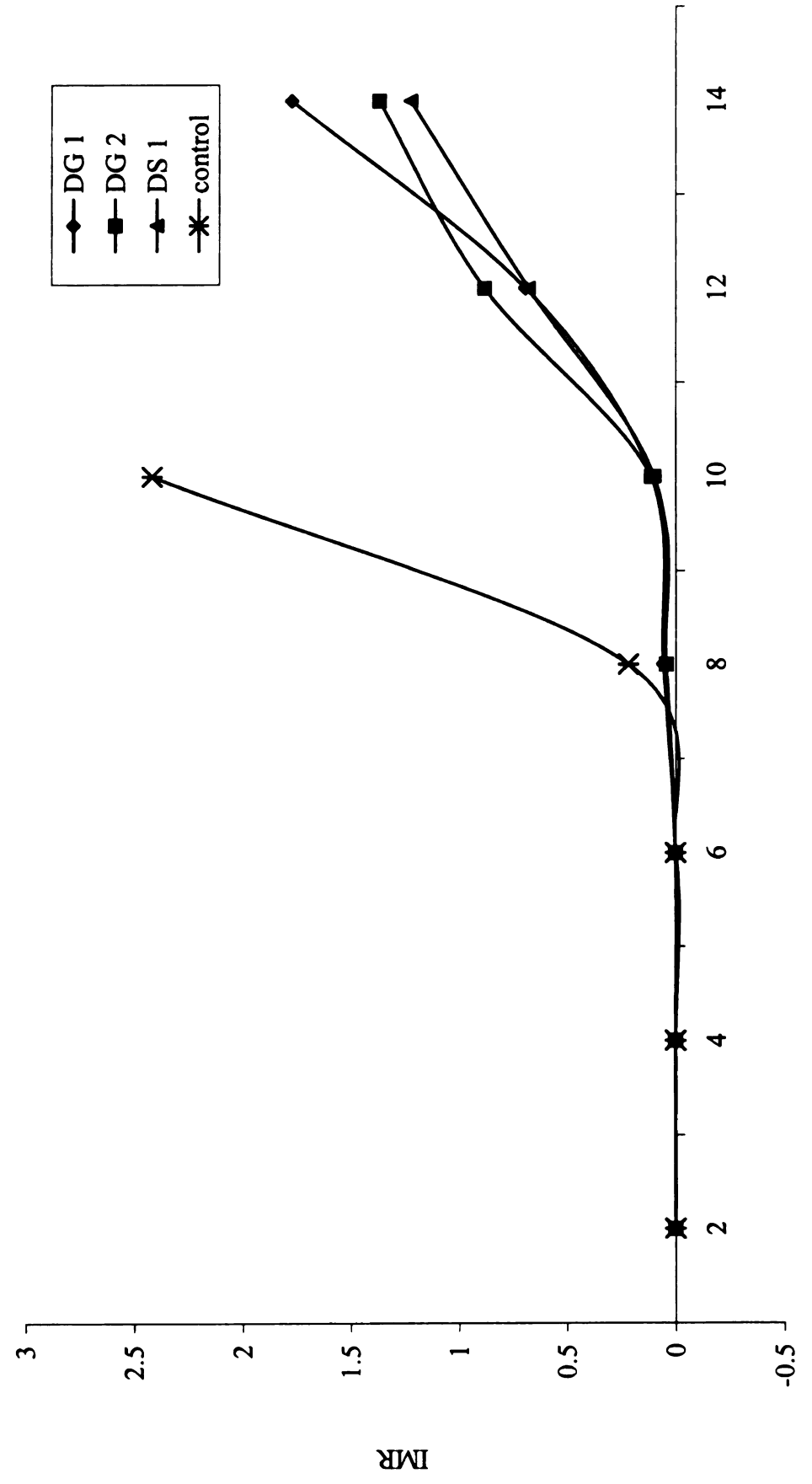

Figure 17. Instantaneous mortality rates (IMR) for larval yellow perch during the first feed trial. Feeding strategies include an *Artemia* control group, Great Salt Lake strain decapsulated *Artemia* cyst groups 1 and 2 (DG 1; DG 2) and San Francisco Bay strain decapsulated *Artemia* cyst group 1 (DS 1).

Trial Two

After the larvae were stocked and allowed one day to adapt to the new surroundings, a period of high mortality was observed. By day 3 and 4, mortality rates approached 96 % to 99 % (Table 45). Between sampling periods 2 and 3, the number of larvae mortalites ranged from 7831 to 8065 (Table 46) and survival dropped to approximately zero (Figure 18). Measurements of TL, GBI, and feed acceptance were not recorded. During the period of high mortality on days 3 and 4, IMR values ranged from 1.8 to 2.9 (Figure 19). By day 6, the IMR values were less than 0.5, but increased to a range of 0.85 to 1.63 by day 10 (Figure 19).

Table 45. Average survival rates over time for each diet strategy during the second feed trial. All values are reported as percents. Feeding strategies are labeled as Great Salt Lake strain *Artemia* nauplii group (G 1), San Francisco Bay strain *Artemia* nauplii group 1 (S 1), San Francisco Bay strain *Artemia* nauplii group 2 (S 2), and the control group.

Diet strategy	day 0	day 2	day 4	day 6	day 8	day 10
Control	99.88	99.86	0.28	0.19	0.14	0.02
G 1	99.81	99.79	0.86	0.56	0.33	0.01
S 1	99.89	99.88	2.45	2.13	1.41	0.20
S 2	99.67	99.61	3.03	2.90	1.36	0.14

Table 46. Average mortality rates over time for each diet strategy during the second feed trial. All values are reported as means for each feeding strategy. Feeding strategies are labeled as Great Salt Lake strain *Artemia* nauplii group (G 1), San Francisco Bay strain *Artemia* nauplii group 1 (S 1), San Francisco Bay strain *Artemia* nauplii group 2 (S 2), and the control group.

Diet strategy	day 0	day 2	day 4	day 6	day 8	day 10
Control	10	1	8065	8	4	9
G 1	15	2	7892	24	19	25
S 1	9	1	7831	26	58	97
S 2	27	5	7998	11	127	101

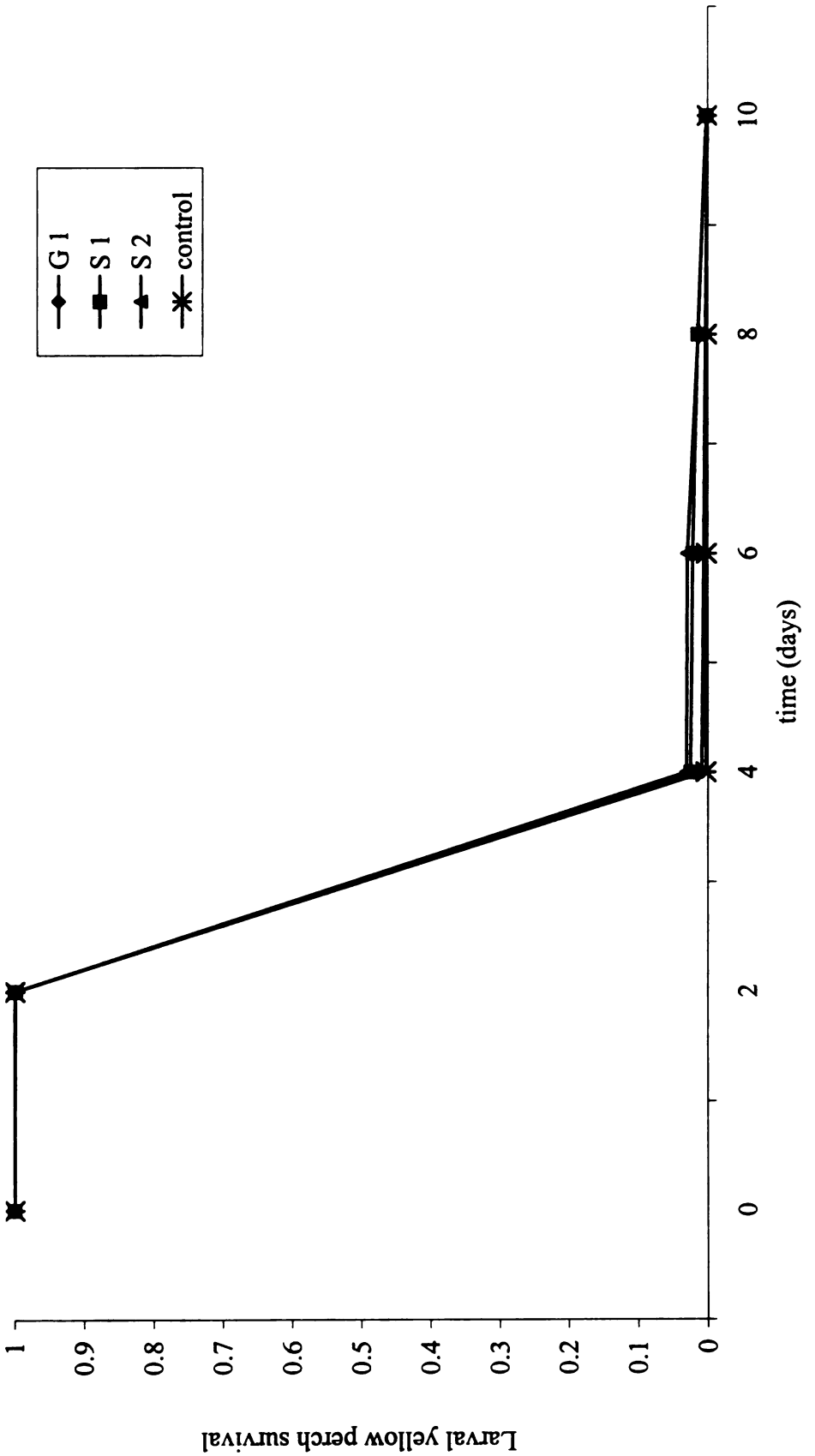

Figure 18. Survivorship curve for larval yellow perch during the second feed trial. Feeding strategies include an *Artemia* control group, Great Salt Lake strain *Artemia* nauplii group 1 (G 1) and San Francisco Bay strain *Artemia* nauplii groups 1 and 2 (S 1; S 2).

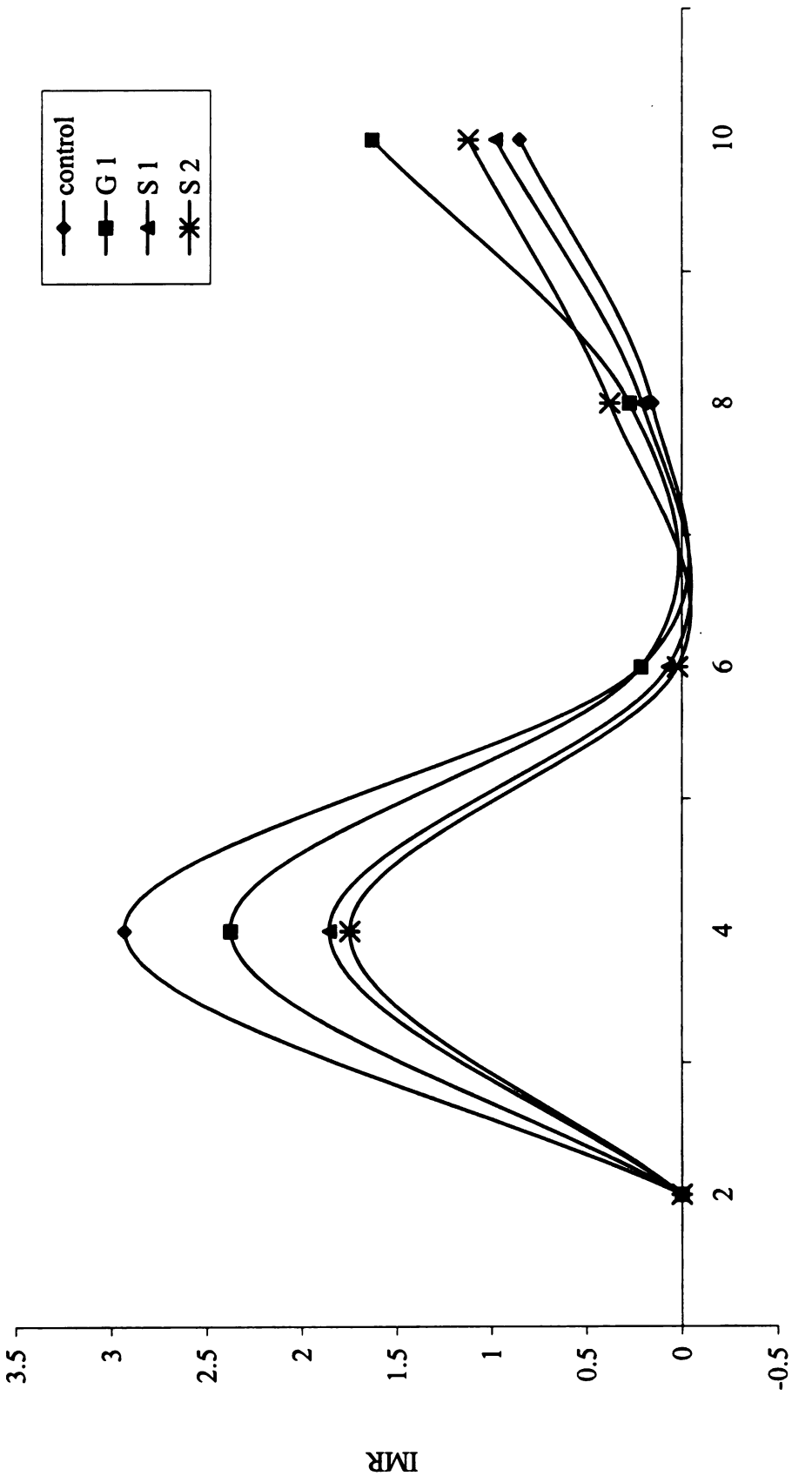

Figure 19. Instantaneous mortality rates (IMR) for larval yellow perch during the second feed trial. Feeding strategies include an *Artemia* control group, Great Salt Lake strain *Artemia* nauplii group 1 (G 1) and San Francisco Bay strain *Artemia* nauplii groups 1 and 2 (S 1; S 2).

DISCUSSION

Although considerable knowledge is known about intensive culture methods for several species of larval fish, including walleye (review Moore et al. 1994a; Moore et al. 1994b; Summerfelt 1996; Moore 1996), less is known about perch and Eurasian perch. Different culture systems have been used to investigate optimal conditions for culturing perch; however, a single, universal intensive culture method has not been accepted. Comparing prey requirements can be hampered by the different experimental designs and rearing conditions used (Buckley et al. 1987). Some optimal conditions of environmental factors, such as; temperature, flow rate, and light intensity have been identified (Houde 1969; Hokanson and Kleiner 1974; Calbert and Huh 1976; Hokanson 1977), but optimal diets and feeding strategies are unknown (Brown et al. 1996).

This study attempted to investigate the growth and survival of larval perch using different diets and strategies. Feed acceptance, GBI, and survival of perch larvae raised on *Artemia* in the first 14 d of life was lower than reported for other previous studies (Hinshaw 1985; Starr, personal communication, 1996). The TL for larval perch stocked into the culture tanks on day 0 were within the range reported by Heidinger and Kayes (1986). By day 9, the yolk sac was almost completely absorbed with only the oil droplet remaining. At this stage, the larvae were approximately 6.2 mm TL. Similarly, Siefert (1972) had observed that larval perch had accepted feed at 6.0 mm TL with complete

yolk sac absorption at 7.1 mm TL under natural conditions. However, egg size (Blaxter and Hempel 1963) and temperature (Ware 1975) can affect the length of time that larvae remain in the yolk sac stage. In this study, the first observation of feed acceptance occurred on day 9 when the larvae were approximately 6.3 mm TL (mean temperature = 13.8 °C).

Larval mortalities were observed in this study beginning on day 8. Hokanson and Kleiner (1974) had observed mortality in non-feeding perch larvae by day 9 at 19.8 °C. The pattern of larval mortality observed was similar to larval walleye mortalities described by Li and Mathias (1982). Although, the larvae had not begun to feed, mortality rates remained low despite complete yolk absorption, which occurred on day 8. The remaining oil globule might have provided a nutritional "buffer" between the onset of exogenous feeding or starvation (Li and Mathias 1982). However, by day 12, the mortality rates ranged from 81 to 100 % in this study. The IGR values calculated for this study support the timing of the endogenous to exogenous feeding transition. The IGR values peaked for all feeding strategies on day 6, but decreased after this point (Figure 14). This may have indicated that the larvae consumed most of the endogenous feeding reserves. Without supplementation from exogenous feed sources, growth decreased and mortality increased. Significant mortality rates were observed for the first time on day 6 (Figure 13). If the larvae had accepted the diet, the IGR values probably would have increased. The trend would have appeared to be similar to the observations for larval lake whitefish (*Coregonus clupeaformis*) IGR values (Brown and Taylor 1992).

The low feed acceptance rate observed in this study may have resulted from an inability by the larval perch to consume the newly hatched *Artemia* nauplii at the onset of

first feeding. This factor alone may indicate the reason for the inability of the larvae to consume the diet. By day 6, the larvae had reached an average TL of 5.94 mm which was close to the TL of first feeding larval perch (Siefert 1972). The linear relationship established between larval TL and mouth gape width indicated that the mouth gape width should have been approximately 310 to 317 μm (review Chapter 2, this thesis). Researchers have reported that the critical dimension for ingestion of oblong prey is the maximum width including all appendages (Blaxter 1965; Arthur 1976). The *Artemia* nauplii used in this study had a maximum appendage width that ranged from 484 to 764 μm (review Chapter 1, this thesis). This indicated that the larvae were mouth gape restricted at the beginning of exogenous feeding. Using this strain of *Artemia*, only larvae longer than 7.69 mm TL at this critical point could have survived (review Chapter 2, this thesis).

Previous studies have reported using *Artemia* nauplii as food for first feeding larval perch with limited success (Mansueti 1965; Hale and Carlson 1972; Hinshaw 1985; Starr, personal communication, 1996). However, differences in nauplii size may have accounted for the different success rates. Differences in nauplii appendage widths have been observed for different *Artemia* strains (review Chapter 1, this thesis). Maximum appendage widths for the SFB strain ranged from 285 to 418 μm. This range of maximum appendage widths would be small enough for 5.52 mm TL, or greater, first feeding larval perch.

Although decapsulated *Artemia* cysts have not been used previously for larval perch culture, the cysts can provide an excellent alternative to newly hatched *Artemia* nauplii. The decapsulated cysts can be handled as an inert diet and have comparable

nutritional qualities (Schauer et al. 1980; Seidel et al. 1982). In addition, the diameters of decapsulated cysts are small enough to be consumed by first feeding larval perch. Vanaecke and Sorgeloos (1980) compared different sources of hydrated, decapsulated cysts. The diameters of the GSL strain averaged between 207.7 to 210.5 μm for the SFB strain and 234.8 to 241.6 μm. Decapsulated *Artemia* cysts have been used for larval culture of carp and African catfish (Verreth et al. 1987; Vanhaecke et al. 1990). Unlike free-swimming *Artemia* nauplii, hydrated decapsulated cysts sink to the bottom. To help alleviate this problem, aeration was used within the tank to keep the cysts suspended in the water column. However, the aeration was discontinued after day 1, because the larvae became disoriented. The air pressure from the aeration system was too high and could not be reduced significantly without affecting the efficiency of the air stones. Although the cysts sank to the tank bottom, 6.7 % of the larvae were feeding by day 9 in the DS 1 diet strategy and 20 % by day 12 in the DG 2 diet strategy (Table 41). A better method might be used to deliver the decapsulated *Artemia* cysts over the entire day rather than during four short feeding periods.

Colesante (1996) discussed a similar method used to deliver *Artemia* nauplii to larval walleye. After hatching, the nauplii were placed into a feeder that dispensed every 8 min for a 3-sec interval, 24 hrs/day. At each feeding, the feeder dispensed approximately 500-600 ml of the nauplii solution into feeder tubes. The feeder tubes directed the solution to different parts of the tank. The amount of nauplii released during each feeding was not reported, but the target feeding rate was 800-1000 nauplii/fish/day. By using this method, the decapsulated cysts would not lose significant nutritional quality, as is the case with nauplii. Newly hatched nauplii continue to grow and use the

energy reserves, which significantly reduces the nutritional content (Sorgeloos et al. 1986).

A second method could have employed the use of dry, decapsulated *Artemia* cysts. Unlike free-swimming nauplii and hydrated cysts, the dry, decapsulated cysts will float on the water surface and sink after hydrating. Vanhaecke et al. (1990) successfully used the dry, decapsulated cysts to raise larval carp. The growth rates of the larvae were comparable to the larvae fed newly hatched nauplii.

Another reason for the differences in survival rates for first feeding larval perch fed *Artemia* nauplii, may be due to differences in broodstocks used in previous studies. Blaxter (1965) observed that differences in mouth gape existed between different races of herring and concluded that these differences could be significant in early survival. The same may be true for perch. Researchers have not utilized a single broodstock source in the various experiments. Some sources of broodstock may be better suited for intensive culture conditions or may have offspring that are larger, have larger mouth gapes, or both. Researchers in the NCR have conducted evaluations of offspring performance taken from different wild stocks as an initial step for a broodstock development program (NCRAC 1997). The research indicated that variations occurred between sources of broodstock and identified geographically disparate populations but did not identify a single source that performed better than all others (NCRAC 1997). Variations within strains of broodstock have been identified, including fast and slow growth rates (Moav 1979; Kamler 1992; review Chapter 2, this thesis). When the gametes from a slow growing female perch and a fast growing male perch were crossed, the combination resulted in

larger larvae (review Chapter 2, this thesis). All of these variables may contribute to differences observed when comparing results between studies.

Two other factors that might have contributed to the high mortality rates observed in the study were the behavior of the larvae and the design of the culture tanks. These two factors combined together may have caused higher mortalities than might be expected. The culture tank included a conical bottom design to collect excess feed and fecal material. This design was expected to help facilitate waste removal. However, when the larvae were not swimming, they sank to the bottom and displayed a resting behavior. Approximately 50 % of the larvae remained on the bottom of the culture tanks. With the design of this culture system, the larvae were concentrated into a small area and were covered with uneaten *Artemia* nauplii or decapsulated cysts. This sinking motion has previously been observed in larval walleyes (Li and Mathias 1982) and perch (Ross et al. 1977) during intensive culture conditions. The reasons for remaining on the bottom of the culture tanks are unclear. This behavior may have been the result of non-inflated gas bladders. GBI averaged approximately 13 % in two of the diet strategy culture tanks and 0 % in the other two by day 6 (Table 6). Although, the GBI rates increased over time, the combination of a resting behavior and uneaten feed collection may have led to early mortalities.

While non-inflated gas bladders may help to explain the swimming behavior exhibited by the perch larvae, there was no explanation of larvae remaining on the bottom for extended periods of time while being covered by uneaten food. The water temperature may have had an effect on the swimming ability of the larvae. Temperature is an important factor. It directly affects organogenesis and metabolism. Teleost larvae

are unable to compensate for temperature induced metabolic alterations (Wang et al. 1987). Lower water temperatures may have reduced the ability to swim. The average temperature was 13.8 °C through the first nine days of the experiment. This average temperature was higher than the 13 °C used by Houde (1969) to observe swimming abilities of larval perch and walleye. The water temperatures in this study (12.0 to 15.0 °C) did not reach the optimal temperature range for larval culture (20 to 23.9 °C) determined by Hokanson (1977). An additional factor that might have had an affect on the swimming behavior was the flow rate. Summerfelt (1996) determined the flow rate used in this study as the optimal flow rate for intensive culture conditions of larval walleye. Although both perch and walleye are poor swimmers as newly hatched larvae, perch have a better relative swimming ability than larval walleye as the yolk is absorbed (Houde 1969). The flow rate used in these experiments should have been within an optimal flow rate range.

The perch larvae exhibited another behavior that may have contributed to the high mortality rates observed in this study. Many of the remaining larvae that did not exhibit the sinking or resting behavior had a tendency to stay very near to the sides of the culture tanks. Often, the larvae appeared to rest with their heads touching the side of the tank. This behavior has also been observed in larval walleyes during intensive culture conditions (Bristow and Summerfelt 1994; Summerfelt 1996). Researchers have suggested that this behavior (also known as clinging behavior) may be a result of stress (Kise and Meade 1986) or may be an attraction to light reflected from the sides of the tanks (Summerfelt 1996). To help reduce this behavior, researchers have investigated the use of turbid water (Bristow and Summerfelt 1994; Bristow et al. 1996) and higher light

intensities that ranged from 100 – 200 lux (Colesante 1996). For the second feeding trial, a higher light intensity was used (estimated to be 400 lux). A previous study used a high light level that supported a mean survival rate of 44 % for perch larvae fed *Artemia* nauplii as the first food (Hinshaw 1985). The higher light levels (95 lux) used in the second feeding trial appeared to eliminate the clinging behavior.

After analyzing the results of the first feeding trial, the design of the culture system and feeding strategy changed. A false, flat bottom was added to the culture tanks (similar to Kim et al. 1993). This change offered a larger surface area for any larvae resting on the bottom. Based on research conducted by Bay Port researchers (review Chapter 1, this thesis), the decapsulated *Artemia* cysts were replaced with newly hatched *Artemia* nauplii as the first food. The GSL strain was offered again as the control, but the newly hatched nauplii from the SFB strain was introduced. While the maximum appendage width of the SFB strain was small enough for first feeding perch larvae to eat, observations were unable to be made since the majority of the larvae did not survive past day 3.

The reasons for the sudden mortalities remain unclear. After stocking the larvae into the culture tanks, the larvae were observed to be actively swimming and possibly inflating the gas bladders. Many of the larvae were swimming at the surface, which caused little ripples to form on the surface. It appeared that the larvae might have been trying to inflate the gas bladders. By day 3, approximately 90 % of larvae were inactive on the tank bottom and by day 4, mortality rates were as high as 99 %. It might be possible that water temperature may have played a significant role. In the first experiment, the initial water temperature was 11 °C and was warmed gradually over a

period of 15 days. In the second experiment, the initial water temperature was 16 °C. Since the larvae were incubated in 11 °C well water, the higher culture temperature may have been too high for the larvae to survive. Higher water temperature could have increased larval metabolism to the point where the endogenous energy reserves were depleted at a faster rate. At higher temperatures, larvae must be fed shortly after the swim-up stage (Hokanson and Kleiner 1974). However, Hokanson and Kleiner (1974) reported a median period of 9 days between swim-up and mortality of unfed larvae at 19.8 °C, and larvae hatched at lower temperatures had an increased chance of survival when transferred to higher temperatures. It remains unclear if temperature played a significant role in the mortalities observed in this study.

The sudden mortality rates observed in this study may have been the result of handling stress induced during transportation. The trip from the Bay Port facility to the MSU aquaculture facility was approximately 2 hours. Steps were taken to minimize stress and damage to the larvae. These steps included packing larvae into coolers and five-gallon containers to minimize shifting and covering the containers to block direct sunlight. Handling stress also seems unlikely since Bay Port researchers transport larvae at higher densities and over longer distances with high success rates.

While this study was unable to culture a significant number of larvae to an advanced fingerling stage, several lessons were learned. First, perch larvae can be raised successfully on *Artemia* as the initial food (Mansueti 1965; Hale and Carlson 1972; Hinshaw 1985; Starr, personal communication, 1996). The small size of the mouth gape and the large size of the diet provided restricted larval perch in this study. A small nauplii strain of *Artemia* is necessary for acceptance. Second, perch larvae can be raised

on decapsulated cysts. While this study produced limited results, refining a feed dispensing system might increase the acceptance of decapsulated cysts. Finally, the selection of broodstock is an important step for successful culture of larval perch. Through genetic selection, important culture characteristics can be enhanced. Freshwater salmonids have gone through the process of selection, which has resulted in higher fecundity rates larger egg sizes, higher hatching percentages and higher growth rates (Moav 1979). Controlled selection experiments should be designed to begin the process of developing perch stocks with traits favorable to intensive culture.

SUMMARY AND CONCLUSIONS

1) Small strain *Artemia* nauplii, such as the SFB strain, should be used for first feeding larval perch.
2) Decapsulated *Artemia* cysts were used with some success as the first food for larval perch. Hydrated, decapsulated cysts can be used with limited aeration, but dehydrated cysts would be better, since they tend to float on the surface and sink over a longer period of time.
3) The flat bottom added to the culture tanks helped to spread out the larvae when they exhibited the resting behavior.
4) Developing intensive culture techniques for perch larvae increases the number of options for culturists. With out-of-season spawning techniques and intensive culture techniques, a supply of perch could be made available year round. Intensive culture of larval perch would reduce the amount of variablity observed in pond culture by controlling the environment and food.
5) A selection program, which identifies perch that grow well in intensive culture conditions, should be started to aid intensive larval culture techniques. This could be started by using perch raised entirely in intensive culture conditions as the broodstock for future cultures.

APPENDICES

APPENDIX A: *Artemia* hatching medium using artificial seawater

Table VII

Artificial seawater used in *Artemia* hatching and culturing

	Hatching medium[a]	Culturing medium[a]
Evaporated sea salt (NaCl)	5.0	31.08
$MgSO_4$[b]	1.3	7.74
$MgCl_2$	1.0	6.09
$CaCl_2$	0.3	1.53
KCl[b]	0.2	0.97
$NaHCO_3$[b]	2.0	2.00

[a] In gram salts (technical grade) per liter tap water.
[b] Should be dissolved separately in warm tap water before addition to the solution of other salts.

APPENDIX B: Analytical methods used for determining hatching percent

APPENDIX 1

Analytical methodology for hatching characterization of *Artemia* cysts.

HATCHING PERCENTAGE – Method A

A.1. Incubate approx. 250 mg of cysts in approx. 100 ml of 25 ppt seawater, under continuous illumination (1,000 lux) at 25 °C in a conical tube (preferentially) or in a graduated cylinder (see schematic drawings under the hatching efficiency section, same appendix); provide aeration from bottom to keep all cysts in suspension (aeration should be low enough to prevent foaming).

A.2. After 1 hour, take 10 subsamples (i=10) of approx. 100 cysts each using an automatic micropipet (preferentially) or a 0.5 ml glass pipette with the tip cut away if the opening is too small for the cysts/nauplii.

A.3. Pipet cysts for each subsample onto a separate paper filter.

A.4. Count cysts on each paper filter using a dissection microscope (c_i); calculate the mean value (c).

A.5. Rinse cysts from paper filters into individual glass petridishes or small plastic tubes (5 ml); add 35 ppt pre-aerated seawater and incubate under continuous illumination (> 1000 lux) at 25 °C.

A.6. After 48 hours, fixate nauplii by adding a few drops of lugol's solution.
Preparation of lugol's solution:
- dissolve 10g KI and 5g I_2 in 20 ml distilled water;
- dissolve 5g Na-acetate in 50 ml distilled water;
- mix the 2 solutions and store in a dark bottle in refrigerator.

The pharmaceutical disinfective iodine tincture can be used as an alternative for the lugol's solution.

A.7. Count nauplii under dissection microscope (n_i) and calculate the mean value (n).
Practical method for nauplii counting:
- construct small filter nets made of PVC-tube (8 cm diameter, 1-2 cm height) which 100µm nylon filter are glued;
- drain contents of hatching tube or petridish over filter; rinse tube or petridish and empty contents over filter;
- place filter in petridish filled with water; shaking and moving filter in and out of water will ensure more even distribution of the nauplii over the filter as to facilitate counting;
- remove filter from petridish, dry filter from underneath using a towel or filter paper;

- place filter under microscope for counting; eventually place filter in plastic petridish which has been graduated as to facilitate counting

A.8. Hatching percentage $H\% = \frac{n \times 100}{c}$

A.9. Practical example

Sample	Number of cysts counted on filter (c_i)	Number of nauplii (n_i)	Hatching %
1	112	76	67.9
2	89	72	80.9
3	102	75	73.5
4	110	79	71.5
5	99	69	69.7
6	108	70	64.8
7	103	76	73.8
8	97	69	71.1
9	98	65	66.3
10	115	80	69.6
n			70.9
Standard deviation (s.d.)			4.5

A.10. Remarks

- this method can only be used when the cysts have been properly processed (i.e. not containing empty cyst shells).
- correct sampling and counting is indicated by a standard deviation of less than 5%.

APPENDIX C: Results of the statistical analysis conducted for objectives 1 and 2

Appendix C. Results of the statistical analysis conducted for objectives 1 and 2.

GSL				
Parameter	Test for normal distribution	Hartley's F-max test	Type of analysis test	p-value
Cyst diameter	0.0001	$p < 0.05$	Kruskal-Wallis	chi-square 0.0001
Nauplii length	0.4897	$p > 0.05$	One-way ANOVA	0.0001
Nauplii width	0.1213	$p > 0.05$	One-way ANOVA	0.0001
Appendage length	0.0507	$p > 0.05$	One-way ANOVA	0.0001

SFB				
Parameter	Test for normal distribution	Hartley's F-max test	Type of analysis test	p-value
Cyst diameter	0.0001	$p < 0.05$	Kruskal-Wallis	chi-square 0.0001
Nauplii length	0.9554	$p > 0.05$	One-way ANOVA	0.0001
Nauplii width	0.7617	$p > 0.05$	One-way ANOVA	0.0001
Appendage length	0.1172	$p > 0.05$	One-way ANOVA	0.0001

APPENDIX D: Calculation of heritability (h^2) estimates

Appendix D. Calculation of heritability (h^2) estimates using the following model (Falconer 1989).

$h^2 = V_G / V_P$

where, V_G was the genotypic variance component;

V_P was the phenotypic variance component;

$V_G = 2 * (V_{SIRE} + V_{DAM})$;

$V_P = V_{SIRE} + V_{DAM} + V_{ERROR}$;

where, V_{SIRE} was the male contribution to the additive variance component;

V_{DAM} was the female contribution to the additive variance component;

V_{ERROR} was the remaining unexplained contribution to the additive variance component.

APPENDIX E: Calculation of dominance (d^2) estimates

Appendix E. Calculation of dominance (d^2) estimates using the following model (Falconer 1989).

$d^2 = V_I / V_P$

where, V_I was the interaction variance component between sire and dam;

$V_I = V_{SIRE \cdot DAM} * 4$;

$V_P = V_{SIRE} + V_{DAM} + V_{SIRE \cdot DAM} + V_{ERROR}$;

where, $V_{SIRE \cdot DAM}$ was the sire by dam interaction of the non-additive variance component;

V_{SIRE} was the male contribution to the non-additive variance component;

V_{DAM} was the female contribution to the non-additive variance component;

V_{ERROR} was the remaining unexplained contribution to the non-additive variance component.

APPENDIX F: Results of the statistical analysis conducted for female spawner total length, weight, age, egg ribbon weight, total fecundity, and number of eggs per gram of eggs (objectives 3, 4 and 5)

Appendix F. Results of the statistical analysis conducted for female spawner total length, weight, age, egg ribbon weight, total fecundity, and number of eggs per gram (objectives 3, 4 and 5).

Parameter	Test for normal distribution	Modified Levene's test[1] for equal variances	Type of analysis test	p-value
Spawner total length	0.0980	$p < 0.05$	One-way AVOVA	0.0001
Spawner weight	0.0074			
log(spawner weight)	0.0874	$p < 0.05$	One-way AVOVA	0.0001
Spawner age	0.0509	$P > 0.05$	One-way AVOVA	0.0001
Spawner total length			Two factor ANOVA	0.0001
log(spawner weight)			Two factor ANOVA	0.0001
Egg ribbon weight	0.0013			
log(egg ribbon weight)	0.3603	$p > 0.05$	Two factor ANOVA	0.0001
Number of eggs per g	0.4726	$p > 0.05$	Two factor ANOVA	0.0834
Total fecundity	0.0028			
log(total fecundity)	0.7372	$p < 0.05$	Two factor ANOVA	0.0001
log(egg ribbon weight)		$p > 0.05$	One-way AVOVA	0.0001
Number of eggs per g		$p > 0.05$	One-way AVOVA	0.4816
log(total fecundity)		$p < 0.05$	Kruskal-Wallis	chi-square 0.0001
Chorion shell diameter	0.8979	$p > 0.05$	One-way AVOVA	0.0381
Egg yolk diameter	0.9915	$p > 0.05$	One-way AVOVA	0.0661
Larval total length	0.0345			
(Larval total length)^2	0.0679	$p > 0.05$	One-way AVOVA	0.0037
Larval mouth gape width	0.3876	$p > 0.05$	One-way AVOVA	0.0049
Larval mouth gape height	0.0942	$p > 0.05$	One-way AVOVA	0.0001

[1] - Critical value for the Modified Levene's test was adjusted using a Bonferroni adjustment based on sample size.

APPENDIX G: Results of Two factor Analysis of Variance (ANOVA) testing for female spawner total length, weight, egg ribbon weight, total fecundity, and number of eggs per gram of eggs (objective 3)

Appendix G. Results of Two factor Analysis of Variance (ANOVA) testing for female spawner total length, weight, egg ribbon weigth, total fecundity, and number of eggs per gram (objective 3).

Parameter	Overall p-value	Group p-value	Age p-value
Spawner total length	0.0001	0.0001	0.1590
log(Spawner weight)	0.0001	0.0001	0.8390
Total fecundity	0.0001	0.0001	0.2351
log(Egg ribbon weight)	0.0001	0.0001	0.7468
Number of eggs per gram	0.0834	0.0576	0.9450

APPENDIX H: Results of simple linear regression analysis testing of female spawner size to egg ribbon weight, total fecundity, and number of eggs per gram of eggs (objective 3)

Appendix H. Results of simple linear regression analysis testing of female spawner total length to spawner weight, egg ribbon weight, total fecundity, and number of eggs per gram. Includes simple linear regression analysis testing for female spawner to egg ribbon weight, total fecundity, and number of eggs per gram (objective 3).

Model	Equation	p-value	r-square
$\log(\text{Weight}) = \beta_o + \beta_x (x) + e$ Where x = log (spawner TL)	$\log(y) = 3.417 - 13.675 \log(x) + e$	0.0001	0.9820
$\log(\text{Egg ribbon weight}) = \beta_o + \beta_x (x) + e$ Where x = spawner TL	$\log(y) = 0.928 + 0.012 (x) + e$	0.0001	0.9089
$\log(\text{Fecundity}) = \beta_o + \beta_x (x) + e$ Where x = spawner TL	$\log(y) = 3.335 + 0.005 (x) + e$	0.0001	0.8771
$\log(\text{Fecundity}) = \beta_o + \beta_x \log (x) + e$ [2] Where x = log(spawner TL)	$\log(y) = -2.958 + 1.365 (x) + e$	0.0001	0.8780
Number of eggs per gram $= \beta_o + \beta_x (x) + e$ Where x = spawner TL	$y = 834.250 - 0.644 (x) + e$	0.0667	0.0983
$\log(\text{Egg ribbon weight}) = \beta_o + \beta_x \log (x) + e$ Where x = log(spawner weight)	$\log(y) = -1.049 + 0.981 (x) + e$	0.0001	0.8938
$\log(\text{Fecundity}) = \beta_o + \beta_x \log (x) + e$ Where x = log(spawner weight)	$\log(y) = 2.550 + 0.900 \log(x) + e$	0.0001	0.8567
Number of eggs per gram $= \beta_o + \beta_x \log (x) + e$ Where x = log(spawner weight)	$y = 948.260 - 53.079 (x) + e$	0.0643	0.1000

[2] - Linear regression model was developed to compare models in literature.

APPENDIX I: Results of factorial analysis testing for the determination of significant differences between areas of the egg ribbon for number of eggs per gram of eggs, chorion shell diameter, and yolk diameter

Appendix I. Results of factorial analysis testing for the determination of significant differences between areas of the egg ribbon for number of eggs per gram, chorion shell diameter, and yolk diameter.

Parameter	Test for normal distribution	Modified Levene's test[1] for equal variances	Type of test	p-value
Number of eggs per gram	0.2829	$p > 0.05$	2 x 3 Factorial analysis	0.9984
Chorion shell diameter	0.4101	$p < 0.05$	2 x 3 Factorial analysis on ranked data	0.0001
Yolk diameter	0.6186	$p < 0.05$	2 x 3 Factorial analysis on ranked data	0.0001

[1] Critical value for the Modified Levene's test was adjusted using a Bonferroni adjustment based on sample size.

APPENDIX J: Results of simple linear regression analysis of female spawner TL and weight on chorion shell and egg yolk diameters

Appendix J. Results of simple linear regression analysis of female spawner TL and weight on chorion shell and egg yolk diameters.

Model	Equation	p-value	r-square
Chorion shell diameter = $\beta_o + \beta_x (x) + e$ where x = spawner TL	$y = 0.3118 + 0.00003 (x) + e$	0.4338	0.0005
Chorion shell diameter = $\beta_o + \beta_x \log (x) + e$ where x = log(spawner weight)	$y = 0.3082 + 0.00228 (x) + e$	0.5009	0.0003
Egg yolk diameter = $\beta_o + \beta_x (x) + e$ where x = spawner TL	$y = 1.3690 + 0.00004 (x) + e$	0.4874	0.0004
Egg yolk diameter = $\beta_o + \beta_x \log (x) + e$ where x = log(spawner weight)	$y = 1.3640 + 0.00291 (x) + e$	0.5009	0.0003

APPENDIX K: Results of One-way Analysis of Variance (ANOVA) testing for larval total length, larval mouth gape width, and larval mouth gape height for each day sampled post-fertilization

Appendix K. Results of One-way Analysis of Variance (ANOVA) testing for larval total length, larval mouth gape width, and larval mouth gape height for each day sampled post-fertilization.

Larval characteristic	Day post-fertilization	p-value	r-square
Larval total length			
	15	0.3926	0.1669
	16	0.7545	0.0344
	17	0.2975	0.0712
	18	0.0127	0.1273
	19	0.0004	0.2648
Larval mouth gape width			
	15	0.1623	0.2423
	16	0.2521	0.0837
	17	0.0622	0.1196
	18	0.0588	0.0952
	19	0.0497	0.1401
Larval mouth gape height			
	15	0.3500	0.2039
	16	0.2081	0.1009
	17	0.2805	0.0732
	18	0.0610	0.0944
	19	0.0849	0.1234

APPENDIX L: Results of simple linear regression analysis of female spawner total length and weight on larval total length, mouth gape width, and mouth gape height

Appendix L. Results of simple linear regression analysis of female spawner total length and weight on larval total length, mouth gape width, and mouth gape height.

Model	Equation	p-value	r-square
Larval $TL^2 = \beta_o + \beta_x (x) + e$ where x = spawner total length	$y = 34.929094 - 0.024424 (x) + e$	0.0007	0.0294
Larval $TL^2 = \beta_o + \beta_x \log (x) + e$ where x = log(spawner weight)	$y = 37.871087 - 1.761268 (x) + e$	0.0023	0.0238
Larval mouth gape width $= \beta_o + \beta_x (x) + e$ where x = spawner total length	$y = 0.322190 - 0.000192 (x) + e$	0.0087	0.0177
Larval mouth gape width $= \beta_o + \beta_x \log (x) + e$ where x = log(spawner weight)	$y = 0.352175 - 0.015091 (x) + e$	0.0100	0.0170
Larval mouth gape height $= \beta_o + \beta_x (x) + e$ where x = spawner total length	$y = 0.325632 - 0.000230 (x) + e$	0.0032	0.0230
Larval mouth gape height $= \beta_o + \beta_x \log (x) + e$ where x = log(spawner weight)	$y = 0.364671 - 0.018654 (x) + e$	0.0029	0.0235

APPENDIX M: Results of simple linear regression analysis of larval total length on mouth gape width and height across all female spawner size groups

Appendix M. Results of simple linear regression analysis of larval total length on mouth gape width and height across all female spawner size groups.

Model	Equation	p-value	r-square
Larval mouth width = $\beta_o + \beta_x (x) + e$ where x = (Larval total length)^2	$y = 0.081599 + 0.006664 (x) + e$	0.0001	0.4330
Larval mouth height = $\beta_o + \beta_x (x) + e$ where x = (Larval total length)^2	$y = 0.060535 + 0.007155 (x) + e$	0.0001	0.4577

Appendix N. Results of linear regression analysis of larval total length on mouth gape width and height for each female spawner size groups (SG)

Appendix N. Results of linear regression analysis of larval total length on mouth gape width and height for each female spawner size groups (SG).

Group	Model	Equation	p-value	r-square
SG 201	Mouth width = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.076105 + 0.007100 (x) + e	0.0001	0.4337
SG 201	Mouth height = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.076380 + 0.009455 (x) + e	0.0001	0.6100
SG 226	Mouth width = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.095317 + 0.006113 (x) + e	0.0001	0.4614
SG 226	Mouth height = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.079476 + 0.006353 (x) + e	0.0001	0.4331
SG 251	Mouth width = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.043924 + 0.007767 (x) + e	0.0001	0.3861
SG 251	Mouth height = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.047744 + 0.007426 (x) + e	0.0001	0.3487
SG 276	Mouth width = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.038060 + 0.008123 (x) + e	0.0001	0.5631
SG 276	Mouth height = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = - 0.002215 + 0.009192 (x) + e	0.0001	0.5907
SG 301	Mouth width = $\beta_o + \beta_x (x) + e$ where x = (Larval total length)^2	y = 0.101377 + 0.005848 (x) + e	0.0001	0.3703
SG 301	Mouth height = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.092632 + 0.005828 (x) + e	0.0001	0.4003
SG 326	Mouth width = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.061349 + 0.007962 (x) + e	0.0100	0.4109
SG 326	Mouth height = $\beta_o + \beta_x (x) + e$ Where x = (Larval total length)^2	y = 0.071121 + 0.007493 (x) + e	0.0054	0.4611

Appendix O. Results of Analysis of Covariance (ANCOVA) testing larval total length on mouth gape width and height between each spawner size group

Appendix O. Results of Analysis of Covariance (ANCOVA) testing larval total length on mouth gape width and height between each spawner size group.

Model	p-value	r-square
Mouth width = $\beta_o + \beta_x (x) + e$ where x = (Larval total length)^2	0.0001	0.4486
Mouth height = $\beta_o + \beta_x (x) + e$ where x = (Larval total length)^2	0.0001	0.4936

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