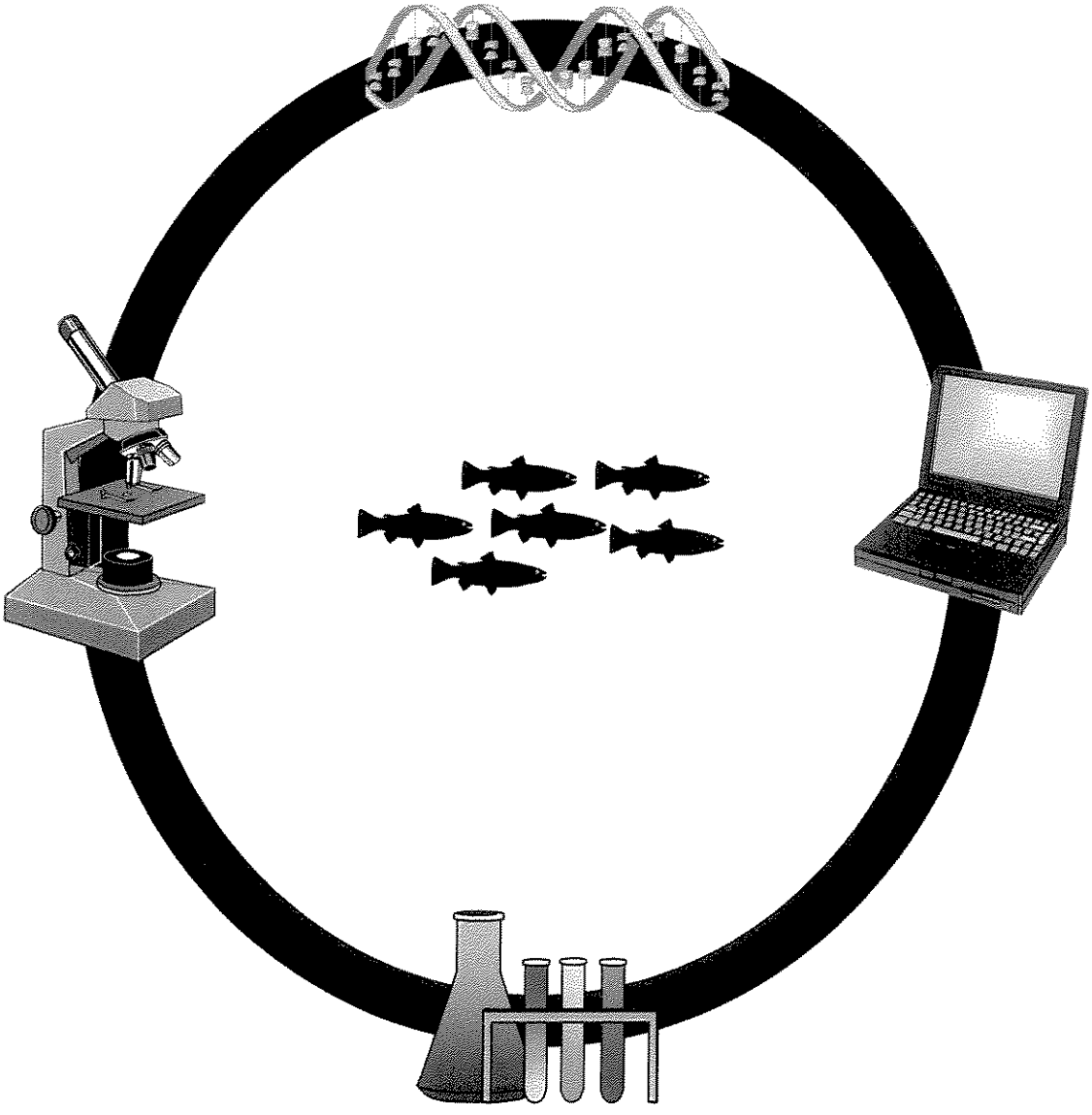
U. S. Fish & Wildlife Service
Fish Technology Centers
Technical Information Leaflet No. BZ-05-92

Effects of Diet on Growth, Survival, and Performance of Rio Grande Silvery Minnow: Larvae through Juvenile and Subadult Stages



U. S. Department of the Interior
U. S. Fish & Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT 59715

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September 2005

Key Words: Rio Grande silvery minnow, diet trial, endangered species culture, larvae culture, and live feeds

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

Captive propagation of the federally endangered Rio Grande silvery minnow has been identified as essential in its recovery efforts. Thus, important elements in captive propagation activities throughout New Mexico center on the fish's life history requirements and the development of standardized culture techniques with the ultimate goal of producing a healthy fish for release into the wild. This document presents a compilation of research which began January 2000 at New Mexico State University on the culture and dietary requirements of the Rio Grande silvery minnow. The research described in this report represents a cooperative effort among U.S. Geological Survey, New Mexico Cooperative Fish and Wildlife Research Unit (Colleen Caldwell), U.S. Fish and Wildlife Service, New Mexico Fisheries Resources Office (Jim Brooks and Jerry Landye), Dexter National Fish Hatchery and Technology Center (Manuel Ulibarri), and Bozeman Fish Technology Center (Frederic T. Barrows and Greg A. Kindschi).

ACKNOWLEDGEMENTS

The funding for this research was provided by the Middle Rio Grande Endangered Species Act Collaborative Program, with additional funding from the U.S. Fish and Wildlife Service Albuquerque Fishery Resources Office, Dexter National Fish Hatchery and Technology Center, Bozeman Fish Technology Center, and the Albuquerque Ecological Services Field Office. W.R. Gould, University Statistics Center, New Mexico State University, provided assistance with statistical analysis. Catherine Sykes assisted in experimental design and completion of the majority of the work described in this report. Jerry Landye, Manuel Ulibarri, Dave Hampton, William Knight, Cody Robertson, Jarred Bowman, and Christiane Friedman provided technical assistance and Terina Niskamen provided a critical review of the report. We appreciate the support of Jim Brooks of the U.S. Fish and Wildlife Service, Albuquerque Fishery Resources Office and Dr. Rudi Schoenmacher and Ron Polka of the Southwest Technology Development Institute, New Mexico State University for continued access and support of the A-Mountain geothermal aquaculture facility. The Department of Fishery and Wildlife Sciences provided space for a portion of the research and the U.S. Geological Survey, Cooperative Research Units provided salary for the principal investigator.

EXECUTIVE SUMMARY

An important component of the Rio Grande silvery minnow (*Hybognathus amarus*) (RGSM) recovery program requires the eventual release or repatriation of the fish into the wild. Proper nutrition becomes essential if fish are to survive while in propagation facilities and upon release to the wild. Thus, the overall goal of the research reported here was to characterize optimum dietary requirements as morphological and physiological needs shift from larvae through maturity to gonadal development and reproduction. The results of four feed trials from 2002 to 2004 evaluating dietary requirements of RGSM throughout early life history stages are reported here.

The objective of the first study was to evaluate a series of commercially available feeds manufactured for larval fish to determine optimum growth (as evaluated by length, weight, condition factor) and survival in RGSM (from day 12 post-hatch through day 40 post-hatch). The benchmark of larval feeds (BioKyowa, Inc.) resulted in survival that was low (61% cumulative mortality) when compared to survival in larvae presented a live diet of *Artemia* nauplii (35% cumulative mortality). Growth, as indicated by length and weight, was detectably greater in larval fish provided either *Artemia* nauplii or Biokyowa compared to those provided a pellet formulation (Razorback sucker feed). Although the live diet resulted in good growth and survival of RGSM larvae, live diets are labor intensive and costly and may not provide the needed vitamins and essential nutrients for growth of larval fish. Thus, the results of this first study indicated that manufactured feed should be obtained and subjected to experimental feed trials to achieve propagation goals of successfully and cost-effectively rearing RGSM for eventual release into the wild.

The objective of the second study was to identify food preferences (manufactured versus live feed) for RGSM larvae (just prior to absorption of yolk sac at day 4 post-hatch through the end of the larval stage to day 23 post-hatch). Survival was low in RGSM larvae fed two manufactured feeds (21% cumulative mortality for RGSM Starter and 9% cumulative mortality for Ziegler) compared to less than 1% cumulative mortality in RGSM larvae fed *Artemia* nauplii. Condition factors (KtL) were detectably higher in larval fish fed *Artemia* nauplii (0.75) than in fish provided the RGSM Starter feed (0.47) or the Ziegler feed (0.69). Survival and growth in RGSM larvae fed *Artemia* nauplii compared to manufactured feeds from time of resorption of yolk suggests that live feed may be important for successful rearing of RGSM larvae within the first few days after exogenous feeding begins.

The objectives of the third study were to determine the optimum time when larval RGSM (day 5 post-hatch) could be shifted from live to manufactured feed and the effects the manufactured diets have on survival, growth, and performance (critical swimming speed; U_{crit}) through the juvenile stage (day 70 post-hatch). The results indicated that timing of introduction of manufactured feed was more important than feed type. Survival was greatly increased in fish fed manufactured diets when initially fed *Artemia* nauplii from the time of yolk resorption to day 15 post-hatch. Cumulative mortality ranged from 1% to 3% in fish fed manufactured diets which represented a considerable increase in survival compared to the previous study in which survival ranged from 21% (RGSM Starter) to 9% (Ziegler) in fish started on the same manufactured diets at the time of yolk resorption. Thus, live food was critical for survival of RGSM larvae within the first two weeks post-hatch and manufactured diets (either an experimental RGSM Starter or the commercial Ziegler AP-100) may be successfully introduced into the larvae's diet as early as 15 days post-hatch. There were no detectable differences in length, weight, condition factor, mortality, or critical swimming speed among treatments when RGSM larvae were moved from

live feed to manufactured feed at either day 15 or day 24 post-hatch and fed their respective feeds through to day 70 post-hatch. Although there were no detectable differences among the treatments for growth, the most promising starter diet appears to be the RGSM Starter feed which demonstrated a growth rate of 13.0%/day and total growth of 266% by the end of the study.

The objective of the fourth study was to rear RGSM from the juvenile stage (day 179 post-hatch) through sub-adult just prior to gonadal maturation and spawning using commercial and experimental feeds at different RGSM propagation facilities. Two feeding trials were simultaneously conducted to determine the effects of diet on survival, growth, and performance (critical swimming speed; U_{crit}) of RGSM at two RGSM propagation facilities having varied water quality and culture conditions. The results of the study clearly indicated varied responses in RGSM given the same feeds at two propagation facilities reflecting differences in culture operations and the importance that culture conditions may have when considering feed requirements. Rio Grande silvery minnow exhibited an overall greater growth rate when fed the experimental RGSM Flake (1.23%/day and total weight gain of 184%) compared to the Silver Cup Pellet (0.69%/day and total weight gain of 104%). No differences were observed for survival or performance in RGSM provided the three feeds. It is apparent from these results that RGSM prefer a flake feed over a pellet feed and that the RGSM Flake feed would fulfill the necessary dietary requirements for growth.

Briefly stated, live food (*Artemia* nauplii) is essential in successfully rearing RGSM larvae from time of yolk resorption through the end of the larval stage (approximately day 15 post-hatch). A manufactured feed can be introduced near the end of the larval stage with negligible effects on survival through the juvenile stage. An experimental flake feed was recommended to maintain the fish through the juvenile stage to adult prior to gonadal development. Additional research, however, is needed to characterize the dietary effects of the experimental flake feed on gonadal development in adult RGSM, egg quality (fertilization rate and viability), and ultimately larvae survival.

INTRODUCTION

Captive propagation of the Rio Grande silvery minnow (RGSM; *Hybognathus amarus*) was identified as essential for recovery efforts and was initiated to specifically address a narrative recovery task in the 1999 Rio Grande Silvery Minnow Recovery Plan: "Refine captive rearing methods, establish captive populations and produce RGSM populations for experimental purposes... with the ultimate goal of reestablishing the species to appropriate locations within its historic range...". Thus, an important element in captive propagation activities is the development of culture programs centered on the fish's life history requirements with appropriate diet regimes to produce a healthy fish. Captive propagation programs for RGSM should take into consideration diet quality and type for optimum growth and long term health throughout the various life stages, especially if the fish are to be returned to the wild. A propagation report (Davenport 2002) highlighted the need to address elevated mortality observed throughout the early life stages of the fish and the nutritional quality of commercial feeds that are used in propagation facilities throughout New Mexico. Given the lack of information regarding nutritional needs for the endangered fish, we identified the following questions to be addressed in the research program: What is the optimum prey size and preferred live food for RGSM in culture conditions? When should larval RGSM begin receiving manufactured feed and thus eliminate or reduce the additional costs and effort in rearing live food in culture conditions? What effects do flake versus pellet feeds and commercially-available versus experimental feeds have on fish survival, growth and performance throughout various life stages (from larvae to sub-adult) in culture conditions? What effect would culture practices among the RGSM propagation facilities have on feed requirements?

Research presented here was initiated through discussions that began in 2000 between the U.S. Fish and Wildlife Service (New Mexico Fishery Resources Office, Dexter National Fish Hatchery and Technology Center), and the U.S. Geological Survey, New Mexico Cooperative Fish and Wildlife Research Unit regarding captive propagation of the federally endangered Rio Grande silvery minnow. The objectives within a five-year plan (from 2000 to 2005) were to (1) develop and test diets formulated for various life history stages (from larvae through reproducing adult) of the Rio Grande silvery minnow for optimum growth and long term health, and (2) develop breeding and rearing protocol for the various propagation facilities involved in augmentation efforts of this species.

CHAPTER I

Survival and Growth of Rio Grande Silvery Minnow From Larvae (Day 12 Post-Hatch) To Juvenile (Day 40 Post-Hatch): Comparison of Manufactured Feeds

INTRODUCTION

Developmental stages of larval Rio Grande silvery minnow (*Hybognathus amarus)* (RGSM) have been described using morphological changes that occur throughout the fish's early life history stages: protolarvae (absence of dorsal, anal and caudal fin spines and rays), from meso- to meta-larvae (begins with the presence of at least one dorsal, anal, or caudal fin spine or ray to a full complement of principal fin rays in all median fins), and juvenile (absorption of fin fold and full complement of fin spines and rays) (Platania 2000). Developmental stages associated with ecological shifts in food and feeding requirements in RGSM, however, have not been fully documented. Presumably, the larval stage begins with absorption of the yolk sac when exogenous feeding begins, followed by a shift in food particle size in later larval stages to a shift in food requirements (from predominantly proteinaceous to herbivory) in the juvenile stage. From subadult to reproductively mature, energetics are shifted from growth to gamete development.

An important component of the RGSM recovery program requires the eventual release or repatriation in the wild. If the fish is to survive through its first winter, proper nutrition will be necessary. New Mexico State University A-Mountain Aquatic Research Facility (NMSU Aquatic Facility) is one of four facilities in New Mexico that has dedicated space and resources to the propagation of RGSM. Thus, the objective of this study was to evaluate a variety of commercially available feeds manufactured for larval fish to determine optimum growth (as evaluated by length, weight, condition factor) and survival in RGSM (from day 12 post-hatch through day 40 post-hatch). The feeds evaluated in the study were used by RGSM captive propagation facilities. Thus, it was necessary to evaluate whether these feeds were providing the most optimum nutrition during the earliest life stage of RGSM.

The experiment took place from 30 May to 9 July 2002 at the NMSU Aquatic Facility which uses treated water from the University Water Treatment Plant within a water reuse system to minimize water loss (see Appendix A). Water quality was optimal for fish growth and maintenance (i.e., water temperature 22-26°C; pH 8.0; electrical conductivity 620 micromhos/cm; total dissolved solids 360 mg/L; chloride 60 mg/L; alkalinity 146 mg/L as $CaCO_3$; hardness 188 mg/L as $CaCO_3$). Eggs were collected from adult RGSM (2001 captive spawn F1 female x 2001 captive spawn F1 male) injected with commercial carp pituitary extract (see Appendix B). Within 96 h post-hatch (just prior to resorption of the yolk when exogenous feeding begins), *Artemia* nauplii were presented to the larvae four times daily until day 12 post-hatch when fish were of sufficient size for netting and handling.

Experimental Design and Feeding Protocol

The experimental design consisted of four feed treatments randomly assigned in eight replications to four blocks. The four blocks represented east side upper and lower levels and west side upper and lower levels of the recirculating system. The feeding trial began with RGSM larvae at day 12 post-hatch when 50 larvae were placed into each of thirty-two 70-liter aquaria (18 gallon) resulting in a density of 1 fish per 1400 ml. The fish were fed 26% of their body weight per day of the three commercial feeds spread among five feedings. In one group (Artemia), fish were provided *Artemia* nauplii three times daily and fish were allowed to graze freely on endemic filamentous algae (*Cladophora* sp.) and diatoms (not described). The feed trial was conducted through the larval life stage for 28 days (day 40 post-hatch). At 40 days post-hatch, larvae performance among treatments were evaluated by comparing growth (length and weight), condition factor (KtL), and survival (% mortality). Temperature (°C) and dissolved oxygen (mg/L) were monitored daily with a Yellow Springs Instrument (YSI) dissolved oxygen meter.

Diet Compositions

The commercial diets were selected based on a series of criteria: (1) current use in RGSM culture, (2) commercially availability and cost effectiveness, and (3) formulation based on protein content.

Artemia nauplii (A. franciscana; GSL, Ogden, UT): Analysis of the *Artemia* by Eurofins (Woodson-Tenet Laboratories Division, Des Moines, IA) resulted in a detailed list of the fatty acid profile (see Appendix C) and protein content of 53.87%. Size (300-450 µm) was determined capturing digital images of the nauplii using a Leica stereomicroscope and Optronics MagnaFire SP (Version 1.0x5). The image was imported into an image analysis program (Scion Image, Fremont, CA) to obtain length (µm).

BioKyowa, Inc. (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan): The major constituents of the FFKB feed (250 µm) for larval fish and shrimp are provided in Table 1.

Sanders Brine Shrimp Co., (Ogden, UT): The company produces flake diets for tropical and freshwater fishes. The study used a flake combination (50:50) brine shrimp:schizochytrium algae. The major constituents of the feed are provided in Table 1.

Nelson and Sons, Inc. (Murray, UT): Razorback Fry "starter" feed formulation (#0301); This was a steam pelleted feed that was manufactured in sizes that ranged from 400 to 700 µm which were broken to smaller sizes for larval fish consumption (approximately 250 µm) by the USFWS Bozeman Fish Technology Center. The starter pellet was chosen for the feed trial because it had been developed for the threatened razorback sucker (*Xyrauchen texanus*; Catastomidae). Although the RGSM belongs to the family Cyprinidae, the two families are more closely related than species for which other commercially available diets have been developed (e.g., salmonids, catfish). The starter feed was developed to provide elevated protein for rearing the razorback sucker. Thus, these needs were initially believed to be similar to those of the RGSM larvae. The major constituents of the feed are provided in Table 1.

Table 1. Major constituents of the three manufactured feeds fed to larval Rio Grande silvery minnow from day 12 post-hatch through day 40 post-hatch. '-' indicates information not available.

	BioKyowa, Inc. (Biokyowa)	Sanders Brine Shrimp Co. (Sanders)	Nelson and Sons, Inc. (Nelsons)
Protein	55%	50%	60%
Fat	10%	15%	18%
Carbohydrate	-	15%	-
Ash	13%	9%	9.2%
Fiber	3%	-	0.85%
Mineral	-	9%	-
Moisture	-	9%	9%

Statistical Analysis

The feed study was designed as a randomized complete block design to allow for analysis of block effects if differences were observed among treatments in aquaria which experience different environmental conditions (i.e., light, temperature, traffic exposure). Statistical analysis was performed as a two-way ANOVA with diet as the fixed factor and block as the random factor. Principal component analysis was conducted to evaluate block effect.

RESULTS

The results indicated important dietary and particle size requirements in RGSM larvae by day 40 post-hatch. The testing of a commercial flake diet (Sanders) resulted in detectably lower growth (Figures 1A and 1B) and poor survival (83% cumulative mortality) which may have resulted from the loss of important vitamins and minerals through leaching (Figure 2) ($P \leq 0.05$). The testing of the commercial pelleted feed (Nelson's) resulted in poor growth (Figures 1A and 1B) and poor survival (77% cumulative mortality) (Figure 2) compared to a commercial benchmark feed (Biokyowa) used in larval fish culture which resulted in detectably greater growth (Figures 1A and 1B) but poor survival (61% cumulative mortality) (Figure 2) ($P \leq 0.05$). Although length (Figure 1A) and weight (Figure 1B) of fish fed live feed was comparable to those fed Biokyowa, survival, however, was greatest (35% cumulative mortality; Figure 2) in larvae fed a live diet throughout the 40-day study.

Anecdotal information suggests that RGSM may have a better chance of surviving when released to the wild with condition factors of 1.0 or greater. Condition factor for larvae at day 40 post-hatch was greatest in Biokyowa (mean = 1.1; standard error (SE) = 0.015) and Artemia (1.0; SE = 0.051) and lowest in the Sanders feed (0.87; SE = 0.016) (Figure 3).

Principal component analysis indicated a slight effect of location on condition factor within the experimental system indicating that location of the fish within the experimental system may have confounded the effects of treatment on condition factor. While there were no effects due to location on length, weight, or survival, analysis indicated that condition factor was negatively affected in fish on the west side of the experimental system. Although activity was minimized around the experimental system throughout the study, disturbance may have been slightly greater on the west side. We also noted diurnal light intensity differed on east and west sides of the experimental system. Light intensity had a positive effect on feeding behavior with increased feeding activity occurring during greater light intensity. We noted fish fed well on the east side compared to fish on the west side of the experimental system with the first morning feeding.

DISCUSSION

Survival and performance of RGSM larvae (day 12 post-hatch through day 40 post-hatch) was greater when fed a live feed compared to three commercially manufactured feeds. While live feed resulted in better survival and growth of the larval fish, rearing live food in large-scale fish culture facilities is costly and labor intensive.

Biokyowa, a proven high quality feed for larval fish, provided acceptable growth albeit reduced survival of RGSM larvae. The feed is a micro-bound particle that uses zein (a protein from corn that is not water soluble) as its binder. The result is greater stability of water-soluble nutrients (e.g., B vitamins and amino acids) thus improving nutritional value as well as reducing water quality degradation. At the time of the report, however, the feed is no longer available for distribution in the United States.

The flake food produced by Sanders Brine Shrimp Company resulted in reduced growth and survival due to its low stability in water. The flake feed arrived from the manufacturer in large (2 cm) flakes and required grinding by hand to obtain smaller particles for the larval fish. This facilitated the disintegration and leaching of valuable nutrients into the water which

presumably reduced the nutritional value of the food and thus growth of the larvae. Water quality deterioration also occurred within tanks receiving the flake feed. Large mats of uneaten food would accumulate each day and, although the tanks were cleaned, water quality was less than optimal. Thus, the presence of a feed low in nutritional quality (due to leaching) and poor water quality may have contributed to the elevated mortality observed within the treatment.

The starter pellet produced by Nelson and Sons is manufactured using a steam pelleting process. Smaller sizes are obtained for feeding larval fishes by crumbling or cracking the larger pellets. This process presumably resulted in a pellet with rough edges reducing its palatability to the RGSM larvae. In addition, the steam pelleting process results in a pellet that dissolves more easily in water resulting in greater leaching and loss of important nutrients. While the same feed may be adequate in rearing the razorback sucker, it was deemed inappropriate for rearing larval RGSM.

Summary and Recommendations

Reducing a commercial flake feed to a smaller particle for feeding larval fish reduced water stability of the feed and increased leaching of nutrients resulting in poor nutritional quality. Reduced water quality was usually associated with this commercial flake feed which may have contributed to reduced survival in RGSM larvae. In contrast, palatability may have been poor in a commercially available pelleted feed resulting in poor performance and survival of RGSM larvae. Although a live diet resulted in good performance and survival in RGSM larvae through the juvenile stage, live diets are labor intensive and costly unless naturally produced plankton (phyto- and zooplankton) are provided to larvae in ponds. If the larvae are to be reared indoors, a suitable manufactured feed must be obtained and subjected to experimental feed trials to achieve propagation goals of successfully and cost-effectively rearing RGSM in propagation facilities.

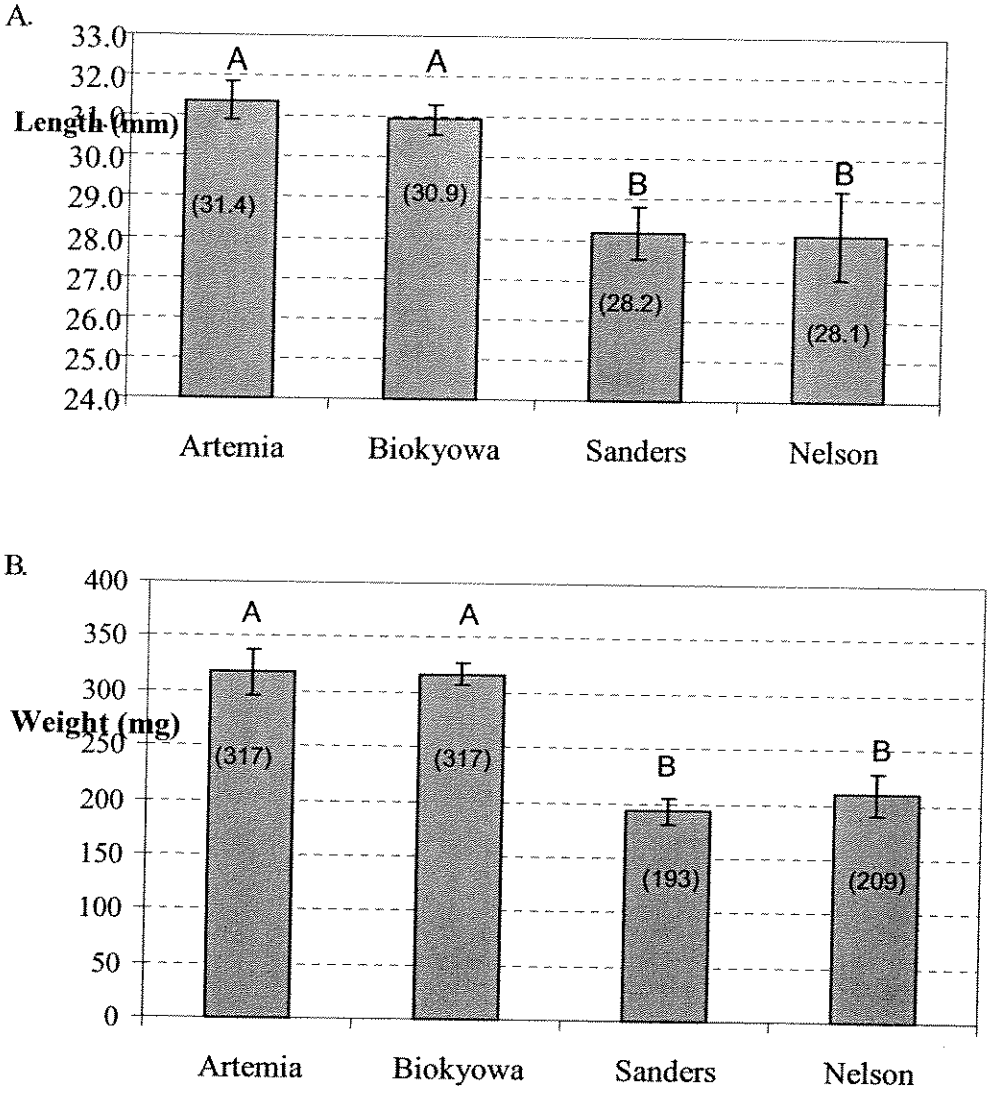


Figure 1A and 1B. Average length (mm) and weight (mg) in Rio Grande silvery minnow larvae fed four diets from day 12 through day 40 post-hatch. Numbers in parenthesis within bars represent the average value. Standard error bars are presented and letters above bars that are different indicate significant differences among treatments ($P < 0.05$). Sample size (n) of 5 for all treatments.

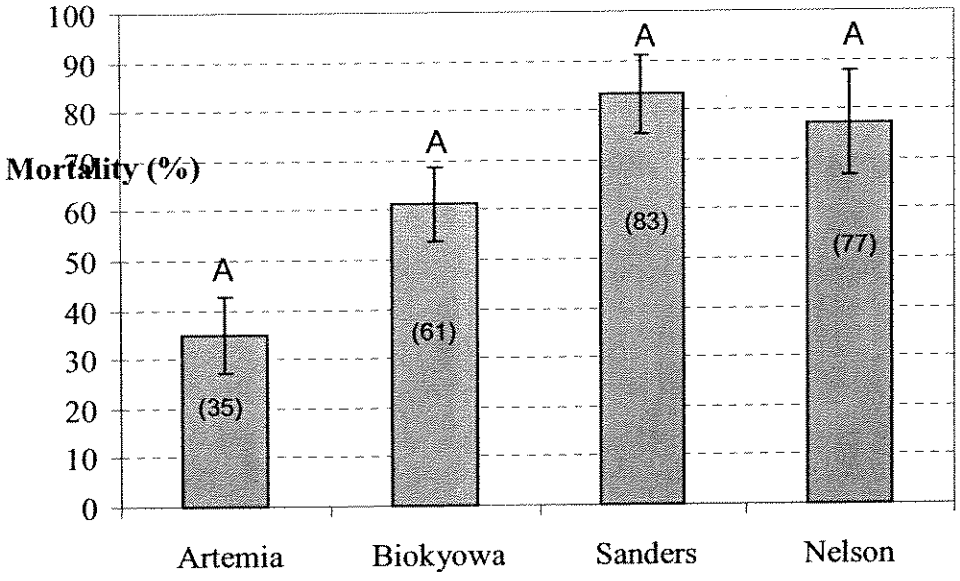


Figure 2. Average mortality (%) in Rio Grande silvery minnow larvae fed four diets from day 12 through day 40 post-hatch. Numbers in parenthesis within bars represent the average value. Standard error bars are presented and letters above bars that are different indicate significant differences among treatments ($P \leq 0.05$). Sample size (n) of 5 for all treatments.

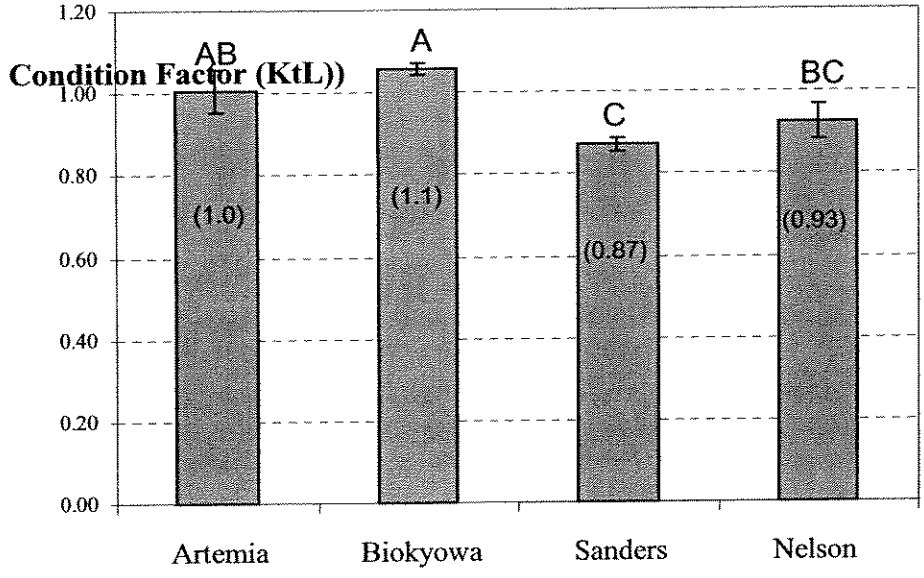


Figure 3. Average condition factor (KtL) in Rio Grande silvery minnow larvae fed four diets from day 12 through day 40 post-hatch. Numbers in parenthesis within bars represent the average value. Standard error bars are presented and letters above bars that are different indicate significant differences among treatments ($P < 0.05$). Sample size (n) of 5 for all treatments.

CHAPTER II

SURVIVAL AND GROWTH OF RIO GRANDE SILVERY MINNOW FROM LARVAE (DAY 4 POST-HATCH) TO JUVENILE (DAY 23 POST-HATCH): COMPARISON OF MANUFACTURED AND LIVE FEEDS

INTRODUCTION

Mouth size has been shown to be a limiting factor in successfully introducing food to larval fish beginning exogenous feeding (Hoff and Snell, 1987). It is not the overall size of prey, but the width of the prey item that is important in prey selection and larvae survival. For example, *Branchionus plicatilis*, a freshwater rotifer, ranges in width from 114 to 199 μm while some commercial *Artemia* nauplii range from 300 to 500 μm in width (Hoff and Snell 1987). Anecdotal observations at the NMSU Aquatic Research Facility at A-Mountain identified increased survival of larval Rio Grande silvery minnow (RGSM) if given freshwater rotifers from day 1 through day 6 post-hatch at 21°C. As the fish larvae increased in size, *Artemia* nauplii were eventually introduced into the fish's diet. In addition, feeding a mixture of smaller and larger live prey through a transitional period was thought to increase survival of larval RGSM.

Larval fish of various species are successfully reared on manufactured feed without live food supplementation (R. Barrows, USFWS; personal comm.). Manufactured feed provides optimum dietary needs while reducing the labor and costs associated with the maintenance and culture of live feed. In addition, the nutritional quality of live feed may vary and while live feed such as *Artemia* nauplii can be enhanced with vitamin supplements, this additional step is costly. Thus, the objective of this study was to identify optimum prey size and food preference (manufactured versus live) for RGSM larvae (just prior to absorption of yolk sac through the end of the larval stage). The following diets were selected: (1) a live diet consisting solely of *Artemia* nauplii from day 4 through day 23 post-hatch (2) a live diet of rotifer (*Branchionus calyciflorus*) from day 4 to day 15 post-hatch followed by *Artemia* nauplii through day 23 post-hatch, (3) a commercial diet for larval fish and shrimp produced by Ziegler Bros., Inc. (AP-100) from day 4 through day 23 post-hatch, and (4) a microparticle diet formulated by the U.S. Fish and Wildlife Service, Bozeman Fish Technology Center (Bozeman FTC) from day 4 through day 23 post-hatch. In addition, we were interested in determining effects of handling larvae shortly after yolk

resorption. Spawning practices among RGSM propagation facilities result in brief handling of young larvae moved from aquaria to rearing ponds or tanks.

MATERIALS AND METHODS

The experiment took place from 20 June to 9 July 2003 at the New Mexico State University (NMSU) Fisheries Research Laboratory (Appendix D). The NMSU Fishery Research Laboratory uses water from a well having optimum water quality for fish growth and maintenance (i.e., constant temperature 20°C; pH 8.2; electrical conductivity 692 micromhos/cm; total dissolved solids 723 mg/L; chloride 54.2 mg/L; alkalinity 169 mg/L as $CaCO_3$; hardness 198 mg/L as $CaCO_3$). Eggs were collected from adult RGSM (2002 captive spawned F1 female x 2002 captive spawn F1 male) injected with commercial carp pituitary extract (Appendix B). The initial phase of the study was conducted using static-renewal in 1-liter beakers to observe and evaluate larvae survival from the initial stress of handling. There was also concern regarding the ability to observe and track survival of the small larvae (4 mm) which lack melanin until the meso- or meta-larvae phase (presence of at least one dorsal, anal, or caudal fin spine or ray to a full complement of principal fin rays in all median fins) (Platania 2000). Once the larvae were of sufficient size and pigmented, they would be transferred to 38-liter aquaria within a water recirculating system.

Experimental Design and Feeding Protocol

Experimental design consisted of four feed treatments that were randomly assigned in eight replications to four blocks (north upper and lower banks, south upper and lower banks) within the experimental system. At 72 h post-hatch, 50 larvae were placed into each 32 1-liter beakers. Each beaker contained 800 mL (1 fish in 16 ml) of water aerated with a 2.5 cm airstone and each beaker was wrapped with black construction paper to reduce photo-positive behavior of the larvae. Uneatened food was siphoned once per day with 1/3 water replacement. Temperature (°C) and dissolved oxygen (mg/L) were monitored daily with a YSI dissolved oxygen meter. Ammonia (mg/L, as total nitrogen) was measured using method 8155 on a HACH DR/2010 model spectrophotometer and pH was measured using a Beckman 34 with an Orion pH probe calibrated prior to use. At day 17 post-hatch, larvae were released from the beakers into 32, 38-liter (10-gallon) aquaria of the recirculating system within the NMSU Fisheries Research

Laboratory. Within 96 h post-hatch (just prior to resorption of the yolk when exogenous feeding begins), freshwater rotifers (*Brachionus calyciflorus*), *Artemia* nauplii, RGSM Starter, and Zeigler feeds were presented to RGSM larvae six times daily. Feed rations were calculated based on a large percentage of the estimated body weight per day (26%). Recommendations for successful rearing of larval fish were to feed in excess (R. Barrows, USFWS; personal comm.). A pre-determined volume of suspended *Artemia* nauplii (approximately 1400 nauplii per larvae) and *B. calciflorus* (approximately 1000 per larvae) were presented to each aquarium using a volumetric pipette following the methods of Hoff and Snell (1987). Larvae were fed similar particle sizes of manufactured feeds throughout the study. The RGSM Starter feed had a particle size of 250 µm and the Zielger AP-100 had a particle size range of 150-250 µm.

Diet Compositions

Freshwater rotifers (B. calyciflorus; 100-200 µm) Aquatic Eco-Systems, Inc. (Apopka, FL) Rotifers were hatched from resting eggs and cultured in laboratory conditions (0:24 dark:light; 20°C; enhanced with a microalgae formulation of *Nanochloropsis* (Hoff and Snell 1987).

Artemia nauplii (A. franciscana; GSL, Ogden, UT): *Artemia* cysts were obtained commercially (Aquatic Eco-Systems, Inc.) and hatched twice daily (0800 and 1200) in laboratory conditions (0:24 dark:light; 27°C; 30 g/L salinity). The young nauplii rapidly lose weight and caloric value as they pass through early instar stages (within 2 hours) (Hoff and Snell 1987). Thus, batches of nauplii were hatched for only the morning or the afternoon feedings. The cysts were from the Great Salt Lake in Utah and newly hatched nauplii varied in size from 350 to 450 µm (confirmed by C. Sykes). Proximate analysis of the *Artemia* used in this study was characterized (Eurofins, Woodson-Tenet Laboratories Division, Des Moines, IA) and resulted in a detailed list of the fatty acid profile (Appendix C) and a protein level of 53.87%.

Rio Grande Silvery Minnow Starter Feed (U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, MT): RGSM Starter #0304 (Table 1).

Ziegler AP-100 (Ziegler Brothers, Inc., Gardners, PA): Microparticle diet formulated as a supplement for larval shrimp and a complete diet for finfish larvae (Table 1).

Table 1. Major constituents of the two manufactured feeds fed to larval Rio Grande silvery minnow (RGSM) from day 4 post-hatch through day 23 post-hatch. '-' indicates information not available.

	RGSM Starter	Ziegler AP-100
Protein	42.2%	50%
Fat	18.2%	12%
Ash	7.8%	7%
Fiber	-	3%
Mineral	-	-
Moisture	7.1%	9%

Statistical Analysis

The feed study was designed as a randomized complete block design to allow for analysis of block effects if differences were observed between treatments in aquaria which experience different environmental conditions (i.e., light, temperature, traffic exposure). Statistical analysis was performed as a two-way ANOVA with diet as the fixed factor and block as the random factor.

Results and Discussion

Very little is known regarding feeding habits of wild RGSM. Gut analysis of adult RGSM obtained from museum holdings indicate the fish were omnivorous feeding on diatoms and detritus (Cowley, NMSU; person. comm.). The testing of two manufactured feeds resulted in high mortality in RGSM larvae fed both RGSM Starter and Ziegler feeds (19% and 9%, respectively) compared to RGSM larvae fed *Artemia* nauplii (<1%) (Table 2). In addition, average condition factor (KtL) was detectably higher in larval fish fed *Artemia* nauplii (mean = 0.75, standard error (SE) = 0.004) than in fish provided Zeigler (0.69, SE = 0.007) or RGSM Starter (0.47, SE = 0.007) feeds (Table 2) ($P \leq 0.05$). Survival and good growth observed in RGSM larvae fed *Artemia* nauplii from time of absorption of yolk sac suggested smaller size rotifers may not be necessary. However, the results from this feed study do suggest that live feed was preferred by RGSM larvae and may be important for successful rearing of RGSM larvae within the first few days after exogenous feeding begins.

By day 6 of the study (day 9 post-hatch), excessive mortality (greater than 10 fish per tank per day) was observed in RGSM larvae fed live rotifers. By day 15 of the study (day 18

post-hatch), survival ranged from 10 to 58% among the eight aquaria receiving rotifers and the rotifer culture was exhibiting poor hatching success. Thus, this treatment was removed from the feed study.

Conducting the initial phase of the study in 1-liter beakers resulted in moderately elevated ammonia levels ranging from 0.60 to 2.69 mg/L throughout all treatments despite daily cleaning and water renewal of all beakers. After fish were transferred to the aquaria, ammonia levels decreased in all treatments throughout the remainder of the study (from 0.25 to 0.66 mg/L). pH ranged from 8.4 to 8.7 among all treatments throughout the study. Elevated ammonia levels, however, did not result in elevated mortality among treatments as indicated by nearly 100% of survival in RGSM larvae fed *Artemia* nauplii. Average dissolved oxygen concentrations throughout all treatments ranged from 5.7 to 6.3 mg/L and temperature throughout all treatments ranged from 20.6 to 21.9°C.

SUMMARY AND RECOMMENDATIONS

Observations throughout this study indicated larval RGSM did not observe the feed particles unless the particles fell close in proximity. *Artemia* nauplii exhibited an extended life span of up to 6 hours in treatment aquaria providing additional time for the larvae to feed. Movement of the nauplii may also have drawn the young fish's attention to feed which may have increased chances of survival through a critical life stage. In contrast, the manufactured diets were often ignored or missed by the young fish and presumably began losing vital nutrients through leaching processes.

We were successful in evaluating handling effects of larvae as early as 72 h post-hatch and demonstrated the use of live feed (i.e., *Artemia* nauplii) was necessary for survival and growth within the first few weeks of development in RGSM. Although initially rearing RGSM larvae in 1-liter beakers provided an opportunity to observe the larval fish through early developmental stages and evaluate potential effects of handling and treatment, effort in maintaining the beakers through static-renewal was great and would not be recommended in future culture efforts of the species. We recommend a timeline be developed when the larval fish can be successfully shifted from live feed to manufactured feed.

Table 2. Mean survival (% cumulative mortality), length (mm), weight (mg), and condition factor (KtL) of Rio Grande silvery minnow (RGSM) from day 4 post-hatch to day 23 post-hatch fed an experimental manufactured feed (RGSM Starter), a commercial feed for larval fish and shrimp (Ziegler), and a live feed of *Artemia* nauplii. Standard error and sample size are in parentheses. Letter superscripts that differ among the three treatments indicate detectable differences ($P \leq 0.05$).

	Mortality (%)	Length (mm)	Weight (mg)	KtL
RGSM Starter	19.0^{a} (2.673, 8)	9.6^{a} (0.167, 8)	4.5^{a} (0.268, 8)	0.47^{a} (0.013, 8)
Ziegler	9.0^{b} (1.250, 8)	11.5^{b} (0.178, 8)	11.1^{b} (0.535, 8)	0.69^{b} (0.020, 8)
Artemia nauplii	0.75^{c} (0.366, 8)	19.1^{c} (0.222, 8)	53.3^{c} (2.252, 8)	0.75^{c} (0.014, 8)

CHAPTER III

SURVIVAL, GROWTH AND PERFORMANCE OF RIO GRANDE SILVERY MINNOW FROM LARVAE (DAY 5 POST-HATCH) TO JUVENILE (DAY 70 POST-HATCH): TIMING FOR INTRODUCTION OF MANUFACTURE FEEDS

INTRODUCTION

Water is a dense viscous medium that imposes the greatest cost on the fish's energy budget as it moves through the water. Swimming long distances at constant speeds requires energy derived from aerobic metabolism (fatty acids), while at higher swimming speeds anaerobic metabolism (anaerobic glycolysis) becomes important (Beamish 1978; Jobling 1996). Diet can alter the physiology of a fish through altered chemical composition of tissues. Thus swimming performance provides an integrated assessment of effects throughout the molecular and cellular level that are manifested at the organismal level. An estimate of performance at the organismal level has been evaluating critical swimming speed (U_{crit}; Beamish 1978). Critical swimming speed, or time to fatigue, reflects maximum aerobic and anaerobic capacity and provides an estimation of the fish's ability to perform in the wild providing ecologically relevant information on ability to forage, avoid predators, and migrate long distances (Plaut 2001).

Beamish et al. (1989) evaluated the effects that dietary protein and lipids have on swimming speeds in juvenile lake trout (*Salvelinus namaycush*) and observed critical swimming speeds (U_{crit}) increased with increased dietary protein levels. While none of the juvenile lake trout fed the various diets exhibited differences in weight, the authors presumed the increased body protein resulted in the potential to do work. When subjected to various dietary lipids (poultry-based, fish-based, or plant-based), U_{crit} of Atlantic salmon (*Salmo salar*) varied greatly (Wagner et al. 2004). While fish performance was poorest when fed poultry fat, diets containing plant-based lipids (sunflower and flaxseed oils) resulted in similar swimming performance compared to the performance of fish fed fish-based lipids (anchovy oil).

The results of an earlier study in which manufactured and live feed were compared in Rio Grande silvery minnow (RGSM) from day 4 through day 23 post-hatch indicated that live feed resulted in better survival and growth. Propagation facilities in New Mexico currently use live *Artemia* nauplii throughout the entire larval stage of the RGSM (approximately 30 days post-hatch). The optimum length of time in which *Artemia* nauplii should be provided to larval

RGSM is uncertain. The costs of culturing live food as well as labor could be reduced if manufactured feed is offered at the earliest stage in which larval RGSM can successfully switch from live to manufactured food. Thus, the objectives of this study were to determine when larval RGSM could be shifted from live to manufactured feed and the effects manufactured diets have on survival, growth, and performance (critical swimming speed; U_{crit}) through the juvenile stage.

MATERIALS AND METHODS

The experiment was conducted at the NMSU Fisheries Research Laboratory (Appendix D) from 7 August 2003 through 11 October 2003. The NMSU laboratory uses recirculated water obtained from a well having optimum water quality for fish growth and maintenance (i.e., constant temperature 20°C; pH 8.2; electrical conductivity 692 micromhos/cm; total dissolved solids 723 mg/L; chloride 54.2 mg/L; alkalinity 169 mg/L as $CaCO_3$; hardness 198 mg/L as $CaCO_3$). Eggs were collected from adult RGSM (2002 wild egg F0 female x 2002 captive spawn F1 male) injected with commercial carp pituitary extract (Appendix B). Within 72 h post-hatch, 40 RGSM larvae were placed into 25, 38-liter aquaria. Each aquarium was wrapped with black construction paper to reduce photo-positive behavior of the larvae. Throughout the study, all aquaria were cleaned daily and temperature (°C), dissolved oxygen (mg/L), pH, and ammonia (mg/L as total nitrogen) were recorded within all treatments weekly.

Experimental Design and Feeding Protocol:

The experimental design consisted of (1) a live diet consisting solely of *Artemia* nauplii until day 15 and day 24 post-hatch when the larval fish were shifted to an experimental food manufactured by the U.S. Fish and Wildlife Service, Bozeman FTC (RGSM Starter) until day 70 post-hatch when the study was terminated, (2) a live diet consisting solely of *Artemia* nauplii until day 15 and day 24 post-hatch when the larval fish were shifted to a commercial diet produced by Ziegler Brothers for larval fish and shrimp (AP-100) until day 70 post-hatch when the study was terminated, and (3) a live diet consisting solely of *Artemia* nauplii until day 70 post-hatch when the study was terminated. At day 30 post-hatch, a subsample of five fish were removed from each aquarium and lengths and weights recorded to determine growth rates; these fish were not placed back into their respective tanks, but euthanized in a lethal dose of anaesthetic (approximately 200 mg/L of MS-222). At the end of the feed study (day 70 post-hatch), eight fish from each

aquarium (five aquaria within each treatment) were placed into a swimming stamina chamber to determine critical swimming speed (U_{crit}; Appendix E), after which RGSM larvae from all treatments were euthanized and lengths and weights recorded for determining condition factor and body length swam per second (BL/sec) (see Appendix E).

Diet Compositions

Artemia nauplii (A. franciscana; GSL, Ogden, UT): *Artemia* cysts were obtained commercially (Aquatic Eco-Systems, Inc.) and hatched daily in laboratory conditions (0:24 dark:light; 27°C; 30 g/L salinity). The cysts were from the Great Salt Lake in Utah and vary in size from 350 to 450 µm. Proximate analysis of the *Artemia* used in this study was characterized (Eurofins, Woodson-Tenet Laboratories Division, Des Moines, IA) and resulted in a detailed list of the fatty acid profile (Appendix C) and protein level (53.9%).

Rio Grande Silvery Minnow Starter Feed (U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, MT): RGSM Starter #0304; constituents of the feed are listed in Table 1.

Ziegler AP-100 (Ziegler Brothers, Inc., Gardners, PA): Microparticle diet formulated as a supplement for larval shrimp and a complete diet for finfish larvae (Table 1).

Table 1. Major constituents of the two manufactured feeds fed to larval Rio Grande silvery minnow (RGSM) from day 5 post-hatch through day 70 post-hatch. "-" indicates information not available.

	RGSM Starter	Ziegler AP-100
Protein	42.2%	50%
Fat	18.2%	12%
Ash	7.8%	7%
Fiber	-	3%
Mineral	-	-
Moisture	7.1%	9%

Feeding Protocol

Within 96 h post-hatch (at time of resorption of the yolk sac when exogenous feeding begins), *Artemia* nauplii was presented to all RGSM larvae eight times daily. Excess uneaten *Artemia* nauplii were removed at the end of each day. At day 15 post-hatch, RGSM Starter and Zeigler feeds were introduced to aquaria receiving *Artemia* nauplii. Larval fish were presented a combination of live and manufactured feeds for two days to allow the larvae to become accustomed to the presence of manufactured feed. Subsequently, the larvae were offered only the respective manufactured feed at 13% body weight per day spread throughout eight feedings each day. At day 24 post-hatch, the RGSM starter feed and Ziegler feed were introduced to a second set of aquaria receiving *Artemia* nauplii. After two days of combined live and manufactured feeds, the larvae were offered only the respective manufactured feed at 13% body weight per day each day. The remaining aquaria received *Artemia* nauplii until the termination of the study.

Larvae were fed similar particle sizes of manufactured feeds throughout the study. The RGSM Starter feed had a particle size of 250 μm and the Zielger AP-100 had a particle size range of 150-250 μm. At day 25 post-hatch, the young fish were shifted to particle sizes that ranged from 400 to 700 μm of the RGSM Starter and from 450 to 600 μm of the Ziegler AP-100 throughout the end of the study.

Statistical Analysis

The feed study was designed as a complete randomized block design to allow for analysis of block effects between treatments in aquaria which experience different environmental conditions (i.e., light, temperature, traffic exposure). Statistical analysis was performed as a two-way ANOVA with diet as the fixed factor and block as the random factor.

RESULTS AND DISCUSSION

Timing of the introduction of manufactured feed was more important than the feed type in promoting growth and survival in RGSM. There were some detectable differences in length (Figure 1A), but no detectable differences in weight (Figure 1B), or condition factor (Figure 2) among treatments when RGSM larvae were moved from live feed to manufactured feed at either day 15 or 24 day post-hatch and fed the respective feeds through to day 70 post-hatch.

Growth rates varied slightly among treatments with the greatest growth rate (15.1%/day) and total growth (294%) in larvae fed *Artemia* nauplii throughout the study (Table 2). The lowest growth rate was exhibited by fish switched to the Ziegler feed at day 15 post-hatch (11.0%/day and 186% total growth) (Table 2). Growth rate increased to 13.2%/day in fish shifted to Ziegler feed at day 24 post-hatch with an overall increase in total growth to 269%. Although there were no detectable differences among the treatments for length or weight, the RGSM Starter feed appeared to be the most promising starter diet with a growth rate of 13.0%/day and total growth of 266%.

In a previous study, survival was increased in RGSM larvae provided live food within the first 23 days post-hatch when compared to larvae fed manufactured diets at the onset of yolk resorption. In this study, survival was greatly increased in fish fed manufactured diets when initially fed *Artemia* nauplii from the time of yolk resorption to day 15 post-hatch. Average cumulative mortality ranged from 0.6% (SE = 0.60) to 4.4% (SE = 1.568) in fish fed manufactured diets (Figure 3) which represented a considerable increase in survival compared to the previous study in which cumulative mortality ranged from 9% (Ziegler) to 19% (RGSM Starter) in fish started on manufactured diets at the time of yolk resorption.

We observed no detectable differences in time to fatigue (U_{crit}) among RGSM fed *Artemia* nauplii throughout the entire study (mean = 36.5 cm/sec, SE = 1.15) or when shifted to either Ziegler (36.2 cm/sec, SE = 0.96) or the RGSM Starter (36.3 cm/sec, SE = 0.46) feeds at day 15 post-hatch (Table 3). The U_{crit} for juvenile RGSM having total lengths (TL) from 3.5 to 4.9 cm were lower than U_{crit} reported for adult RGSM by Bestgen et al. (2003). These authors observed U_{crit} ranging from 50.2 to 52.8 cm/sec for adult RGSM of larger sizes (5.25 – 7.5 cm, TL). In general, larger fish are more capable of obtaining greater swimming speeds due to greater muscle mass and thus the potential to do work (Beamish 1978). It should also be pointed out that Bestgen et al. (2003) acclimated or preconditioned their fish to the swimming chamber for 133 minutes prior to increasing the flow which was longer than our acclimation period of 10 minutes prior to increasing flow. Although Bestgen et al. (2003) found no significant differences in U_{crit} of RGSM acclimated for shorter (133 min) or longer acclimation times (860 min), the acclimation period may be important in minimizing the confounding effects of stress due to netting and handling of fish during the transfer to the swimming chamber.

Live food is critical for survival of RGSM larvae within the first two weeks post-hatch and that manufactured diets (either an experimental RGSM Starter or the commercial Ziegler AP-100) may then be successfully introduced into the larvae's diet as early as 15 days post-hatch. Although not statistically significant, growth rate was best when larvae were provided the experimental RGSM feed compared to the Ziegler AP-100. We recommend acclimation trials be conducted on RGSM for both short and longer acclimation periods and for RGSM of varying size and age prior to challenge to increased swimming speeds to eliminate the question of confounding effects of handling on U_{crit}.

Table 2. Growth rates (%/day) and total growth (%) in Rio Grande silvery minnow (RGSM) provided *Artemia* nauplii (Artemia) and two manufactured feeds. RGSM 15 and Ziegler 15 represent RGSM larvae switched to the experimental RGSM Starter feed and the Ziegler Brothers larval fish and shrimp diet (AP-100) at day 15 of the study. RGSM 24 and Ziegler 24 represent RGSM larvae switched to the experimental RGSM Starter feed and the Ziegler feed at day 24 of the study.

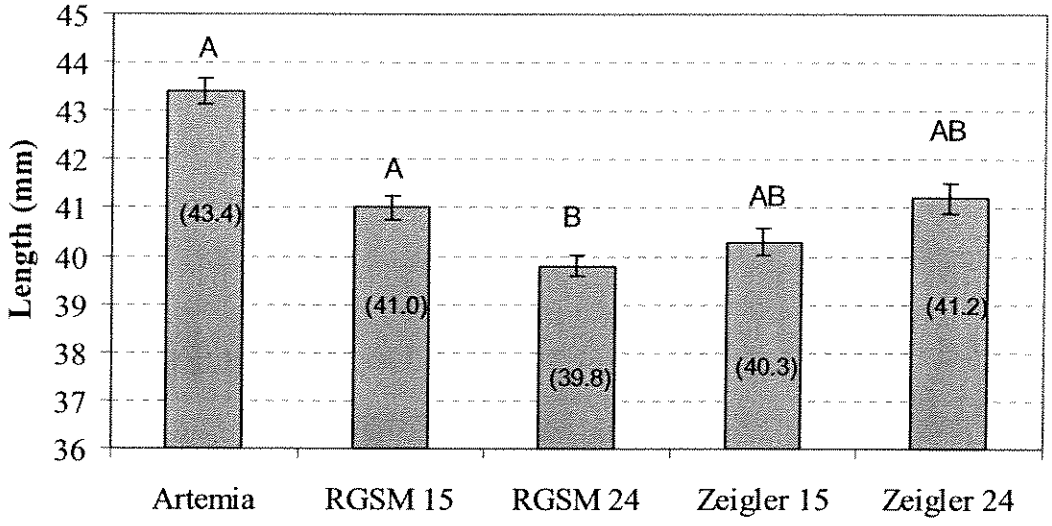
	5 Sep – 11 Oct (%/day)[1]	Total Growth (%)[2]
Artemia	15.1	294
RGSM 15	13.0	266
RGSM 24	11.0	226
Ziegler 15	11.0	186
Ziegler 24	13.2	269

[1] Growth rates were computed from the difference in average weight for each diet collected at the beginning and at the end of the time interval. Growth rates were then normalized to represent percent increase on a per day basis by dividing the growth rate for the time interval by the total days within the time interval (35 days).
[2] Total growth (%) was computed by subtracting the beginning weight from the ending weight and then dividing by the beginning weight.

Table 3. Average critical swimming speed (U_{crit}) and swimming rate (body length/sec) in Rio Grande silvery minnow (RGSM) at day 70 post-hatch. Artemia represent RGSM larvae fed *Artemia* nauplii throughout the duration of the study. RGSM 15 and Zielger 15 represent RGSM larvae switched to the experimental RGSM Starter feed and the Ziegler Brothers larval fish and shrimp diet (AP-100) at day 15 of the study. Standard error and sample size are in parenthesis. Superscripts that differ indicate significant differences among treatments ($P \leq 0.05$).

	U_{crit} (cm/sec)	Body length/sec
Artemia	36.54^{a} (1.15, 5)	8.48 (0.279, 5)
RGSM 15	36.26^{a} (0.457, 5)	8.83 (0.066, 5)
Ziegler 15	36.25^{a} (0.955, 5)	8.93 (0.249, 5)

1 A.



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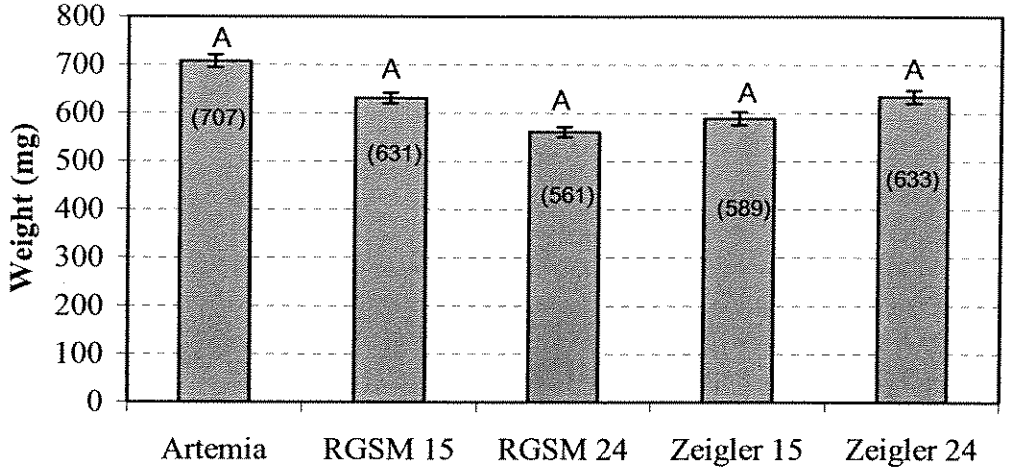

Figures 1A and 1B. Average length (mm) and average weight (mg) of Rio Grande silvery minnow (RGSM) at day 70 post-hatch. Artemia represents RGSM larvae fed Artemia nauplii throughout the duration of the study. RGSM 15 and RGSM 24 represent RGSM larvae switched to the experimental RGSM feed at day 15 and at day 24 of the study. Ziegler 15 and 24 represent RGSM larvae switched to Ziegler (AP-100) at day 15 and at day 24 of the study. Numbers in parenthesis within bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P < 0.05$).

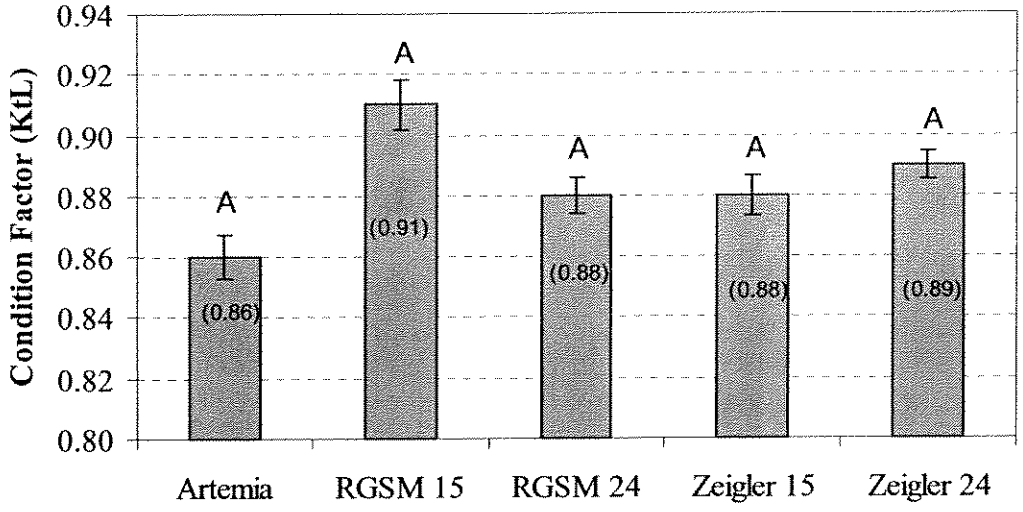

Figure 2. Average condition factor (KtL) in Rio Grande silvery minnow (RGSM) at day 70 post-hatch. Artemia represents RGSM larvae fed *Artemia* nauplii throughout the duration of the study. RGSM 15 and RGSM 24 represent RGSM larvae switched to the experimental RGSM feed at day 15 and at day 24 of the study. Ziegler 15 and 24 represent RGSM larvae switched to Ziegler (AP-100) at day 15 and day 24 of the study. Numbers in parenthesis within bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P \leq 0.05$).

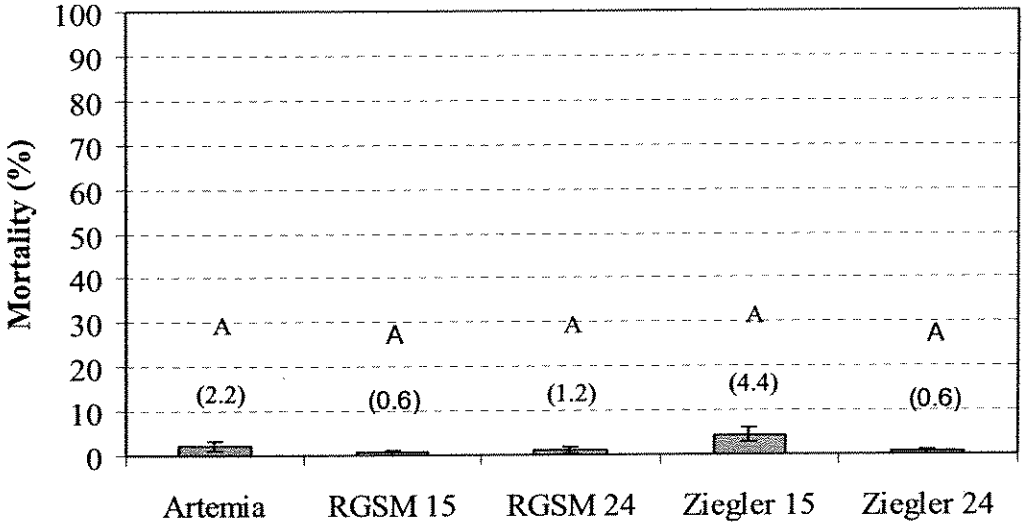

Figure 3. Average mortality (%) in Rio Grande silvery minnow (RGSM) at day 70 post-hatch. Artemia represents RGSM larvae fed *Artemia* nauplii throughout the duration of the study. RGSM 15 and RGSM 24 represent RGSM larvae switched to the experimental RGSM feed at day 15 and at day 24 of the study. Ziegler 15 and 24 represent RGSM larvae switched to Ziegler (AP-100) at day 15 and day 24 of the study. Numbers in parenthesis above bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P \leq 0.05$).

CHAPTER IV

SURVIVAL, GROWTH, AND PERFORMANCE OF RIO GRANDE SILVERY MINNOW FROM JUVENILE (APPROXIMATELY DAY 179 POST-HATCH) TO SUB-ADULT (APPROXIMATELY DAY 300 POST-HATCH): COMPARISON OF MANUFACTURED FEEDS BETWEEN TWO CULTURE FACILITIES

INTRODUCTION

The U.S. Fish and Wildlife Service Dexter National Fish Hatchery and Technology Center (Dexter NFHTC) and the NMSU Aquatic Facility are two of four facilities in New Mexico that have dedicated a large portion of their space and resources to the propagation of Rio Grande silvery minnow (RGSM). However, if captive propagation is to successfully produce healthy fish for eventual release into the wild, then captive rearing methods must be developed and adopted throughout all the facilities. This research was designed to provide information on the effects variables such as diet, water quality or culture techniques have on growth and survival of RGSM. The NMSU Facility is an experimental facility that uses treated water (NMSU water treatment plant) within a water reuse system to minimize water loss and optimal water quality for fish growth and maintenance (i.e., pH 8.0; electrical conductivity 620 micromhos/cm; total dissolved solids 360 mg/L; chloride 60 mg/L; alkalinity 146 mg/L as $CaCO_3$; hardness 188 mg/L as $CaCO_3$). In contrast, Dexter NFHTC is a relatively large propagation facility with water quality that resembles the brackish water typical of the Pecos watershed within which the facility is located (i.e., pH 8.1; electrical conductivity 3480 micromhos/cm; total dissolved solids 3474 mg/L; chloride 454.6 mg/L; alkalinity 200 mg/L as $CaCO_3$; hardness 2925 mg/L as $CaCO_3$).

Previous research characterized feeding requirements of larval RGSM and the time frame with which feeding should be accomplished to increase survival of the RGSM through early life stages. The results indicated that live food (*Artemia* nauplii) is critical for survival of RGSM larvae within the first two weeks post-hatch and that manufactured diets (either an experimental RGSM starter or the commercial Ziegler AP-100) may then be successfully introduced into the

larvae's diet as early as 15 days post-hatch. After that time, the brand of manufactured feed may not be as important. Ideally, the feed should provide the most optimum quality for optimum growth and survival. Thus, the objective of the research was to rear RGSM from the juvenile stage (day 179 post-hatch) through sub-adult just prior to gonadal maturation and spawning using commercial and experimental feeds at RGSM propagation facilities. Two feeding trials were conducted simultaneously to determine the effects of diet on survival, growth, and performance on RGSM at two RGSM propagation facilities having varied water quality and culture conditions. Growth, survival, and performance of RGSM were evaluated in fish reared from juvenile through adult (just prior to reproduction) using: (1) a commercial steam-pelleted feed supplemented with brine shrimp and spirulina produced by Nelson & Sons, Inc., (2) a steam-pelleted diet formulated for RGSM by the Bozeman FTC, Bozeman, MT and (3) a flake feed formulated by the Bozeman FTC having the same ingredients as the pellet feed. A second flake feed was selected for testing only at the NMSU facility. This feed represented a mixture of feed types (krill, spirulina, brine shrimp) sold through a commercial distributor (Aquatic Eco-Systems, Inc.). This feed has been used in other RGSM propagation facilities as well as other fish culture facilities throughout the USFWS. Flake feeds were selected for study because a flake feed will float having a wider dispersal area and thus may be the preferred formulation over a sinking pelleted feed. A sinking pelleted feed may be the preferred formulation if extended nutritional quality in water is required.

Methods and Materials

The experiments took place concurrently at the NMSU Facility (Appendix A) and the Dexter NFHTC (Appendix F). Eggs were collected from adult RGSM (02 wild egg F0 female x 2002 captive spawn F1 male) injected using commercial carp pituitary (Appendix B). From eggs, larvae were reared to the juvenile stage at the Fisheries Research Laboratory on the NMSU campus (Appendix D) until day 178 post-hatch, at which time, fish were transported to NMSU Facility and Dexter NFHTC. Fish were handled similarly at both facilities until the feed studies began at day 179 post-hatch. Experimental design consisted of three feed treatments with six replications randomly placed throughout the aquaria. Fish densities were maintained at 1 fish/5 L (1.3 fish/1 gallon) at both facilities. Aquaria were siphoned daily and temperature (^{o}C) and dissolved oxygen (mg/L) were monitored daily with a YSI dissolved oxygen meter. Ammonia

(mg/L, NH_3-N, salicylate method) and nitrite (mg/L; NO_2-N, diazotization method) were measured using a HACH DR/2010 spectrophotometer and pH was measured using a Beckman 34 with an Orion pH probe calibrated prior to use. Temperature (°C), dissolved oxygen, pH, nitrite, and ammonia (total N) were characterized in all treatments weekly. Once each month, fish were counted, weighed and feed ration (8% body weight) adjusted for all treatments at both facilities. The study at the NMSU facility began 11 December 2003 and was terminated 9 April 2004 (day 296 post-hatch) after 119 days. The study at Dexter NFHTC began 10 December 2003 and was terminated 29 April 2004 (day 320 post-hatch) after 149 days. At the end of each study, eight fish from each aquarium within each treatment were subjected to a performance test using a swimming stamina chamber to determine critical swimming speed (U_{crit}, Beamish 1978; see Appendix E). Fish from all treatments were euthanized and lengths and weights were recorded. Fish from the NMSU facility were evaluated for gonadal development and scored for visceral fat (0-3; with 0 indicating no fat and 3 indicating viscera completely covered by fat).

Diet Compositions

Silver Cup Fish Feed (Nelson & Sons, Inc., Murray, UT): A steam-pelleted diet (#1: 36 mm–48 mm and # 2: 48 mm–61 mm) was augmented with 110 kg of krill and 110 kg of spirulina to 4,400 kg of feed by the manufacturer at the request of Dexter NFHTC. Proximate analysis was conducted by Eurofins (Woodson-Tenent Laboratories Division, Des Moines, IA) and percent moisture and ash were confirmed by the Bozeman FTC, Bozeman, MT. The addition of krill and spirulina by the manufacturer increased protein of the feed by 1.5% (from 35% to 36.5%) and increased fat content by 6.4% (from 7% to 13.4%) (Table 1).

Rio Grande silvery minnow Fish Flake and Pellet Feeds (Bozeman Fish Technology Center, Bozeman, MT): RGSM #0304 (Table 1).

Aquatic Ecosystems Flake Feed (Aquatic Eco-Systems, Inc., Apopka, FL): This diet formulation has been used by USFWS fisheries biologists for fish culture and consists of (1) two parts spirulina (F30S) with spirulina as the primary ingredient; (2) one part brine shrimp (F30B) fortified with vitamins and minerals; and (3) one part plankton/krill/spirulina (F30K) (Table 1).

Table 1. Major constituents of four manufactured feeds fed to juvenile Rio Grande silvery minnow (RGSM) from day 179 post-hatch through approximately day 300 post-hatch. "-" indicates information not available.

	Silver Cup Pellet	RGSM Starter Pellet	RGSM Starter Flake	Aquatic Ecosystem Flake
Protein	36.5%	42.2%	43.3%	41%-45%
Fat	13.4%	18.2%	17.6%	3%
Ash	6.5%	7.8%	8.1%	-
Fiber	-	-	-	3%
Mineral	-	-	-	-
Moisture	6.1%	7.1%	5.8%	-

Statistical Analysis

Both feed studies were analyzed using a complete randomized block design to allow for analysis of block effects of environmental conditions (i.e., light, temperature, traffic exposure). Statistical analysis was performed using a two-way ANOVA with diet as the fixed factor and block as the random factor and Tukey's HSD (honestly significantly different) was used to identify differences in means.

RESULTS

Growth

At the end of the study, there were no detectable differences among fish fed different diets at the NMSU Facility for either length ($F_{3,15} = 2.49$, $P = 0.10$) or weight ($F_{3,15} = 1.85$, $P = 0.18$; Figure 1). Growth rates, calculated for each collection period, showed the greatest daily increase in fish fed the RGSM flake with an increase in growth rate of 1.21%/day and a total weight gain of 144% by the end of the study (Table 1). The lowest growth rates occurred in fish fed Silver Cup pellet with lowest daily growth rate of 0.16%/day and the lowest weight gain of 108% by the end of the study.

At the Dexter NFHTC, detectable differences were observed among the feeds for both length ($F_{2,15} = 6.19$, $P = 0.018$) and weight ($F_{2,15} = 6.57$, $P = 0.015$; Figure 2). Mean lengths and weights were greater in fish fed RGSM flake and lowest in fish fed the Silver Cup pellet ($P<0.05$).

No differences in length or weight were detected between fish fed RGSM pellet and the Silver Cup pellet or in fish fed the RGSM pellet and RGSM flake ($P \geq 0.05$). Growth rates, calculated for each collection period, showed the greatest daily increase in fish fed the RGSM flake with an overall growth rate of 1.23%/day and a total weight gain of 184% by the end of the study (Table 2). An increase in 1.85%/day was observed in fish fed the flake diet within the first 46 days of the study. The lowest growth rates were observed in fish fed the Silver Cup pellet with the lowest daily growth rate of 0.69%/day and lowest total weight gain of 104% by the end of the study. It is worth noting that growth rates at the facility declined the latter half of the study with daily growth rates that ranged from 0.24%/day in fish fed the RGSM flake to as low as 0.06%/day in fish fed the RGSM pellet.

Visceral fat was scored for only the NMSU study and reflected greater fat content in fish fed the Aquatic Ecosystems feed (mean = 2.69, standard error (SE) = 0.038) when compared to the RGSM flake (2.40, SE = 0.072), RGSM pellet (2.40, SE = 0.095), or Silver Cup pellet (2.45, SE = 0.063) (Table 3). The ratio of male to female fish in all aquaria was nearly 50:50 with the majority of fish exhibiting well developed ova (but not ovulating) and developed testes (but no running milt).

Condition Factor

There were no detectable differences in condition factor among fish fed four diets at the NMSU facility at the end of the study ($F_{3,15} = 1.19$, $P = 0.35$; Figure 3A). There were detectable differences, however, among fish fed three diets at the Dexter NFHTC ($F_{2,15} = 6.00$, $P = 0.019$; Figure 3B). Condition factor was significantly greater in fish fed the RGSM flake (0.89, SE = 0.011) compared to fish fed Silver Cup pellet (0.82, SE = 0.007) ($P \leq 0.05$), but not in fish fed the RGSM pellet feed (0.85, SE = 0.010) ($P = 0.09$).

Survival

There were no detectable differences in survival among RGSM fed four diets at the NMSU Facility ($F_{3,15} = 0.75$, $P = 0.54$; Figure 4A). Total cumulative mortality ranged from 5% in fish fed the RGSM flake to 13% in fish fed the flake feed from Aquatic Ecosystems. Similarly, there were no detectable differences in survival among RGSM fed three diets at the Dexter NFHTC ($F_{2,15} = 1.77$, $P = 0.22$; Figure 4B). Midway through the study at the Dexter facility, a bacterial outbreak of *Aeromonas* sp. occurred in the majority of the aquaria resulting in elevated mortality. Of note, 62% of the fish died in one aquarium receiving RGSM flake and 45% of the fish died in

one aquarium receiving RGSM pellet. When these two aquaria were treated as outliers and removed from the analysis, a marginal difference was detected for survival among the three diets at the Dexter facility ($P = 0.09$).

Performance: Critical Swimming Speed

At the end of both studies, diet had no effect on critical swimming speed of RGSM at the NMSU Facility ($F_{3,15} = 0.89$, $P = 0.47$, Table 5) or the Dexter NFHTC ($F_{2,15} = 0.48$, $P = 0.63$; Table 6). Although no statistical differences were detected for U_{crit} among RGSM at both facilities, fish fed the RGSM flake performed best by obtaining greater U_{crit} at both facilities while fish provided the RGSM pellet had the lowest U_{crit}. Of interest, U_{crit} differed greatly between the two facilities with fish fatiguing at speeds that ranged from 51.9 to 58.2 cm/sec at the NMSU Facility and from 33.6 to 38.4 cm/sec at the Dexter NFHTC.

DISCUSSION

Diet had no detectable effect on growth, condition factor, survival or performance (U_{crit}) in adult RGSM provided four feeds at the NMSU facility for 119 days (from juvenile to sub-adult). Detectable differences were observed, however, for growth and condition factor in adult RGSM provided three feeds at the Dexter NFHTC for 149 days (from juvenile to sub-adult). Growth rates for RGSM reared at the Dexter NFHTC were greater in fish fed the RGSM flake feed (overall growth rate of 1.23% and a total growth of 184%) when compared to fish fed the RGSM pellet feed (overall growth rate of 0.88%/day and a total growth of 131%) or the Silver Cup pellet (overall growth rate of 0.69% and a total growth of 104%).

Diet had no detectable effect on survival or swimming performance (U_{crit}) in RGSM at either facility. The lower critical swimming speeds in RGSM at the Dexter facility (33.7 cm/sec – 35.0 cm/sec) were due to the smaller size fish having average total lengths that ranged from 5.0 cm to 5.5 cm. In comparison, RGSM reared at the NMSU Research Facility had average total lengths that ranged from 6.0 cm to 6.1 cm which allowed them to obtain greater U_{crit} (51.9 – 58.2 cm/sec). Larger fish are capable of obtaining greater swimming speeds due to greater muscle mass and thus the potential to do work (Beamish 1978). Average U_{crit} for adult RGSM reared at the NMSU Research Facility (51.9 - 58.2 cm/sec; 6.0 – 6.1 cm, total length, TL) were similar to U_{crit} reported for adult RGSM by Bestgen et al.

(2003). These authors observed U_{crit} ranged from 50.2 to 52.8 cm/sec for adult RGSM similar in size (5.25 – 7.5 cm, TL).

Exercise and preconditioning were shown to increase swimming performance in both hatchery-reared and wild striped bass (Young and Cech 1993). We observed RGSM preferred to swim against the current of the large circular tanks at the NMSU facility (11,355 L; 3,000 gal). Although anecdotal, we subjected 10 fish to swimming stamina challenge and observed U_{crit} ranged from 62.3 to 85.0 cm/sec in adult RGSM (6.5 – 7.6 cm, TL). One fish (6.7 cm, TL) out swam the stamina challenge by achieving maximum speed of the swim tunnel (81.6 cm/sec) and maintaining this speed for 10 minutes before the test was terminated. Of interest, one gravid female (8.9 cm, TL) subjected obtained U_{crit} of only 39.0 cm/sec.

Unlike fish fed RGSM Flake, RGSM Pellet, and Silver Cup Pellet, fish fed the Aquatic Ecosystem flake feed experienced whirling and erratic behavior that was usually followed by death. Review of the literature indicated this behavior was symptomatic of a vitamin deficiency (pyridoxine, B_6). Elemental analysis was conducted on RGSM exhibiting erratic whirling behavior and on fish not exhibiting whirling that had been reared in outdoor ponds at the Dexter NFHTC. Magnesium (1,100 mg/L) and zinc (160 mg/L) were slightly lower in fish exhibiting whirling compared to those reared in an outdoor pond (1,300 and 180 mg/L, respectively).

Summary and Recommendations

An experimental flake feed (RGSM Flake) produced by the Bozeman FTC resulted in detectably better growth and condition than either the experimental pellet (RGSM Pellet) of the same composition or a commercial pellet (Silver Cup Pellet) at one RGSM propagation facility. Based on these results and overall growth observed for RGSM provided the RGSM Flake at the NMSU Research Facility, it appears RGSM may prefer a flake formulation over a pellet formulation. The results of the study clearly indicated varied responses in RGSM given the same feeds at two propagation facilities reflecting differences in culture operations and the importance that culture conditions may have when considering feed requirements. Long term use of a commercial flake feed resulted in a vitamin deficiency in adult RGSM thereby reducing survival and is not recommended for RGSM propagation.

Table 2. Growth rates (%) for Rio Grande silvery minnow provided four feeds at the NMSU A-Mountain Aquatic Research Facility from 11 December 2003 through 9 April 2004.

	11 Dec–13 Jan (%/day) [1]	14 Jan – 20 Feb (%/day) [1]	21 Feb – 9 Apr (%/day) [1]	Overall Growth Rate (%/day) [2]	Total Growth (%) [3]
RGSM Flake	0.94	0.60	0.68	1.21	144
RGSM Pellet	0.73	0.95	0.31	0.98	117
Silver Cup Pellet	0.55	1.32	0.16	0.91	108
Aquatic Eco Flake	0.88	0.96	0.31	1.09	130

[1] Growth rates were computed from the difference in average weight for each diet collected at the beginning and at the end of each time interval. Growth rates were then normalized to represent percent increase on a per day basis by dividing the growth rate for the time interval by the total days within the time interval.
[2] Overall growth rate was calculated by dividing the total growth rate (from beginning of the study to the end of the study) by the total number of days in the study (119 d).
[3] Total growth (%) was computed by subtracting the beginning weight from the ending weight and then dividing by the beginning weight.

Table 3. Growth rates (%) for Rio Grande silvery minnow (RGSM) provided three feeds at the Dexter National Fish Hatchery and Technology Center from 1 December 2003 through 29 April 2004.

	11 Dec–16 Jan[1] (%/day)	17 Jan–17 Feb[1] (%/day)	18 Feb–29 Apr[1] (%/day)	Overall Growth Rate[2] (%/day)	Total Growth[3] (%)
RGSM Flake	1.85%	0.95%	0.24%	1.23%	184%
RGSM Pellet	1.41%	1.12%	0.06%	0.88%	131%
Silver Cup Pellet	0.97%	0.96%	0.11%	0.69%	104%

[1] Growth rates were computed from the difference in average weight for each diet collected at the beginning and at the end of each time interval. Growth rates were then normalized to represent percent increase on a per day basis by dividing the growth rate for the time interval by the total days within the time interval.
[2] Overall growth rate was calculated by dividing the total growth rate (from beginning of the study to the end of the study) by the total days of the study (149 d).
[3] Total growth (%) was computed by subtracting the beginning weight from the ending weight and then dividing by the beginning weight.

Table 4. Mean viscera fat scores (standard error; maximum and minimum range) in Rio Grande silvery minnow (RGSM) given four feeds at New Mexico State University A-Mountain Aquatic Research Facility from 11 December 2003 through 9 April 2004. Numeric ratings of 0 - 3 with "0" indicting no fat present in visceral cavity to "3" indicating viscera covered entirely in fat.

Feed	Fat Score
RGSM Flake	2.40 (0.072, 2.10 - 2.58)
RGSM Pellet	2.40 (0.095, 2.00 - 2.68)
Silver Cup Pellet	2.45 (0.063, 2.17 - 2.54)
Aquatic Eco Flake	2.69 (0.038, 2.61 - 2.85)

Table 5. Average critical swimming speed (U_{crit}) and swimming rate (body length/sec) in Rio Grande silvery minnow (RGSM) provided four feeds at New Mexico State University A-Mountain Aquatic Research Facility from 11 December 2003 through 9 April 2004 (day 119 post-hatch). Standard error and sample size are in parenthesis. Superscripts that differ indicate significant differences among treatments ($P \leq 0.05$).

	U_{crit} (cm/sec)	Body length/sec
RGSM Flake	58.2^a (2.83, 6)	9.6^a (0.42, 6)
RGSM Pellet	53.2^a (2.31, 6)	9.1^a (0.41, 6)
Silver Cup Pellet	55.5^a (2.99, 6)	9.5^a (0.48, 6)
Aquatic Eco Flake	51.9^a (3.34, 6)	8.6^a (0.58, 6)

Table 6. Average critical swimming speed (U_{crit}) and swimming rate (body length/sec) in Rio Grande silvery minnow (RGSM) provided three feeds at the U.S. Fish and Wildlife Service National Fish Hatchery and Technology Center from 1 December 2003 through 29 April 2004 (day 149 post-hatch). Standard error and sample size are in parenthesis. Superscripts that differ indicate significant differences among treatments ($P \leq 0.05$).

	U_{crit} (cm/sec)	Body length/sec
RGSM Flake	38.4^a (2.92, 6)	7.0^a (0.72, 6)
RGSM Pellet	33.6^a (3.43, 6)	6.6^a (0.67, 6)
Silver Cup Pellet	35.7^a (4.08, 6)	7.2^a (0.88, 6)

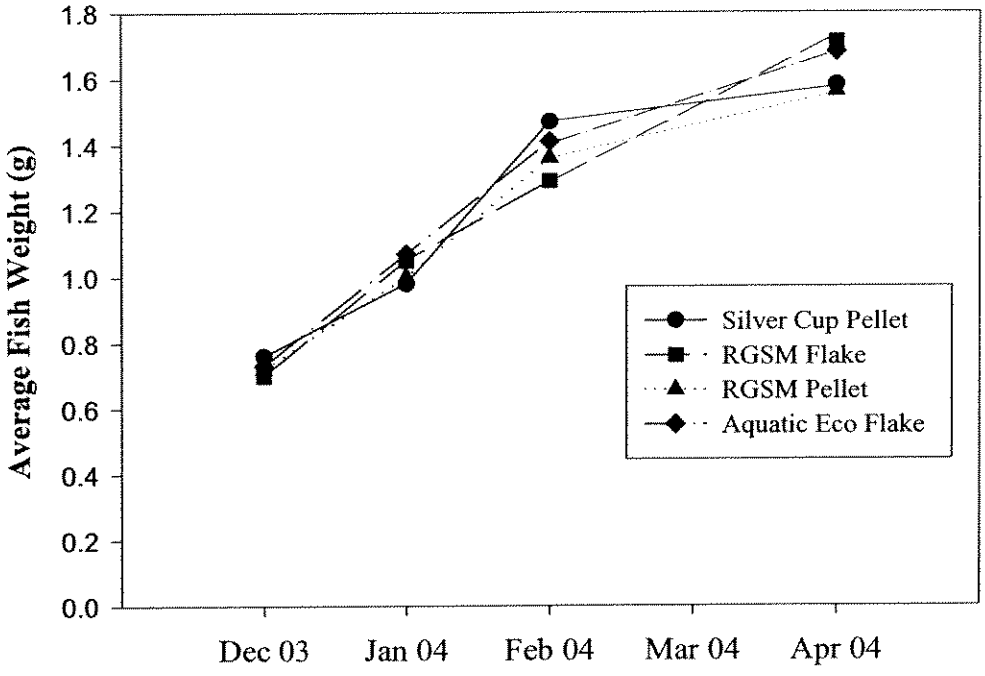

Figure 1. Average weight (g) of Rio Grande silvery minnow (RGSM) provided four feeds from juvenile to adult at the New Mexico State University A-Mountain Aquatic Research Facility from 11 December 2003 through 9 April 2004 (day 119 post-hatch).

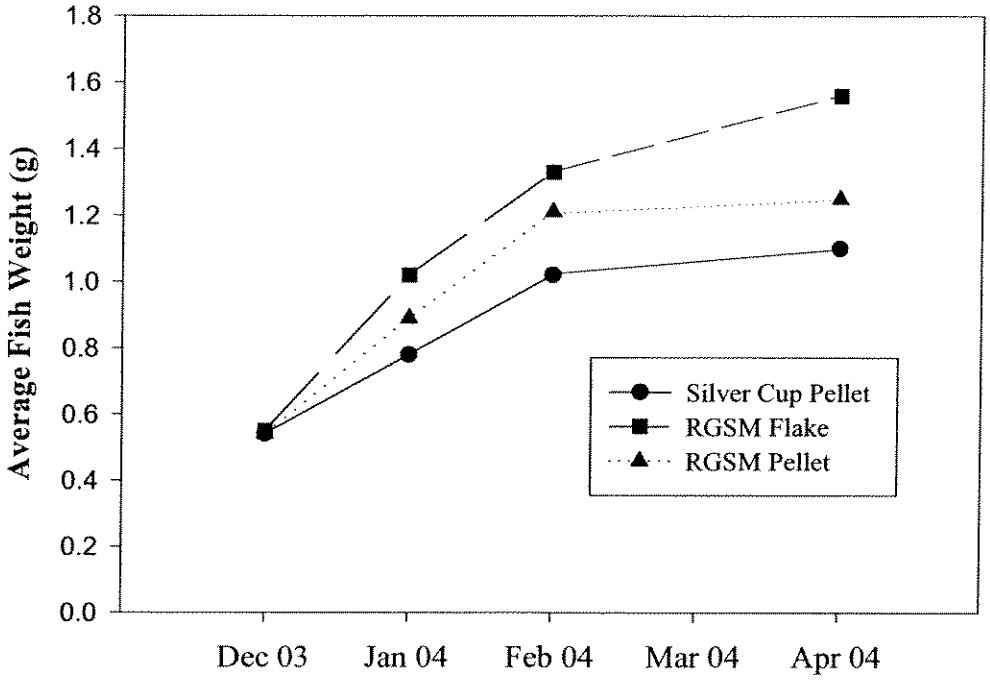

Figure 2. Average weight (g) of Rio Grande silvery minnow (RGSM) provided three feeds from juvenile to adult at the U.S. Fish and Wildlife Service Dexter National Fish Hatchery and Technology Center from 1 December 2003 through 29 April 2004 (day 149 post-hatch).

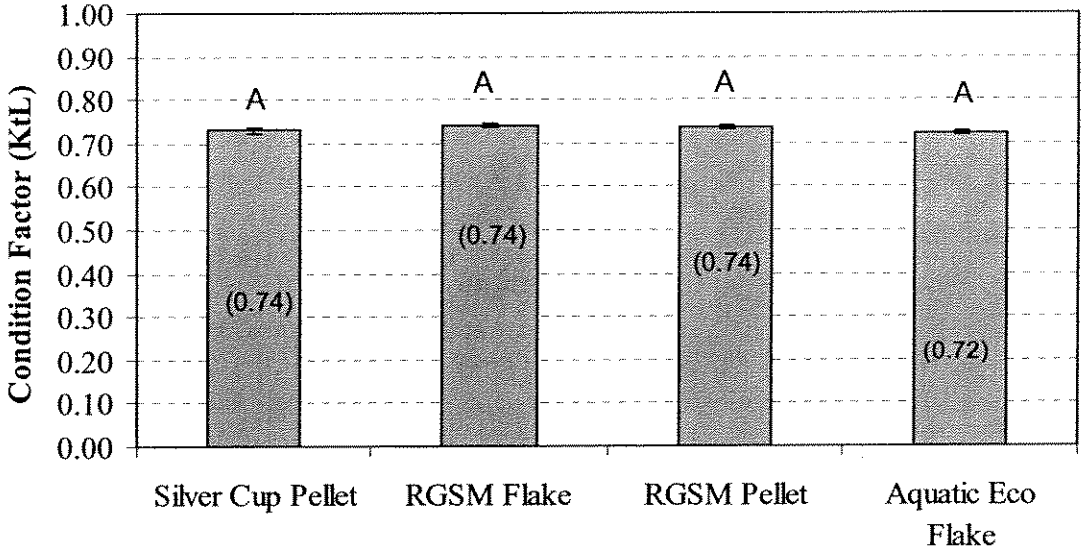

Figure 3A. Condition factor (KtL) of Rio Grande silvery minnow (RGSM) provided four feeds from juvenile to adult at the New Mexico State University A-Mountain Aquatic Research Facility from 11 December 2003 through 9 April 2004. Numbers in parenthesis within bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P \leq 0.05$).

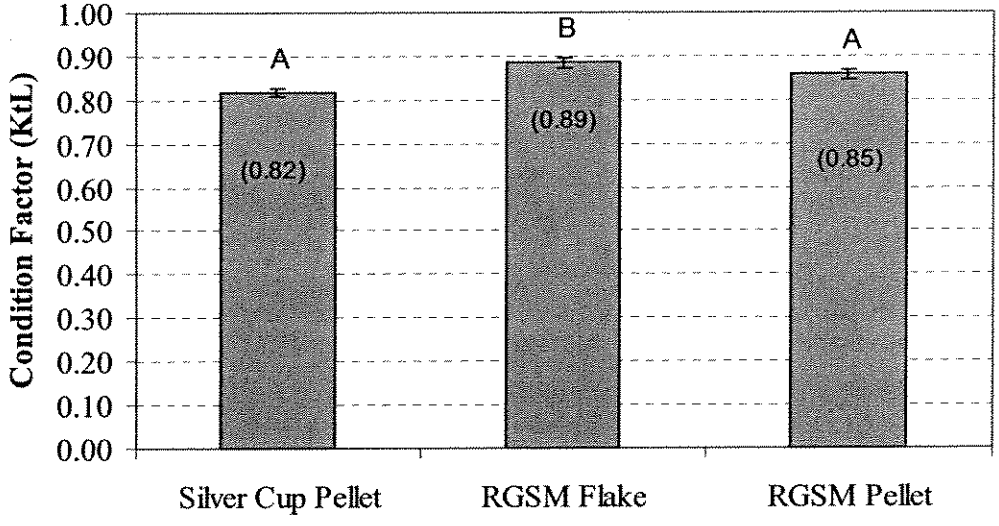

Figure 3B. Condition factor (KtL) of Rio Grande silvery minnow (RGSM) provided three feeds from juvenile to adult at the U.S. Fish and Wildlife Service Dexter National Fish Hatchery and Technology Center from 1 December 2003 through 29 April 2004. Numbers in parenthesis within bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P \leq 0.05$).

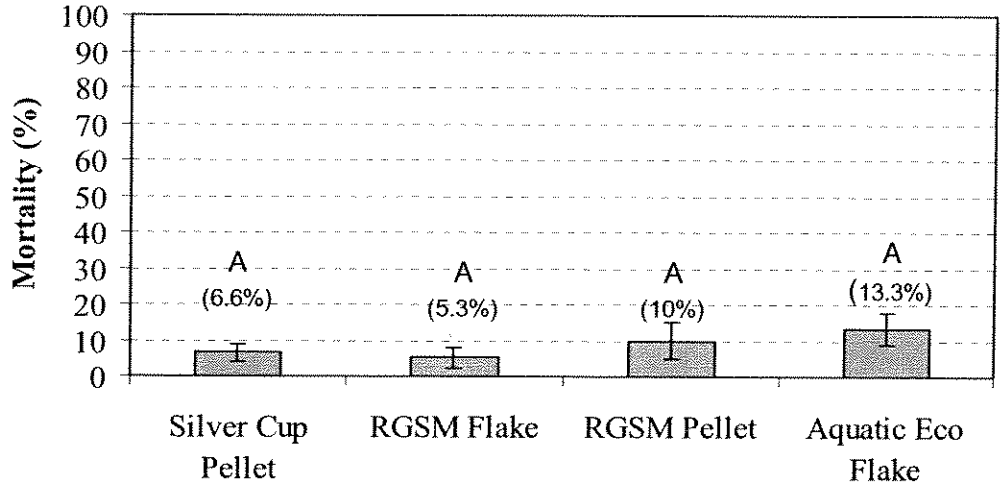

Figure 4A. Mortality (%) of Rio Grande silvery minnow (RGSM) provided four feeds from juvenile to adult at the New Mexico State University A-Mountain Aquatic Research Facility from 11 December 2003 through 9 April 2004. Numbers in parenthesis within bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P \leq 0.05$).

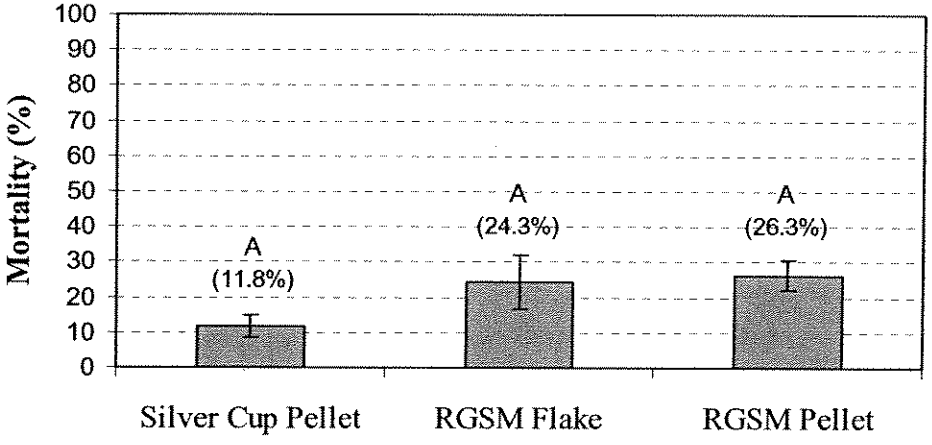

Figure 4B. Mortality (%) of Rio Grande silvery minnow (RGSM) provided three feeds from juvenile to adult at the U.S. Fish and Wildlife Service Dexter National Fish Hatchery and Technology Center from 1 December 2003 through 29 April 2004. Numbers in parenthesis within bars represent the average value. Standard error bars are included. Letters that differ above each bar indicate significant differences among treatments ($P \leq 0.05$).

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APPENDIX A

NEW MEXICO STATE UNIVERSITY A-MOUNTAIN AQUATIC RESEARCH FACILITY

The entire culture system is enclosed in a double-wall arched greenhouse structure (9.2-m x 29.3-m; 270m^2). Recirculated water is treated by either an artificial wetland filter or bead and sand filters designed to remove hazardous nitrogenous wastes prior to reuse. Water quality is improved (greater than 96%) by more complex treatment of ultra-violet sterilizers to irradiate and control potential pathogens. A centrifugal air blower provides oxygen input via air stones and circulation via air lift pumps to the tanks. The research system consists of thirty-two 69.5-liter (18 gal) glass aquaria sitting on two wooden shelves. Sixteen aquaria face east and west on one shelf and sixteen face north and south on the second shelf. Each aquarium is equipped with individual water and airline valves. Light conditions are natural seasonal dark:light cycles. A screen is positioned above both aquarium racks to lower the intensity of light when the sun is directly overhead. Hobo Tidbit Dataloggers™ are installed throughout the research aquaria and greenhouse facility to monitor temperature and ambient light. The data is downloaded weekly to a series of files on a laptop PC. The principal investigator currently holds federal (TE 046517-0) and state (#3033) endangered species permits to conduct research with RGSM at NMSU. In addition, the investigator has received approval from the Institutional Animal Welfare and Use Committee (IAWUC) for conducting research with live animals at the Geothermal NFCF. The facility is inspected biannually by the IAWUC committee and receives an excellent rating for animal welfare and maintenance.

APPENDIX B

RIO GRANDE SILVERY MINNOW SPAWNING AND BREEDING PROTOCOL

Spawning efforts are coordinated with USFWS Dexter NFHTC. The American southwest can experience about a three week variation in weather patterns, thus, pond fertilization will follow this pattern. Also, the spawning of other species at Dexter has to be considered. When it has been decided when spawning should begin, the following guidelines are observed:

On the Monday of the week in which spawning will occur:

1. Fill aquaria within the research recirculation system. Turn on the air supply to each unit. If additional aquaria are needed, these are aerated using a Sweetwater portable air pump. All aquaria are numbered consecutively. Each week during the spawning season, each aquarium will receive new numbers starting with the last number of the last aquarium from the previous week. This will assign a unique number to each aquarium which will represent a lot number to each breeding pair of fish.
2. On a separate table, two aquaria (15 gal with partition) are used to sort female and male fish. Each has a set of air stones using the electric air pump. Just prior to beginning the injections, aquaria are filled with fresh water and salt is added to obtain 0.5% NaCl. Approximately 3 to 5 ml of PolyAqua (stress cost) is added to each aquaria.
3. A portable pump is used to slowly draw down the tank containing adult fish to be used for spawning. Once the tank is down to approximately 1,000 gal (about 1/3), the fish can be seined. If we are to use the same year class within a two week period, then 100 males (running with milt) and 100 females (extended abdomen) will be needed.
4. Fifty males will be placed into the 15 gal aquarium with the partition and 50 females can be placed into the other 15 gal aquarium with a partition. The other 100 fish can be placed in one of the 350 gal tanks for the following week's spawn.

Materials and Methodology for Injections:

Target time of injections to begin is at 4 pm. Fish will spawn within 6 to 8 hours of injection resulting in eggs the following morning.

Items needed:

1) Small plastic tubs or containers to hold 3 to 4 fish at a time (one for MS-222 solution, two for recovery in fresh water).
2) Syringes and needles: 1.0 cc with 27 g needles
3) Carp Pituitary Extract (CPE) (Sigma #P3034) – Diluted 42 mg/20 ml. This should be made fresh each week and kept refrigerated. Our experience is that the CPE loses its potency after three weeks.
4) Balance for obtaining weights
5) Small measuring board for obtaining lengths
6) Book to record all spawning data
7) MS-222
8) Poly Aqua or Stress Coat
9) Salt
10) 2 to 3 dip nets (8")
11) At least 3 people, but 4 is preferred

Steps for injection:

1. Fill four syringes with 0.9 ml of CPE and place near injection area to allow them to come to room temperature which should be the temperature of the fish (± 2 °C). Leave the remaining CPE out of the refrigerator for refilling when needed.
2. One person will capture one fish at a time, beginning with male fish, and place into the MS-222 (75-100 mg/l). After the fish rolls to its side, immediately remove and check for running milt. If milt is present, record weight.
3. A general rule of thumb will be to inject fish less than 5 g with 100 µL of CPE. If the fish weighs more than 5 g, but less than 10 g, inject the fish with 200 µL of CPE. The resulting dosage is 210 µg/100 uL for a 5 g or less fish or 420 µg per 200 µL for a fish greater than 10 g.
4. Allow the water to circulate in the aquaria until 8 pm when the individual water supplies are turned off plus the main valve. If this is not done there will be some siphoning out of the aquariums during the night and the potential loss of eggs.

Some notes about this process.

- It is best if one person records data and assigns the amount of CPE needed based on the weight of the fish. One person will inject the CPE while another person moves fish from the recovery container and place in the assigned aquarium. The person recording the data will keep track of where the fish is to be placed (aquarium number), the amount of CPE used, and weight of the fish.

- It is important to collect lengths and weights on the first 20 fish of both sexes to determine condition factor for the lot of fish.

- Anaesthetize one fish at a time and remove from MS-222 before they are in deep anesthesia. We have seen fish that are down too long could suffer from delayed mortality several days later.

- Pair males that are larger with large females so that they can mechanically complete the spawning process which is to wrap around the female.

- At the end, check the condition of each fish in the aquaria and replace a newly injected fish if the fish in the aquaria appear to be overly distressed, disoriented, or discolored.

Tuesday-- Egg Counting Begins
Items Needed:

1) 4 to 5 thumb counters
2) 4 to 5 spoons with holes
3) Large (100 ml) and small (50 ml) beakers
4) 2- 100 ml graduated cylinders
5) 4 to 6 Petri dishes
6) Data Book for recording data
7) At least 3 people, but 4 is better
8) Egg Cups
9) Calculator
10) Ice chest with crushed ice

Spawning Protocol:

1. Fill two 350 gallon tanks; one tank will be for fish that spawned (contributing to the genetic pool and will subsequently be released to the wild) and the other for those fish that did not spawn. Often male fish that did not spawn may spawn at a later date. Thus, the male fish can be used more than once in the season.
2. Evaluate each aquarium and record whether the spawn was negative (no eggs), partial (some or few eggs) or full spawn (several thousand).
3. Remove all males from aquaria. The negative-spawn fish should be returned to a holding tank for monitoring and recovery and possibly later use.
4. Remove the female fish and carefully check to see if eggs remain within her abdominal cavity. Gently squeeze the abdomen from anterior to posterior to strip remaining eggs. This will confirm the full or partial spawn and facilitate recovery. Place these female fish with their male counterpart to an assigned tank for observing recovery.
5. Once all fish are removed, turn up the air in each aquarium so that the eggs are gently tumbling.

Egg Counts:

1. Utilizing a dip net, gather sufficient eggs for three estimates.
2. To the 100 ml graduated cylinder, add 10 ml of water and add eggs to the cylinder until 20 ml is obtained. This will provide a 10 ml egg sample for counting.
3. Repeat this process three times so that an average egg count can be made.
4. Once an average has been calculated, collect all eggs from the aquarium and obtain a volumetric estimate using either a 100 ml or 250 ml graduated cylinder.
5. Multiply total ml (volumetric amount) by the sample count.
   - Be sure to add in the total number of eggs obtained from the three estimates to get the total egg count for the paired mating.
   - All egg counts, averages, and total egg count for each paired mating should be recorded.
6. Return all eggs to the original aquarium making sure that there is adequate circulation for tumbling of the eggs.
7. Maintain a maximum of 22-23°C to prevent hatching until final transport.

Wednesday--Determining Fertilization Rates and Hauling Eggs

Five people are needed to assist with determining fertilization rates. Since time is crucial, four people evaluating eggs and one person gatherings eggs and bookkeeping are needed.

Items needed:

1. Stereoscopes w/lights – 4
2. Counters – 8
3. Recording book
4. Dip Net
5. Forceps for handling eggs
6. Cyrovials-one for each egg group-prelabeled with aquarium number and date for archiving and genetic analysis

7. Fine Sharpie marking pen for marking vials
8. Egg hauling bags – enough for 5,000 - 6,000 eggs / bag (double bagged)
9. Green rubber bands to close bags and tool to close bags
10. Oxygen cylinder (full) and flexible hose for filling bags
11. Coolers – for holding bags
12. Small cooler with ice for extra ice and to transport genetic samples
13. Pre-labeled egg cups – one for each aquarium with eggs
14. Rack for cryovials
15. 2- 100 ml graduated cylinders
16. Petri dishes
17. Flat ice block

Determining Fertilization Rates:

1. Places 5 ml of water in a graduated cylinder.
2. Add 10 ml of eggs to the graduated cylinder.
3. Transfer eggs to the pre-labeled aquarium egg cup which is then placed on a flat ice block.
4. The evaluators will take a cup and pour a portion in a Petri dish. Utilizing two thumb-counters, one counter will be used to record fertilized eggs while the other counter will record unfertilized eggs.
    - The criterion for fertilized eggs is the neural fold. If the neural fold is present, the egg is considered fertilized.[1]
5. The first 50 fertilized eggs will be placed in the pre-labeled cryovial and the vial and refrigerated.
6. Once all aquaria are completed, tabulate fertilized and non-fertilized eggs and record in the book.
7. Divide the fertilized eggs by the total eggs counted to obtain percent fertilization.
8. Multiple percent fertilization for each aquarium by total egg count for the respective aquarium.
9. It is important to determine which lots of eggs to be shipped to Dexter NFHTC for rearing. A decision was made of 900[2] fertilized eggs from one paired matting as the lower cut-off for shipping to Dexter for rearing in ponds. Thus, if one paired mating of 900 fertilized eggs or less is obtained then these eggs are not shipped to Dexter and are retained at the A-Mountain facility for research purposes or disposed of.
10. Arrange the numbers of eggs from each aquarium so that approximately 5,000-6,000 fertilized eggs will be placed in each bag.
11. Number each plastic bag and placed in coolers (double bagged), fill each bag (*no salt or Stress-Coat*) with1/3 water.
12. Fill the pre-assigned bag with eggs from the pre-assigned aquarium.
13. Once all bags are filled and in the cooler, inflate each bag with the flexible tubing connected to the compressed oxygen gas cylinder and secure with green rubber bands. Secure both inner and outer bags with green rubber bands.
14. If it is a particularly hot day, place a small handful of ice at outside corner of each bag.
15. Place cryovials in a zip-lock bag and place in small ice chest.
16. Check for bag inflation and temperature several times during trip.

Thursday and Friday: Clean Aquaria and Prepare for Next Spawning and Egg Collection

[1] Footnote: The decision to evaluate egg viability in addition to fertilization rate will be made at the beginning of each spawning season. Viability represents movement and will allow us to obtain better estimates of hatch.

[2] Footnote: Total fish to be determined each year by Dexter NFHTC Personnel.

APPENDIX C

FATTY ACID PROFILE ARTEMIA NAUPLII (% RELATIVE)

(Artemia franciscana) GSL, Inc. Ogden, UT

C08:0Octanoic (Caprylic)	<0.10
C10:0 Decanoic (Capric)	<0.10
C11:0Undecanoic (Hendecanoic)	<0.10
C12:0Dodecanoic (Lauric)	<0.10
C13:0Tridecanoic	<0.10
C14:0Tetradecanoic (Myristic)	0.71
C14:1Tetradecanoic (Myristoleic)	1.14
C15:0Pentadecanoic	0.20
C15:1Pentadecenoic	0.22
C16:0Hexadecanoic (Palmitic)	10.87
C16:1Hexadecenoic (Palmitoleic)	3.57
C16:2Hexasecadienoic	<0.10
C16:3Hexadecatrienoic	0.75
C16:4Hexadecatetraenoic	0.90

APPENDIX D

NEW MEXICO STATE UNIVERSITY FISHERIES RESEARCH LABORATORY

The Fisheries Research Facility at New Mexico State University contains a 3293-liter (870-gallon) recirculating system. High quality well water is fed into a 946-liter (250-gallon) sump tank. A 1/8 h.p. pump moves the water through a 113-lpm (30-gpm) BBF2P bubble bead filter (Aquatic Eco-Systems, Inc.) using a filter media of low-density polyethylene beads with a 56.6 cm^3 (2 ft^3) bead capacity. A Rainbow Lifegard UV97 sterilizer is attached behind the bead filter with 3 UV bulbs totaling 120 watts and an exposure of 30,000 µws. From the sterilizer, water is pumped into a 378-liter (100-gallon) head tank which is aerated with a 122-cm (4-ft) bio-weave type diffuser hose (supports 7.26-kg [16-lbs] of fish). Water is then gravity fed into fifty-two 38-liter (10-gallon) glass aquaria resting on stainless steel racks. Each aquarium has a globe valve for individual water adjustments and an airline with a 10-cm (4-inch) diffuser (supports 0.772-kg [1.7-lbs] of fish). Aquaria drain from bulkhead fittings centered in the front glass panel and gravity feeds the water through a 7.62-cm (3-inch) slotted PVC pipe back into the sump tank. Nitrite (mg/L) and ammonia (nitrogen; mg/L) are monitored using HACH spectrophotometer (DR/2010) and HACH methods 8507 and 8155, respectively. Dissolved oxygen and temperature are monitored using a Yellow Springs Instrument dissolved oxygen meter. Both light and temperature are monitored continually using Hobo Tidbit™ Dataloggers. The principal investigator currently holds federal and state endangered species permits to conduct research with the RGSM at NMSU (TE#046517-0). In addition, the investigator has received approval from the Institutional Animal Welfare and Use Committee (IAWUC) for conducting research with live animals. The Fisheries Research Laboratory is inspected biannually by the IAWUC committee and receives an excellent rating for animal welfare and laboratory maintenance. Protocols have been developed and strictly adhered to for disease prevention and maintaining daily logs for animal care and treatment.

APPENDIX E

OBTAINING CRITICAL SWIMMING SPEED (UCRIT; CM/SEC) USING SWIMMING STAMINA TUNNEL

A fish's normal swimming performance will be altered by exposure to environmental stressors and contaminants (Beamish 1978). Swimming capacity is regulated by the metabolic capacity of the fish to mobilize stored energy into mechanical energy through muscular contractions. Thus, the premise of determining time to exhaustion (critical swimming speed; U_{crit}) allows one to compare swimming speeds among various fishes and the effects the environment will have on those speeds. Swimming speeds of individual fish of the same species would have different swimming speeds due to differing physiological states. Sustained swimming (200 min or longer) is fueled by the formation of ATP through aerobic processes or the catabolism of carbohydrate and lipid stores. Anaerobic and aerobic processes (through both lipid and carbohydrate metabolism) will provide sufficient energy for prolonged or endurance swimming (20 sec to 200 min). While burst swimming (10 to 20 sec) relies on anaerobic mobilization of energy from carbohydrate or glycogen stores. If swimming becomes prolonged or severe, energy used to fuel the muscles is shifted to anaerobic glycolysis which results in lactic acid build up and eventually fatigue.

Critical swimming speed is obtained by placing a fish in a stamina tunnel. The stamina tunnel (11.4 cm or 4.5 in) and its apparatus consist of a plexi-glass tunnel having a front-half and a rear-half separated mid-way by a screen. The tunnel has two openings which are covered by moveable hatch covers, a paddle wheel flow meter with a digital readout, a 380-L water supply tanks with a Remcor™ water circulator to control water temperature, and a variable speed 1.5 horsepower water pump to control water velocity. Water is pulled through the Plexiglas tunnel by the variable speed pump from the supply tank. The variable speed pump is controlled by a computer program loaded into a central control unit so that water flow is automatically increased at specified time intervals. The central control unit has three LED readouts that give the interval cycle number, operation time in the current cycle, and the flow rate. A typical swimming test program will consist of nine cycles of increasing flow rate from 38.75 L/min (10 gallons/min) to 348.75 L/min (90 gallons/min) at 5 minute intervals.

Fish are deprived of food for 24 prior to the test and placed within the front and rear half of the tunnel. These fish are allowed to acclimatize to the tunnel for 10 min at the lowest flow rate

(38.75 L/min = 10 cm/sec). When flow is generated, a fish will typically react by swimming against the flow. Flow is increased at increments of 10 cm/sec in which the fish swim against the current for a pre-determined time interval (300 sec). Ultimately, as the flow velocity increments increase, a flow is reached at which fish are unable to swim. When a fish falls against the screen for more than 5 seconds, the test is considered over for this fish and the time within the cycle is recorded. After all the fish have completed the challenge, weight (g) and both total length (mm; from the tip of the snout to the end of the caudal fins when compressed dorso-ventrally) and body length (mm; from the tip of the snout to the caudal peduncle) are recorded. The following calculations are performed to obtain (1) critical swimming speed [U_{crit} (cm/sec)] and (2) body lengths per second (BL/sec) swam (Beamish 1978):

(1) $U_{crit} = U_i + [(t_i/t_{ii}) * U_{ii}]$; where

U_i = highest velocity (cm/sec) maintained for entire cycle

U_{ii} = velocity increment (10.2 cm/sec)

t_i = time (sec) fish swam before fatigue in final cycle

t_{ii} = duration of the interval (sec)

Critical swimming speed is measured by interpolating between the beginning and end of a prescribed swimming period. Note: the velocity increment (10.2 cm/sec) is equivalent to a 10 gallon/min flow rate which is the incremental flow rate for each time interval for the 3.5 inch stamina tunnel.

10 gal/min x 1 cm^3/0.000264 gal x 1 min/60 sec = 631/313 cm^3/sec

(3.5 in dia. Tunnel x 1 cm/0.394 in) / 2 = 4.4416 cm radius

$A = pr^2$; 3.14 $(4.4416 cm)^2$ = 61.945 cm^2

631.313 cm^3/sec / 61.945 cm^2 = 10.19 or 10.2 cm/sec

(2) Body lengths per second (BL/sec): U_{crit} / total length (cm)

Body lengths per second swam provides a standard metric to compare swimming rates of fish subjected to varied environmental conditions. As an example, U_{crit} will decline as temperature departs from the fish's optimum range. Critical swimming speed also will decline as a fish grows (Beamish 1978). Thus, changes in swimming rates indicate altered physiological mechanisms. One can compare across species and amongst varied environmental factors (e.g., temperature, flow).

APPENDIX F

U.S. Fish and Wildlife Service, Dexter National Fish Hatchery and Technology Center

The culture system is situated on the North end of a 25-m by 18.5-m room within the insulated metal fish culture building. The system itself is comprised of (23) glass 14-L aquaria set side-by-side on top of (4) 1-m high tables. All aquaria faced south (slender wall of rectangular tanks). Air was supplied to each aquarium by a centrifugal blower and air stones. Water was recirculated through the system using a centrifugal water pump to push the water through the bead filter then into the aquaria. No pathogen treatment was used. Light conditions were ambient. Fluorescent lights used during the work hours (0630 – 1600) and wall-mounted windows provided natural light. Temperature (°C) was monitored and recorded throughout the study. The facility currently holds a federal endangered species permit (TE 676811-8).