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Spawning and Culture of Gila Chub

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Summary

The information needed to effectively culture imperiled native fishes for recovery efforts is lacking for certain species, yet is critical for proper management and conservation. Culture techniques and requirements are virtually unknown for Gila chub *Gila intermedia*, a species federally listed as endangered.

1. We identified methods to spawn and rear Gila chub in captivity. Our results provide the first published data on spawning and selected reproductive characteristics of larval Gila chub. Fish were brought to the laboratory in March 2003 from Sabino Creek, Arizona (12.3°C). Fish were then warmed slowly and spawned at 14.93°C, 10 d following collection. Following this initial spawning, Gila chub spawned consistently in the laboratory without hormonal, chemical, photoperiod, or drastic temperature manipulation, during all times of the year. Spawns were noted at temperatures ranging from about 15 to 26°C; however, we noted that Gila chub were more reluctant to spawn at temperatures above 24°C. Multiple spawning attempts per year per individual are likely. There was a strong, inverse relationship between time to hatch and incubation temperature. Hatch rate of eggs was high (mean = 99.43%) and larval Gila chub accepted a variety of natural and formulated diets at first feeding.

2. We investigated the effect of different feed types on growth, survival, and overt health of larval and juvenile Gila chub. Larval Gila chub fed a commercial larval fish diet grew the same or slightly better than those fed thawed *Artemia* sp. nauplii, and significantly better than those fed chicken *Gallus domesticus* egg-yolk powder, but survived significantly better when fed *Artemia*. Despite the latter, observations suggest *Artemia* nauplii may be difficult for first-feeding larval Gila chub to handle. Thawed

chironomid sp larvae clearly outperformed prepared commercial feeds for small and large juvenile Gila chub with respect to growth; however, survival was 100% for all feed treatments. Overt health of larval and juvenile Gila chub remained largely unchanged during all experiments. Our results have shown first-feeding larval Gila chub may be reared on a natural or prepared diet but we recommend larval Gila chub be fed a natural feed if survival is paramount to objectives. Based on diets tested we recommend juvenile Gila chub be fed a natural diet if faster growth is paramount to objectives. Further work is suggested to define the nutritive requirements and identify the most efficient feeding regimen for Gila chub.

3. We tested the effect of four different water temperatures on growth, survival, and overt health/appearance of larval (20, 24, 28, and 32ºC) and two sizes of juvenile (20, 23, 26, and 29ºC) Gila chub. Growth of larval Gila chub was highest at 28ºC and lowest at 32ºC, while survival of larval Gila chub was highest at 24ºC and lowest at 20ºC. Spinal deformities were common for larval Gila chub reared at 32ºC but generally rare for those reared at lower temperatures. Although growth of small (32-49 mm TL) and large (52-72 mm TL) juvenile Gila chub increased with temperature, differences were not statistically significant. Survival was 100% (one accidental mortality) and no external abnormalities were noted in any experiment testing small and large juveniles. Water temperatures from 20-28ºC appear suitable for rearing larval Gila chub, with temperatures from 24-28ºC more optimal. Water temperatures from 20-29ºC appear suitable for rearing juvenile Gila chub, with temperatures at the higher part of this range likely better for faster growth.

4. We tested the effect of three different rearing densities on growth, survival, and overt health of larval Gila chub (0.065 g/L and 38.9 fish/L, 0.540 g/L and 319.5 fish/L,

and 1.343 g/L and 795 fish/L), small juvenile Gila chub (3.618 g/L and 4.0 fish/L, 16.986 g/L and 20.1 fish/L, and 60.145 g/L and 68.3 fish/L), and large juvenile Gila chub (1.681 g/L and 0.4 fish/L, 14.346 g/L and 2.7 fish/L, and 53.942 g/L and 8.4 fish/L). Mean length and weight gain appeared inversely related to rearing density for larval and large juvenile Gila chub. Survival of larval Gila chub was significantly greater for those groups reared at low densities. Survival for juvenile Gila chub approached 100% for all density treatments. Few oddities in overt fish appearance/health were noted during the experiments and development for larval Gila chub largely followed growth rates. Our data strongly support increasing density having a negative effect on growth and survival (larval only) of Gila chub. Results may assist in formation of preliminary guidelines for initial stocking and loading densities for Gila chub, with possible relevance to other similar species.

The future of Gila chub may someday depend in part on hatchery propagation to provide specimens for restocking formerly occupied habitats and establishing refuge populations. The guidelines we present here can be used to successfully spawn and rear Gila chub.

Introduction

Gila chub *Gila intermedia* is a moderate-sized cyprinid endemic to the Gila River basin of central and southeast Arizona, southwest New Mexico, and northern Sonora, Mexico (Rinne 1976; Minckley and DeMarais 2000). Populations of Gila chub have been reduced or extirpated throughout the species range primarily due to loss and modification of aquatic habitats (Hendrickson and Minckley 1984; Vives 1990; Weedman et al. 1996) and the introduction of nonnative species (Minckley et al. 1977; Minckley and Deacon 1991; Dudley and Matter 2000). This species is currently limited to about 29 isolated streams, cienegas, and springs (USFWS 2005); only one of which contains a population that was considered stable and secure by Weedman et al. (1996). Gila chub is listed as endangered with critical habitat under the United States Endangered Species Act (USFWS 2005).

The ability to culture the Southwest's threatened native fishes for recovery efforts is lacking for certain species, yet is critical for proper management and conservation. The natural-history strategies and requirements of Gila chub are poorly understood (Weedman et al. 1996). Information on reproductive ecology of Gila chub is largely limited and qualitative. Previous observations (Ken Wintin, personal communication, Arizona-Sonora Desert Museum; Jeanette Carpenter, personal communication, U.S. Geological Survey; and personal observation) confirm that Gila chub have the ability to spawn and be maintained in non-natural conditions but culture techniques and requirements are largely unknown. The limited information available on culture techniques and general life-history of Gila chub hampers recovery of this species (Vives 1990). The future of Gila chub may someday depend in part on hatchery propagation to

provide specimens for restocking formerly occupied habitats and establishing refuge populations.

Objectives

The purpose of our study was to develop spawning and rearing techniques for Gila chub. We had four major objectives for our study.

1. Our first objective was to establish a broodstock of adult Gila chub in the laboratory, identify methods to successfully spawn Gila chub in captivity, and develop Gila chub eggs through post-hatch to the larval phase. This study was integral to the acquisition of first-feeding larval and juvenile Gila chub for other research.

2. The type of feed and feeding regimen imposed has a direct link to the development and health of captive reared animals. Comparative information on the responses and limitations of Gila chub with respect to feed type is largely unknown. Thus, our objective was to investigate the effect of different feed types on growth, survival, and overt health/appearance of Gila chub larvae and juveniles under laboratory conditions.

3. Growth is affected by many factors; however, none may be as important as water temperature (Dwyer et al. 1983). Our objective was to identify the effect of different water temperatures on growth, survival, and overt health/appearance of Gila chub larvae and juveniles under laboratory conditions.

4. The density at which fishes have been cultured influences growth. Our objective was to investigate the effect of different rearing densities on the growth, survival, and overt health/appearance of Gila chub larvae and juveniles under laboratory conditions.

Methods

Spawning and Hatching

In March 2003 we collected Gila chub from Sabino Creek, Arizona to serve as broodstock. Fish were the transported to the laboratory at the University of Arizona in aerated containers and then acclimated to laboratory temperatures. Because the temperature of Sabino Creek was 12.3°C, we cooled the laboratory to about 15°C and allowed fish to slowly warm. After their first spawn (at 14.93°C), we varied temperatures to estimate the range of temperatures at which fish Gila chub would spawn. Most spawning trials were conducted between 18-24°C. Approximate length range of adults was 110-175 mm TL and sex ratio was unknown. Groups of 5-9 adult Gila chub were maintained and spawned in rectangular glass tanks filled with treated municipal water and capacities from about 110-330 L, with a maximum density of about 0.08 chub/L. All spawning/holding and egg-incubation tanks were aerated and fitted with recirculating bio-filters that returned water to create a surface disturbance and slight flow within the tank. The main diet of adults consisted of thawed natural feeds (commercially prepared), mainly chironomid larvae. We fed adult Gila chub in slight excess twice daily at an interval of anywhere from about 6-9 hours. Adult Gila chub were observed at least twice daily and tanks checked for signs of spawning activity. We thoroughly cleaned tanks of all debris at least twice daily, which resulted in a water exchange of about 5-20% daily. Water quality was monitored daily.

We placed 11 x 11-cm glazed, beige colored ceramic tiles on the bottom of the spawning tanks each time we needed a spawn. A fairly rigid, hard plastic grating (pattern was 15 x 15-mm [open space] squares, 8 mm high and 2 mm thick) cut to fit the

dimensions of the tank sides was raised 2-4 inches off the tile substrate using 4-6 pieces of 1.27-mm diameter PVC pipe glued directly to the underside of the grating. Following spawning, tiles were removed from spawning tanks, tiles with eggs were rinsed clean of debris, and the number of eggs present on the tiles was recorded. Tiles with eggs were then placed vertically in small metal racks located in 57 L aquaria. We counted newborn larval Gila chub following hatch (usually within 24 h or less).

We used an ocular micrometer to measure diameter of spawned eggs and length (to nearest 0.1 mm) of larval Gila chub. We measured wet-weight (to nearest 0.0001 g) of Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from larval Gila chub prior to measurement. Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement.

Feed Type

We randomly assigned three size classes of Gila chub to each treatment group (feed type) and replicate tank (39-L recirculating aquarium tanks). Feed treatments for first feeding larval Gila chub (6.1-7.7 mm TL) included an enriched natural feed (frozen *Artemia* sp. nauplii, Hikari Bio-Pure Baby Brine Shrimp, Hikari, Inc.), a prepared feed (chicken *Gallus domesticus* egg-yolk powder, John Oleksy, Inc.), and a commercial larval fish diet (Hikari First-Bites, Hikari, Inc.) fed to excess four times daily (Table 1). We defined “feeding to excess” to mean that there was feed left in the tanks 15 min following a feeding. Feed treatments for small (22-29 mm TL) and large (44-68 mm TL) juvenile Gila chub included an enriched natural feed (frozen chironomid larvae, Hikari Bio-Pure, Hikari, Inc.) and the following complete commercial feeds (Hikari Micro

Pellets, Hikari, Inc.; Wardley Staple Food Flakes [small juveniles only] and Wardley Premium Shrimp Pellets Formula [large juveniles only], Hartz Mountain, Co.; Golden Pearls Weaning and Juvenile Diet, Brine Shrimp Direct, Inc.; Silver Cup, Nelson and Sons, Inc.), respectively, fed to excess three times daily (Table 1). Feedings were spaced by 2-3 hours between about 6AM and 8PM. Initial biomass of Gila chub per tank was 0.008 g/L or less for larval chub, 0.083 g/L or less for small juveniles, and 0.396 g/L or less for large juveniles. Tanks varied with laboratory temperature, which rarely deviated from 20-22ºC. Experiments ran for 14 d for Gila chub larvae and 21 d for Gila chub juveniles.

We used an ocular micrometer to measure initial length (to nearest 0.1 mm) of larval Gila chub and calipers to measure final length (to nearest 0.1 mm) of larval Gila chub. We measured length (to nearest 1 mm) of juveniles using a measuring board. We measured wet-weight (to nearest 0.0001 g) of all Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from all larval Gila chub prior to measurement. Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement. Initial larval length and weight measurements were derived from a random subsample (n = 20) acquired within 24-hr of hatching. Final larval length and weight measurements were derived from a random subsample (n = 10) of survivors for each treatment group. For large juvenile fish, we measured lengths and weights of all individual fish. For small juveniles we measured lengths of all individuals but compared batch weights of tanks for the analysis.

We used analysis of variance (ANOVA) to test for significant differences in mean weight and length gain, and percent survival of larval and juvenile Gila chub among test

temperatures. Due to limited numbers larval treatments equally included fish from different spawns. To account for this possible confounding factor, spawning origin was used as a blocking factor for analysis of larval data. If a statistically significant ($P \leq 0.05$) difference was detected in ANOVA tests we used a Tukey-Kramer HSD Multiple Comparison Procedure to identify which means differed.

Temperature

We tested the effect of water temperature on growth, survival, and overt health of Gila chub larvae and juveniles. We randomly assigned Gila chub to each of four different treatment levels (test temperatures) and three replications (tanks) per treatment level. Initial biomass of Gila chub was 0.004 g/L larval chub (6.0-7.5 mm TL), 0.19 g/L small juveniles (32-49 mm TL), and 0.49 g/L large juveniles (52-72 mm TL). Gila chub were acclimated by increasing water temperature in equally divided intervals over a five-day period until reaching the desired test temperature. Larval Gila chub were tested at 20, 24, 28, and 32ºC. Juvenile Gila chub were tested at 20, 23, 26, and 29ºC. Test temperatures were monitored daily for accuracy and adjusted when necessary. Experiments ran for 29-30 days.

Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement. Initial larval measurements were derived from a random subsample ($n = 20$) acquired within 24-hr of hatching. Final larval measurements were derived from a random subsample ($n = 10$) of survivors from each treatment group. We measured wet-weight (to nearest 0.0001 g) of all Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from all larval Gila chub

prior to measurement. We used an ocular micrometer to measure initial length (to nearest 0.1 mm) of larval Gila chub and calipers to measure final length (to nearest 0.1 mm) of larval Gila chub. We measured length (to nearest 1 mm) of juveniles using a measuring board.

Each replicate group of larval Gila chub was fed to excess four times daily using a combination of thawed *Artemia* sp. nauplii (Hikari Bio-Pure, Hikari, Inc.) and Hikari First-bites (Hikari, Inc.). Each replicate group of juvenile chub was fed to excess three times daily using a combination of unfrozen chironomid larvae and Hikari Micro-pellets (Hikari, Inc.) (small juveniles) or Silver Cup (Nelson and Sons, Inc.) (large juveniles).

We used one-way analysis of variance (ANOVA) or Welch's ANOVA test (when group variances were significantly different) to test for significant differences in mean weight and length gain, and percent survival of larval and juvenile Gila chub among test temperatures. If a statistically significant ($P \leq 0.05$) difference was detected in ANOVA tests we used a Tukey-Kramer HSD Multiple Comparison Procedure to identify which means differed. We used Pearson's chi-squared test to determine if the incidence of spinal deformity of larval Gila chub was different among test temperatures.

Density

We randomly assigned Gila chub to each of three different treatment levels (test densities) and four replications (tanks) per treatment level. Mean initial biomass and density (low, moderate, and high, respectively) of Gila chub was 0.065 g/L and 38.9 fish/L, 0.540 g/L and 319.5 fish/L, and 1.343 g/L and 795 fish/L for larval chub (6.3-6.8 mm TL); 3.618 g/L and 4.0 fish/L, 16.986 g/L and 20.1 fish/L, and 60.145 g/L and 68.3

fish/L for small juveniles (36-47 mm TL); and 1.681 g/L and 0.4 fish/L, 14.346 g/L and 2.7 fish/L, and 53.942 g/L and 8.4 fish/L for large juveniles (57-95 mm TL). All experiments were conducted within closed recirculating systems. Larval Gila chub were tested in 11 x 11 cm cylindrical, acrylic, floating pods set to contain about 0.25 L of water. Experimental pods were set within a 340-L rectangular glass tank which gravity fed water to a smaller 189-L rectangular glass tank in which water was then pumped back to the larger tank. The smaller tank was fitted with 2 recirculating bio-filters with a maximum combined filtering capacity of 3784 L/h. Pod bottoms consisted of stainless steel mesh (0.25-mm open-space). A drip system allowed each pod to receive a flow of at least 2.4 mL/s. Small juvenile Gila chub were tested in floating hard plastic pods (9.6 x 9.6 x 9.6 cm) set to contain 0.25 L water. Pods were contained within 38-L aquarium tanks. Large juvenile Gila chub were tested in 4.75-L (8.5 x 22 x 25.4 cm) sections of standard 38-L aquarium tanks. All juvenile tanks were fitted with a recirculating bio-filter with a filtering capacity of 1135 L/h. Tanks for all experiments were maintained near 24ºC. Experiments ran for 33 d for Gila chub larvae, 48 d for small juveniles, and 45 d for large juveniles.

Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement. Initial larval measurements were derived from a random subsample (n = 20) acquired within 24-hr of hatching. Final larval measurements were derived from a random subsample (n = 10) of survivors from each treatment group. We measured wet-weight (to nearest 0.0001 g) of all Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from all larval Gila chub prior to measurement. We used an ocular micrometer to measure initial length (to nearest

0.1 mm) of larval Gila chub and calipers to measure final length (to nearest 0.1 mm) of larval Gila chub. We measured length (to nearest 1 mm) of juveniles using a measuring board.

Each replicate group of larval Gila chub was fed to excess four times daily using a combination of thawed *Artemia* sp. nauplii (Hikari Bio-Pure, Hikari, Inc.) and Hikari First-Bites (Hikari, Inc.). Each replicate group of juvenile chub was fed to excess three times daily using a combination of thawed chironomid larvae and Hikari Micro Pellets (Hikari, Inc.) (small juveniles) or Silver Cup (Nelson and Sons, Inc.) (large juveniles).

We used one-way analysis of variance (ANOVA) to test for significant differences in mean weight and length gain, and percent survival, of larval and juvenile Gila chub among test temperatures. If a statistically significant ($P \leq 0.05$) difference was detected in ANOVA tests, we used a Tukey-Kramer HSD Multiple Comparison Procedure to identify which means differed.

Results

Spawning and Hatching

Gila chub taken from Sabino Creek, Arizona in March at a temperature of 12.3°C spawned at 14.93°C within 10 days of initial introduction into the lab. Gila chub consistently spawned in the laboratory thereafter without hormonal, chemical, photoperiod, or drastic temperature and substrate manipulation, during all times of the year. Spawns were noted at temperatures ranging from about 15 to 26°C; however, we noted that Gila chub were more reluctant to spawn at temperatures above 24°C. Most trials were conducted between 18-24°C and groups of Gila would usually spawn within

14 d of tanks being set up for spawning within this range.

Spawning behavior of Gila chub was observed several times in the laboratory and for those acclimated, behavior appeared little affected by observers. Before spawning, several presumed males chased what appeared to be a lone female. Presumed males were often noted to have more vivid spawning colors than females. In addition to orange/red spawning colors, strong, dark-colored lateral banding was noted on the most active fish. Nudging and possible nipping of the female posteriorly by males was noted. The actual release of gametes was often immediately preceded by a slight upward turn and then a light to violent shudder by the female, especially when against a rough surface or wedged between in-tank structures. Roughly 30 eggs were released during each act. Following the act, nearby fish, including perhaps those involved in the act, immediately began eating available eggs. Such spawning acts were repeated several times by what appeared to be the same female. Video footage taken in the laboratory confirmed the aforementioned behavior. Spawning events often lasted over an hour.

Total number of viable eggs counted following a spawn ranged from 106 to 2750 (mean = 1044; SD = 667) and egg counts had no obvious relationship to temperature at time of spawn. Mean percent of non-viable eggs counted from total following a spawn was 6.36 % (SD = 8.8). Eggs of Gila chub were demersal, adhesive, ovoid, and translucent with about the inner 80-90% of the egg a light yellow cream color and the remaining colorless. Mean diameter of fertilized eggs about 24 h after spawn was 2.16 mm (SD = 0.05). Not including spawns affected by fungal outbreaks, mean hatch rate was 99.43% (SD = 1.39). We found a strong inverse linear relationship ($r^2 = 0.88$; df = 1, 32; $P < 0.001$) between mean incubation temperature and time to hatch for the

temperature range examined (Figure 1). The regression equation for this relationship was:

Time to Hatch = 21.77 – 0.72 Mean Incubation Temperature

Mean length and weight of larval Gila chub (n = 20) within 6 h or less of hatch was 6.55 mm TL (SD = 0.12) and 1.69 mg (SD = 0.29), respectively. Larval Gila chub remained benthic upon emergence. Slight yolk present upon hatch was quickly reduced and swim-up appeared to occur within the first 48 hours. Larval Gila chub accepted several types of natural and prepared/commercial feeds upon exogenous feeding.

Feed Type

Mean length gain of larval Gila chub was significantly different (ANOVA = 6.649 df = 2, 13; P = 0.010) among feed types with the commercial feed outperforming the others (Figure 2). Mean weight gain showed a similar pattern with respect to feed types but the difference was not found to be statistically significant (ANOVA = 1.208; df = 2, 13; P = 0.330) (Figure 3). Mean percent survival of larval Gila chub was significantly different (ANOVA = 6.087 df = 2, 13; P = 0.013) among feed types with a consistently higher survival for those groups fed *Artemia* sp. nauplii (Figure 4). Few oddities in overt fish health were noted during the experiment, and development largely followed growth rates.

Mean length gain of small juvenile Gila chub was significantly different (ANOVA = 9.096 df = 4, 5; P = 0.016) among feed types with chironomid larvae strongly outperforming the remaining commercial feeds (Figure 5). As in the larval experiments, mean weight gain for small juveniles showed a similar pattern with respect

to feed types but the difference was not found to be statistically significant (ANOVA = 3.011; df = 4, 5; $P = 0.128$) (Figure 6).

Mean length and weight gain of large juvenile Gila chub was significantly different (ANOVA = 7.076 and 11.725; df = 4, 5; $P = 0.027$ and 0.009, respectively) among feed types with chironomid larvae strongly outperforming the remaining commercial feeds (Figure 7 and 8). Outside of two escapees for both small and large juvenile experiments, survival was 100% for all replicate tanks and no oddities in overt fish health were noted during either experiment.

Temperature

Mean weight and length gains of larval Gila chub were significantly different (ANOVA = 6.87 and 11.05; df = 3, 8; $P = 0.05$ and 0.03, respectively) among test temperatures. Growth of larval chub increased as temperature increased up to 28ºC but decreased markedly at 32ºC (Figure 9 and 10). Mean weight gain of larval Gila chub was significantly greater at 28ºC than 20ºC and 32ºC. There was weak evidence (ANOVA = 2.76; df = 3, 8; $P = 0.11$) that survival of larval chub differed among test temperatures, with larval chub surviving best at 24ºC (Figure 11). There was strong evidence (Chi-square = 31.11; $P < 0.001$) that spinal deformities of larval Gila chub differed among test temperatures. Spinal deformities were present in almost half (47%) of the larval chub reared at 32ºC, less common (23%) for those reared at 24ºC, and non-existent for those reared at 20ºC and 28ºC. No other overt abnormalities were noted.

Although a positive trend with increasing temperatures was sometimes apparent (Figures 12–15) there was no statistical evidence of a difference in mean weight and

length gain for small (ANOVA = 0.17 and 1.80; df = 3, 8; P = 0.91 and 0.22, respectively) or large (ANOVA = 0.47 and 0.67; df = 3, 8; P = 0.70 and 0.59, respectively) juvenile Gila chub among temperatures. Mortalities were all but non-existent (one accidental) for either juvenile size-class. All juvenile Gila chub tested appeared overtly healthy throughout the experiment.

Density

There was convincing evidence that mean length and weight gain of larval Gila chub was significantly different (ANOVA = 66.201 and 15.637; df = 2, 9; $P < 0.001$ and 0.001, respectively) among rearing densities. Mean length and weight gain deceased as rearing density increased (Figure 16 and 17). There is convincing evidence that mean percent survival of larval Gila chub was significantly different (ANOVA = 25.258; df = 2, 9; $P < 0.001$) among rearing densities with a consistently higher survival for those groups reared at a low density (Figure 18). Few oddities in overt fish appearance/health were noted during the experiment and development largely followed growth rates.

Mean length gain of small juvenile Gila chub was significantly different (ANOVA = 5.025; df = 2, 9; P = 0.034) among rearing densities being least for those reared at a high density. However, the multiple comparisons procedure used was unable to identify which treatments statistically differed (Figure 19). Mean weight gain of small juvenile Gila chub was significantly different (ANOVA = 7.418; df = 2, 9; P = 0.012) among rearing densities, being greatest for those reared at a moderate density (Figure 20). Survival was 100% for all density treatments with small juvenile Gila chub and no oddities in overt fish appearance/health were noted. There is convincing evidence that

mean length and weight gain of large juvenile Gila chub was significantly different (ANOVA = 22.241 and 88.155; df = 2, 9; $P < 0.001$, respectively) among rearing densities. Mean length and weight gain deceased as rearing density increased (Figure 21 and 22). For large juvenile Gila chub, survival and lack of oddities in fish health/appearance was at or approached 100% for all density treatments. Evidence of reproductive activity (eggs) was noted in one moderate and high density treatment tank.

Discussion

Although maximizing production is likely not a main goal in the culture of many imperiled native fishes at this time, there are distinct benefits to an efficient grow-out phase when producing fish for stocking and other efforts. Faster grow-out to a certain size allows stocking for a greater part of the year, may lower feed and labor costs, and may increase available rearing space. Where piscivores are present, stocking of larger-size individuals may be necessary to lower their loss due to predation (Marsh and Brooks 1989).

Much life-history information can be learned when spawning and culturing a species. Often this life-history information is difficult to observe in nature. Life-history information can help identify factors limiting natural and introduced populations. Former culture studies have provided vital information for many federally-listed threatened or endangered species (Johnson and Jensen 1991).

Spawning and Hatching

The highly adhesive nature of Gila chub eggs created challenges when first trying to efficiently process the eggs and develop the embryos in a timely, efficient, space-saving fashion. Preliminary efforts to remove the adhesive eggs of Gila chub and subsequently rear them were largely unsuccessful. Rakes et al. (1999) were able to remove adhesive fish eggs and incubate them. Other spawning substrates proved difficult to clean thereby leading to higher losses of eggs due to fungal outbreaks. Our described spawning set up allowed most of the spawned eggs to fall through the grating and adhere to the glazed ceramic tiles. The grating allowed for protection of the eggs from adults and the tiles provided an easily cleaned and handled system for transfer and counting. Some eggs were cannibalized prior to falling through the grating. Cannibalization of eggs may be reduced by having spawning tanks contain only a single brood pair. It is unknown how such pairing would affect spawning behavior. Debris was easily rinsed off tiles with eggs and the slick nature of the surface may have been a contributing factor. Rakes et al. (1999) used unglazed ceramic tiles to facilitate spawning in species that spawn in crevices or angled spaces behind current. If accessible to fish, an unglazed or rough tile surface may offer a more natural feel and potential spawning stimulus than glazed tiles, or allow for a stronger attachment point for eggs. However, in situations where contact is unnecessary the more easily cleaned substrate is advantageous, and Gila chub eggs strongly adhered to the slick glazed surface. The equipment needed for our spawning set-up was inexpensive and most parts could be found at a typical hardware store and easily modified to fit varying needs. Construction, maintenance, and

monitoring procedures related to our spawning system did require a good deal of labor however.

Schultz and Bonar (2006) stated reproduction of Gila chub in Bonita Creek and Cienega Creek, Arizona commenced in February, peaked at the beginning of spring, and dropped off as summer began. Additional spawning activity in the fall was suggested by some of the data. Our observations suggest that spawning of Gila chub in captivity is possible year-round. Multiple spawnings per year per individual are likely given our observations. However, it is unknown at this time what mechanism triggered Gila chub to spawn out of season within the laboratory. We first collected Gila chub broodstock from Sabino Creek, Arizona at 12.3°C and began acclimating them to laboratory conditions. Within ten days of collection these fish had spawned at 14.93°C. Because Gila chub first spawned without much of a temperature increase and readily spawned at a variety of temperatures without inducement afterwards, we cannot say that temperature manipulation is necessary to spawn Gila chub in captivity. However, temperature manipulation was helpful to spawn other similar species in captivity, including Yaqui chub *Gila purpurea* and Mohave tui chub *Gila bicolor mohavensis* (J. Kline, T. Archdeacon, and S. Bonar, University of Arizona, Unpublished Data). Minckley (1973) noted Gila chub had an extended spawning regime in a relatively constant spring-fed pond. The goal of maximizing fitness via reproductive effort and success of future progeny is central to evolutionary theory. The cost of reproductive efforts may be lessened over time within environmentally stable environments having moderate, less variable temperatures, consistent high quality food resources, consistent access to mates, and/or reduced predator threats.

Gila chub often take on brilliant orange/red colors when in a heightened reproductive state. A previous field study we participated in described reproductive colors and a subsequent rating system for Gila chub (Schultz and Bonar 2006). We found those Gila chub releasing gametes upon collection in the field ranged in spawning color from moderate to very strong. The most intensely colored Gila chub (≥ strong spawning colors) were found at daytime water temperatures from about 12-28 °C in two different streams. Spawning colors for Gila chub were noted throughout the year in the laboratory but often failed to achieve the intensity of colors in the field. Gila chub presumed to be males (due to spawning behavior and growth in the laboratory) expressed a greater intensity in spawning coloration than other captive Gila chub. This is supported by field data as males dominated the catch of Gila chub having strong and very strong spawning coloration (Schultz and Bonar 2006). Based on spawning coloration patterns, Nelson (1993) hypothesized Gila chub in Cienega Creek, Arizona greater than 75 mm could spawn. Qualitative observations in the laboratory support this claim that the Gila chub can mature quickly under intensive conditions. Although spawning coloration is undoubtedly related to the reproductive cycle it is not clear if a definitive relationship exists between intensity of spawning colors and time before spawning

Chasing behavior attributed to spawning activity of Gila chub in the wild (Bonita Creek, Arizona) was similar to that observed in the laboratory (Schultz and Bonar 2006). Minckley (1973) described similar behavior for Gila chub in a pond where large presumed females were followed by numerous smaller presumed males.

The total counts of eggs following a spawn in our study should be considered slight to moderate underestimates due to cannibalization of eggs prior to falling through

the protection grid, and any loss of eggs from tiles during transfer. In addition, heavy disturbances (e.g., cleaning activity) could arrest spawning activity and may account for occasional spawns of low magnitude. There was a marked disparity between estimates of fecundity from the enumeration of actual spawns in the laboratory and extrapolation of total ova from ovaries of sacrificed Gila chub in a related field study (Schultz and Bonar 2006) that could not be explained by size differences or partial cannibalization in the laboratory. The actual production of viable oocytes (functional fecundity) may differ from true reproductive potential due to incomplete spawning or degeneration and resorption of oocytes (Crim and Glebe 1990). In spite of the strong relationship noted between mean incubation temperature and time to hatch, measurement of time to hatch was likely biased at times as detection of a spawning occurrence or final hatch was dependent on visual observation.

Roundtail chub *Gila robusta*, a closely related but larger species, had a larger mean fertilized egg diameter and length at hatch (Muth et al. 1985) than our results for Gila chub. A formal description of Gila chub larvae was not undertaken as part of our study but given the consistency with which Gila chub will spawn in the laboratory and the proven ability to rear young to the juvenile stage, specimens needed for a larval developmental series should be possible to obtain.

The ability to domesticate and spawn adult fish of a species without inducement may greatly reduce effort and costs in production, and be deemed advantageous when the synchronicity and timing of cohorts is not a priority. Our results provide the first published data on spawning and selected reproductive characteristics of larval Gila chub. Our observations have shown that given proper care and environmental conditions, Gila

chub have the ability to spawn year-round without inducement or natural surroundings, with likely multiple spawning attempts per year per individual possible. In addition hatch rate of eggs is often high and larval Gila chub accept a variety of natural and formulated feed types at first feeding.

Feed Type

It is not uncommon for natural feeds to outperform prepared/commercial feeds with respect to growth (Barrows and Hardy 2001). Larval stages of many species of fishes grow and survive better on natural feed (Mischke et al. 2001; Mohler et al. 2000; Bardi et al. 1998). While survival of larval Gila chub was greater for those fed a natural feed, growth was comparable to slightly better for those fed the commercial diet. Mischke (2001) had similar results for larval bluegill *Lepomis macrochirus*. It is possible that some *Artemia* nauplii are too large for first-feeding larval Gila chub to handle which may account for it not outperforming the commercial diet with respect to growth. We observed several unsuccessful feeding attempts of larval Gila chub before they found an *Artemia* they could ingest. Alternative feeds that are smaller or co-feeding (Rosenlund et al. 1997) may prove necessary to optimize growth and survival of first-feeding larval Gila chub.

Although differences in growth of juvenile Gila chub among natural and commercial diets were obvious, we did not identify a commercial feed that consistently outperformed other commercial feeds. A more lengthy experiment may be needed to reveal differences among prepared/commercial feed types.

Prior to our feeding experiments we discovered larval Gila chub would consume thawed *Artemia* nauplii with similar enthusiasm to live *Artemia* nauplii. It is unknown if live or thawed *Artemia* affect growth of Gila chub differently. Mohler et al. (2000) found Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* fed thawed *Artemia* nauplii grew slower than, but had similar survival to, those fed live *Artemia*. We noted that thawed *Artemia* drifted similarly to live *Artemia* when a slight flow was present in tanks. The use of frozen natural feeds produced off site meant that *Artemia* was readily available, and we did not have to culture *Artemia* on site, which is labor intensive. While an economic evaluation was not included in our study, it is likely commercially available frozen natural feeds are more costly per nutritive value than most prepared/commercial feeds. Maximum survival and health of larval cohorts is often valued over short-term cost disadvantages and may be more pronounced for imperiled species such as Gila chub. While growth was comparable to slightly less for larval Gila chub fed a natural rather than a commercial larval fish diet, survival was significantly higher for larval chub fed the natural diet. Both growth and survival may have been increased for larval Gila chub had a smaller natural feed been given for the first few days of exogenous feeding.

Although comparing growth to specific nutritive properties of diets was not central to our design, trends in growth with respect to nutritive differences among feed types (e.g., protein) were unclear. Our study provides initial guidelines for the feeding of larval and juvenile Gila chub. Further studies will be needed to identify proximate compositions of diet that will optimize the growth, survival, and health of Gila chub.

In summary our investigation demonstrated that larval Gila chub survived significantly better, but grew comparably to slightly less, when fed a natural diet (i.e.,

Artemia nauplii) versus a commercial larval fish diet and chicken egg-yolk powder. However, further investigation of the efficiency upon which first-feeding larval Gila chub handle and ingest *Artemia* nauplii is warranted given observations made. It appears prepared or commercial feeds can be used to rear larval Gila chub but longer-term growth, survival, and health was not studied. Juvenile Gila chub clearly grew better when fed a natural diet (i.e., chironomid larvae) versus any of the commercial diets we tested. However, survival and overt health was similar for both commercial and natural diets. Based on feeds tested, we recommend larval Gila chub be fed a natural diet if survival is paramount to objectives. Based on feeds tested, we recommend juvenile Gila chub be fed a natural diet if faster growth is paramount to objectives. Further work is suggested to define the nutritive requirements and identify the most efficient feeding regimen for Gila chub.

Temperature

Of the temperatures we tested, optimal temperature for larval Gila chub growth was 28ºC, while optimal temperature for their survival was 24ºC. Juvenile Gila chub seemed to grow best between 26–29ºC but statistical differences were not apparent. A statistically significant difference in growth among test temperatures for juveniles may have been revealed employing a more lengthy experiment, a wider range of test temperatures, or more replicates for a more powerful test. The temperature at which highest growth rate occurs is probably optimal for most physiological processes (Harrelson 1988). However, factors independent of growth can shape criteria when

determining optimal culture temperature. Diseases, and resistance to certain diseases, can vary with temperature (Harrelson 1988) and are always a concern.

Higher growth as temperatures approach an optimum is well known and likely related to an increase in food intake, metabolism, and nutrient absorption, as well as other factors (Brett 1979; Harrelson et al. 1988; Kroll et al. 1992; Jobling and Baardvik 1994; Koskela et al. 1997; Deng et al. 2002). Our tests were conducted under relatively well-controlled laboratory conditions. Study of growth and other factors under more variable conditions, such as outdoor ponds, is needed for Gila chub. Growth rates can be greater in a cyclic than a static temperature regime (Harrelson 1988).

Although other factors are certainly involved, certain rearing temperatures may result in an increase in size variability (Britz and Hecht 1987), which may promote cannibalism of siblings in certain species (Coutant and DeAngelis 1983). We found no pattern with regard to size variability and range within temperature groups but significant differential growth of a certain individuals within a cohort of Gila chub over time has been noted repeatedly in our laboratory.

Based on the parameters of our study, for optimal growth and survival we recommend larval and juvenile Gila chub be reared from 24-28ºC and 23-29ºC, respectively.

Density

Our data strongly supported that rearing density affected growth of larval and large juvenile Gila chub. The relationship of density to small juvenile growth was less clear. Mean length gain of small juvenile Gila chub decreased as density increased;

however we cannot explain why there was not the same relationship was not present with regard to weight gain. The inverse relationship between rearing density and growth of larvae and juveniles shown in our study has been noted for other species of fishes (Rahman 2005; Sahoo et al. 2004; Anderson et al. 2002; Jodun et al. 2002; Irwin et al. 1999).

Larval Gila chub survived better at low rearing densities. This has been noted for other fishes as well (Sahoo et al. 2004; Alvarez-Gonzalez 2001). We found little effect of rearing densities we tested on survival of either small or large juvenile Gila chub over the course of our experiment. Anderson et al. (2002) found no effect of rearing density on the survival of juvenile bluegill *Lepomis macrochirus* in a longer study. The low percentage of mortalities (other than accidental) noted during our experiments with juvenile Gila chub took place in high density treatments. In addition, high density treatments for large juveniles actually caused a loss in weight over a 45-d period. Thus, it is possible the mechanism(s) affecting growth of Gila chub in high density treatments may have eventually led to a significant increase in mortality rates during a more lengthy experiment.

Irwin et al. (1999) correctly mention relationships between density and growth may not always be uniformly negatively or positively linear, and that a threshold level may exist for certain species. Our study was conducted at three broadly separated rearing densities and it is unknown how growth and survival of Gila chub between these ranges would be influenced and what type of relationships exist therein. It is unknown by what mechanism(s) rearing density effects the growth and survival of larval and juvenile Gila

chub as observations of social interactions, individual behaviors, and physiological measurements were not conducted, or were limited, during our study.

As referred to prior, the effect of rearing density upon Gila chub is undoubtedly influenced by surrounding factors. The effect of density upon Gila chub in more natural and/or variable conditions such as outdoor ponds will likely vary from our results. The probable interactive effects between density and vital factors such as feeding regime, temperature, and water quality, warrants study. Furthermore, our results are for closed recirculating systems, other types of systems may affect growth patterns in relation to rearing densities differently. Given the increasing limitations on space, water use, and funding often encountered by hatchery managers, recircualting systems may become more prevalent in the future.

Our results provide the first published data on the effects of certain rearing densities upon growth and survival of Gila chub. These results may assist in formation of preliminary guidelines for initial stocking and loading densities for Gila chub, with possible relevance to other similar species. Recommended initial stocking densities for Gila chub are dependent upon management objectives. However, based on the densities we tested, if growth and/or survival of larval Gila chub are desired over other considerations we recommend initial stocking density stay near 39 fish/L. For juvenile Gila chub all densities tested appear acceptable (at least in the short term) with regard to survival. If maximizing growth rate of juvenile Gila chub versus initial stocking density is important we recommend approximately 16.986 g/L for small juveniles and approximately 1.681 g/L for large juveniles be used. Further research is needed to further define the relationship(s) and any thresholds between rearing density and growth/survival

for early life stages of Gila chub and will undoubtedly affect recommendations. We recommend further research for closed recirculating systems concentrate on testing densities within the range of the low to moderate treatment levels employed during our study.

The future of Gila chub may someday depend on culture of the species. The increasing prevalence and importance of culturing imperiled fish species as a conservation and management strategy (Johnson and Jensen 1991; Modde et al. 1995) is a regrettable reality. Nonetheless it can be a powerful tool when needing stock to repatriate extirpated populations or establish refuge populations. Culture techniques can also be used to perpetuate a species during a crisis. Lack of such knowledge has led to the extinction of certain species (Minckley and Deacon 1991).

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FIGURE 1.−Time to hatch plotted against mean incubation temperature (with linear regression fit) for larval Gila chub *Gila intermedia*.

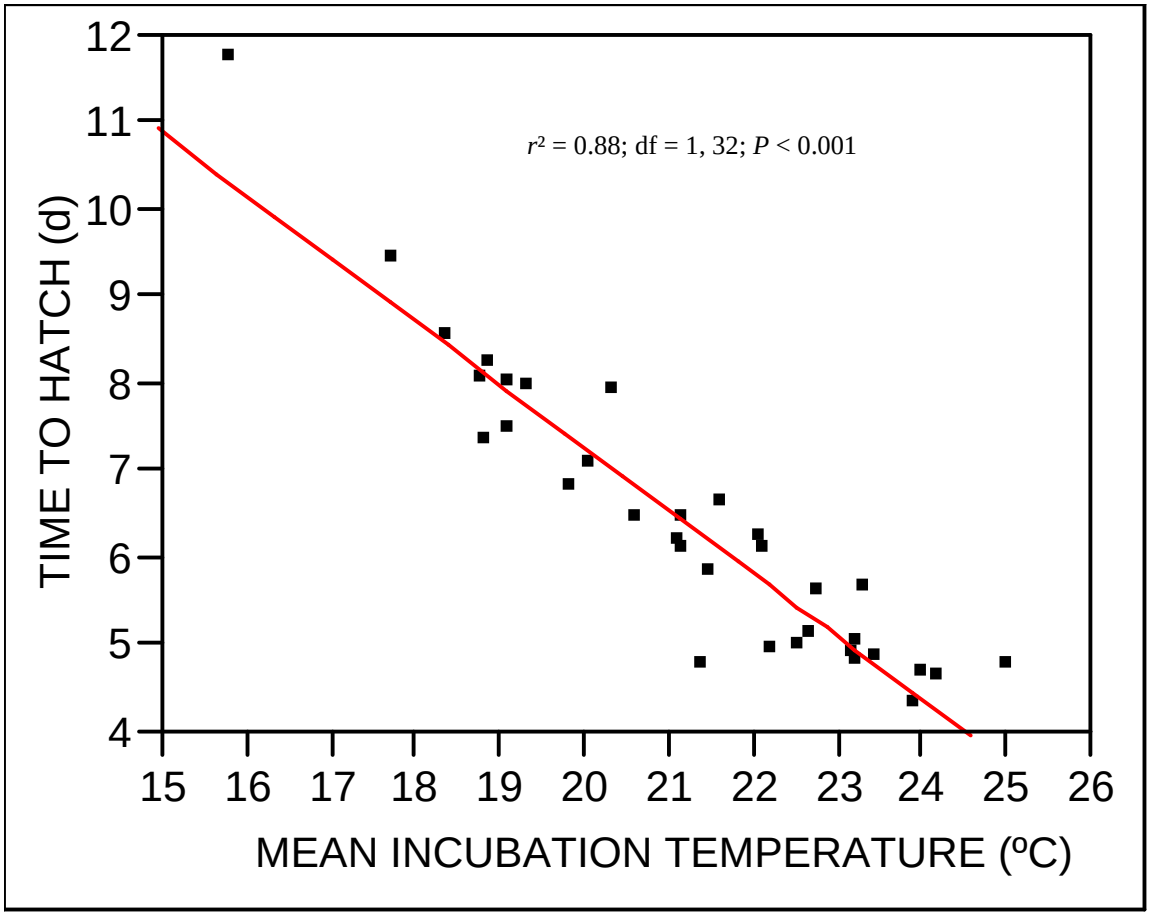

FIGURE 2. − Mean length gain (with standard error of the mean) per feed type for larval Gila chub *Gila intermedia*. Feed types include thawed *Artemia* sp. nauplii (Hikari Bio-Pure Baby Brine Shrimp), chicken *Gallus domesticus* egg-yolk powder, and a commercial larval fish diet (Hikari First-Bites). Feed types not connected by the same letter are significantly different ($P \leq 0.05$).

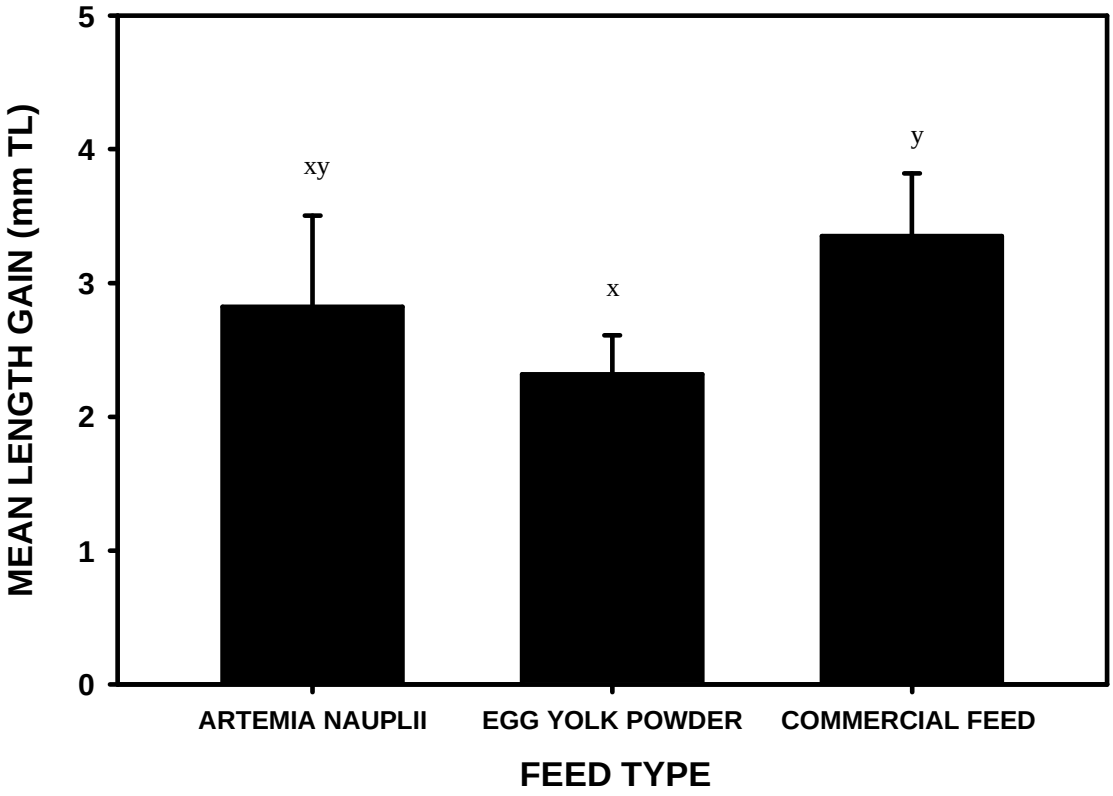

FIGURE 3. − Mean weight gain (with standard error of the mean) per feed type for larval Gila chub *Gila intermedia*. Feed types include thawed *Artemia* sp. nauplii (Hikari Bio-Pure Baby Brine Shrimp), chicken *Gallus domesticus* egg-yolk powder, and a commercial larval fish diet (Hikari First-Bites).

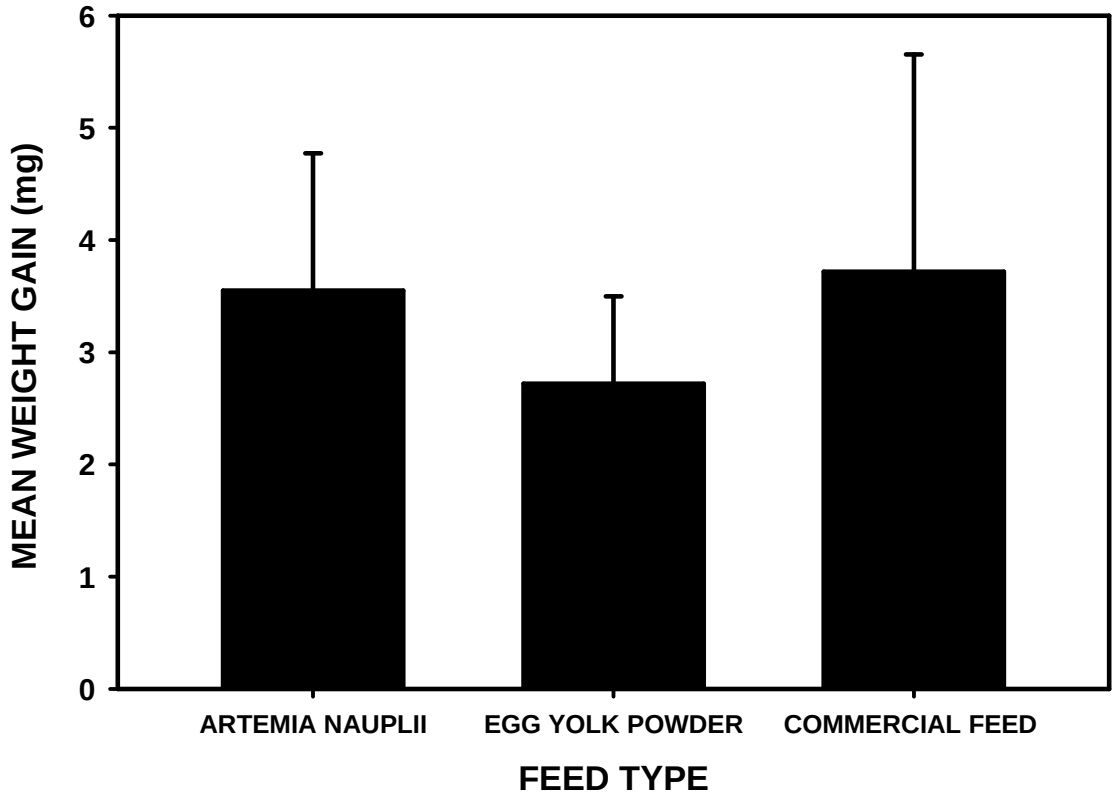

FIGURE 4. − Mean percent survival (with standard error of the mean) per feed type for larval Gila chub *Gila intermedia*. Feed types include thawed *Artemia* sp. nauplii (Hikari Bio-Pure Baby Brine Shrimp), chicken *Gallus domesticus* egg-yolk powder, and a commercial larval fish diet (Hikari First-Bites). Feed types not connected by the same letter are significantly different ($P \leq 0.05$).

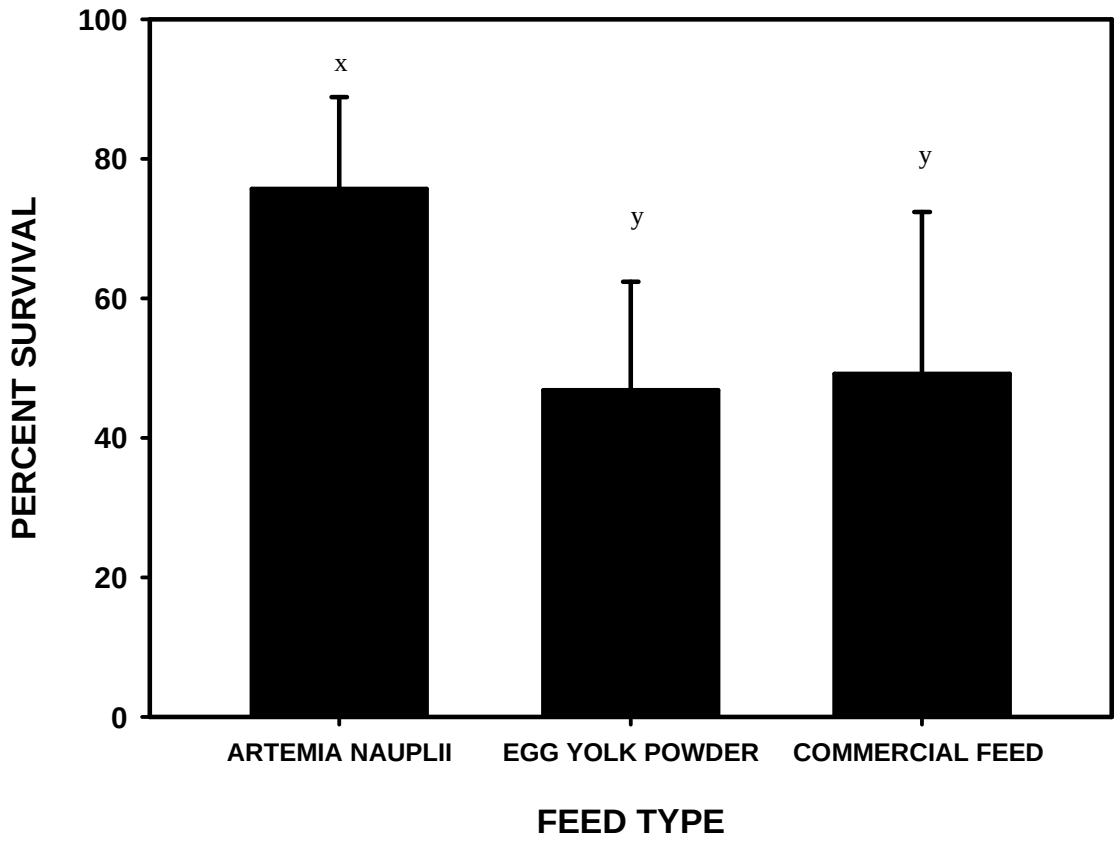

FIGURE 5. − Mean length gain (with standard error of the mean) per feed type for small juvenile Gila chub *Gila intermedia*. Feed types include thawed chironomid larvae (Hikari Bio-Pure Blood Worms) and four commercial feeds (Wardley Staple Food Flakes [Feed 1], Golden Pearls Weaning Diet [Feed 2], Hikari Micro Pellets [Feed 3], and Silver Cup [Feed 4]). Feed types not connected by the same letter are significantly different ($P \leq 0.05$).

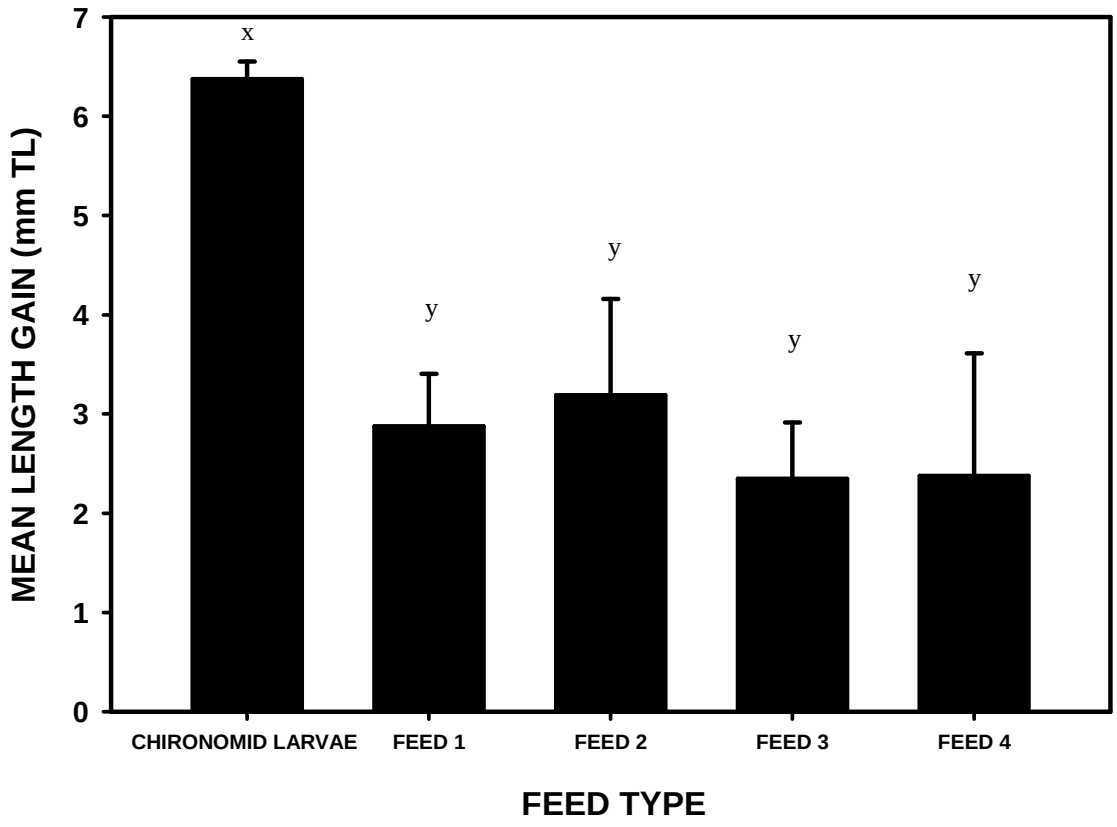

FIGURE 6. − Mean weight gain (with standard error of the mean) per feed type for small juvenile Gila chub *Gila intermedia*. Feed types include thawed chironomid larvae (Hikari Bio-Pure Blood Worms) and four commercial feeds (Wardley Staple Food Flakes [Feed 1], Golden Pearls Weaning Diet [Feed 2], Hikari Micro Pellets [Feed 3], and Silver Cup [Feed 4]).

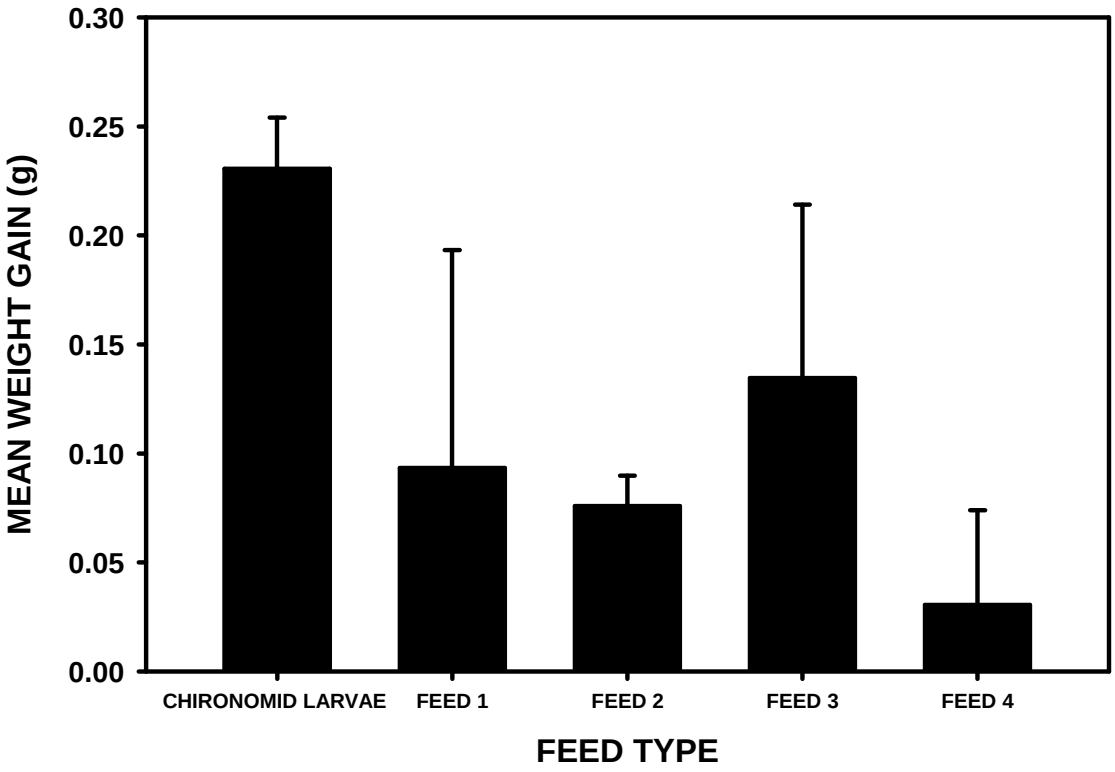

FIGURE 7. − Mean length gain (with standard error of the mean) per feed type for large juvenile Gila chub *Gila intermedia*. Feed types include thawed chironomid larvae (Hikari Bio-Pure Blood Worms) and four commercial feeds (Golden Pearls Weaning and Juvenile Diet [Feed 1], Hikari Micro Pellets [Feed 2], Wardley Premium Shrimp Pellets [Feed 3], and Silver Cup [Feed 4]). Feed types not connected by the same letter are significantly different ($P \leq 0.05$).

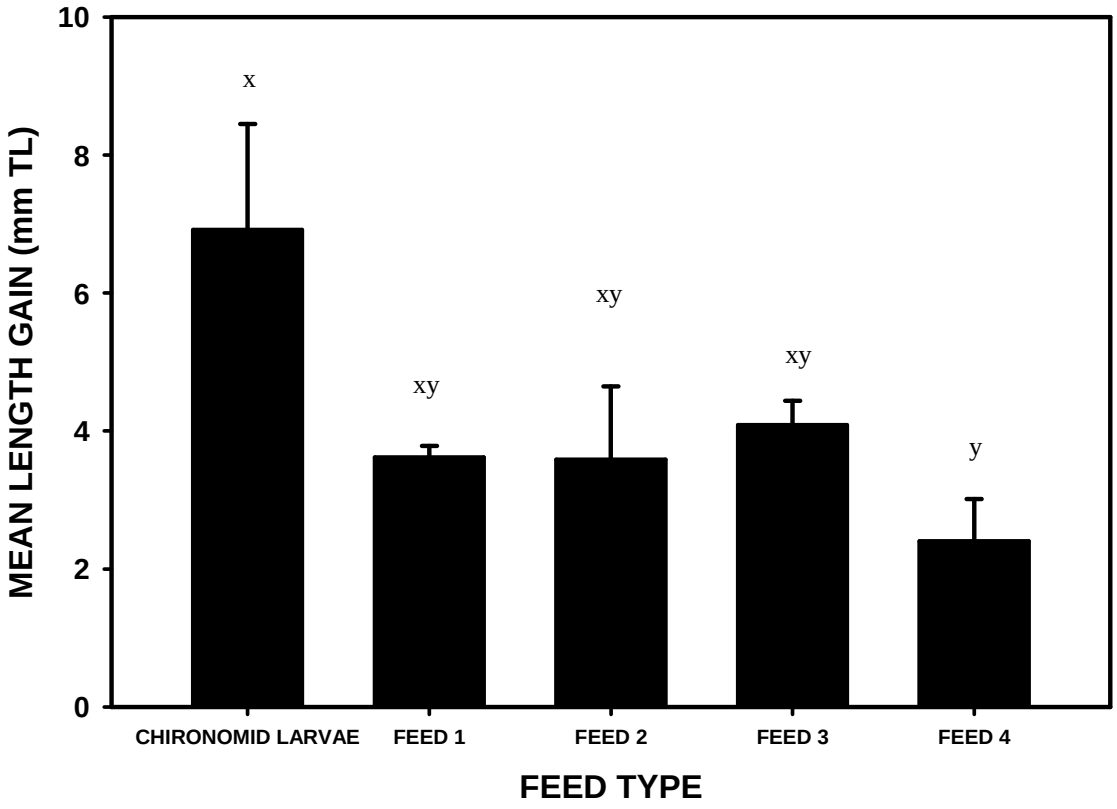

FIGURE 8. − Mean weight gain (with standard error of the mean) per feed type for large juvenile Gila chub *Gila intermedia*. Feed types include thawed chironomid larvae (Hikari Bio-Pure Blood Worms) and four commercial feeds (Golden Pearls Weaning and Juvenile Diet [Feed 1], Hikari Micro Pellets [Feed 2], Wardley Premium Shrimp Pellets [Feed 3], and Silver Cup [Feed 4]). Feed types not connected by the same letter are significantly different ($P \leq 0.05$).

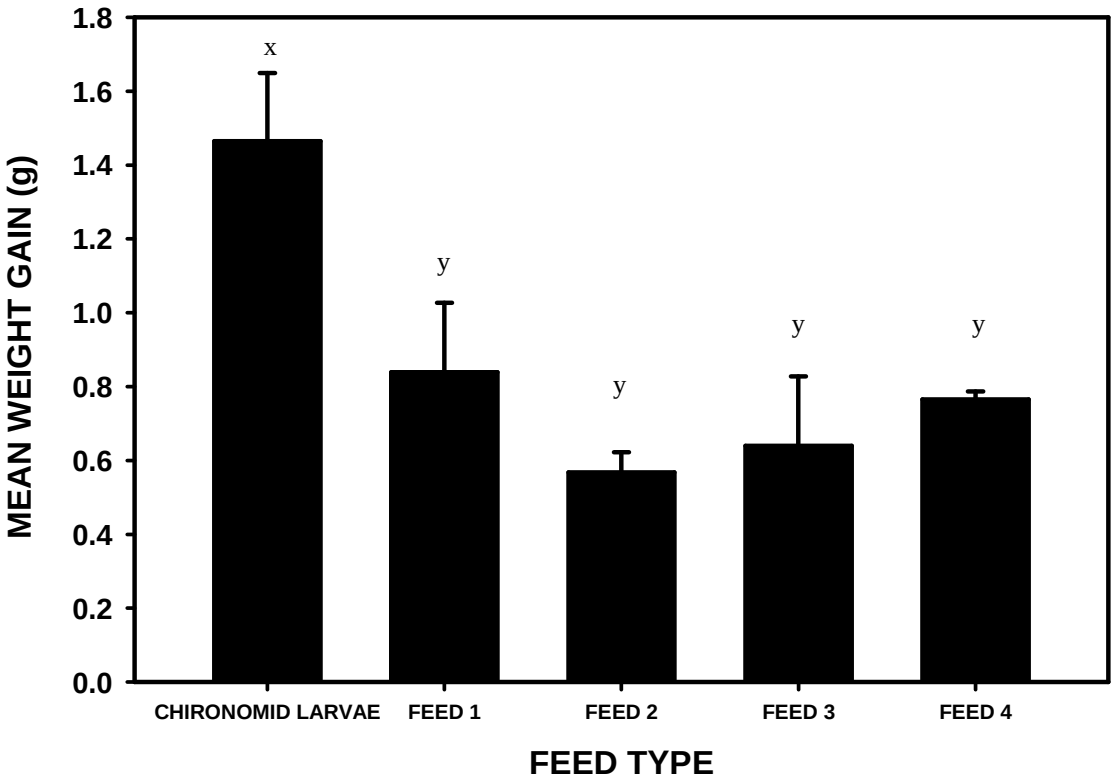

FIGURE 9.−Mean weight gain (with standard error of the mean) per test temperature for larval Gila chub *Gila intermedia*.

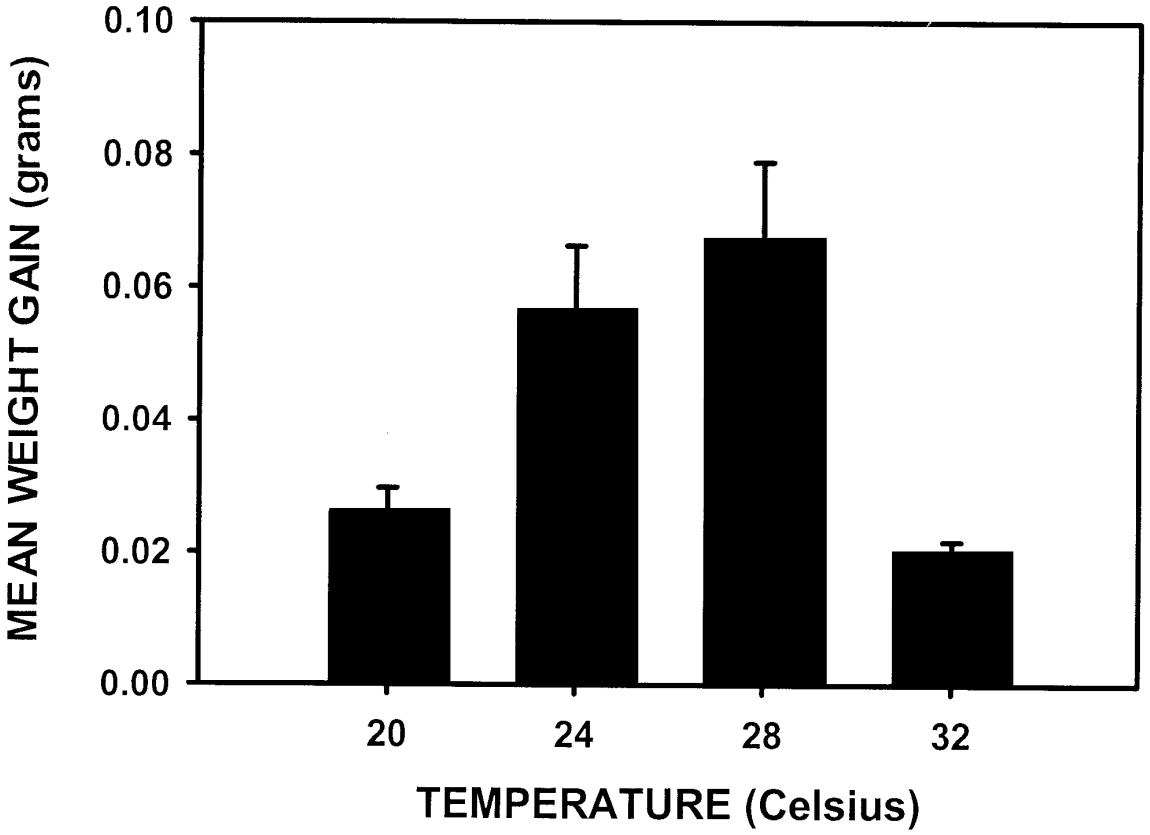

FIGURE 10.−Mean length gain (with standard error of the mean) per test temperature for larval Gila chub *Gila intermedia*.

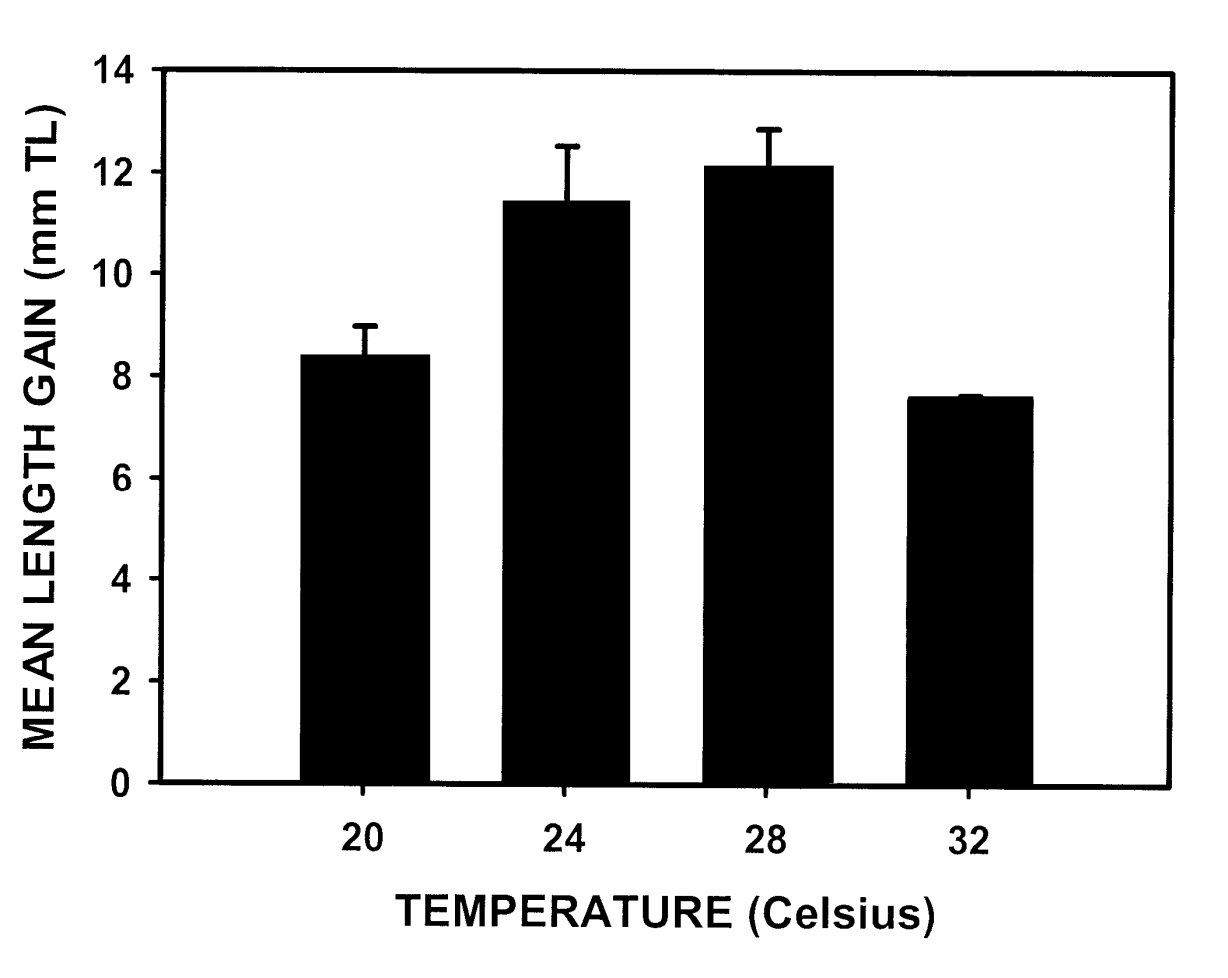

FIGURE 11.−Mean percent survival (with standard error of the mean) per test temperature for larval Gila chub *Gila intermedia*.

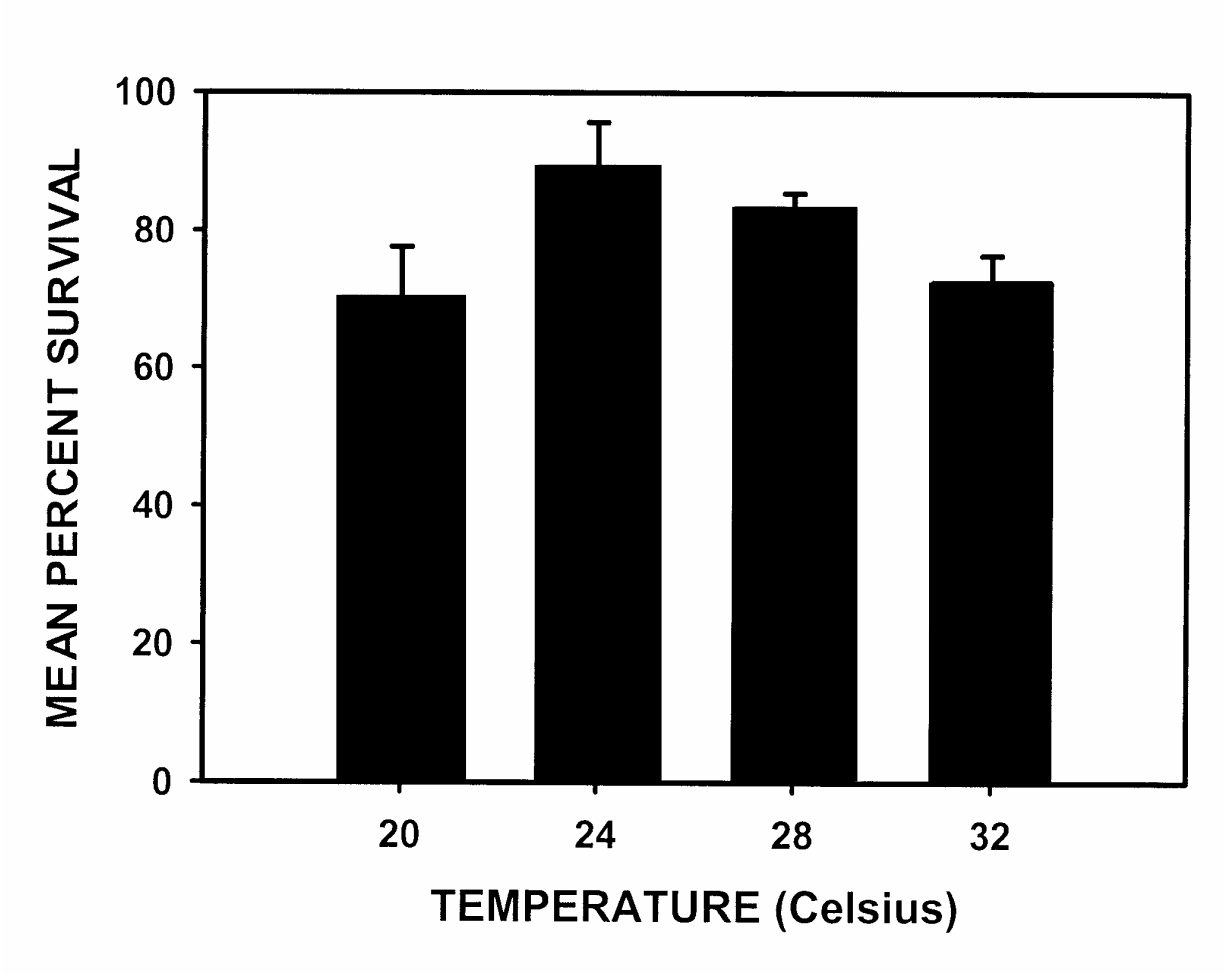

FIGURE 12.−Mean weight gain (with standard error of the mean) per test temperature for small juvenile Gila chub *Gila intermedia*.

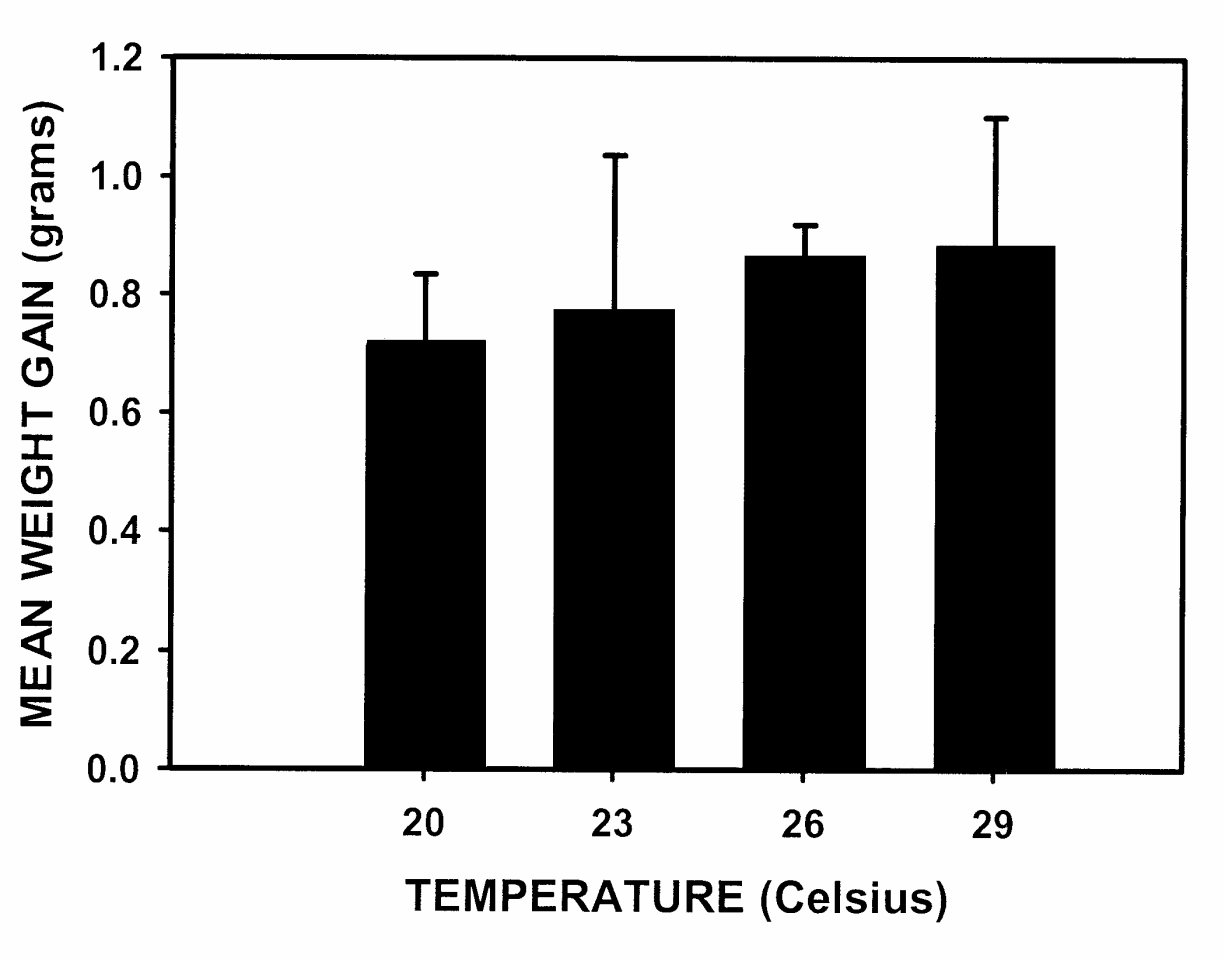

FIGURE 13.−Mean length gain (with standard error of the mean) per test temperature for small juvenile Gila chub *Gila intermedia*.

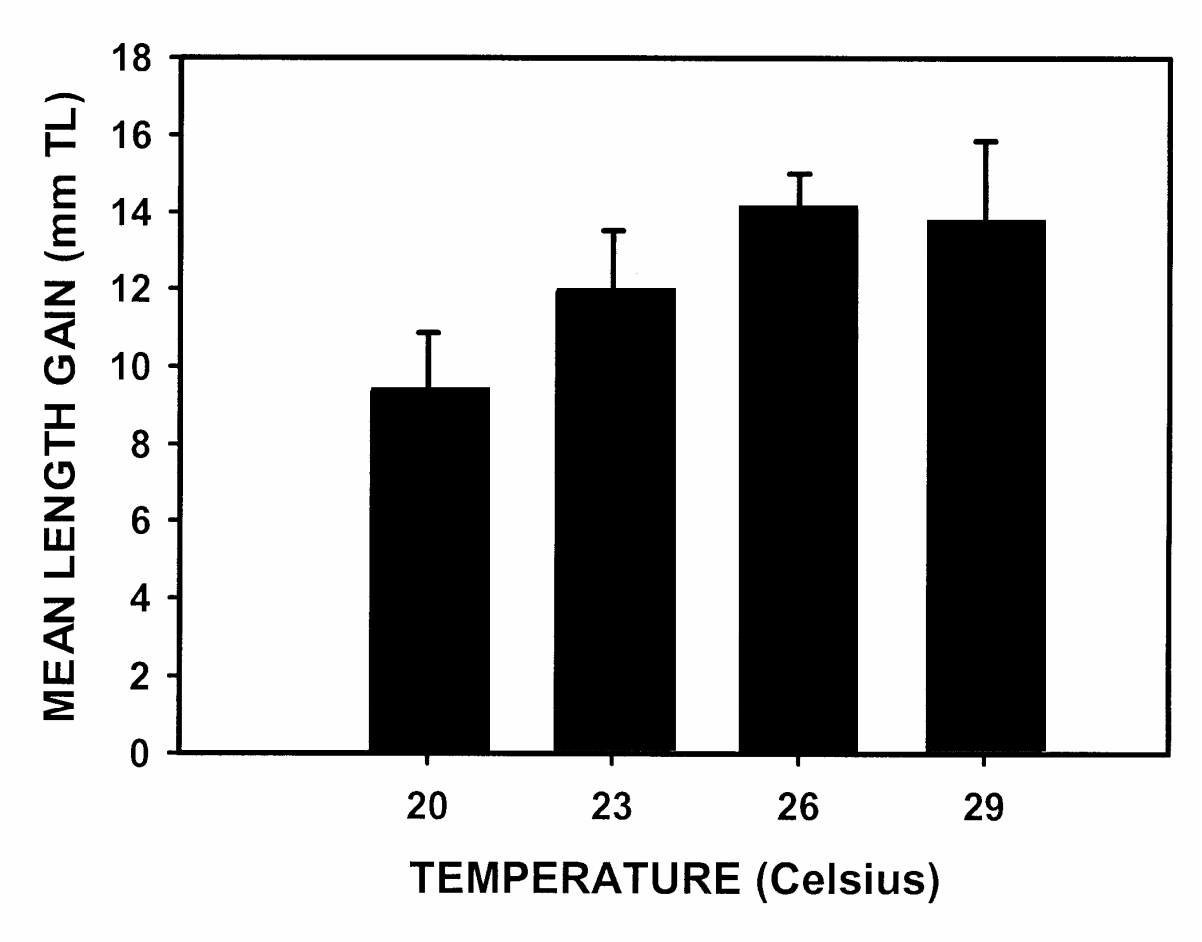

FIGURE 14.−Mean weight gain (with standard error of the mean) per test temperature for large juvenile Gila chub *Gila intermedia*.

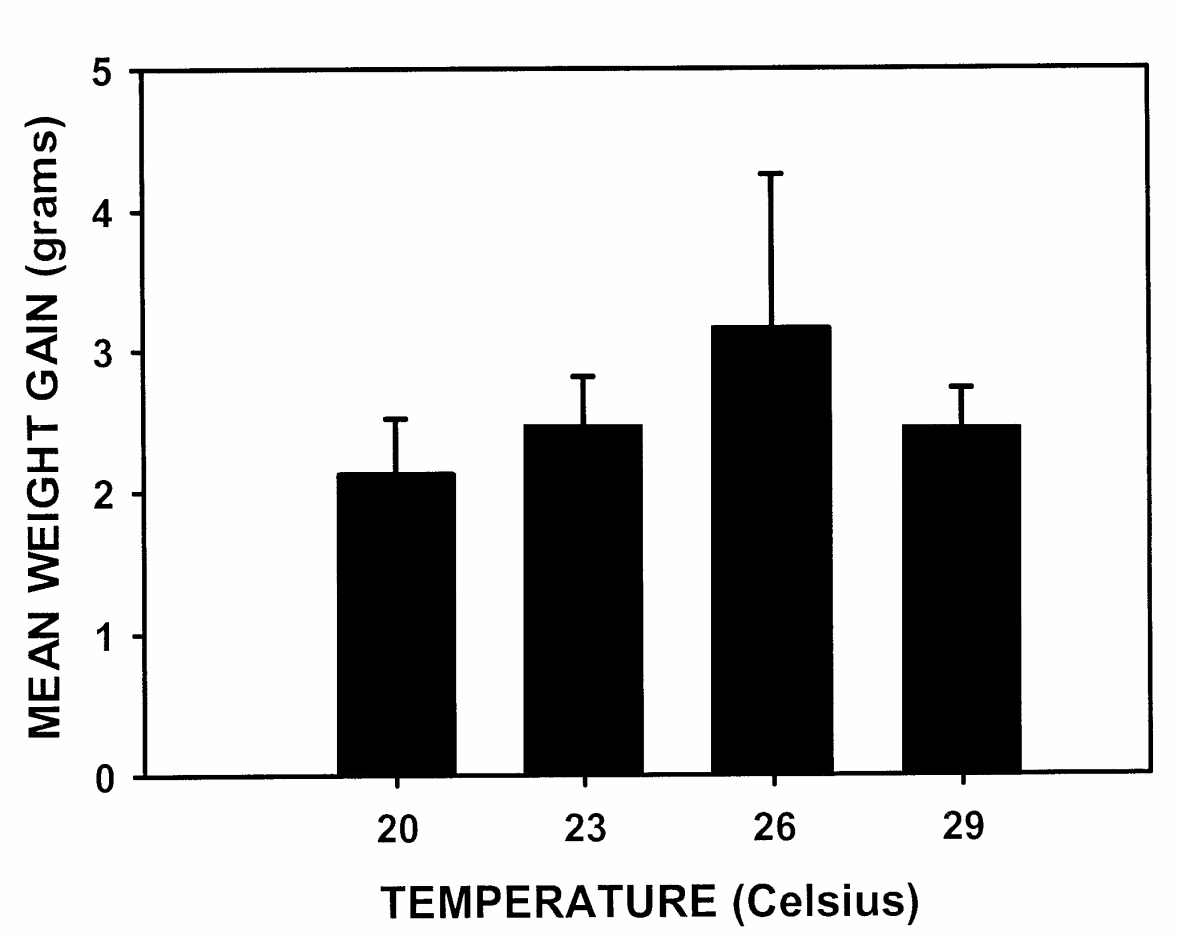

FIGURE 15.−Mean length gain (with standard error of the mean) per test temperature for large juvenile Gila chub *Gila intermedia*.

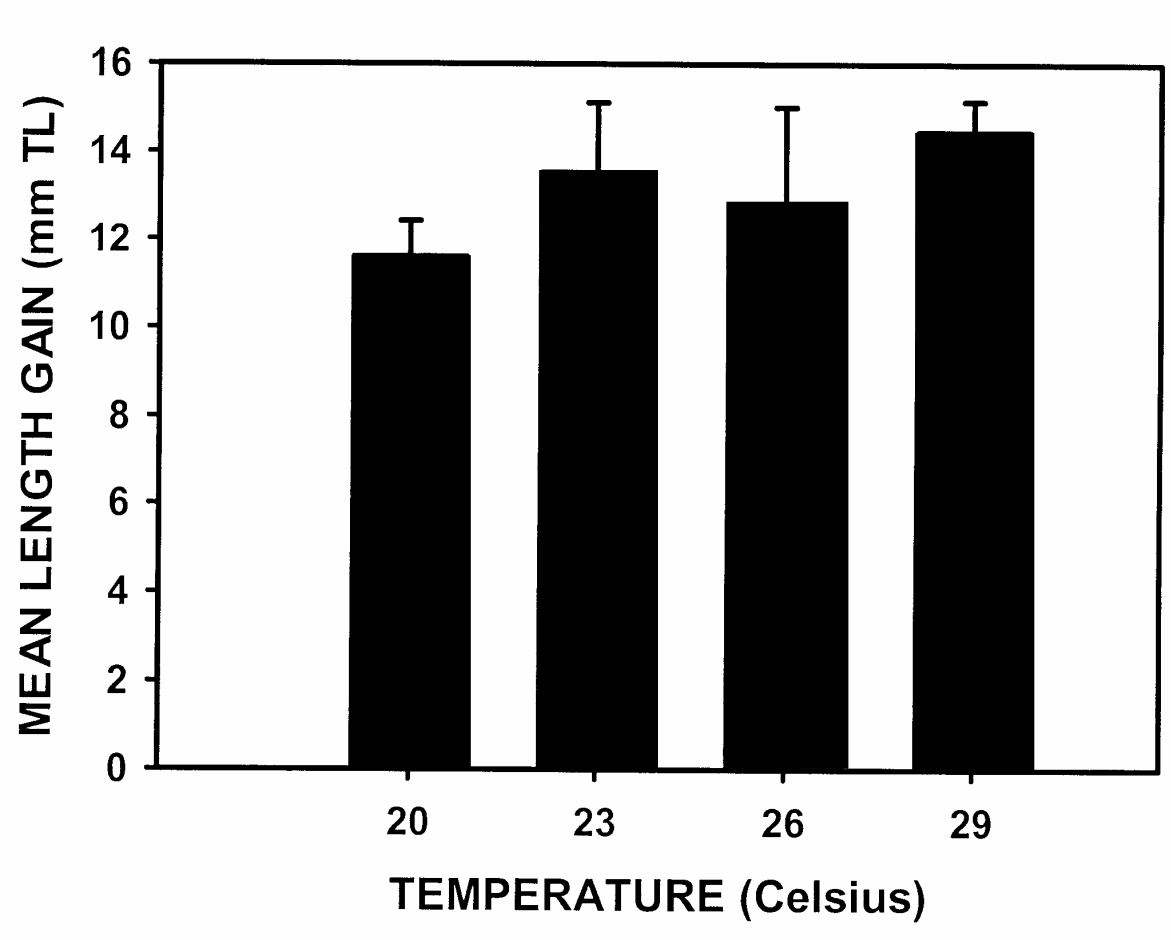

FIGURE 16.−Mean length gain (with standard error of the mean) per rearing density for larval Gila chub *Gila intermedia*. Density levels not connected by the same letter are significantly different ($P \leq 0.05$).

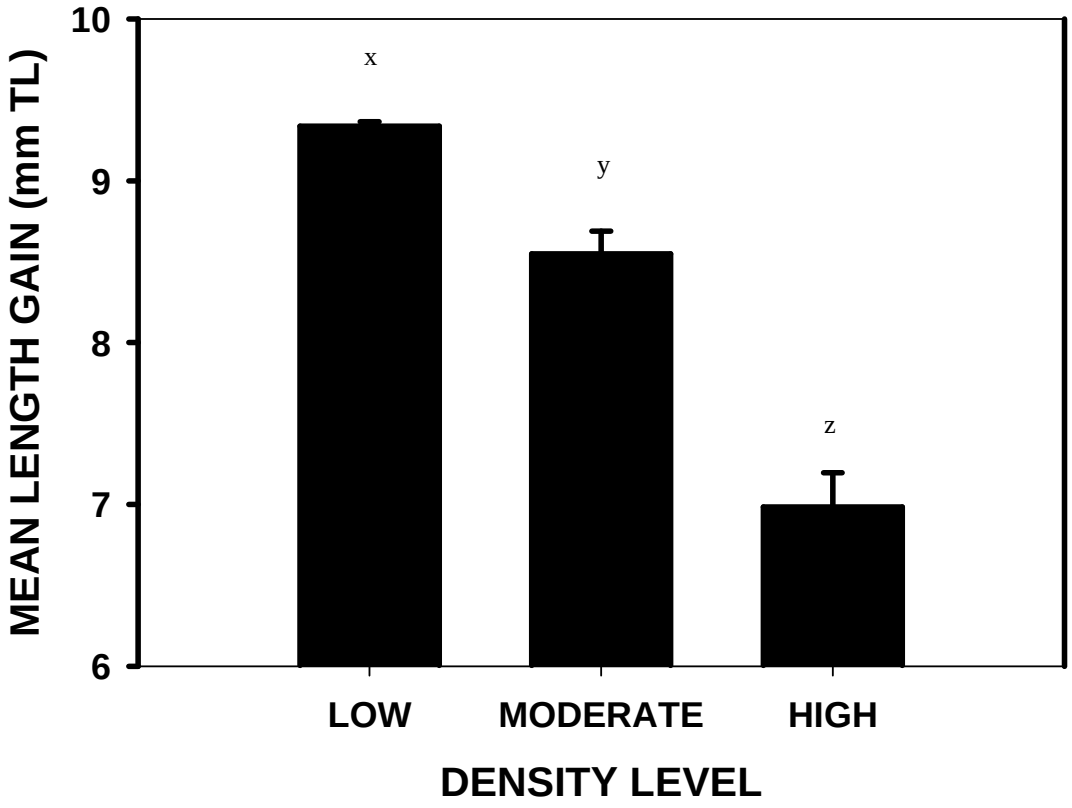

FIGURE 17.−Mean weight gain (with standard error of the mean) per rearing density for larval Gila chub *Gila intermedia*. Density levels not connected by the same letter are significantly different ($P \leq 0.05$).

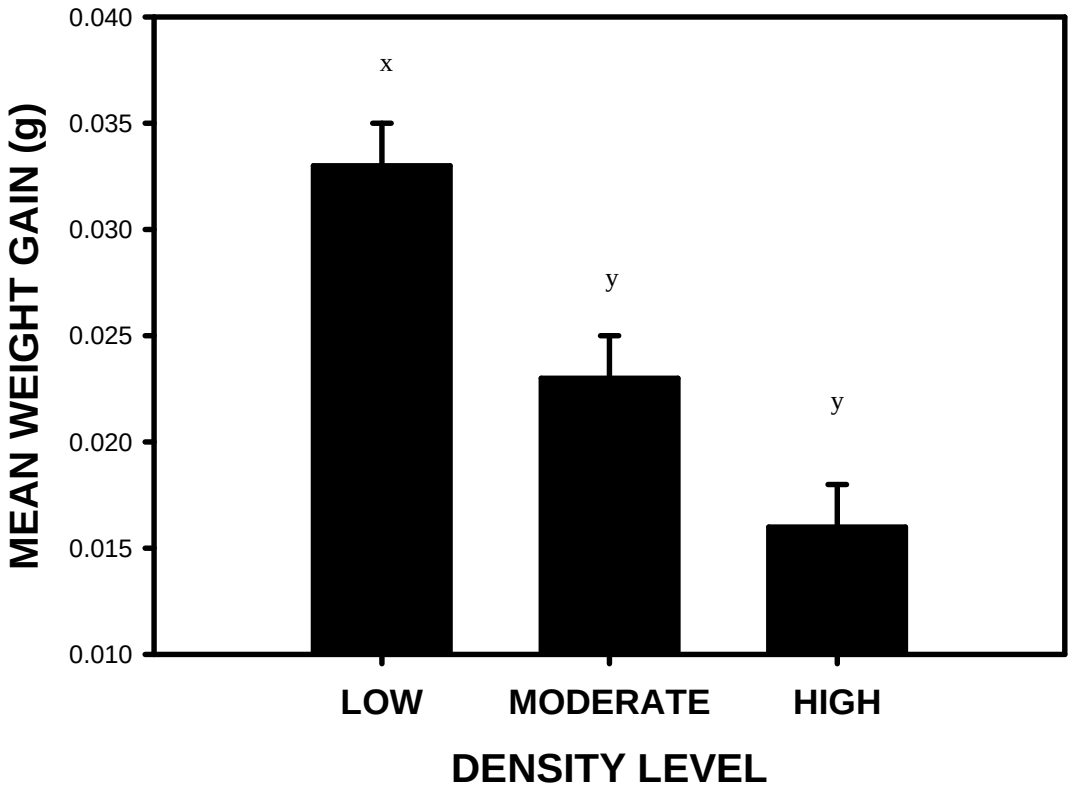

FIGURE 18.−Mean percent survival (with standard error of the mean) per rearing density for larval Gila chub *Gila intermedia*. Density levels not connected by the same letter are significantly different ($P \leq 0.05$).

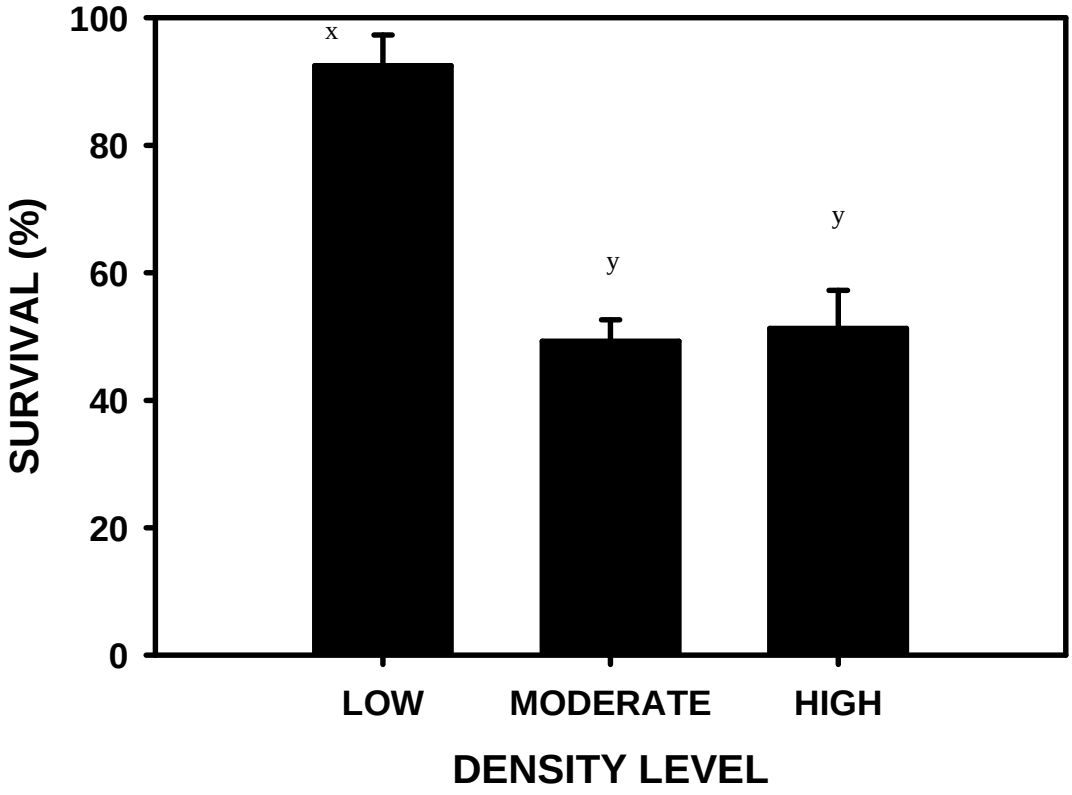

FIGURE 19.−Mean length gain (with standard error of the mean) per rearing density for small juvenile Gila chub *Gila intermedia*.

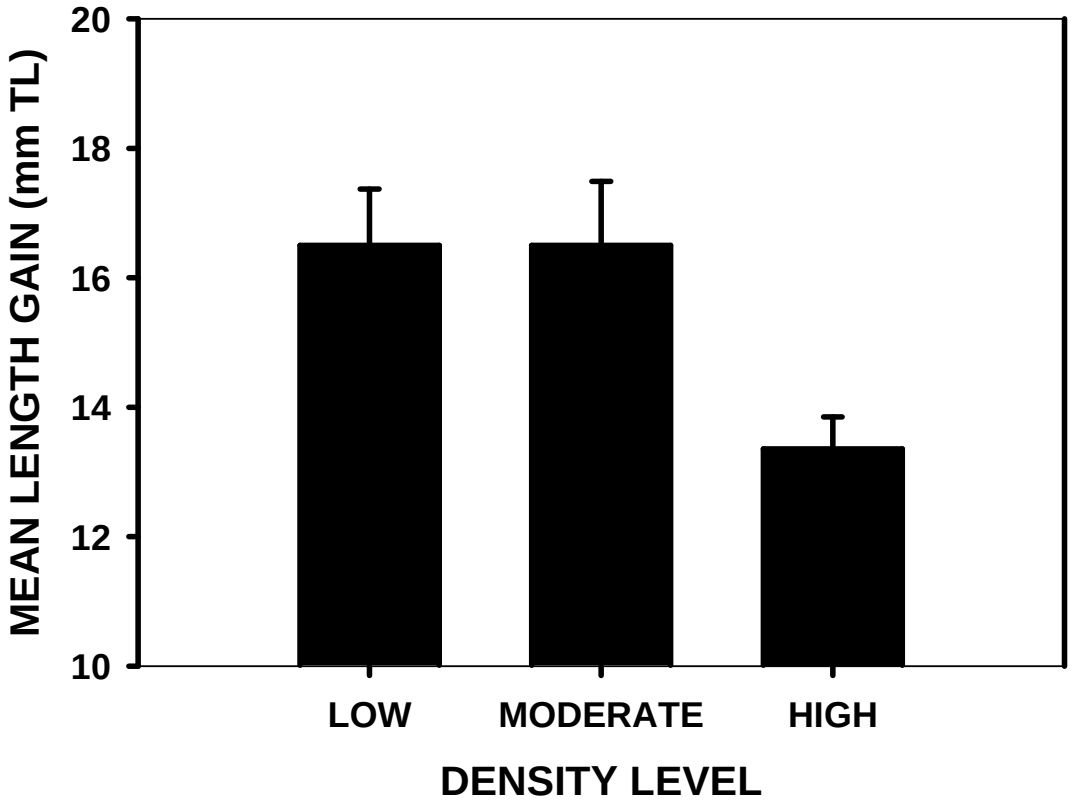

FIGURE 20.−Mean weight gain (with standard error of the mean) per rearing density for small juvenile Gila chub *Gila intermedia*. Density levels not connected by the same letter are significantly different ($P \leq 0.05$).

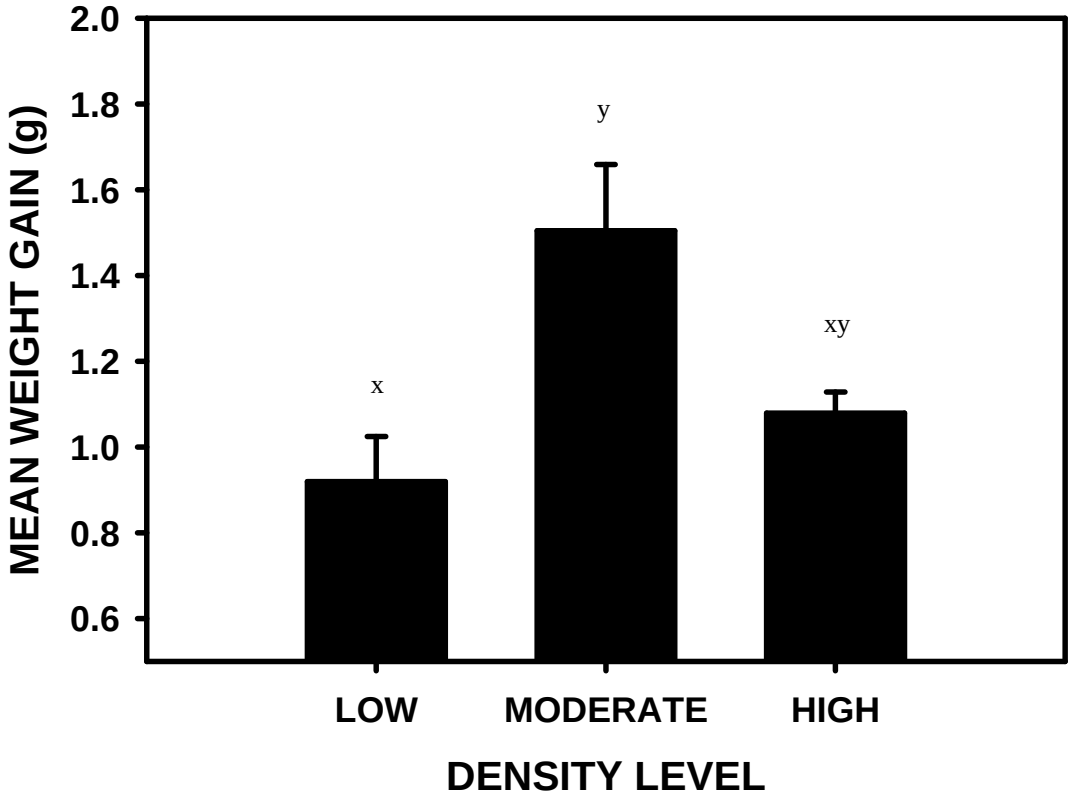

FIGURE 21.−Mean length gain (with standard error of the mean) per rearing density for large juvenile Gila chub *Gila intermedia*. Density levels not connected by the same letter are significantly different ($P \leq 0.05$).

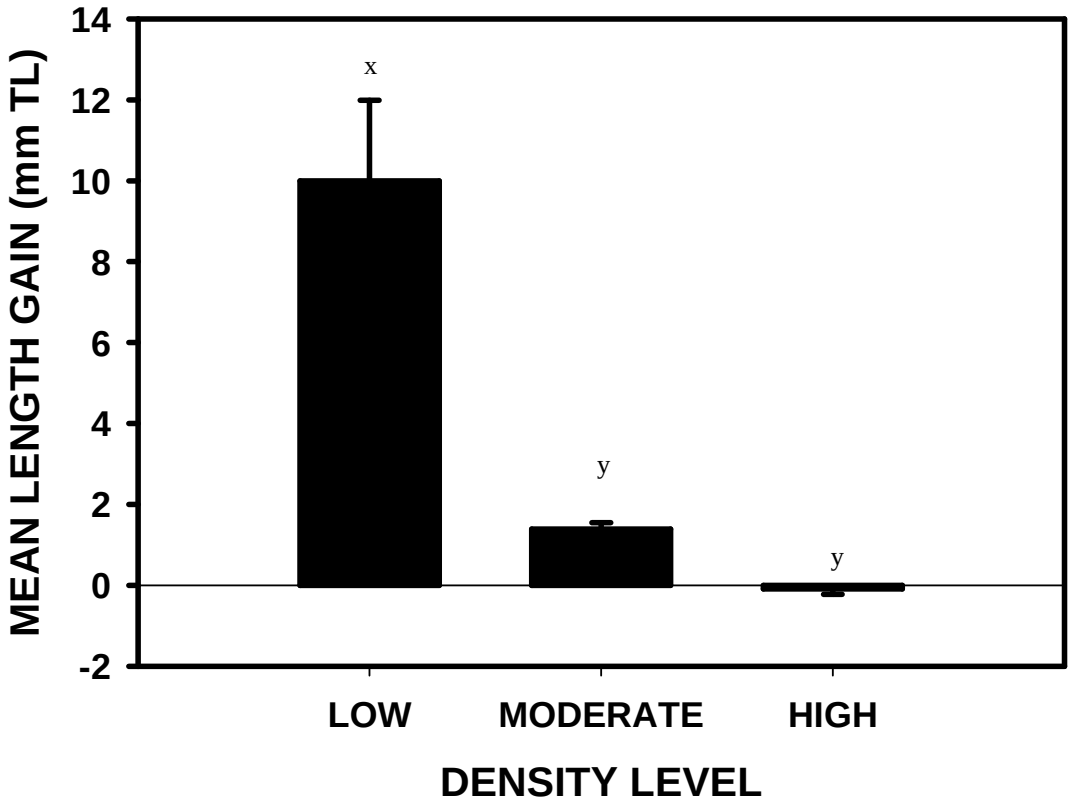

FIGURE 22.−Mean weight gain (with standard error of the mean) per rearing density for large juvenile Gila chub *Gila intermedia*. Density levels not connected by the same letter are significantly different ($P \leq 0.05$).

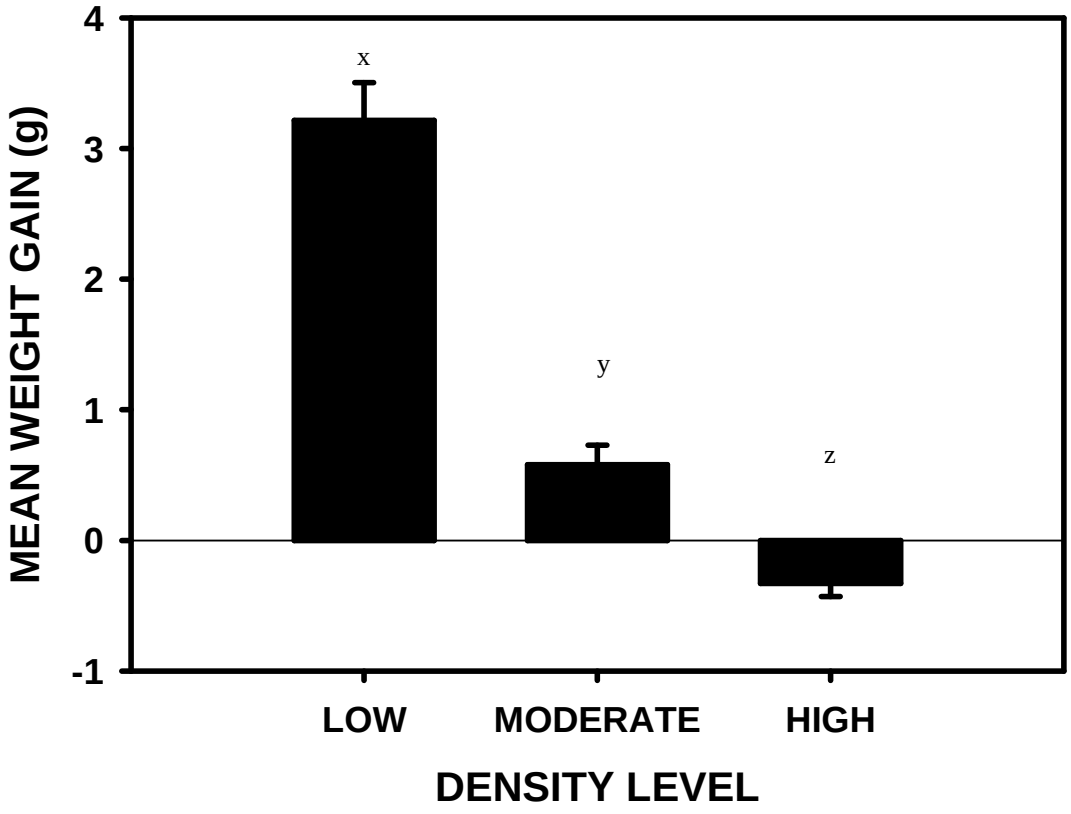

TABLE 1. − Nutrient analysis (percent, by weight; from data supplied by feed manufacturers) of 2 natural diets (enriched and processed by manafacturers; frozen *Artemia* sp. nauplii and frozen chironomid larvae, Hikari Bio-Pure, Hikari, Inc.) and 7 prepared/commercial diets (chicken egg-yolk powder, John Oleksy, Inc.; Hikari First-Bites and Hikari Micro Pellets, Hikari, Inc.; Wardley Staple Food Flakes and Wardley Premium Shrimp Pellets Formula, Hartz Mountain, Co.; Golden Pearls Weaning and Juvenile Diet, Brine Shrimp Direct, Inc.; Silver Cup, Nelson and Sons, Inc.) fed to three size classes of Gila chub *Gila intermedia* . Values for protein and fat represent minimum guaranteed levels, and fiber, phosphorus, and moisture represent maximum guaranteed levels. Values specific to Silver Cup represent a range of minimum or maximum and typical guaranteed levels.

Diet	Protein	Fat	Fiber	Ash	Phosphorus	Moisture	Size Class Fed
Artemia sp. nauplii*	6.8	1.5	1.2			86	Larval
	(47)	(5.5)	(0.5)		(0.1)	(6)	
Chironomid larvae*	6	0.5	0.9			89	Sm. and Lg. Juvenile
	(65)	(5)	(3.5)		(0.1)	(6.5)	(6.5)
Egg-yolk powder	34.25	55.8		3.4	<1	2.95	Larval
Hikari First Bites	48	3	1	15	1.3	10	Larval
Hikari Micro Pellets	42	4	3	12		10	Sm. and Lg. Juvenile
Wardley Staple Flakes	40	4	5			8	Sm. Juvenile
Wardley Shrimp Pellets	30	3	10			10	Lg. Juvenile
Golden Pearls	60	18		15		8	Sm. and Lg. Juvenile
Silver Cup	48-51	14-16	3-1	12-9		<10	Sm. and Lg. Juvenile

* Values in parentheses are for for a dried version of the feed type.