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Captive Breeding and Culture of Gila Chub *Gila intermedia*, Headwater Chub *Gila nigra* and Roundtail Chub *Gila robusta*

Scott A. Bonar, Andrew A. Schultz, and Erica A. Sontz

Fisheries Research Report 01-11

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Executive Summary

Captive breeding and maintenance of genetic stocks has long been used for recovery of a variety of imperiled taxa (Philippart 1995; Snyder, et al. 1996). It is often used as a last resort when populations can no longer sustain themselves in the wild and through natural means (e.g. California condor *Gymnogyps californianus,* giant panda *Ailuropoda melanoleuca,* black-footed ferret *Mustela nigripes*). The practice of maintaining captive breeding stocks has also been incorporated into fisheries work, with the establishment of captive and supportive breeding programs at state and federal hatcheries to provide refugia for imperiled species and to supplement wild populations. In the southwestern United States, breeding programs have typically focused on restocking fishes from hatchery-reared offspring of wild-caught broodstocks (Johnson and Jensen 1991). Generally, fish are held in these facilities and spawned in artificial habitats such as tanks, pools, raceways and ponds. The young are grown out in captivity to a minimum stocking size and then released into the wild in accordance with species recovery plans (USFWS 2002a; USFWS 2002b).

Many critically endangered Colorado River basin fishes have been taken into captivity for propagation and repatriation. Artificial spawning and culture techniques may be necessary for species that face imminent extinction due to extremely low population sizes or lack of recruitment and are increasingly popular aids to recovery and management efforts for native fishes throughout the Southwest whose populations continue to decline and whose habitats become increasingly scarce.

The headwater chub *Gila nigra,* roundtail chub *G. robusta,* and Gila chub *Gila intermedia* are three imperiled species existing in streams, rivers and cienegas of the Southwest. All have declined substantially in abundance and geographic range in recent years. Roundtail chub are distributed throughout the Colorado River basin, and headwater chub are confined to a few localities in the Verde River basin, the Tonto Creek subbasin and the San Carlos River basin in Arizona and a few headwater reaches of the Gila River in New Mexico. Gila chub are currently limited to about 29 isolated streams, cienegas, and springs (USFWS 2005a); only one of which contains a population that was considered stable and secure by Weedman et al. (1996). The Gila chub is listed as endangered with critical habitat under the United States Endangered Species Act (ESA; USFWS 2005a). In response to a petition to list both headwater and roundtail chubs under the ESA, the headwater chub was granted candidate status, but the roundtail chub was denied status (USFWS 2006). Following a further petition and a second review, a distinct population segment (DPS) of the roundtail chub in the Lower Colorado River basin was determined to warrant listing and designation of critical habitat. The roundtail chub, along with the headwater chub, will be raised to candidate status, "warranted but precluded," under the Listing Priority System (USFWS 2009).

Culture techniques have been developed for many fishes in the *Gila* genus (Hamman 1981, 1982a, 1982b; Kline and Bonar 2009), but, other than a single reported artificial spawn of roundtail chub (Muth et al. 1985) and anecdotal evidence for captive spawning (Doug Sweet, personal communication 2006), no one has developed such techniques for roundtail or headwater chub. Previous observations (Ken Wintin, Arizona-Sonora Desert Museum, personal communication; Jeanette Carpenter, U.S. Geological Survey, personal communication; and Andrew Schultz, personal observation) confirm that Gila chub will spawn and rear in captivity but culture techniques and requirements are largely unknown for this species as well. The future of headwaters chub, roundtail chub and Gila chub may ultimately rest in part on the ability of resource managers to properly incorporate captive propagation and culture techniques into recovery efforts to augment existing populations.

Our objectives for this project were to (1) collect individuals from wild populations and establish a broodstock for each species at the University of Arizona's Fish Propagation Laboratory in Tucson, AZ, (2) induce spawning of the captive broodstock under controlled conditions, and (3) culture offspring spawned in the laboratory under different hatchery environments to identify conditions for maximum growth and survival to assist agencies in management efforts. The most important findings of our work are highlighted below.

- Headwater chub collected from Fossil Creek, AZ; roundtail chub collected from Aravaipa Creek, AZ; and Gila chub collected from Sabino Canyon, AZ were successfully transported to the University of Arizona to create captive broodstocks.

- Fish were stocked into aquaria of 110-454 L various sizes for captive breeding. Aquaria were outfitted with equipment to manipulate water temperature and photoperiod. To collect the adhesive eggs from spawning events, we placed 10-cm square, white, ceramic tiles on the tank bottom. We placed a plastic, light-diffusing, "egg-crate" grating over the tiles to prevent fish from consuming the eggs after spawning. The grating was weighted with rocks to prevent the fish from getting underneath. Following spawning, tiles were transferred to aerated rearing tanks and placed in racks until eggs hatched.

- Headwaters chub were successfully bred in captivity. Headwater chub spawned only in response to temperature manipulations. Fish not subjected to temperature manipulations did not spawn. Fish spawned after water temperatures decreased to 16^{o}C or lower for at least 3 days in a row before warming above 16.5-17^{o}C. Over the next 7-10 days, water temperatures fluctuated between 16.5^{o}C and 18^{o}C, and after water temperature finally held above 17^{o}C and remained between 17.5-18^{o}C

for 3-5 days, the fish spawned. Photoperiod was set at 14 hours of light and 10 hours of dark when the first of the two headwaters chub tanks spawned.

- Gila chub were also successfully bred in captivity. Fish were brought to the laboratory in March 2003 from Sabino Creek, Arizona where water temperatures were 12.3°C. Fish were then warmed slowly and spawned at 14.9°C, 10 days following collection. Following this initial spawning, Gila chub spawned consistently in the laboratory without hormonal, chemical, photoperiod, temperature, and substrate manipulation, during all times of the year. Gila chub spawns were noted at temperatures ranging from about 15 to 26°C; however spawns at temperatures above 24^{o}C were less common.

- We were unable to successfully breed roundtail chub in our aquariums or small plastic stock watering tanks. Temperature manipulations similar to those used to successfully breed headwater chub were tried in addition to other techniques. Fish showed signs of stress at being held under laboratory conditions. Because one inadvertent spawning of roundtail chub, in another study, occurred in large, public aquarium holding tanks of much larger volume than what we had available, roundtail chub propagation may be better suited for larger tanks or ponds. Further investigation with larger tanks or ponds or even with renovated stream systems may prove successful where our small-scale operation was not. In addition the small number of fish we were allowed to bring into captivity may have had insufficient numbers of females. Increased sample size to ensure adequate numbers of males and females for broodstocks will be necessary for successful propagation.

- We were able to observe headwater chub spawning behavior. The first two spawns occurred at night or in early morning. Fish spawned in the afternoon in a later event. During spawning, headwater chub females darted about the tank, moving quickly back and forth. They approached the rocks or gravel and brushed against them. Male chub followed females as they moved about the tank. Females preferentially spawned over rocks used to weight the egg-crate grating, often rubbing against the rocks as they released eggs. Spawning females repeatedly passed over the spawning area, releasing eggs each time. Spawning males followed, releasing milt. Although fish preferred to spawn around rocks placed over the grating, neither rocks nor other types of artificial environmental enrichment such as plastic plants, artificial cover or structure appeared to induce spawning or related behaviors under static temperatures.

- We were also able to observe Gila chub spawning behavior. 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. Spawning colors were present to varying degrees near ventral and pectoral fin

bases, ventral body areas, opercle, and mouth, with strong, dark-colored horizontal banding noted on the most active fish. Nudging and 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, perhaps including 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. Spawning events often lasted over an hour.

- Headwater chub eggs were translucent white, adhesive, and averaged approximately 2.5 - 3 mm in diameter. Headwater chub females released thousands (even tens of thousands) of eggs. Eggs were so dense that on some tiles they were stacked up to 1 cm deep. Eggs hatched in about one week. Eggs were usually eyed after 4-5 days and hatched by 5-6 days.

- Gila chub eggs were demersal, adhesive, ovoid, and translucent with the inner 80-90% of the egg a light yellow cream color and the remainder colorless. Mean diameter of fertilized eggs about 24 h after spawn was 2.16 mm (SD = 0.05). There was a strong, inverse relationship between time to hatch and incubation temperature. Hatch rate of eggs averaged 99.4%. 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.

- We tested effects of temperature, feed type, and density on the growth and survival of headwater and Gila chubs. Size groups of headwater chub tested included larvae; small juveniles (mean total length [TL] mm ± SE at start of experiment: 19.2 ± 0.24); and large juveniles (46.7 ± 0.59 mm TL). Size groups of Gila chub tested included larvae, small juveniles (32-49 mm TL) and large juveniles (52-72 mm TL).

- Suitability of feeds for headwater chub differed. Feeds tested were based on consultation with U.S. Fish and Wildlife Service (USFWS) hatchery personnel familiar with rearing endangered desert fishes Feed types for larval headwater chub were designated natural (mix of decapsulated brine shrimp eggs and plankton); mixed (mix of plankton and Zeigler's Larval AP100); and artificial (Hikari First Bites). Larvae fed the natural diet grew largest followed by those fed mixed feed, while those fed artificial feed grew slowest. Small juvenile headwater chub, were fed either frozen “natural” feeds, (mostly Hikari Bio-pure brine shrimp, *Spirulina*-fed brine shrimp, and occasionally bloodworms); fine-ground Rangen catfish pellet (Rangen Catfish EXTR 350, 35% protein); or fine-ground Aquatic Ecosystems Finfish starter pellet (Aquatic Ecosystems Dense Culture Feed, 43% protein). Natural feed produced the lowest rate of growth

while finfish feed produced above average growth Large juvenile headwater chub were fed either "natural" feed (Hikari Bio-pure bloodworms), coarse-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein) or finfish starter pellets (Aquatic Ecosystems Dense Culture Feed, 43% protein). Large juvenile headwater chub grew largest on the finfish diet; average on the catfish diet; and below-average on the natural diet of bloodworms.

- Feeds for Gila chub also varied in suitability. Larvae fed a commercial 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 finding, 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. Our results demonstrate first-feeding larvae may be reared on a natural or prepared diet but natural feed will maximize survival. Based on diets tested, fastest growth will be achieved if juvenile Gila chub are fed a natural diet.

- Growth and survival of headwater chub varied with rearing temperature. Larvae exhibited the most growth, both in length and weight, and least mortality at 27^{o}C. There was some evidence that small juveniles grew longer at 20^{o}C and heavier at 24^{o}C than at other temperatures but these trends were only significant at the P = 0.10 – 0.15 level. Large juvenile headwater chub growth (both length and weight was highest at 20^{o}C .

- Growth of larval Gila chub was highest at 28ºC, while survival of larvae was highest at 24ºC. Spinal deformities were common (about 47%) for larvae reared at 32ºC but generally uncommon for those reared at lower temperatures. Water temperatures from 20-28ºC appear suitable for rearing larvae, with temperatures from 24-28ºC optimal. Water temperatures from 20-29ºC appear suitable for rearing juvenile Gila chub.

- In small juvenile headwater chub, growth was inversely related to rearing density but there was no statistically significant relationship with mortality. We found no significant differences in larval or large juvenile headwater chub growth or survival at the densities we tested. Densities tested were 180 fish/L, 90 fish/L, and 60 fish/L for the first larval experiment; 90 fish/L and 30 fish/L for the second larval experiment; 40 fish/L or 30 fish/L for the small juvenile experiment; and 6 fish/L or 12 fish/L for the large juvenile experiment. Cages were used to house fish in our density experiments, so using the exact densities we tested to set densities in pond or large aquarium culture should be approached with caution.

- Our data strongly support that increasing density has a negative effect on growth and survival (larval only) of Gila chub. We tested the following densities to rear Gila chub: 0.065 g/L (38.9 fish/L), 0.540 g/L (319.5 fish/L), and 1.343 g/L (795 fish/L) for larval chub (6.3-6.8 mm TL); 3.618 g/L (4.0 fish/L), 16.986 g/L (20.1 fish/L), and 60.145 g/L (68.3 fish/L) for small juveniles (36-47 mm TL); and 1.681 g/L (0.4 fish/L), 14.346 g/L (2.7 fish/L), and 53.942 g/L (8.4 fish/L) for large juveniles (57-95 mm TL). Mean length and weight gain of larval and large juvenile Gila chub were inversely related to rearing density. Survival of larval Gila chub was significantly greater for those groups reared at low densities. Juvenile Gila chub survival approached 100% for all density treatments. Cages were used to house fish in our density experiments, so using the exact densities we tested to set densities in pond or large aquarium culture should be approached with caution.

- As captive breeding is used with increasing regularity to aid in native fish recovery, understanding the limitations of hatcheries and tailoring hatcheries to species' needs could increase recovery program success. Overriding causes of species declines must first be addressed or captive and supportive breeding programs will suffer from limited success. Recovery efforts targeted at the causes of decline have been much more effective than hatchery support alone.

Captive Propagation and Culture of Headwater Chub *Gila nigra*

Erica A. Sontz and Scott A. Bonar

The headwater chub is a cyprinid which generally grows up to 300 mm total length (TL) and occasionally larger. It is endemic to the Gila River basin of Arizona and New Mexico and occupies mid- to headwater stream reaches (925-2000 m elevation above sea level). Until this past decade (Minckley and DeMarais 2000), the headwater chub was classified as a subspecies of the roundtail chub *Gila robusta*. It is believed to have arisen through hybridization between the roundtail chub and the Gila chub *Gila intermedia*, as it shows morphology and characteristics intermediate to these two species. Minckley and DeMarais (2000) list a number of morphometric characters used to separate roundtail chub and headwater chub, but field identification is challenging. The headwater chub is often characterized as similar to the roundtail chub in much of its biology including habitat preferences, diet, reproductive biology and general behavior (Neve 1976). Like other members of the genus, they are omnivorous, opportunistic feeders, consuming primarily plants, detritus, and arthropods, both terrestrial and aquatic. They will consume smaller fishes and are often considered the top predator in the aquatic habitats in which they occur. Research has suggested that roundtail chub diet may vary by fish size. Individuals smaller than 100 mm TL are unlikely to have fish remains in the gut, whereas individuals greater than 100 mm TL are more likely to incorporate fish into the diet (Vanicek and Kramer 1969). Additionally, headwater chub diet may vary seasonally with the availability and abundance of prey items (Neve 1976).

Here we describe the first known attempts to breed and rear headwater chub in captivity. Our primary objectives were to (1) induce spawning in captive adult fish and (2) test the effects of different water temperatures, feed types and fish densities on the growth and survival of the offspring at three life stages: newly hatched larvae (5.4-9.5 mm TL), small juveniles (12-27 mm TL), and larger juveniles (~47 mm TL). Results will inform development of captive breeding and culture protocols for this species.

Methods

Collection and Housing of Broodstock

Adult headwater chub were collected from Fossil Creek, Arizona above the diversion dam in February, 2006. We placed baited hoop nets in deep pools with slow or no current, below undercut banks, and under large woody debris. We anchored hoop nets so that a small portion of the net remained above the water surface, to allow any captured turtles access to air. We used opened cans of generic brand cat food for bait.

We left hoop nets in the water overnight and checked for fish the following morning. Almost all nets caught fish of varying sizes. We chose to minimize handling stress to the fish by not counting, weighing, measuring or otherwise overly handling any

of them, except to compare them to a minimum adult size. We selected the 20 largest fish, all over 150 mm TL, and used the guidelines established by Widmer et al. (2005) to transport broodstock to the Fish Propagation Laboratory at the University of Arizona in Tucson.

Fish were stocked in three 454-L tanks of seven, seven, and eight individuals each; we mixed all sizes in tanks to maximize the chance each tank contained both males and females. Tanks were initially left bare so that tiles could be added during spawning to collect eggs. Each tank had two large aquarium filters (AquaClear 500, Hagen, Mansfield, Massachusetts), one on each end of the long, back wall of the tank. Each tank also had two airstones, positioned near the filters, to provide additional aeration. Fish acclimated quickly to being in the tanks and started taking artificial feeds immediately. Gravel was later added to the tanks to give the fish a more natural setting. Black plastic garbage liners and foam insulating panels were attached to the exterior of each tank to help prevent fish from being disturbed from human activity in the laboratory, especially during expected spawning times. As one of the tanks became increasingly sensitive to human activity, we provided a plastic craft drawer, that when turned upside down and weighted with river stones, provided a cave-like structure with a single entrance in which the fish could hide. We found that the fish would often congregate in this box, which made observing the fish for signs of stress or illness difficult, and we eventually had to remove the box. Gravel, stones and artificial pond plants were added to encourage spawning and provide environmental enrichment.

We administered prophylactic treatment of Kordon Rid-Ich (Kordon LLC, Hayward, California) per instructions on the bottle upon arrival and fish were fed to satiation twice per day starting on the second day; however, approximately 10-20% percent of the time, fish were fed only once per day. Fish readily accepted pelleted feeds (Rangen trout feed and catfish feed, Rangen Incorporated, Buhl, Idaho; Aquatic Eco-systems finfish feed, Aquatic Eco-systems Incorporated, Apopka, Florida) and thawed, frozen natural feeds such as Bio-Pure Bloodworms, Brine Shrimp, Spirulina Brine, Mysis and Krill (Hikari, Hayward, California). Fish were later treated with praziquantel for Asian fish tapeworm *Bothriocephalus acheilognathi*, and tapeworm presence was confirmed by this treatment.

One tank of headwater chub was lost to an *Ichthyophthirius* outbreak in December 2006. This tank was disinfected and the individuals were replaced from a second collecting trip in February 2007. Individuals (N = 14) from two tanks were then relocated into a 1135-L circular plastic stock tank on 29 September 2006 to try a different tank design for inducing spawning.

We placed a shallow bed of gravel, larger rocks and concrete cinder block slabs to provide structure within the stock tank. Roundtail chub are known to jump from tanks (Mike Childs, US. Fish and Wildlife Service, personal communication; Doug Sweet, London State Fish Hatchery, personal communication; Erica Sontz, personal observation), so the stock tank was topped with a wooden frame covered with shade cloth to keep fish from jumping out of the tank.

A 75-L plastic pail set above the tank on the wooden frame provided filtration for the stock tank. The pail was filled with bioballs with a large, plastic filter pad placed on top of these. Water was pumped into the pail from the tank using a submersible Pondmaster magdrive utility pump (Danner Manufacturing, Islandia, New York) with a filtered intake. Water was returned to the tank through a hose that ran from the bottom of the biofilter pail to a polyvinyl-chloride (PVC) bar that sprayed water across the surface of the tank to provide aeration. An additional overflow hose was attached to the pail. The filter intake was cleaned periodically to prevent buildup of organic material that would block the filtration system. The design was modeled after Kline and Bonar (2009).

Spawning

Fish were held in the above system for 10.5 months (25 February 2006 – 6 January 2007) before they spawned, which included the end of one winter and all of the next. During the first winter, water temperatures were maintained above 20°C and photoperiod was maintained at 10 hours light: 14 hours dark (10L: 14D). Tanks were lighted by full-spectrum tank lights, which provided a more direct light to the tank, in addition to the ambient overhead room lighting, and both were set to provide the same photoperiod.

The following winter, we turned off the building heating, which allowed the building temperature to change with ambient outdoor Tucson, AZ conditions, and tank temperatures cooled with winter temperatures (see Figure 1). A recirculating water chiller was also placed on the larger tank to aid in cooling. On 18 October 2006, room lights were set for 10L: 14D and all tank lights for 11L: 13D. On 22 November 2006, all lights, room and tank, were reset to 14L: 10D and kept at those settings.

To collect eggs from spawning events in aquaria, we first cleaned the tank of debris and organic waste, performed a 50% water change, and then placed 10-cm square, white, ceramic tiles on the tank bottom. Initially, we would begin to prepare for egg collection when fish started to show breeding coloration, but we soon learned that fish could maintain this coloration for several weeks without spawning. We tiled the tanks many times in anticipation of spawning only to have to remove the tiles between one and five days later due to debris buildup. One day prior to the first spawn, we observed agitated behavior in the broodstock, consisting of darting and flashing against rocks and gravel, chasing behaviors, and general increased activity, and we subsequently used this as an indication of readiness to spawn. We filled in areas not covered by tile with naturally colored, 6 - 6.5 mm diameter pea gravel and placed a plastic, light-diffusing, "egg-crate" grating over the tiles (Schultz and Bonar 2007) to prevent fish from consuming the eggs after spawning. The grating was weighted with rocks to prevent the fish from getting underneath.

To collect eggs from the large stock tank, we placed tiles inside plastic planter trays of varying sizes and shapes, which had the grating attached to them by zip-ties through holes drilled into the edges of the trays. This arrangement allowed us to place and to remove several tiles at once, because the bottom surface area of the stock tank was

much larger than that of the aquarium tanks. The planter trays could easily be placed in areas that would be hard to reach with individual tiles, and the tiles could be removed from the trays for incubation. We also set a small indicator tray in place with an attached line that allowed it to be pulled from the tank easily. We used this indicator tray to create minimal disturbance to determine if spawning had occurred.

Egg Development and Hatching

Tiles with attached eggs were rinsed with clean, non-chlorinated, well water to remove debris and then transferred to separate tanks (37.9 and 75.7 L) with a small rack to hold tiles vertically (see Kline and Bonar 2009 for basic design). We added one handful (roughly 40g) of rock salt to the tank and watched for development of embryos and signs of fungus *Saprolegnia*. Infertile or infected eggs were removed daily by pipette in the first several hatching trials. These eggs were clouded, floating, or displayed fungal growth. In the last spawning attempt (summer 2008) tanks were treated with Kordon Rid-Ich (Kordon LLC, Hayward, California) to help eliminate fungus.

Larval Culture of Headwater Chub

Once all viable eggs had hatched, we transferred fish from the hatching tank to 7.5- or 19-L plastic buckets for easier handling. We measured lengths and weights from 20 randomly-sampled fish from each hatching tank to establish a starting size for headwater chub larvae. To remove the effect of handling stress, we did not include these larvae in the experiment. We chemically euthanized larvae prior to taking measurements.

We randomly selected fish from buckets and placed ten each into a treatment cage. Treatment cages for larval fish were made of nylon filter netting, assembled in a 6 cm-diameter cylinder with a closed bottom and open top (FIGURE 2, Appendix). The mesh kept fish contained within a small area while still allowing water circulation. A ring of colored foam was attached to the outside of the cage and secured at one of three heights. The color of the foam indicated to technicians what feed type to use for that cage while the height of the foam ring created different water volumes within the cage, depending on how much of the cage volume was submerged. This allowed for three different densities even though the same number of larval fish was stocked in each cage. Each cage was assigned to a density treatment: high (0.18 fish/cm^3), medium (0.09 fish/cm^3), and low (0.06 fish/cm^3), and to one of three feed types. Feed types were designated "natural," which was a brine shrimp egg/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecoystems; "mixed," which was a plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; and "artificial," which was Hikari First Bites. Feeds for all experiments were based on consultation with US Fish and Wildlife Service (USFWS) hatchery personnel who were familiar with rearing endangered desert fishes (Chester Figiel, U.S. Fish and Wildlife Service, Willow Beach National Fish Hatchery, personal communication).

Larval and small juvenile experiments were conducted in 37.9-L tanks. One replicate of 10 fish was placed directly into the tank as a "control" treatment; they had free movement throughout the tank, except for the cages, and were fed only what feeds passed through the cages and what debris was pipetted out of the cages. The purpose of the "control" treatment was to simulate, on a small scale, a sort of free-form pond-rearing type habitat, where they were allowed full range of the tank and fed on debris and detritus. This gave us ten treatments per tank (9 cages and 1 control), for a total of 100 fish per tank. (See FIGURE 3).

Larval experiments were conducted twice. For the first experiment, we replicated this set-up in 15 tanks for a temperature component of the study. Each tank was held at one of five different temperature treatments with three replicates per temperature: 17°C, 19.5°C, 22°C, 24.5°C and 27°C. Every treatment combination (temperature x feed x density) was replicated three times in the experiment.

Because of some cage failures, and to improve information about larval rearing, we eliminated some treatment types and reduced the number of fish per cage in the second experiment. We tested two densities, high (0.09 fish/cm3) and low (0.03 fish/cm3), with three feed types (see first larval experiment above for feed types) at five temperatures: 18°C, 21°C, 24°C, 27°C and 30°C. We had three replicate tanks for each temperature and five fish were placed into each cage for a total of 30 fish per tank and 450 in the entire experiment.

We fed fish three times per day in the first experiment and twice per day in the second experiment, and cleaned cages prior to each feeding. Fish were fed a pinch to a ¼-teaspoon of feed each feeding, which was usually in excess of what they could consume. After allowing ~10 minutes for feeding, the excess was removed. To clean cages, pipettes were used to remove large, clumped debris while being careful not to capture larval fish, and mesh skimmers were used to remove floating debris. We removed dead fish at every feeding as necessary and recorded the location (tank and cage) in which they were found.

Initial temperature for each tank was the same at which eggs hatched (21°C for the first experiment and 18°C for the second experiment). We then increased or decreased temperatures over 3-5 d until tanks reached test temperatures, and then kept tanks within 0.5°C of the test temperature. Fish remained in cages for 34-35 d to give them a full 28-30 d at test temperatures before ending the experiment. At the close of the experiments, we anesthetized fish and measured length (mm TL) and weight (g) of each, and then averaged these measures to obtain a mean weight and length of fish in each cage. We compared the mean starting TL and weight with the mean ending values for each cage to obtain measures of growth by cage.

Rearing of Small Juvenile Headwater Chub

The procedure for testing rearing densities, temperature and feed effects on growth and survival of small juvenile headwater chub was similar to that of larval experiments. Small juvenile fish were four months old and averaged 19.19 mm (± 0.24

mm [95%C.I.] TL) and 0.156 g (± 0.0056 g) at the start of the experiment. The fewer small juvenile fish available precluded testing as many treatment levels for the three factors as we did in larval experiments. Each fish was weighed (g) and measured (mm TL) before being placed into a cage.

We placed fish into the same types of cages as used for the larval experiment at two densities (medium [0.04 fish/cm^3] or low [0.03 fish/cm^3]) and fed them one of three feeds (frozen "natural" feeds, which was mostly Hikari Bio-pure brine shrimp, *Spirulina*-fed brine shrimp, and occasionally bloodworms; fine-ground Rangen catfish pellet [Rangen Catfish EXTR 350, 35% protein]; or fine-ground Aquatic Ecosystems Finfish starter pellet [Aquatic Ecosystems Dense Culture Feed, 43% protein]). Pellets were ground using a combination of electric food chopper and mortar and pestle and then sifted through fine mesh netting to separate fine from coarse grounds. Because the small juvenile fish were considerably larger than the larvae (roughly 2.5 times the length), we only put five juveniles into each cage. Cages were placed in one of nine tanks for replicates of three different temperatures (19-20^{o}C, 24-25^{o}C and 28-29^{o}C). A panel of egg-crate grating (the type that was used in spawning to cover the tiles) was suspended in front of the filter outflow to prevent cages from being pulled under the falling water and swamped. Water levels in the tank were maintained so that cages could not pass below this panel. Fish were fed and cages cleaned as described above for the larval experiment. The experiment was conducted for two months, and was ended as described for the larval experiments, with length and weight measurements being taken and averaged per cage.

Rearing of Large Juvenile Headwater Chub

Large juvenile experiments were conducted in 76-L tanks. These fish were too large, mean 46.75 mm TL (± 0.59 mm) and 2.32 g (± 0.84 g), to use the fish cages, so we used plastic fish cubes (10 cm x 10 cm x 10 cm) available from Aquatic Eco-systems to produce density replicates. The fish cubes were floated at different heights in the water column by zip-tying pieces of foam at two heights: At the top (1000 cm^3) and midway (500 cm^3), on the cubes. Each individual fish was weighed and measured before being placed into a fish cube.

Fish cubes were designated as one of two densities, determined by float height: low (0.006 fish/cm^3) or medium (0.012 fish/cm^3), and one of three feeds ("natural" Hikari Bio-pure bloodworms, coarse-ground catfish pellets [Rangen Catfish EXTR 350, 35% protein] or finfish starter pellets [Aquatic Ecosystems Dense Culture Feed, 43% protein]). We placed six fish cubes into each of six tanks. Three tanks were held at 19-20^{o}C, and three were held at 28-30^{o}C to test the effects of two different temperatures. We did not clean the fish cubes as we did the cages because the fish cubes had large holes in them that passed debris. Fish in cages were fed by sprinkling pellets or by pipetting bloodworms through the cage holes. The experiment continued for 98 days and then fish were anesthetized, weighed and measured in a manner similar to the small juvenile and larval experiments. Growth was again averaged per cage.

Statistical Analysis

We followed Milliken's (2000) modification of the basic split-plot design to test for differences in growth across treatments, and analyzed the results using multiple ANOVAs to test the effects of each treatment individually and regression to look for interactions between treatments and overall significance. Temperature was the main-plot factor, and feed type and fish density were completely randomized as full factorial sub-plot factors, rather than using the more traditional split-split-plot design. This gave us one cage for every possible feed x density combination in every tank, and three tanks at each temperature. This design allowed us to give equal weight to feed type and density. We calculated change in average length and weight as the main response variables. We also calculated percent mortality and used the arcsine transformed values with logistic regression as a third response variable to test if mortality varied across treatment types. An individual fish was only counted as a mortality if we found it dead within a cage and removed it from the tank. This is of particular importance in the first larval experiment where a large number of fish escaped the cages into the main tank. Escaped fish were not counted as mortalities, and this often left us with fewer fish per cage at the end of the experiment than at the start. We compensated by using averages of weight and length per cage rather than per fish. *P*-values were set to a standard 0.05 level to determine significance of effects. The data analysis and data presentation for this paper were generated using JMP software, Version 8 and JMP trial software, Version 9 of the SAS System for Windows. Copyright © 2009 and 2010, respectively, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

Results

Collection and Housing of Broodstock

Headwater chub were easily collected via hoop nets. A range of size classes inhabiting the deeper pools where nets were set were found in the hoop nets the following day.

Fish acclimated readily to captivity and began feeding as soon as feeds were offered. Fish readily accepted all types of feeds offered to them, including pellets, flakes, frozen natural feeds and live feeds. Initially, fish were placid, allowing observers to approach the tank without fleeing and generally not showing signs of agitation. Over time, fish behavior changed, with individuals becoming skittish and shy and then returning to their previous behavior. In addition, fish initially fed aggressively on all food types, but over time, appetites would vary for days or weeks at a time, and fish would not eat while under observation. Fish readily used shaded areas of tanks or in-tank cover such as the plastic box placed in one of the tanks.

Spawning

Fish did not spawn the first year when held at a constant temperature, but did so during the second year, after we were able to lower water temperatures. Both tanks of fish spawned after water temperatures decreased to 16°C or lower for at least 3 days in a row before warming above 16.5-17°C. Over the next 7-10 days, water temperatures fluctuated between 16.5°C and 18°C, and after water temperature finally held above 17°C and remained between 17.5-18°C for 3-5 days, the fish spawned (Figure 1). The first spawn occurred at night or in the early morning. Eggs had been deposited by the time we arrived in the morning, around 10 am. The second spawn followed the same pattern, with eggs deposited in the tank by morning. During one later spawn, fish spawned over several hours in the afternoon. Although headwater chub spawned first in a tank that had cooled due to ambient air temperatures and then warmed, they also spawned in the tanks where temperature was lowered by a water chiller. Room lights and tank lights were set at 14 hours of light and 10 hours of dark when the first of the two tanks spawned.

During spawning, females darted about the tank, moving quickly back and forth. They approached the rocks placed over the tiles or gravel and brushed against them. Male chub followed females while they moved about the tank. Females preferentially spawned over the rocks used to weight the egg-crate grating, often rubbing against the rocks as they released eggs. Non-spawning fish were much less active, often remaining on the opposite side of the tank from the spawning activity, hovering over the grating. Spawning females repeatedly passed over the spawning area, releasing eggs each time. Spawning males followed, releasing milt. Although fish preferred to spawn around rocks placed over the grating, neither rocks nor other types of artificial environmental enrichment such as plastic plants, artificial cover or structure appeared to induce spawning or related behaviors under static temperatures.

Egg Development and Hatching

Eggs were translucent white, adhesive, and averaged approximately 2.5 - 3 mm in diameter. Headwater chub females released thousands (even tens of thousands) of eggs and we did not have the personnel available to count eggs on tiles while moving them to hatching tanks or calculate viability ratios. Because of the unevenness of the egg deposition across the tiles, and the need to get them into hatching tanks rapidly, we also were unable to extrapolate egg density based on a subsample. Eggs were very dense around the rocks and decreased further from the rocks and towards the edges of the tanks. Eggs were so dense that on some tiles they were stacked up to 1 cm deep.

Eggs hatched in about one week following a similar progression to that observed by Muth et al. (1985). Eggs were usually eyed after 4-5 days and hatched by 5-6 days.

Rearing Of Larval Headwater Chub

In the control treatment in the initial larval experiment, there were many escapees from the larval fish cages. Any fish that escaped from cages had to be left with the control fish because we were unable to distinguish individual fish. These escapes were caused primarily by swamping of cages by filter outflow, and occasionally, tears in the cage edges. We did not have any outright cage failures such as complete separation of the cage bottom from the sides. In addition, all fish in one of the 17^{o}C tanks died within one day of starting the experiment. We suspected some sort of contamination of the equipment and replaced most tank equipment (filter, cages, tank heater), and thoroughly washed equipment that could not be replaced (tank, chiller hoses, pump). The tank was then refilled and stocked with additional larvae from the hatching tanks. Again, all of these fish were found dead the next day. Since we could not locate the cause of the mortality, we decided to eliminate this tank, leaving two remaining replicates at this temperature.

In the first larval experiment, high mortality and escapes from cages decreased the power of the statistical analysis, and the numbers were too low to obtain *P*-values for the effect tests (Tables 1-3). In the multiple regression models, the models for growth (mm TL) and weight gain (g) were both significant ($P < 0.0001$ in both models), but we could not determine significance of the individual factors because of the few data available (see also Figures 4-6). The logistic regression of mortality indicated that mortality was not significantly impacted by any of the experimental variables (X^2 [324] = 295.74, $P = 0.8682$). In the second larval experiment (Tables 4-6, Figures 7-9), when all factors were considered together, only feed had a consistently significant effect on larval growth ($P < 0.0001$), although temperature had a strong, but not significant effect at the level set ($P = 0.0618$ and 0.0773 for length and weight, respectively, Tables 4 and 5). There were significant interactions between feed type and density on both types of growth ($P = 0.0023$ and $P = 0.0051$), and temperature and density interacted at almost significant levels ($P = 0.058$) on growth (mm TL).

Analyzing the factors in individual ANOVAs more clearly identified the effects of each. Figures 7, 8, and 9 illustrate larval growth (mm TL) in response to temperature, feed type and density, respectively; figures 10, 11 and 12 illustrate larval weight gain (g) in response to the same variables and figures 13, 14 and 15 represent mortality. In the first larval experiment, larval fish growth and survival was strongly affected by temperature (Figures 7 [$P < 0.0001$], 10 [$P < 0.0001$], 13 [$P = 0.0016$]). The most growth and least mortality occurred at 27^{o}C, while 19.5^{o}C produced the lowest growth and above average mortality. Fish on natural feeds tended to grow larger followed by those fed mixed feed, while those fed artificial feed exhibited the slowest growth. However, this trend was only statistically significant for growth (mm TL) of larval headwater chub ($P = 0.0768$, Table 16). Density appeared to have no significant effect on larval headwater chub growth ($P = 0.2026$ and 0.9002) or survival ($P = 0.5928$).

For the second larval experiment, regression results are illustrated in Tables 4-6 and Figures 16-18. Figures 19, 20 and 21 represent larval growth (mm TL); figures 22,

23 and 24 illustrate weight gain (g) and figures 25, 26 and 27 show mortality, all in response to temperature, feed type and density, respectively, for each dependent variable. Increase in length (mm TL) and weight (g) as well as mortality were strongly affected by feed type (Figures 20 [P <.0001], 23 [P<.0001], 26 [P = 0.0005]) and both types of growth were strongly affected by temperature (Figures 19 [P = 0.0005], 22 [P = 0.0016]), although mortality was not (P = 0.2836). Density had no significant effect on these two factors. Again, 27^{o}C produced the most growth in larval headwater chub, with growth dropping off again at 30^{o}C, a temperature not tested in the first larval experiment. Mortality was also higher at 30^{o}C than the other temperatures. Natural feeds produced larval chub with above average growth and below average mortality, while artificial feed produced less growth in larval headwater chub than the mixed feed, but this trend was not statistically significant.

Rearing Of Small Juvenile Headwater Chub

The models for small juvenile growth (mm TL) and (g) also provided clear results, but were not quite as strong as the models for larval headwater chub (P = 0.0042 and 0.0411, Tables 7 and 8, Figures 28-30). Density had the strongest impact on small juvenile growth (P = 0.0883 [mm TL] and 0.0334 [g]), but not on mortality (P = 0.1650), even though four of five mortalities were from the low density treatment (Figure 30), which produced stronger growth responses. Feed type significantly affected weight gain (Table 8, P = 0.0302) and there was a significant interaction between temperature and density on length increase (Table 7, P = 0. 0013).

Small juvenile growth responses are represented in figures 31, 32 and 33; weight gain in figures 34, 35 and 36 and mortality in figures 37, 38 and 39. The independent variables are temperature, feed type and density in each group of three responses. Small juveniles showed no significant responses to temperature or feed type when factors were examined separately (Figures 31, 32, 34, 35, 37 and 38). There was a non-significant trend indicating that small juveniles grew longer at 20^{o}C (Figure 31, P=0.0886) and heavier at 24^{o}C (Figure 34, P = 0.156) than at other temperatures. Natural feed produced the lowest rate of growth while finfish feed produced above average growth (Figure 35, P=0.0724)). There was a slight significant affect of density on small juvenile growth (g, Figure 36, P=0.0332).

Rearing Of Large Juvenile Headwater Chub

We lost one tank of large juvenile headwater chub a few days into the experiment. The cause appeared to be a filter failure and subsequent water quality issues. We did not replace the fish because of the time into the experiment and lack of available fish. There were no other mortalities throughout the large juvenile experiment.

Temperature and feed type significantly affected large juvenile growth, but density did not, both in regression (Tables 10 and 11 and Figures 40 and 41) and separate ANOVAs (Figures 42-47). There was a significant interaction between temperature and

density (P = 0.0274) and the three way interaction between temperature, density and feed type was strong (P = 0.07) for growth (g, Table 11) but did not meet the criteria for significance.

Large juvenile headwater chub grew largest at 20^{o}C and on the finfish diet; but temperature had the strongest effect. Large juvenile headwater chub demonstrated below-average growth at both 28^{o}C and on the natural diet of bloodworms. Catfish diet produced average growth, midway between the other two feed types.

Statistical Analysis

The "control" treatment of fish (those fish initially placed within the tank but not within a density treatment cage) confounded the regression for the first larval culture experiment because we could not distinguish escaped fish from "control" fish, and we had to remove this group from analysis. We did not anticipate as much larval cage failure as we observed (cages developing holes and tears and cages being swamped by the filter outflow, both of which allowed fish to escape). Any fish that escaped from a treatment cage was relegated to the control group by default, because we could not distinguish individual larvae. This greatly reduced the number of fish in each treatment cage, and in some cases, eliminated treatment cages entirely, and limited the statistical power of our analysis.

All factors were analyzed together using multiple (length and weight) or logistic (mortality) regression and then subjected to individual ANOVAs to look at the separate effects of each treatment on growth and survival. In some cases, the sample data were too small to determine the significance of individual effects on the model, but the whole model significantly explained the results (e.g. the first larval experiment, where lost degrees of freedom prevented P-value calculations). In many cases, P-values between 0.1 and 0.05 indicated trends in the data without actually reaching the P = 0.05 cutoff for significance. Such trends, while not significant, may still be important and relevant to the artificial culture of headwater chub.

Discussion

This, to the best of our knowledge, was the first documented successful attempt to spawn headwater chub in captivity. We were able to spawn two different tanks of adults on multiple, separate occasions. Headwater chub were relatively easy to hold in captivity and appeared to do well with little or no specialized husbandry.

Spawning seems to be mostly a function of water temperature cycles, that is, a cooling followed by a warming, with spawning ceasing when temperatures rise too high (above 19-20^{o} C). In the winters when we did not lower and then raise the water temperature, we did not get any of the fish to spawn. Likewise, in subsequent attempts to spawn fish for the second larval experiment (spawn date = 7 April 2008), where we did not manipulate photoperiod, but did manipulate water temperature, we still managed to successfully produce spawns. Kline and Bonar (2009) found that Yaqui chub *G.*

purpurea also required a decrease and then an increase in temperature to a critical value to induce spawning. Schultz and Bonar (2009) found that Gila chub *G. intermedia* spawned continuously in the laboratory without manipulation beyond setting up a tank for spawning. This behavior followed an initial warming from 12.3°C (Sabino Canyon, AZ) to 14.9°C (laboratory tanks). Archdeacon and Bonar (2009) successfully spawned Mojave tui chub *G. bicolor mohavensis* using a combination of photoperiod and temperature manipulation that simulated winter conditions, followed by spring. It is unclear whether the fish spawned as a result of the combination of effects or if temperature alone would have induced spawning in Mohave tui chub.

An initial drop beyond a threshold temperature followed by a subsequent warming and holding above this threshold may be required for the onset of spawning in *Gila* species, possibly with additional photoperiod manipulation in some species. This may be of special interest for those attempting to spawn chub that have evolved in constant-temperature spring systems, such as the headwater chub of Fossil Creek. Responses to cooling and warming may be ancestral traits within the *Gila* complex of fish, and even those in seemingly constant temperature springs, such as the Fossil Creek population, may be induced to spawn because of spring runoff and influx of cooler temperatures from snow melt. Our work also suggests global climate change trends could impact the spawning behavior of these fishes, either through overall change in stream temperature regulation or in the ability of snow melt and runoff to affect stream temperatures. Also, changes in riparian shading and the response of riparian vegetation to global climate change events and other human-induced impacts could change the frequency or ability of these fishes to spawn.

Density patterns of eggs around the spawning substrate indicate a definite preference of headwater chub for spawning around rocks and other large substrate materials. Fish spawned over gravel, tiles and egg crate grating, but gravitated towards the larger pieces of substrate, often rubbing or flashing against these materials during the spawning ritual. We had no fish that were held in entirely substrate-barren tanks, so it is still unknown if headwater chub would spawn under such conditions. Ideally, any streams into which they would be reintroduced would have a mixed substrate surface that includes rocks, cobbles and other large spawning substrates.

Larval and juvenile headwater chubs showed several growth trends in response to the treatment factors in which they were reared. The effects observed were not always consistent across life stages; in fact, sometimes what produced the most growth in larval fish was least effective for juveniles and vice versa. This could indicate shifting temperature, density and feed requirements at different stages of development, or may simply indicate that our selection of experimental test factors did not adequately capture all growth responses.

Larval fish showed the most growth at 27°C, and the juveniles at 20° and 24°C. Similar trends were found for Gila chub by Schultz and Bonar (2009) suggesting patterns that may mimic habitat availability and could be part of the selection processes that young fish use to find the right habitat. Larval fish in natural environments tend to inhabit shallow waters at stream or pond edges. Barrett and Maughan (1995) showed that

juvenile chub in Fossil Creek (then *G. robusta*, now *G. nigra*) preferred shallower, slower velocity water than adults and were seen using riffles. Personal observation while collecting fish from Fossil Creek showed that juvenile chub frequented the shallow, near-shore habitat. Barrett and Maughan (1995) suggest that ontogenetic changes in habitat preferences may be indicative of differential foraging strategies and prey types. While this helps them avoid predation from the larger fishes that cannot enter these shallower areas, it also puts them in areas that are likely to be warmer. Shallower areas have less water to heat, and when they receive the same amount of solar radiation as the rest of the surface area of the body of water, they get warmer (Schlosser 1995). Young fish thus have the protection from predation and the higher temperatures they need to grow well and survive.

As fish grow larger, they move into deeper, colder water, gaining access to larger food sources. Larger fish are able to avoid some of the predation pressures that might have forced them into shallow waters previously.

As temperatures rise, growth of larval fish increases until it reaches an upper tolerance, and growth of juvenile fish reaches that peak at a lower temperature (*e.g.* compare Figure 7 with Figure 31). Headwater chub stopped spawning when temperatures rose above about 19^{o}C (Figure 1). Eventually temperatures can reach a point where growth and development will cease and reproduction will no longer occur. Fossil Creek is a perennial stream that maintains a constant temperature for most of its length, due to the geothermal springs that feed it. In other southwestern streams, temperatures fluctuate more due to a variety of factors. Reduced stream flow, water diversions, decreased riparian vegetation or other factors increasing temperature could hamper the ability of headwater chub or other native fishes to reproduce and/or grow and develop properly. These may indicate that as global climate change affects the southwestern U.S. it could significantly impact the health and survival of native fish populations. Global climate change is a looming threat worldwide, and conservation efforts directed at imperiled desert fishes, many of which already exist at the limits of their habitat tolerances, would benefit from considering the added effects of climate change.

Larval fish tended to grow larger, and in some cases, exhibited lower mortality on "natural" feeds, which were a combination of plankton and decapsulated brine shrimp eggs, than they did on the "artificial" and "mixed" feed types, which consisted of either a commercial larval fish food (Hikari First Bites) or a combination of a larval feed supplement (Larval AP100) and the same plankton used for the "natural" feeds. This indicates that live culture of feeds for larval fish or processed natural feeds such as the type we used may be optimal for early development of headwater chub in captivity. Perhaps commercial larval feeds are less suited for headwater chub because they are primarily formulated for tropical aquarium fishes.

Both small and large juveniles grew larger on the higher protein finfish feed than on either of other two feeds (catfish and frozen live feeds) to varying degrees of significance. In all cases, the natural feed performed the worst, which could have been due to any number of factors including inadequate selection of frozen feed type,

inadequate nutrition for development in these feed types or manufacture issues that we did not consider. More interesting is that the fish did best on a diet higher in protein and containing more animal and fish sources of protein than what they might eat in the wild. Developing fish may need higher sources of protein than adults, and juvenile fish may consume more animal-based proteins in the form of invertebrates and microscopic animal planktons, since adults are known to feed more on plants and detritus. Our natural feeds were designed to mimic a natural diet, and consisted of frozen invertebrates including brine shrimp, chironomid larvae (bloodworms), mysis shrimp and others. Pilger et al (2010) found that headwater chub in the upper Gila River basin (New Mexico, USA) fed on chironimids (40.4% by volume) and benthic invertebrates (30.6% by volume) as juveniles (< 70 mm TL) on fish (53.8% by volume) and ephemeropterans (18.5% by volume) as subadults (70-150 mm TL) and on algae (46.8% by volume) and fish (19.7% by volume) as adults (> 150 mm TL). Neve (1976) found that chub < 50mm in size in Fossil Creek fed exclusively on diatoms and filamentous algae and that older chub fed opportunistically and exploited all habitats and corresponding food sources available to them.

Maximum growth may not be the single, best metric for choosing which food to provide for fishes destined for stocking in the wild; feeds that more closely replicate those from natural environments, even though they result in slower growth, may ultimately provide better nutrition and more optimal growth for headwater chub. Studies in the Atlantic silverside *Menidia menidia* suggest that there may be a tradeoff between increased growth rates and high levels of energy acquisition with swimming performance, and that such tradeoffs may maintain what appear to be inferior genes for growth within wild populations (Billerbeck et al 2001). Arendt (1997) provides a summary of literature that suggests that a variety of organisms might increase growth rates at the cost of numerous factors including immune system development and response and altered behavioral responses. Alternatively the natural food sources available to developing headwater chub juveniles may not adequately supply all the nutritional components for maximum growth. To improve success of captively-reared headwater chub released into wild environments, further studies could examine how suitable diets designed for optimum growth and survival in captivity compare to what is optimal for headwater chub stocked into the wild. Additionally, post-release performance evaluations of fish grown at hatchery growth rates could be compared to the performance of fish grown at wild growth rates to determine if accelerated hatchery growth impacts fish after being repatriated.

Overall, temperature and feed types had the most effect on growth and survival of subadult headwater chub. These are often the easiest two factors to manipulate in a hatchery or artificial culture environment but two of the hardest to control in a natural setting. This could have several consequences for captive propagation, growout and release of headwater chub. Cooper (1961) found that hatchery brook trout *Salvelinus fontinalis* in five groups grew to twice the length and about ten times the weight of wild brook trout in three populations. Growth in wild populations appeared to be limited by non-optimal temperatures and lack of food, which shows that hatcheries are a poor

imitation of wild conditions and do not adequately mimic natural selection pressures. While fish may grow larger in a hatchery, this may not translate into success after stocking. Reconstruction of three-generation pedigrees show that two generations of captive-bred steelhead trout *Oncorhynchus mykiss* showed 37.5% reduced fitness per captive-reared generation after being released back into the wild (Araki et al. 2007). Hatchery steelhead trout from the Hood River, Oregon left fewer adult offspring per parent than wild steelhead trout, but local, wild fish brought in as supplementary broodstock left more offspring than the traditional, multi-generation hatchery broodstock. However, reproductive fitness in the supplementary broodstock declined rapidly with multiple hatchery breedings (Araki et al. 2008). Fritts et al. (2007) found that wild Chinook salmon *Oncorhynchus tshawytscha* fry had a 2.2% survival advantage against rainbow trout *Oncorhynchus mykiss* and torrent sculpin *Cottus rhotheus* predators than did state-of-the-art hatchery-reared fry. Even one generation of state-of-the-art hatchery domestication is enough to affect survival; although, the effect is very small.

Additionally changes in the natural stream habitats of headwater chub could profoundly affect growth, development, spawning, recruitment and success of wild and repatriated populations. Global climate change, changing riparian communities - even down to a microscopic level -, and other anthropogenic changes could potentially alter the way in which headwater chub grow and develop.

Several significant interaction effects were observed, which underscores why we chose to use a multivariate and split-plot approach rather than testing different rearing conditions separately as Schultz (2009) did. Fish, in the wild or in a hatchery, experience all of these conditions at the same time, and it is important to know if one factor, or stressor, might impact the way the fish respond to another factor. For example, feed type significantly affected growth in larval headwater chub, but density did not. However, there was a significant interaction between the two, which might indicate that under varying levels of density, fish might respond differently to a given food type. Different feeds might become more or less accessible under highly crowded or lightly crowded conditions. If this effect occurs on the smallest scale, then even the hatchery induced "feeding frenzy" observed at hatchery feeding time might have an impact on how fish grow when fed different types of feed.

In all instances where a significant interaction term was observed, one of the two interacting factors did not play a significant role on its own. For larval and large juvenile fish, rearing density alone, and for small juveniles, temperature alone did not significantly affect growth and survival. However, interactions with other factors caused an overall significant effect. Lack of main effects for a factor may indicate that we did not adequately distinguish between levels of effect, particularly for density in hatchery conditions. Repeating some of the culture experiments with more levels of effect or more variation between them could help determine the importance of the roles these environmental factors play in fish health, growth and survival. The rearing cages we used were much smaller than those used by Schultz and Bonar (2007), and the densities used may not adequately represent typical hatchery densities. Increased numbers of fish per treatment would increase the power of the effects tests, particularly for the main-plot

factor in this split-plot design. The rearing conditions in the experiments involved small cages within small tanks, and interactions could have resulted from incidental influences not accounted for or measured in this study.

Deformities were observed in approximately 19% of the small juveniles, and 5% of the large juveniles; they were also present in larval fish, but due to the smaller sizes and larger numbers of fish handled, were not quantified. We did not attempt to correlate appearance of deformities with treatment types as deformities also appeared in fish that were being held in grow-out tanks and were not run through the culture trials. There is some evidence from other species that this may be a normal part of *Gila* biology and development (Jason Kline, personal communication). Spinal deformities were most common, ranging from barely noticeable to nearly 90^{o} bends in the spinal column, affecting mobility and ability to feed. Other deformities included abnormal development of the head and mandible, which may have impeded feeding, growth and locomotion. Schultz (2009) observed that spinal deformities were "common" in Gila chub larvae reared above 30^{o}C; however, we did not rear fish at such high temperatures in non-experimental conditions. We could not determine if the deformities were genetic in origin or if they were induced by our culture techniques. If the former, care must be taken not to introduce adults grown in a hatchery that would normally be culled from wild populations before they could breed. If the deformities are a result of hatchery conditions, then causative factors must be determined, and conditions modified in the laboratory to produce healthier fish.

Overall, spawning and rearing headwater chub in captivity will not be technically difficult, as the fish spawn readily under proper conditions, which are not hard to meet. Having accomplished the task several times, we now face the challenge of refining the techniques so that the fish produced in a captive breeding program fulfill reintroduction goals. That is, they need to be healthy and competent in the wild setting and as genotypically diverse and as minimally diverged from wild populations as possible. Further investigations could try live feeds which, in addition to nutritional value, might force fish to manipulate and capture feed items. Beyond maximizing hatchery growth, future projects might look into post-release performance and success of maximized hatchery-grown fish as compared to more naturally-grown hatchery fish and wild fish. In addition, a genetic baseline for the wild population, captive broodstock and hatchery offspring would be collected through genetic analysis to determine the robustness and variability of a captive population. There is a large and growing body of literature that suggests that inadvertent selection pressures are placed on individuals in hatcheries or other captive systems that can later affect wild populations after reintroductions (Gilligan and Frankham 2002; Jonsson and Jonsson 2006; Kelley et. al. 2006; Salonen and Peuhkuri, 2006). Kostow (2004) suggests that phenotypic changes may occur in as few as one hatchery generation, and while these traits are not genetic and will not be introduced into the population, they can still be subject to selection pressures, both in the hatchery and after release. Reintroducing fish that cannot survive or survive poorly in the wild is ineffective, and may actually harm wild populations.

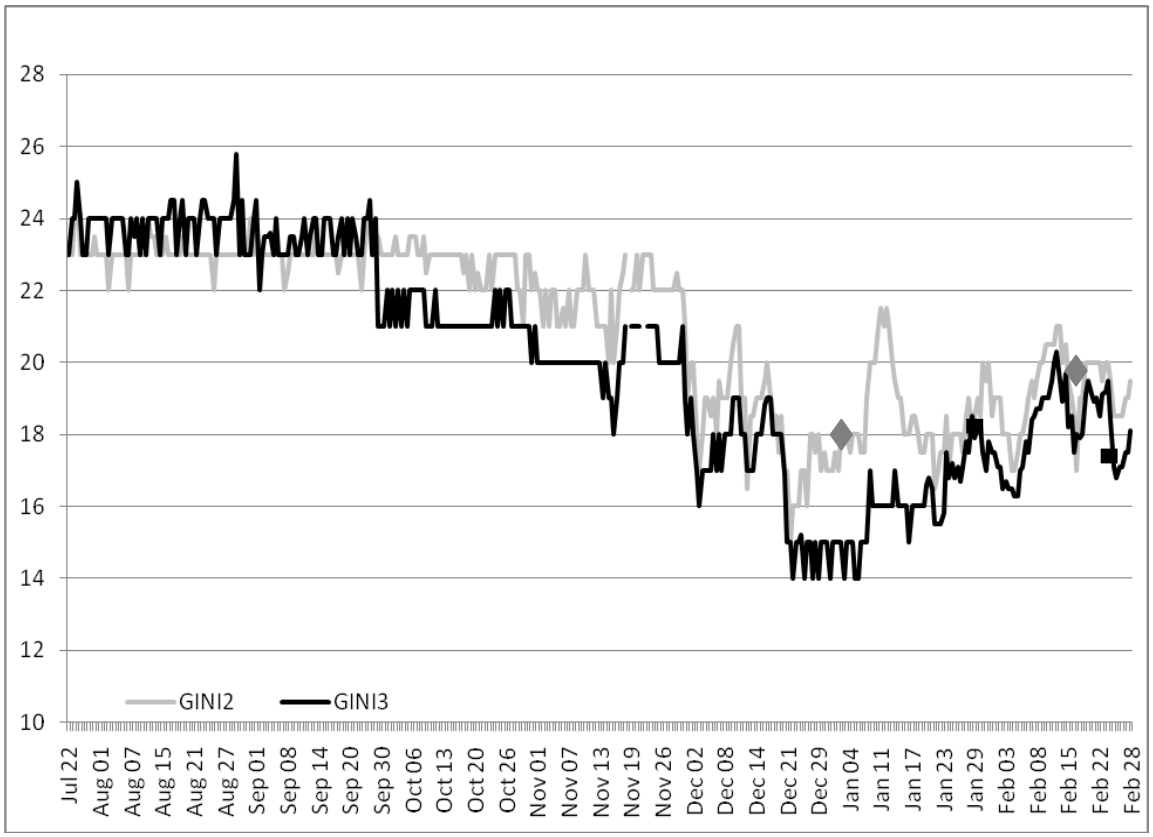

Figure 1.- Spawning times and temperatures for two tanks of headwater chub *Gila nigra*. Spawning occurred between about 17 and 19°C. GINI2 was a glass aquarium tank and GINI3 was a plastic stock tank; see Methods: Collection and Housing of Broodstock for descriptions. Gray diamonds are the spawn dates for GINI2; black squares are the spawn dates for GINI3

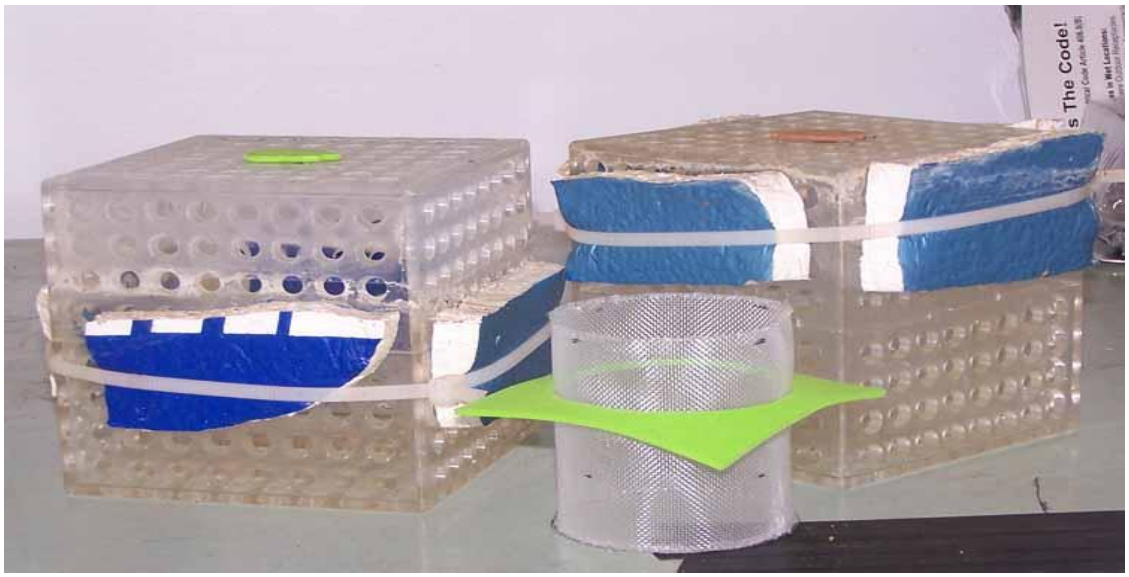

Figure 2.- Cages used to hold headwaters chub during experiments.
Fish cubes used for the large juvenile experiment with floats set at the two different heights for the two density treatments. The small cage in the front is the larval and small juvenile cage with the float height set for medium density. The black marks above and below the green foam float indicate the heights at which the floats were anchored for the low and high density treatments, respectively.

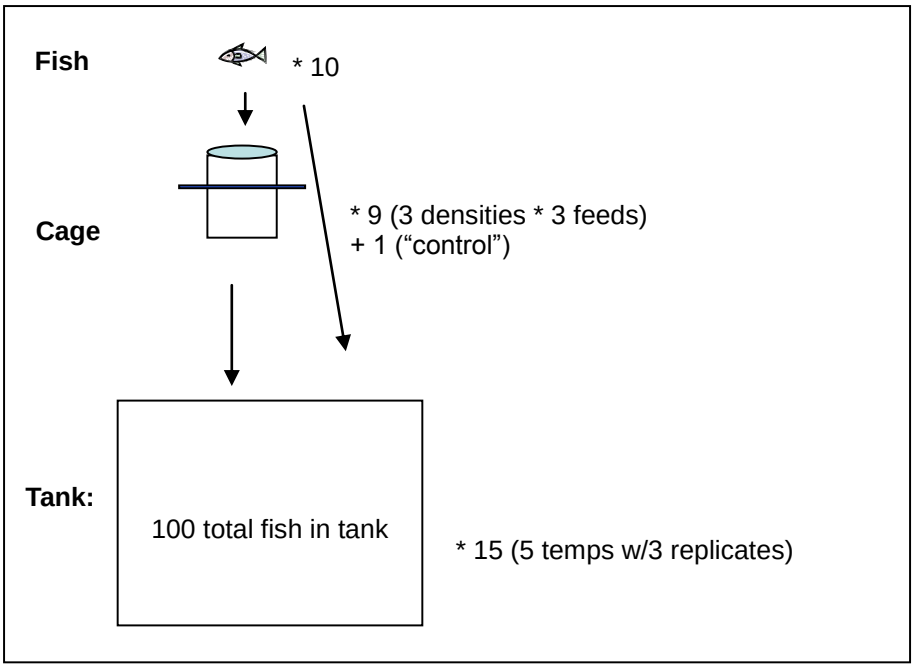

Figure 3: Experimental Design for First Larval Culture Study: Fish were sorted into cages and cages were assigned a feed x float height (density $^{-1}$) treatment. Every combination of feed x float height (density $^{-1}$) was represented within a group of cages which were then placed into a tank that was assigned a temperature treatment. Each temperature was replicated three times. Every possible temperature x feed x float height had three replicates at the start of the experiment. The same design was used for all other fish culture experiments with different numbers of fish/cage, density and temperature treatments depending on the number of fish available, and space and equipment. Only the first larval experiment had a group of fish not assigned to a cage and left free-swimming in the tank.

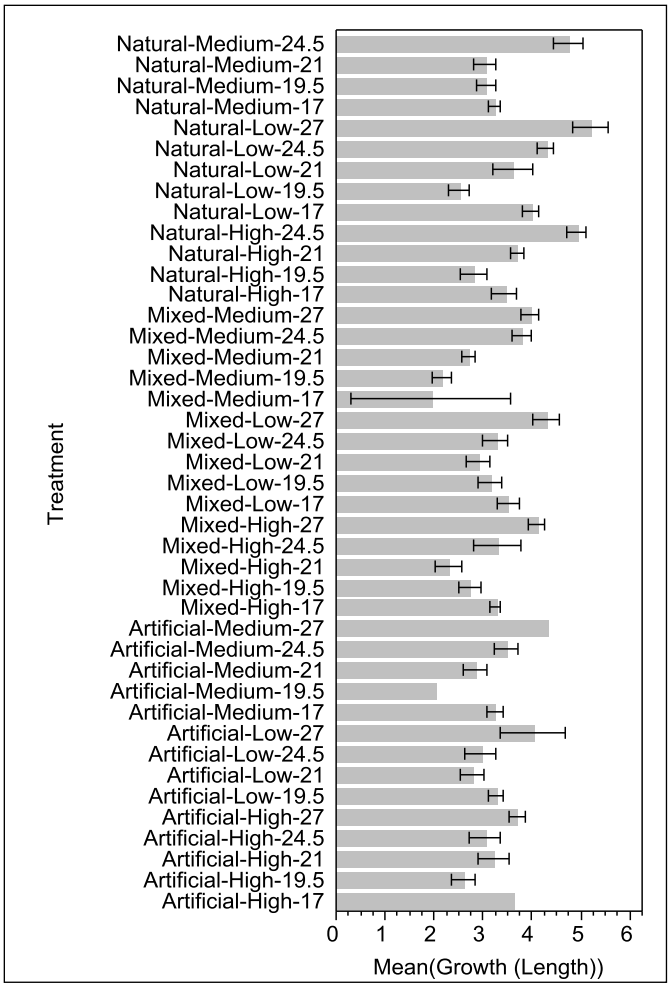

Figure 4. Mean growth (mm) ± SE of larval headwater chub by treatment type for the first larval experiment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Temperatures were 17°C, 19.5°C, 22°C, 24.5°C and 27°C and densities were low (0.006 fish/cm^3), medium (0.09 fish/cm^3) and high (0.18 fish/cm^3).

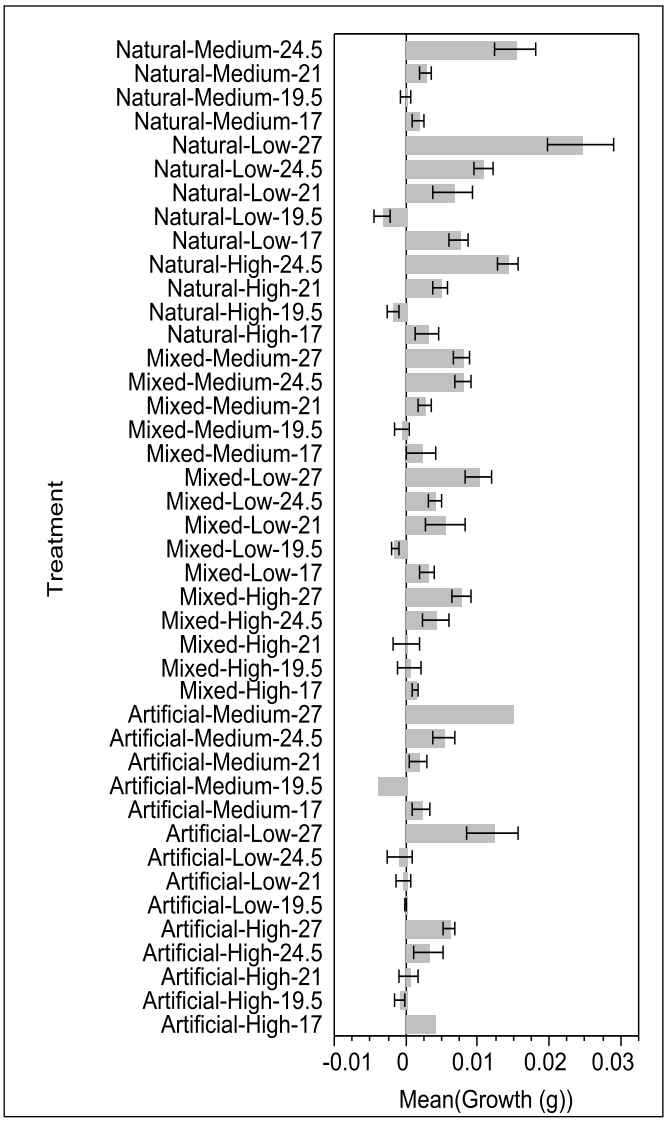

Figure 5. Mean growth (g) ± SE of larval headwater chub by treatment type for the first larval experiment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Temperatures were 17°C, 19.5°C, 22°C, 24.5°C and 27°C and densities were low (0.006 fish/cm^3), medium (0.09 fish/cm^3) and high (0.18 fish/cm^3).

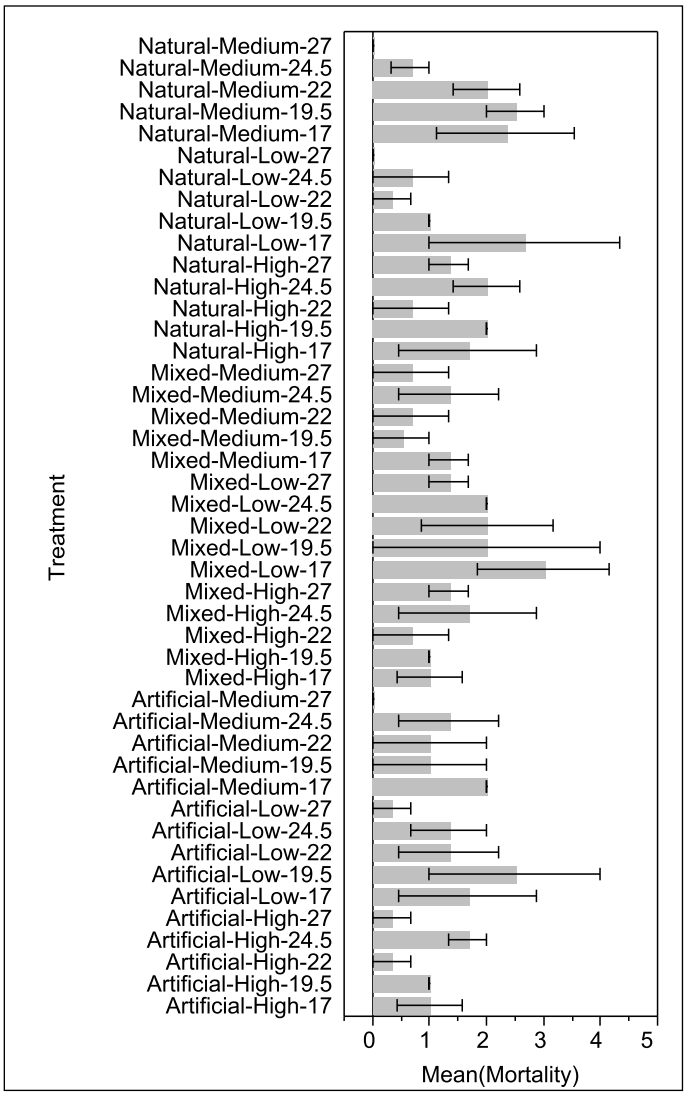

Figure 6. Mean mortality (number of fish per cage of ten) ± SE of larval headwater chub by treatment type for the first larval experiment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Temperatures were 17°C, 19.5°C, 22°C, 24.5°C and 27°C and densities were low (0.006 fish/cm^3), medium (0.09 fish/cm^3) and high (0.18 fish/cm^3). Mortality does not include numbers of fish lost from cages due to escapes, and total number of fish per cage at the end of the experiment was often less than ten.

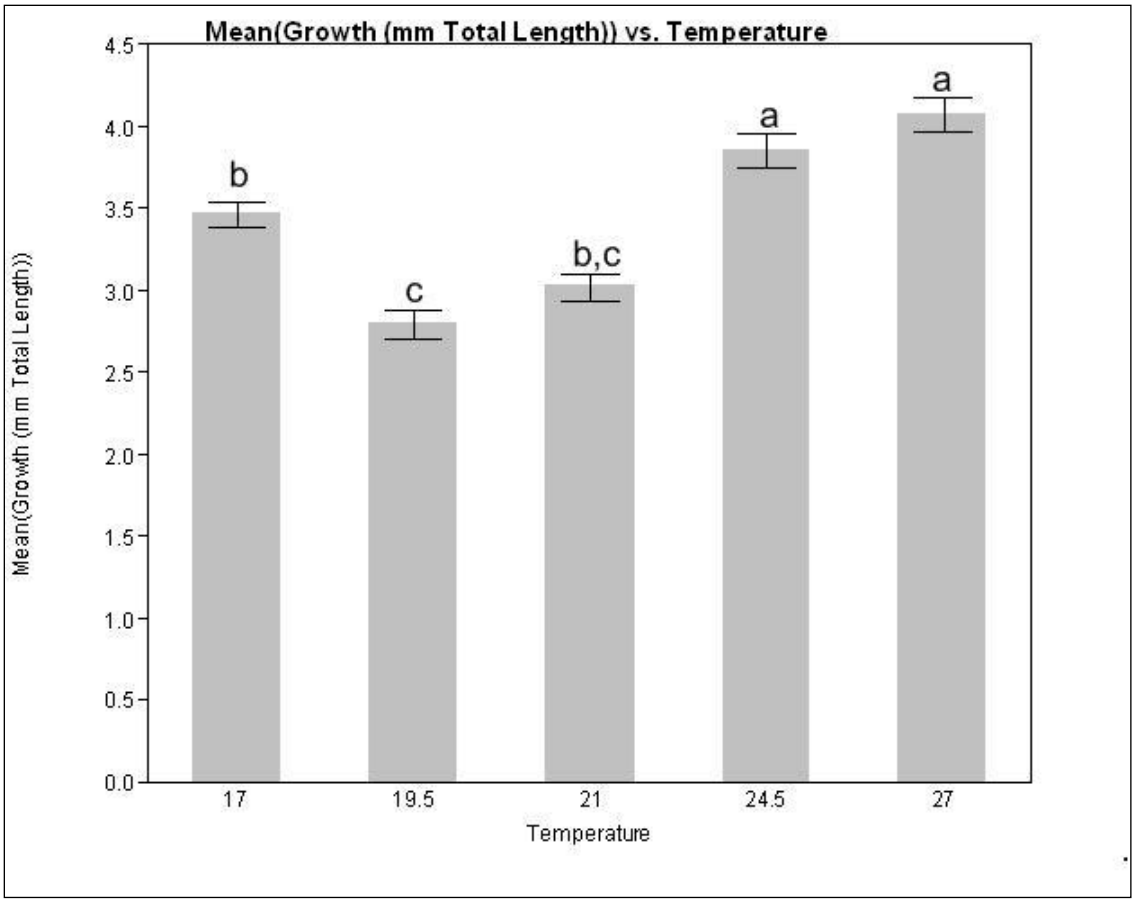

Figure 7. Growth (mm) of larval headwater chub in the first larval experiment by temperature treatment. Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

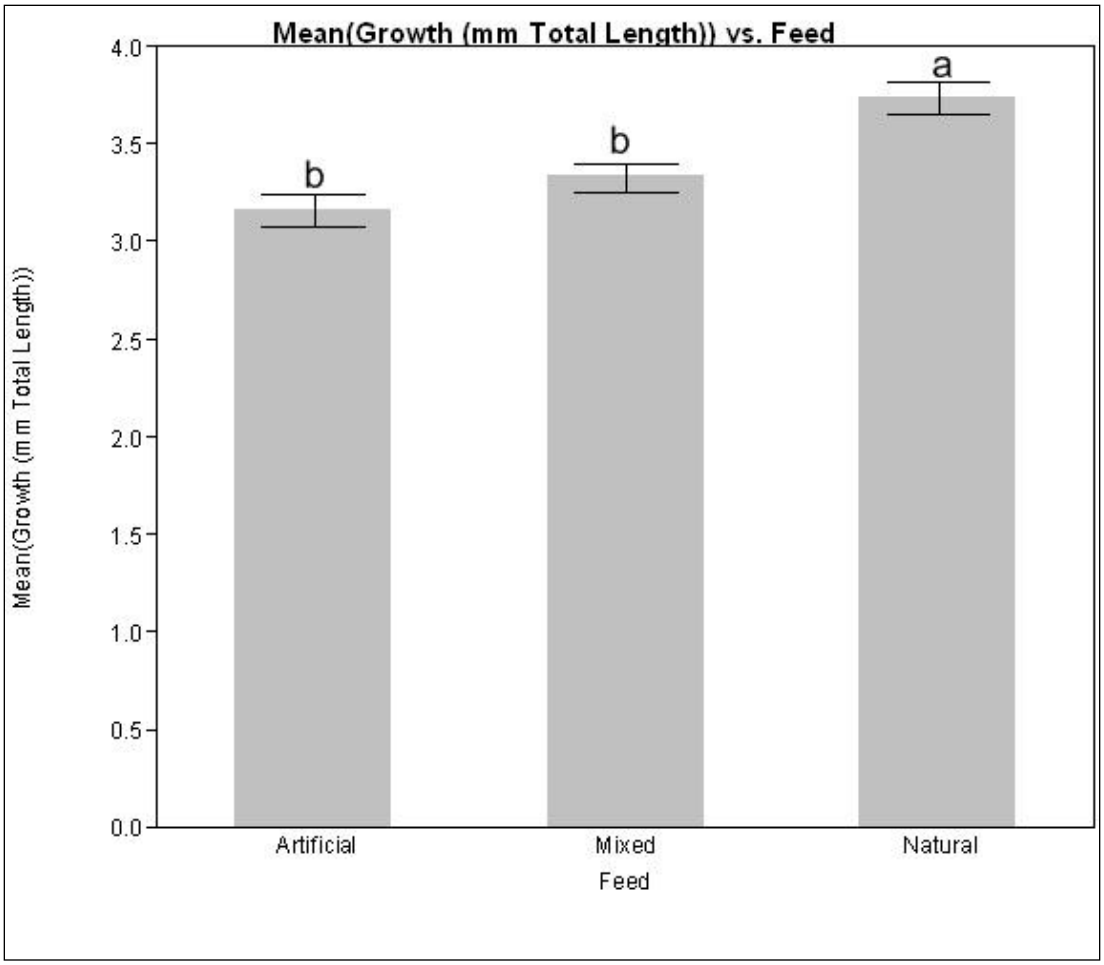

Figure 8. Growth (mm) of larval headwater chub in the first larval experiment by feed type treatment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

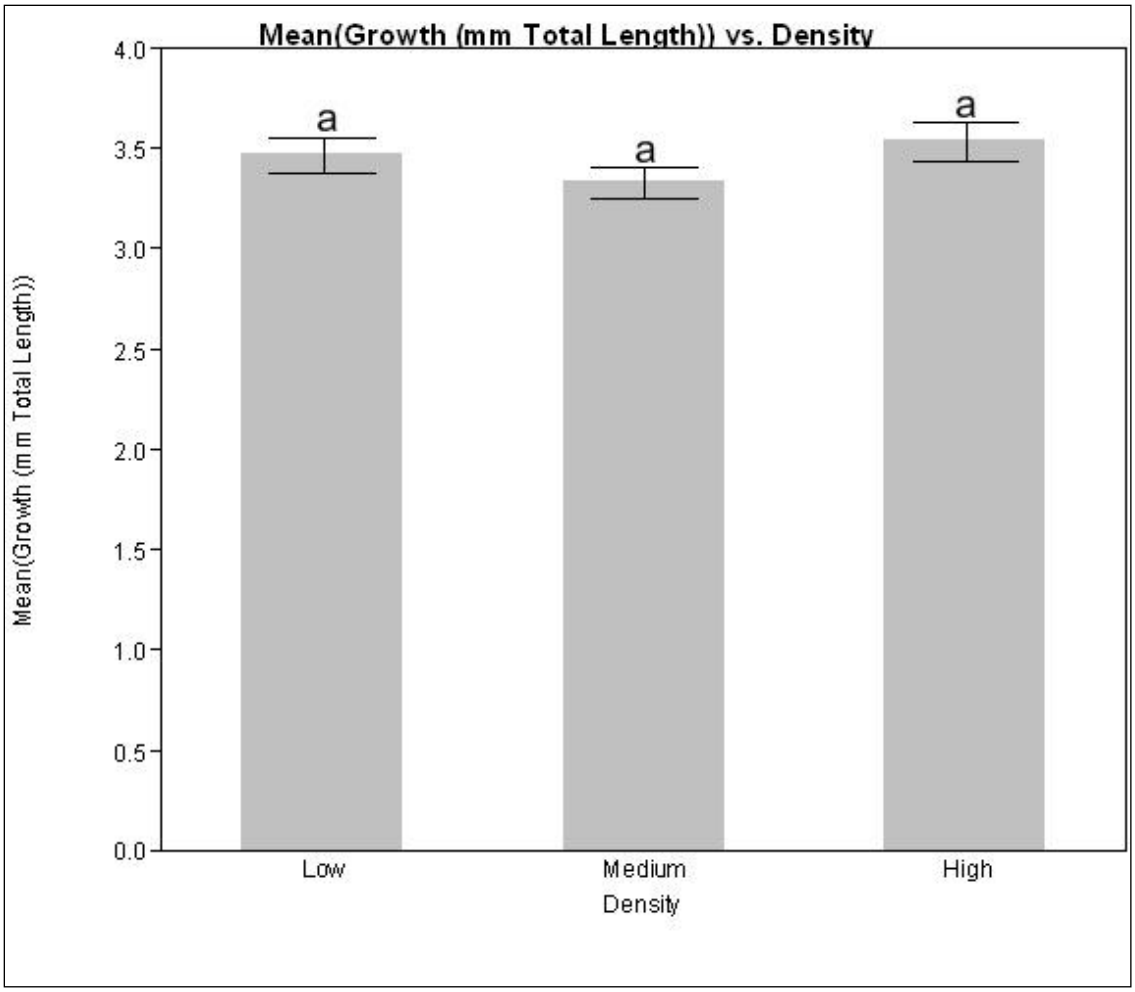

Figure 9. Growth (mm) of larval headwater chub in the first larval experiment by density treatment. Densities were low (0.006 fish/cm^3), medium (0.09 fish/cm^3) and high (0.18 fish/cm^3). Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

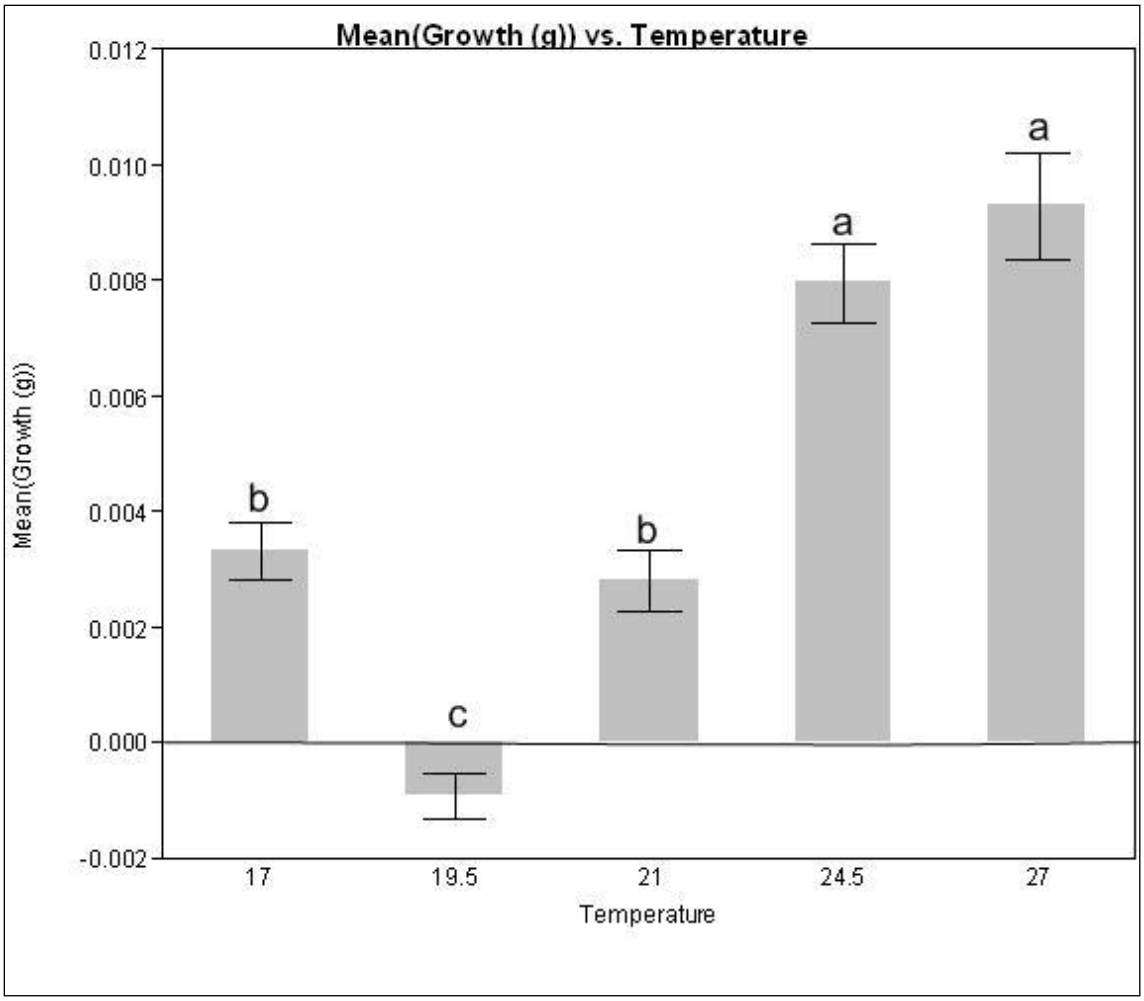

Figure 10. Growth (g) of larval headwater chub in the first larval experiment by temperature treatment. Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

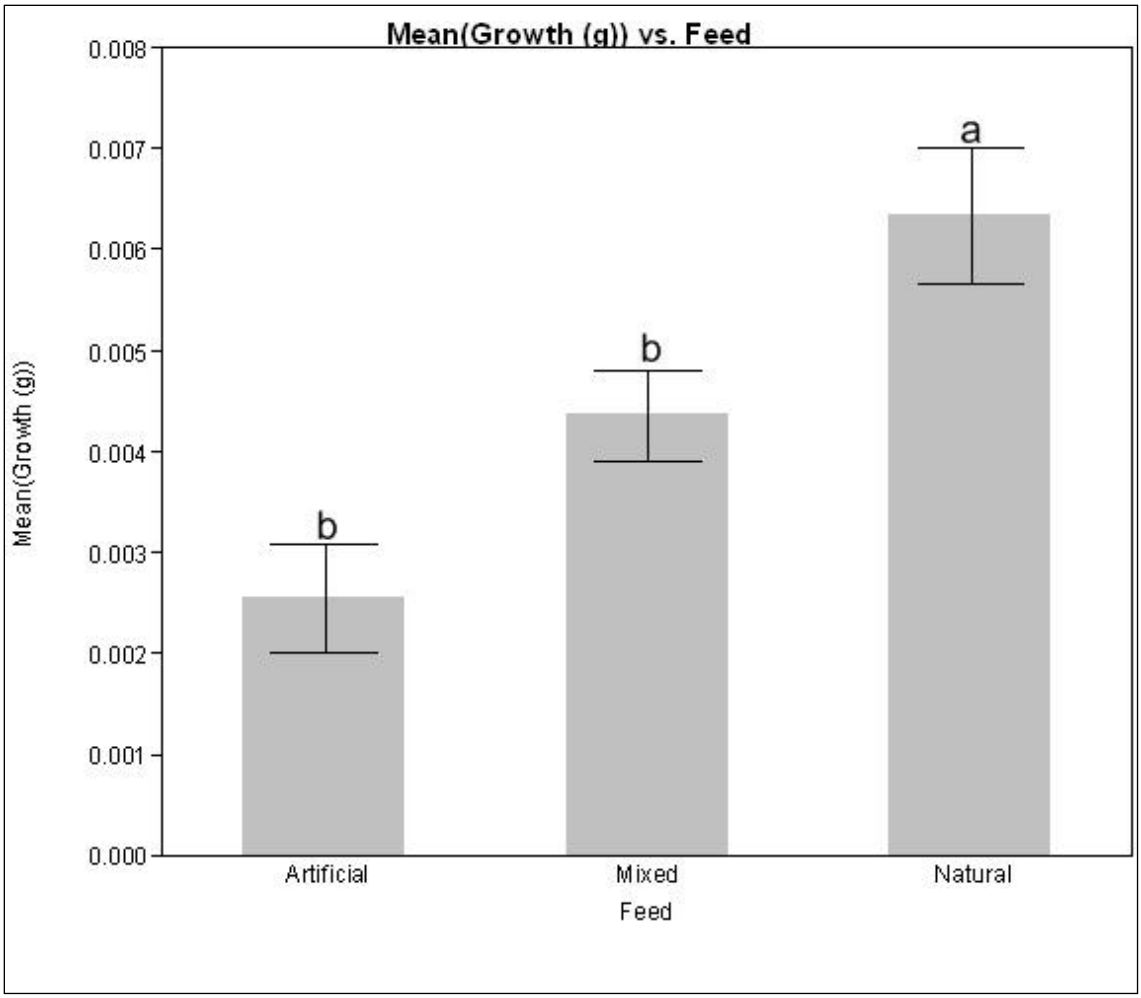

Figure 11. Growth (g) of larval headwater chub in the first larval experiment by feed type treatment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

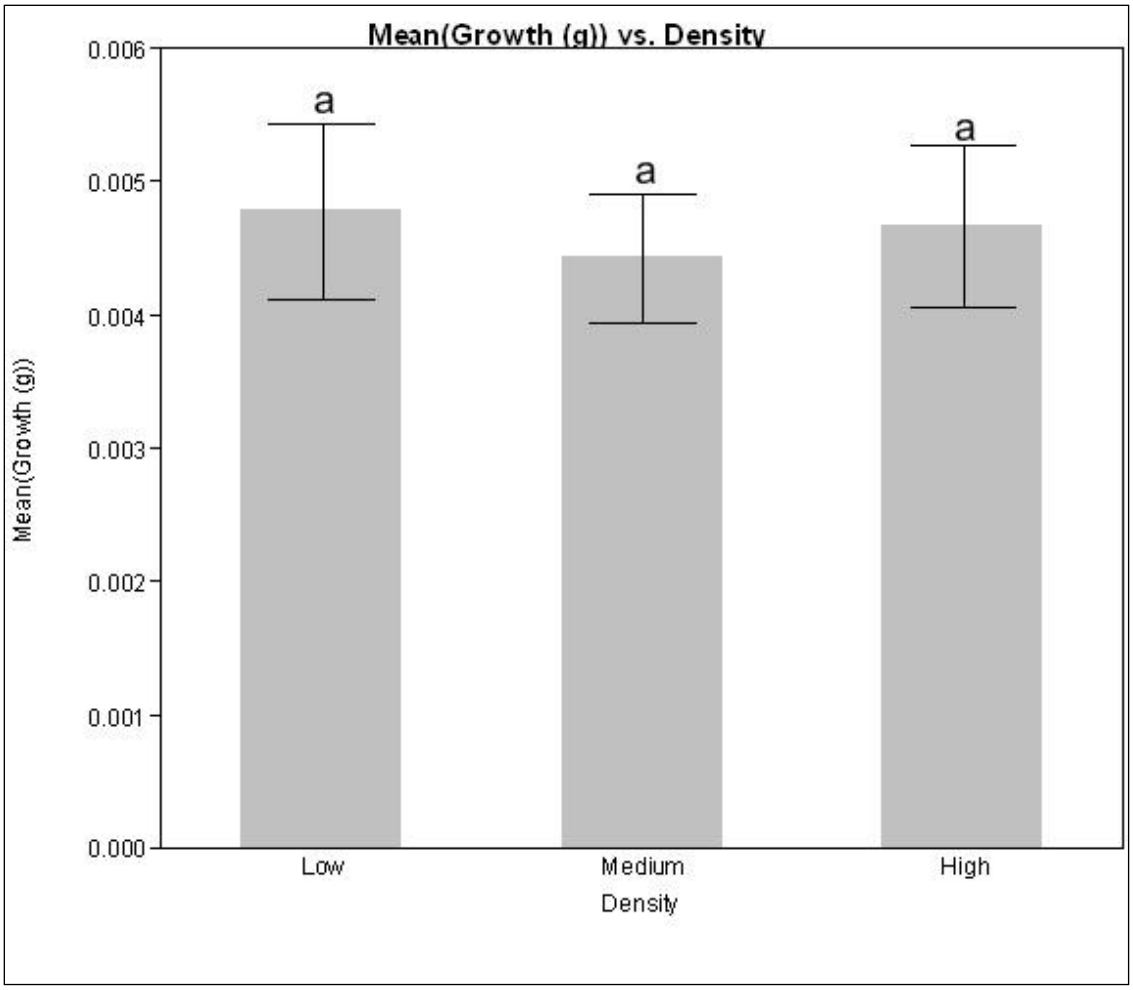

Figure 12. Growth (g) of larval headwater chub in the first larval experiment by density treatment. Densities were low (0.006 fish/cm^3), medium (0.09 fish/cm^3) and high (0.18 fish/cm^3). Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

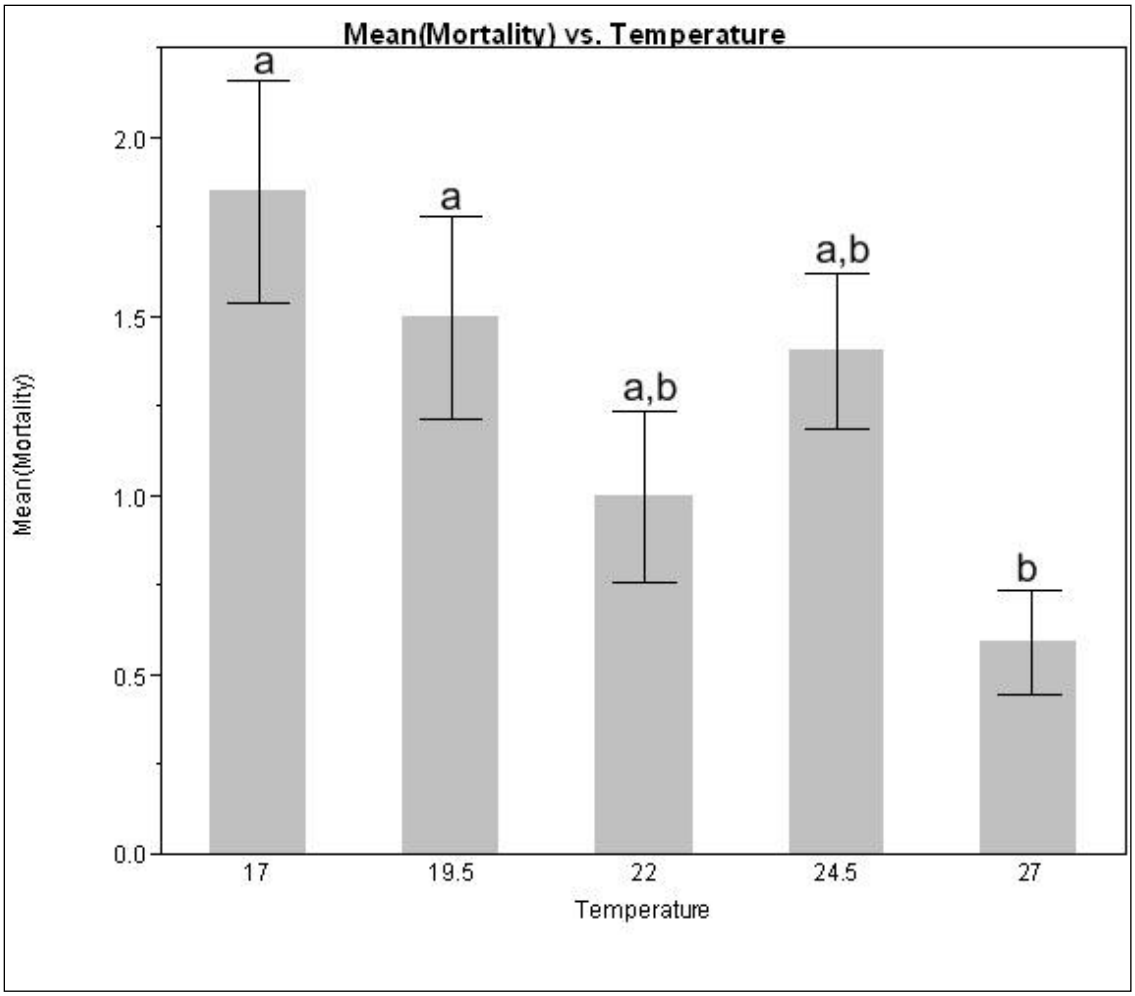

Figure 13. Mortality of larval headwater chub in the first larval experiment by temperature treatment. Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

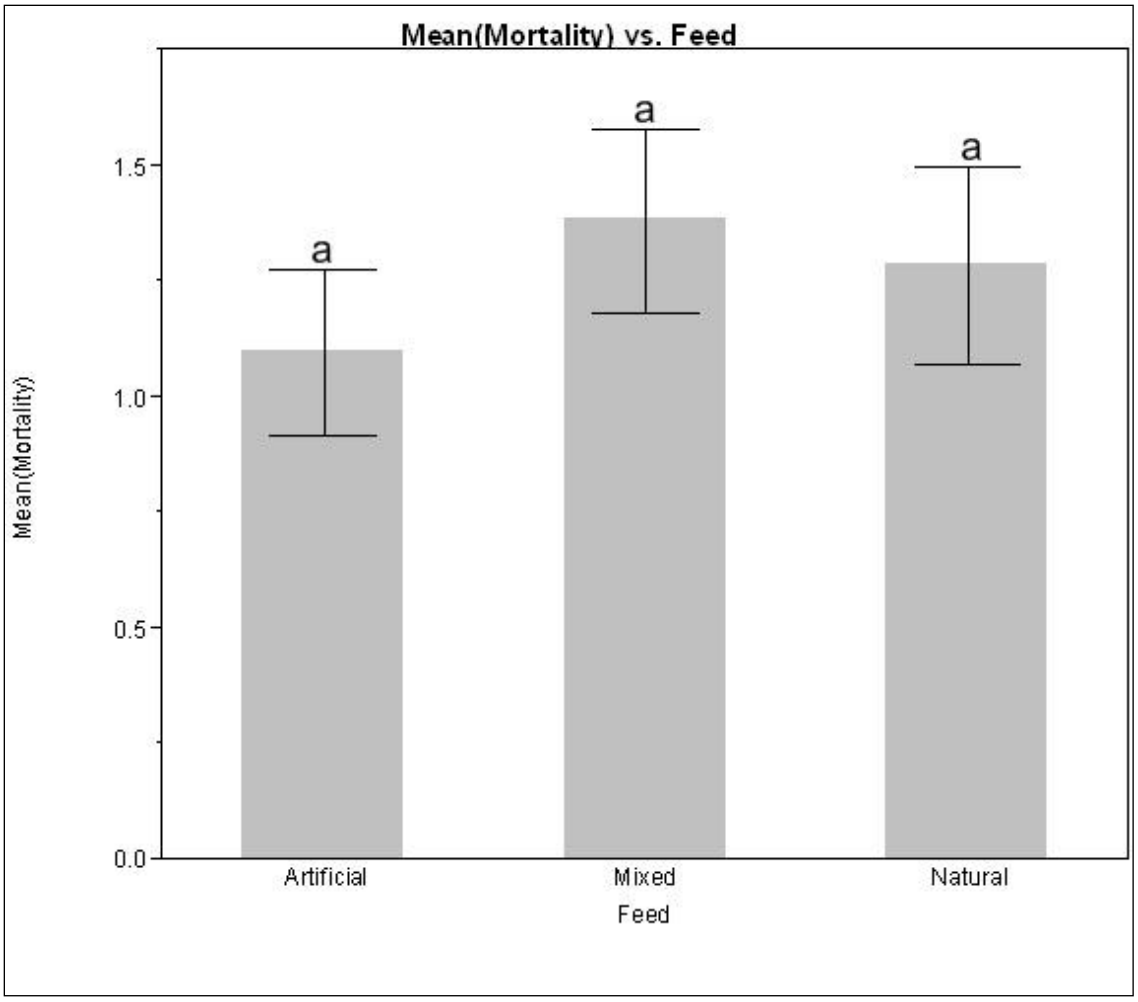

Figure 14. Mortality of larval headwater chub in the first larval experiment by feed type treatment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

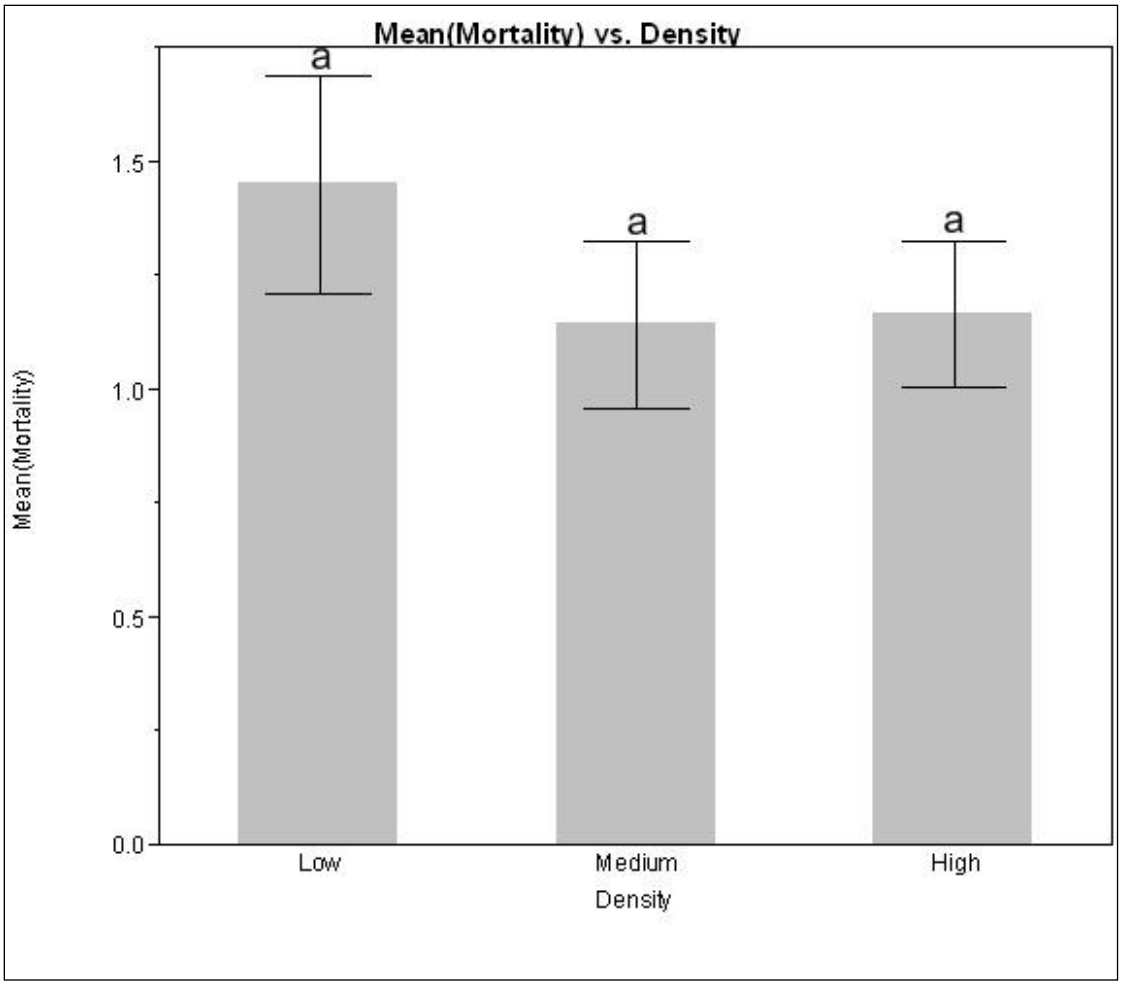

Figure 15. Mortality of larval headwater chub in the first larval experiment by density treatment. Densities were low (0.006 fish/cm^3), medium (0.09 fish/cm^3) and high (0.18 fish/cm^3).Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

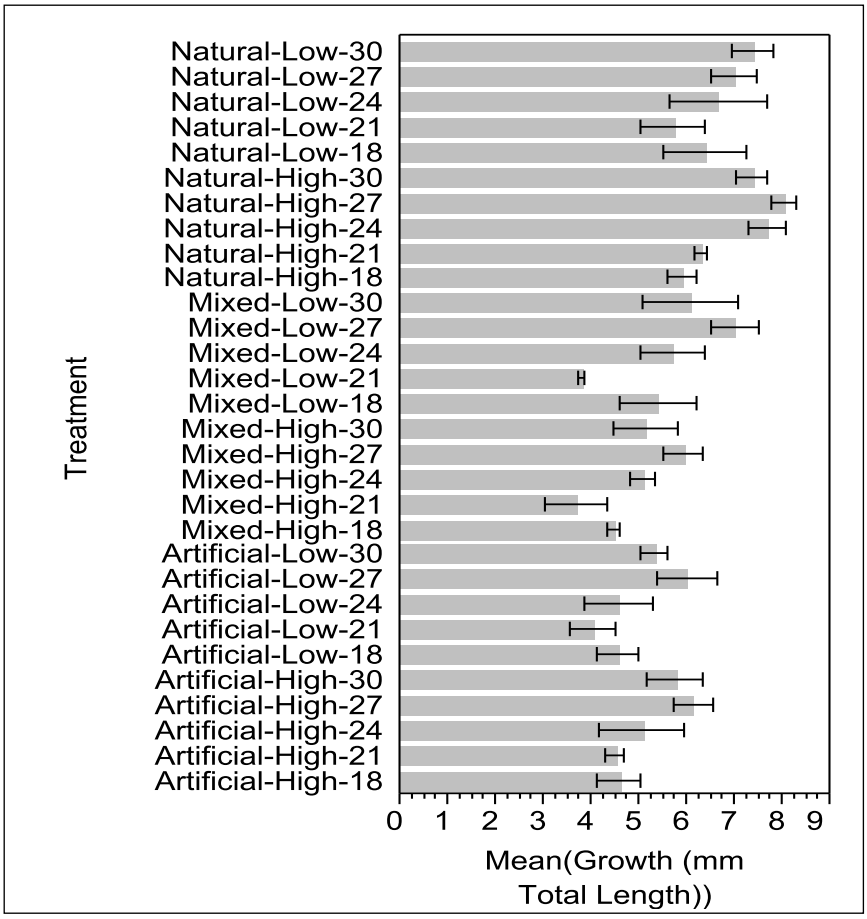

Figure 16. Mean growth (mm) ± SE of larval headwater chub by treatment type for the second larval experiment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Temperatures were 18°C, 21°C, 24°C, 27°C and 30°C and densities were low (0.03 fish/cm^3) and high(0.09 fish/cm^3).

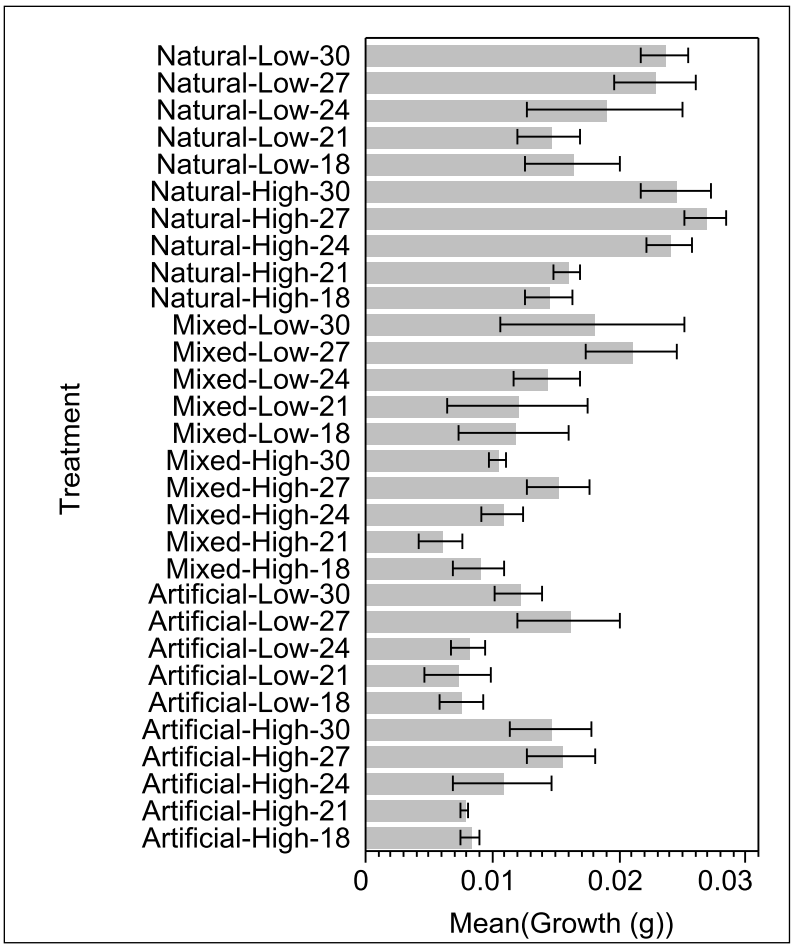

Figure 17. Mean growth (g) ± SE of larval headwater chub by treatment type for the second larval experiment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Temperatures were 18°C, 21°C, 24°C, 27°C and 30°C and densities were low (0.03 fish/cm^3) and high (0.09 fish/cm^3).

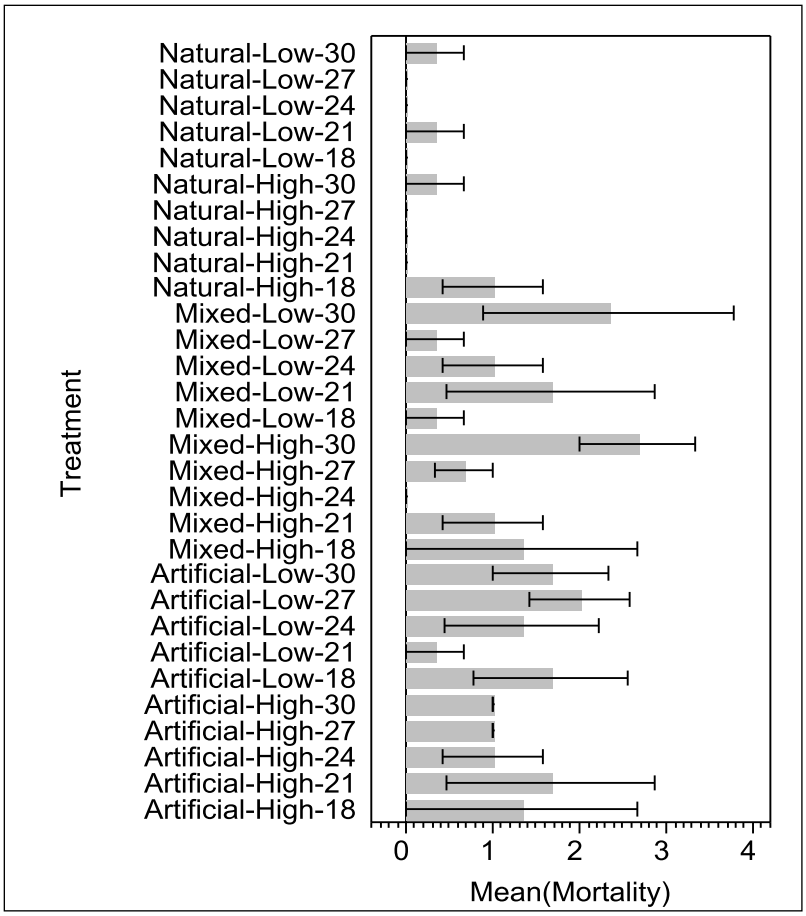

Figure 18. Mean mortality (number of fish per cage of 5) ± SE of larval headwater chub by treatment type for the second larval experiment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Temperatures were 18°C, 21°C, 24°C, 27°C and 30°C and densities were low (0.03 fish/cm^3) and high (0.09 fish/cm^3).

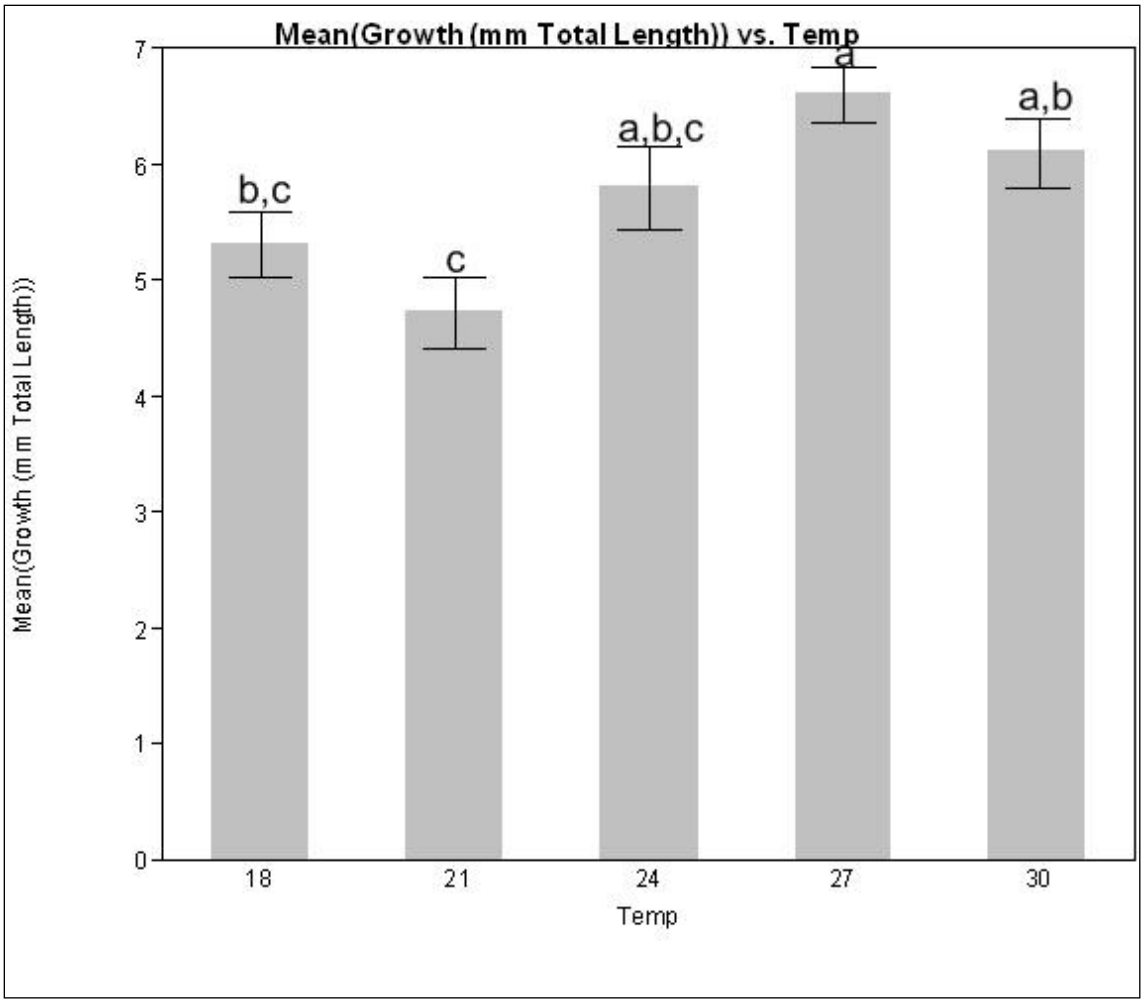

Figure 19. Growth (mm) of larval headwater chub in the second larval experiment by temperature Error bars represent one standard error from the mean. .Bars not connected by the same letter are significantly different ($P \leq 0.05$).

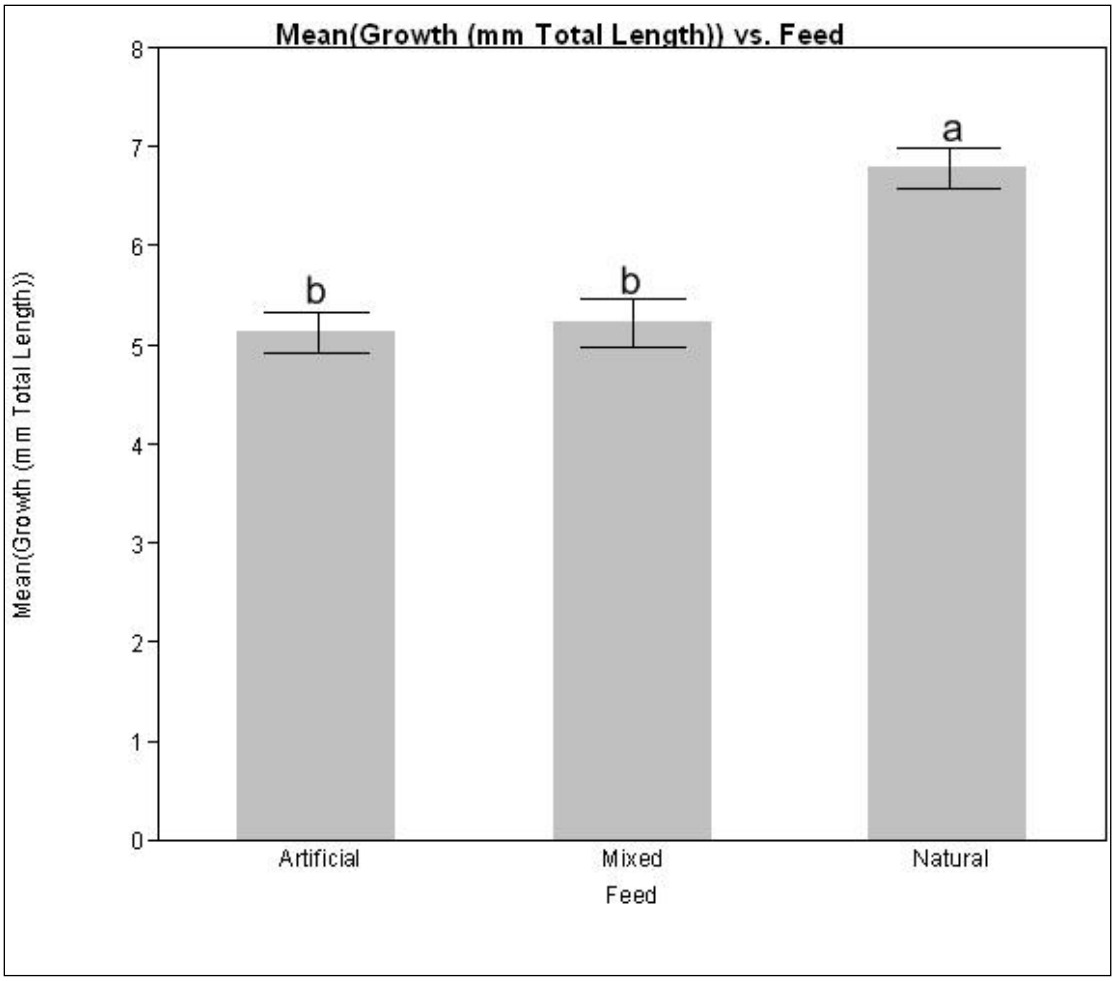

Figure 20. Growth (mm) of larval headwater chub in the second larval experiment by feed type treatment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

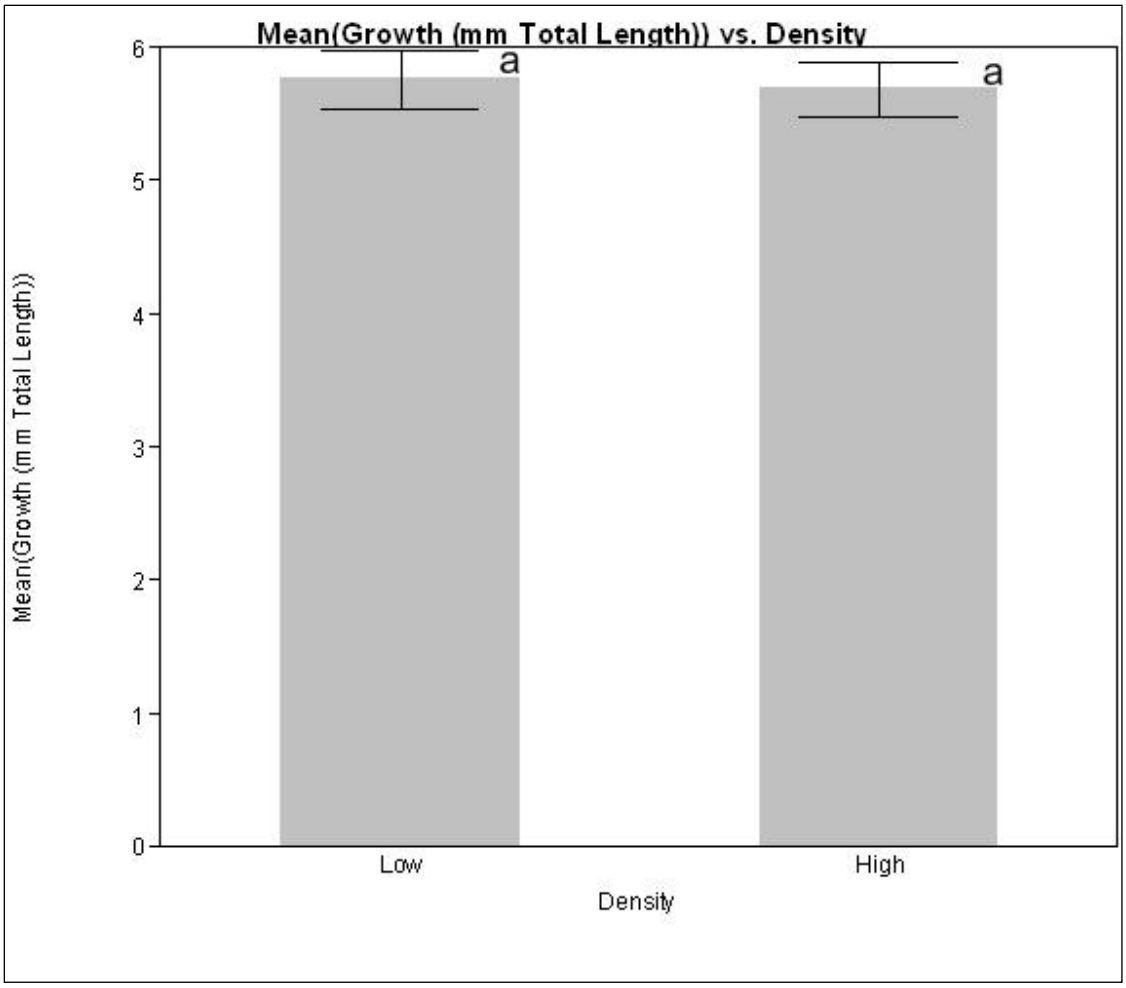

Figure 21. Growth (mm) of larval headwater chub in the second larval experiment by density treatment. Densities were low (0.03 fish/cm^3) and high(0.09 fish/cm^3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

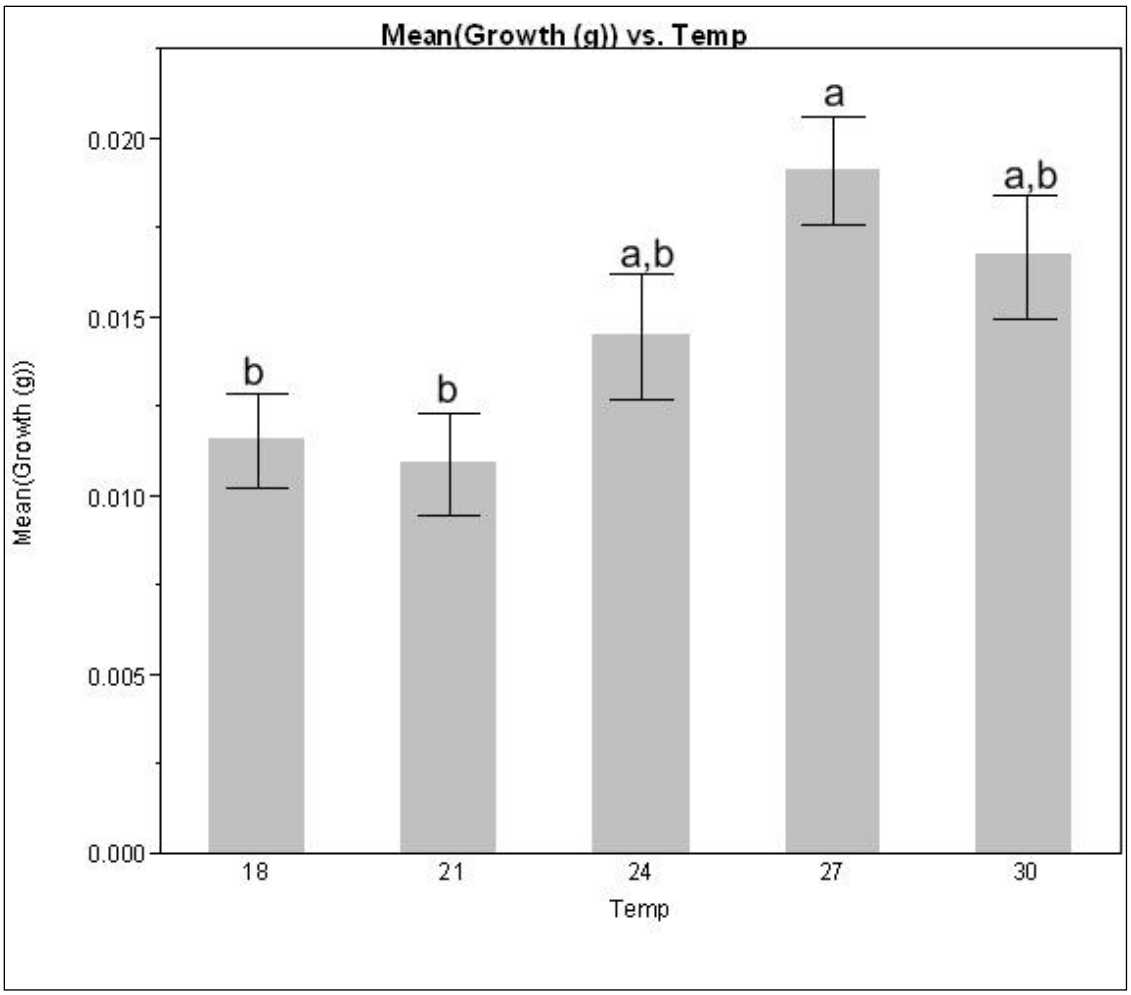

Figure 22. Growth (g) of larval headwater chub in the second larval experiment by temperature treatment. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

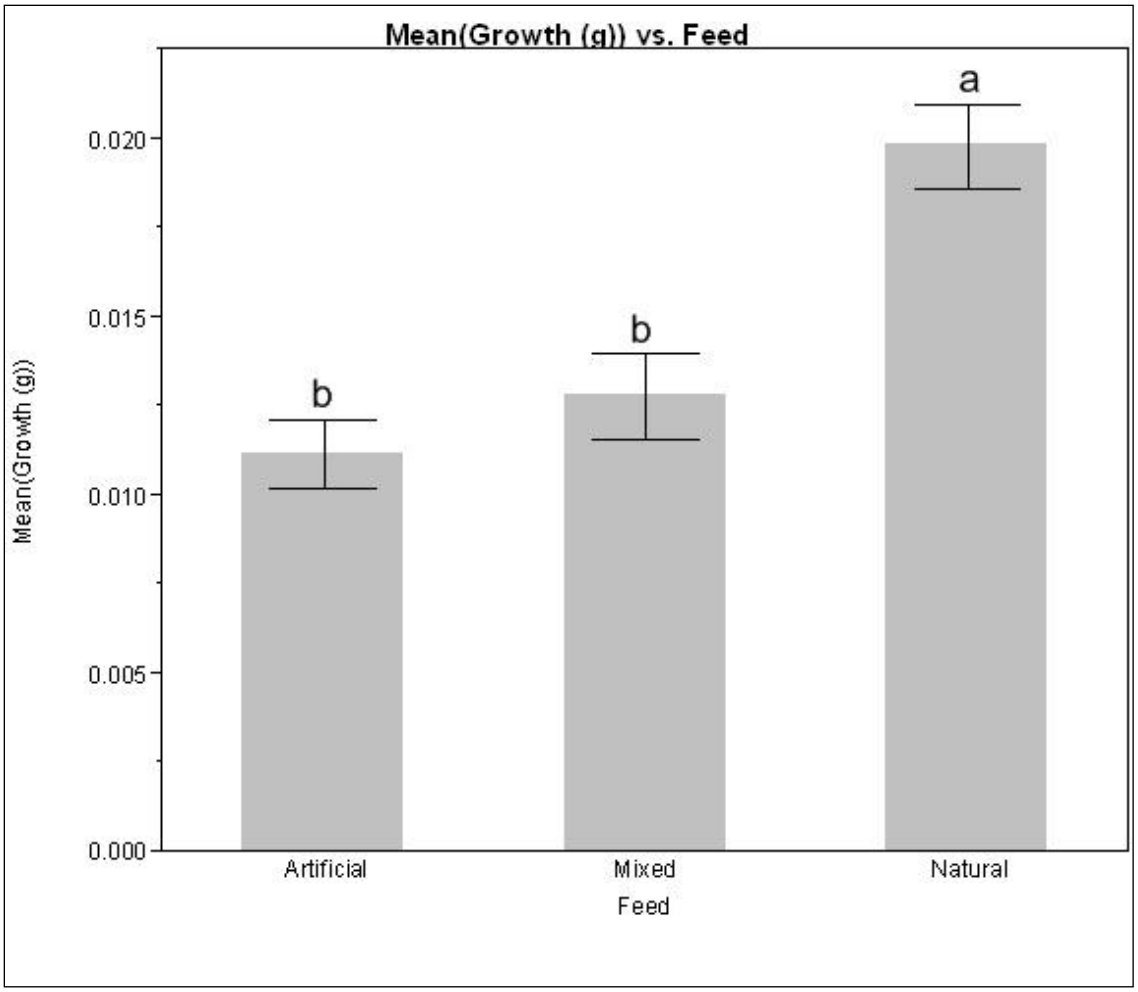

Figure 23. Growth (g) of larval headwater chub in the second larval experiment by feed type treatment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

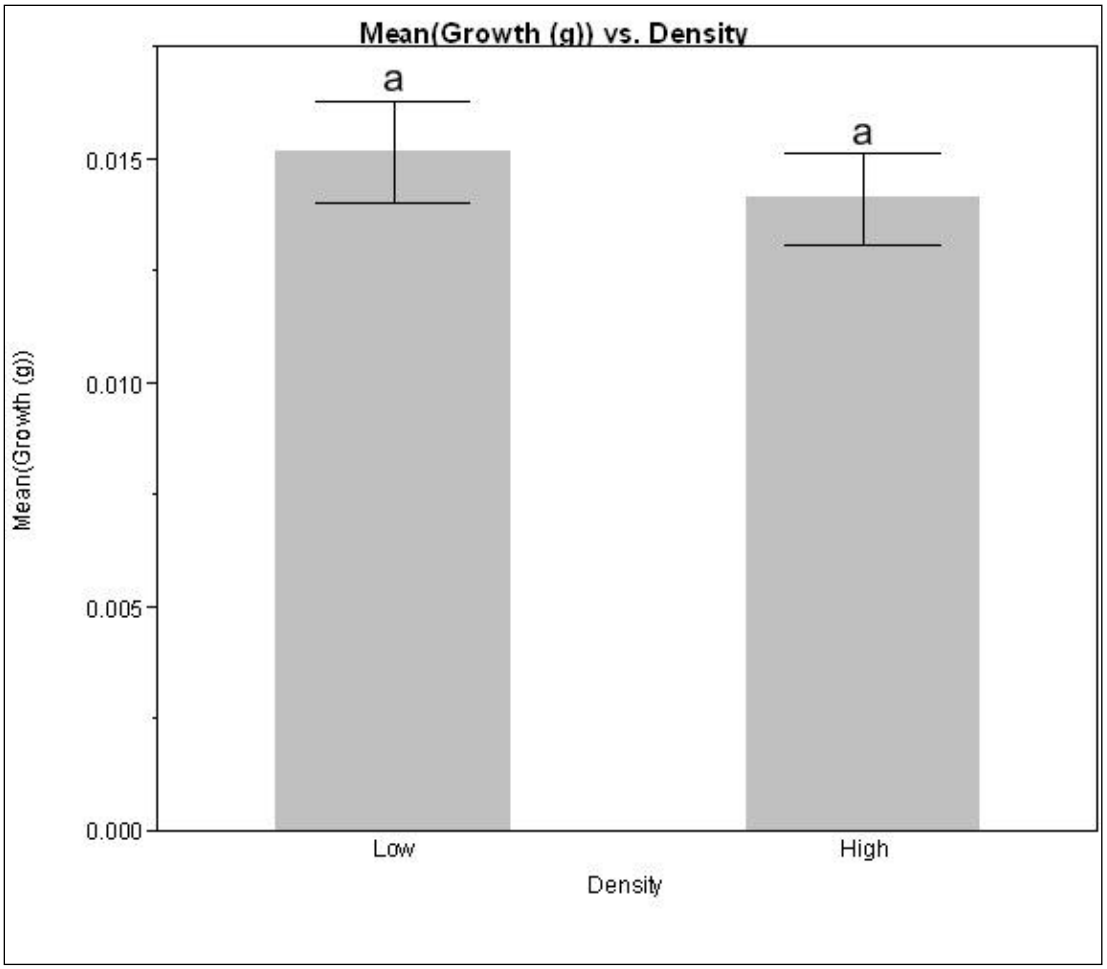

Figure 24. Growth (g) of larval headwater chub in the second larval experiment by density treatment. Densities were low (0.03 fish/cm^3) and high(0.09 fish/cm^3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

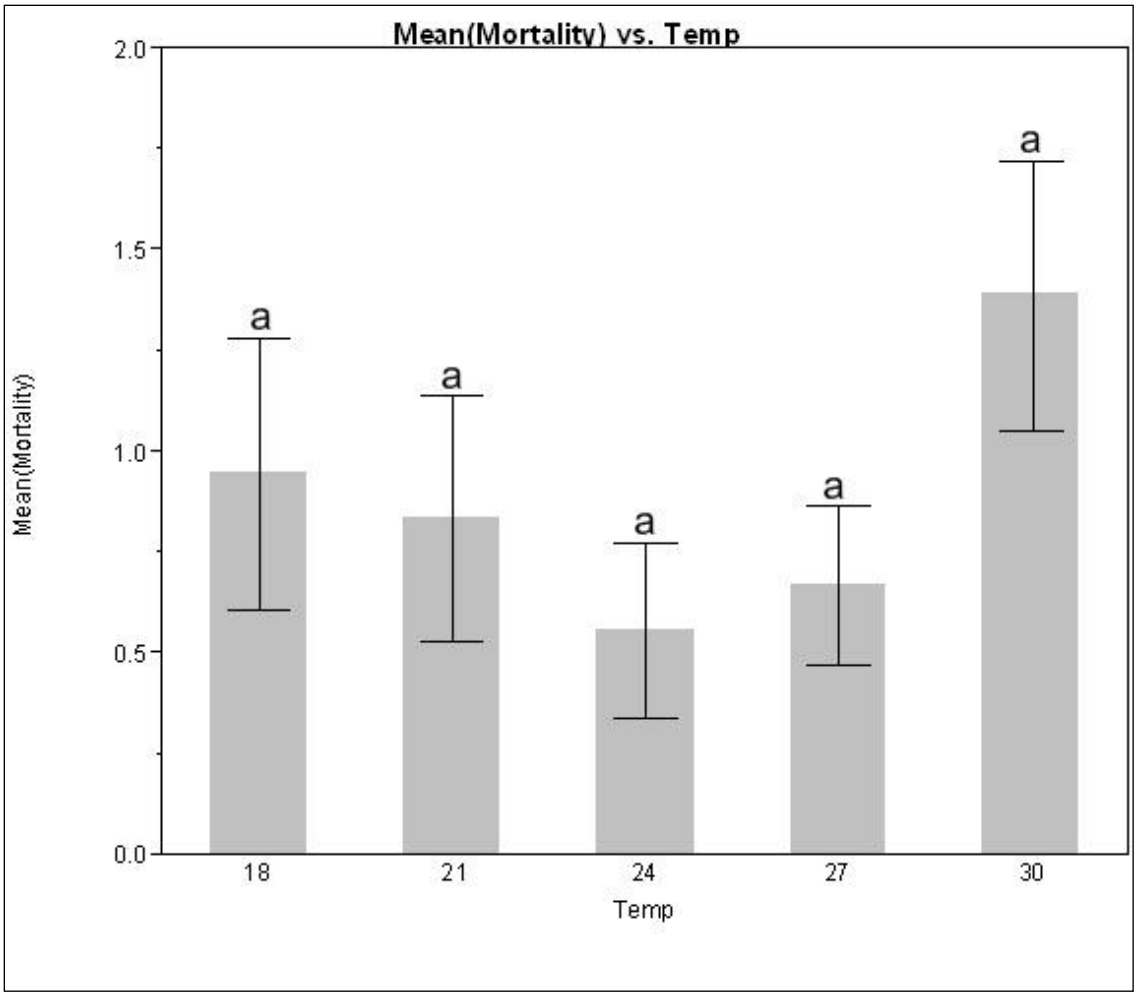

Figure 25. Mortality of larval headwater chub in the second larval experiment by temperature treatment. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

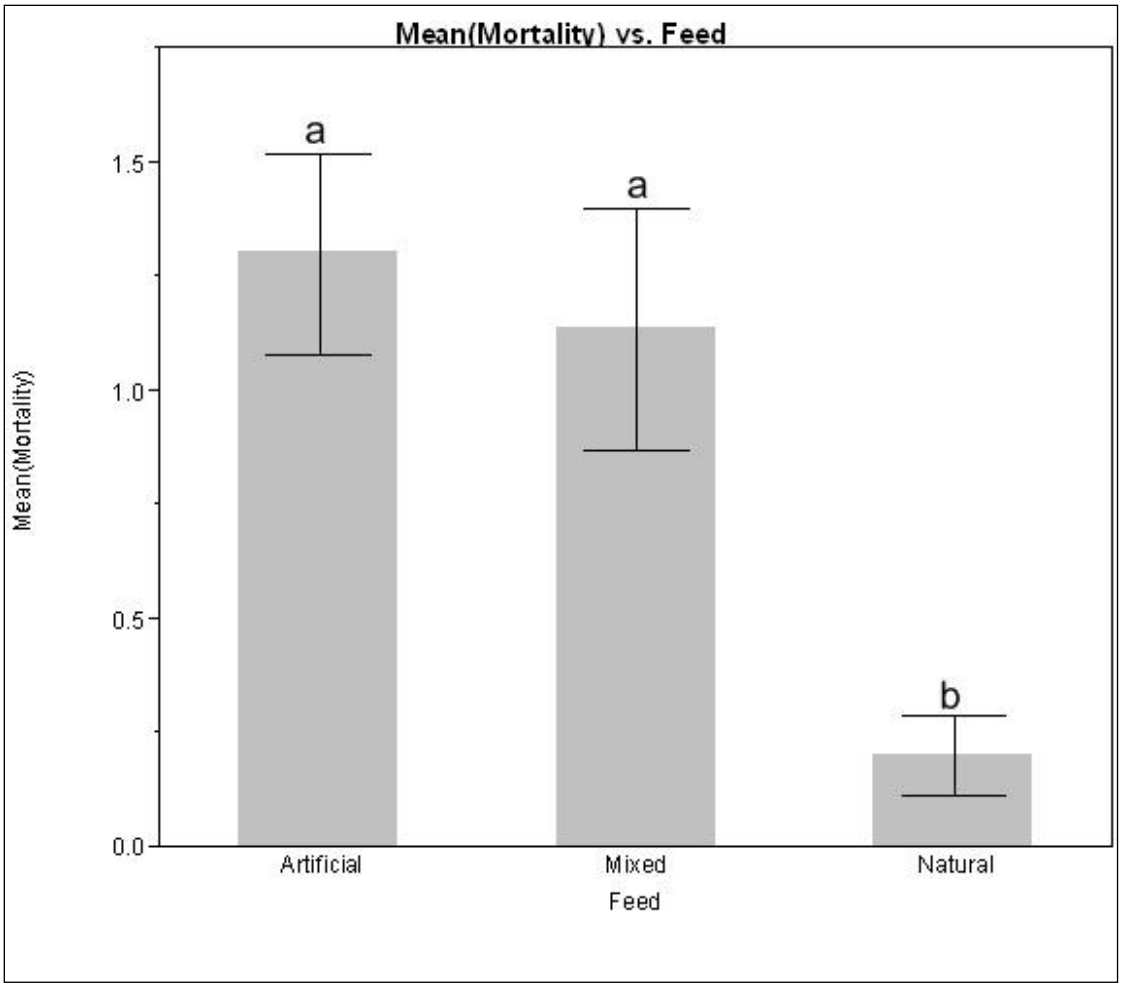

Figure 26. Mortality of larval headwater chub in the second larval experiment by feed type treatment. "Natural" feed was a brine shrimp/plankton mix consisting of decapsulated brine shrimp eggs and plankton from Aquatic Ecosystems; "Mixed" was plankton/AP100 mix consisting of plankton from Aquatic Ecosystems and Zeigler's Larval AP100 from Aquatic Ecosystems; "Artificial" was Hikari First Bites. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

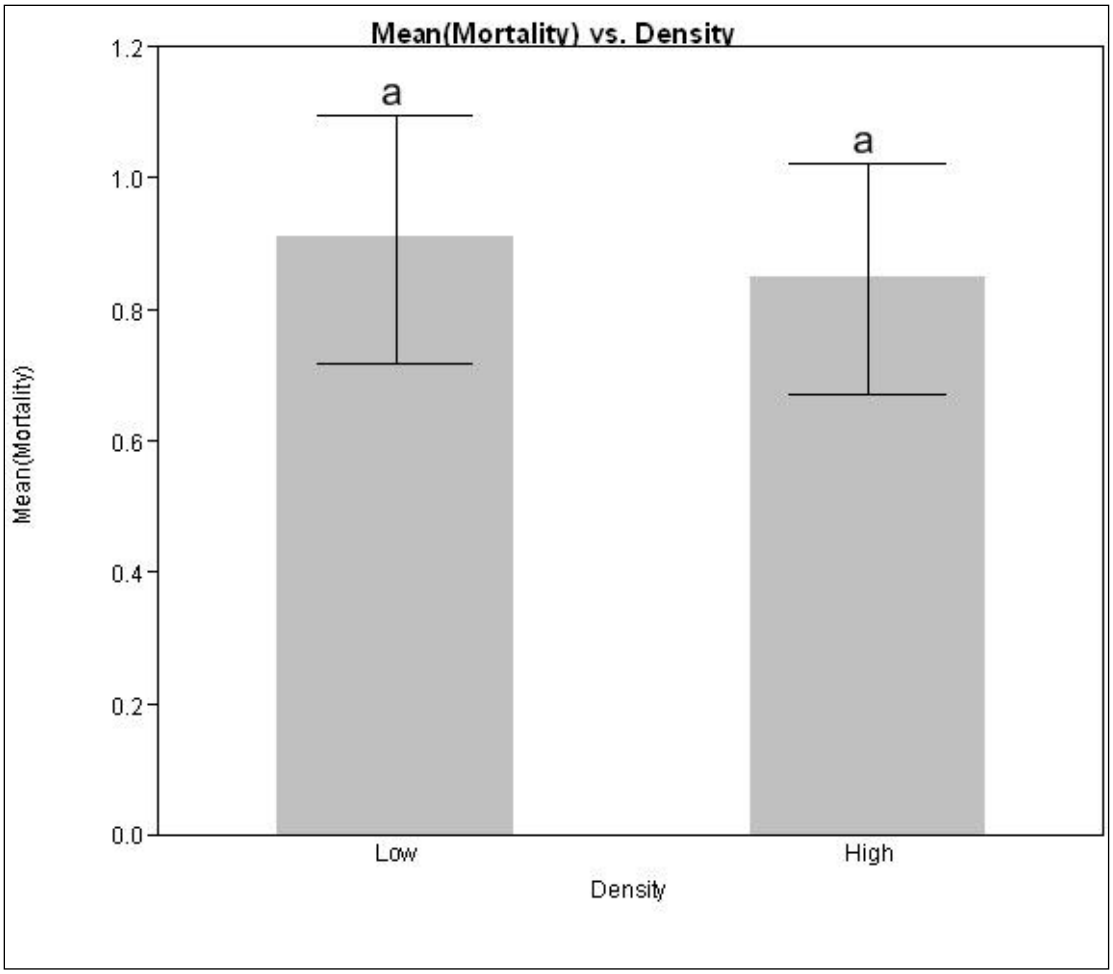

Figure 27. Mortality of larval headwater chub in the second larval experiment by density treatment. Densities were low (0.03 fish/cm^3) and high(0.09 fish/cm^3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

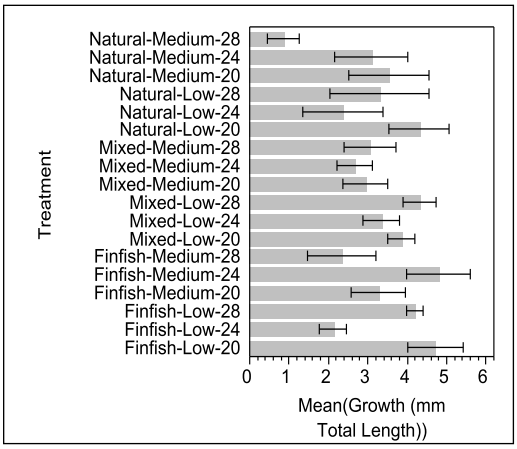

Figure 28. Mean growth (mm) ± SE of small juvenile headwater chub by treatment type. Natural feed was Hikari Bio-pure brine shrimp, Spirulina brine shrimp and bloodworms; "Catfish" was fine-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was ground Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Temperatures were 20, 24 and 28°C and densities were low (0.03 fish/cm^3) and high (0.04 fish/cm^3).

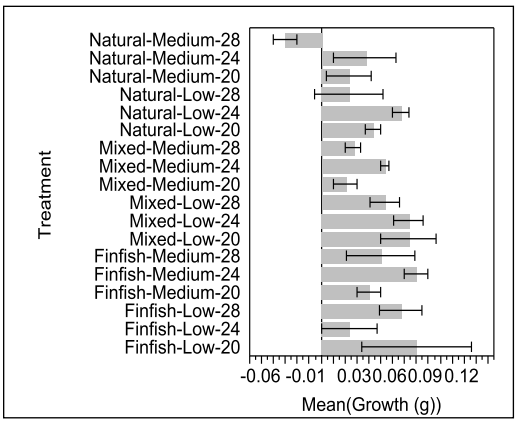

Figure 29. Mean growth (g) ± SE of small juvenile headwater chub by treatment type. Natural feed was Hikari Bio-pure brine shrimp, Spirulina brine shrimp and bloodworms; "Catfish" was fine-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was ground Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Temperatures were 20, 24 and 28°C and densities were low (0.03 fish/cm^3) and medium (0.04 fish/cm^3).

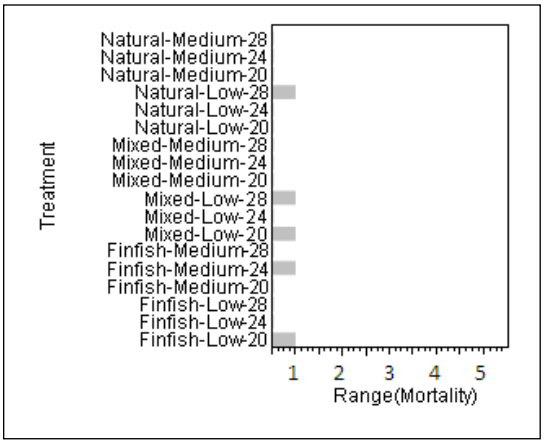

Figure 30. Mortality (number of fish found dead per cage of five) of small juvenile headwater chub by treatment type. Bars represent a single mortality; any treatment types with no bars did not experience mortality.

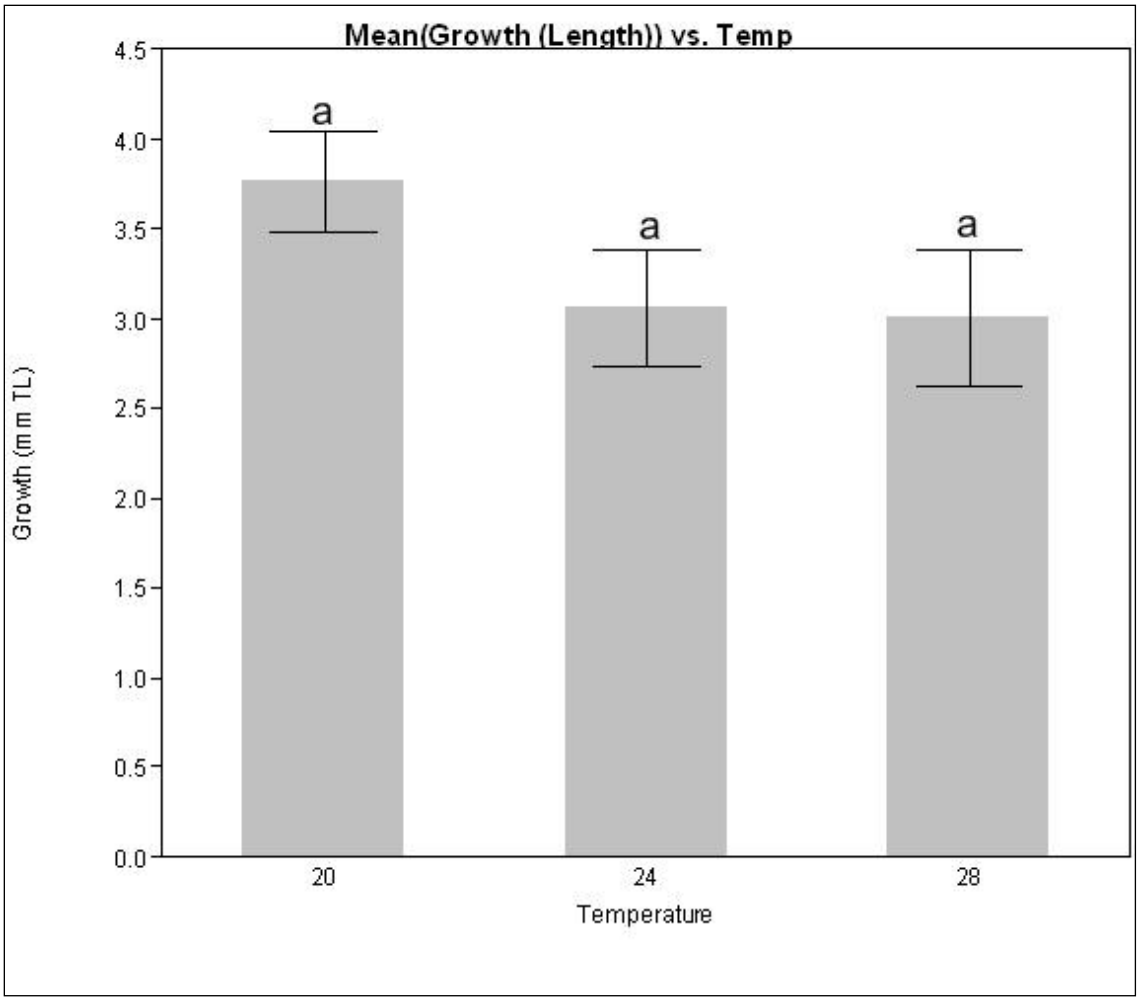

Figure 31. Growth (mm) of small juvenile headwater chub by temperature treatment. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

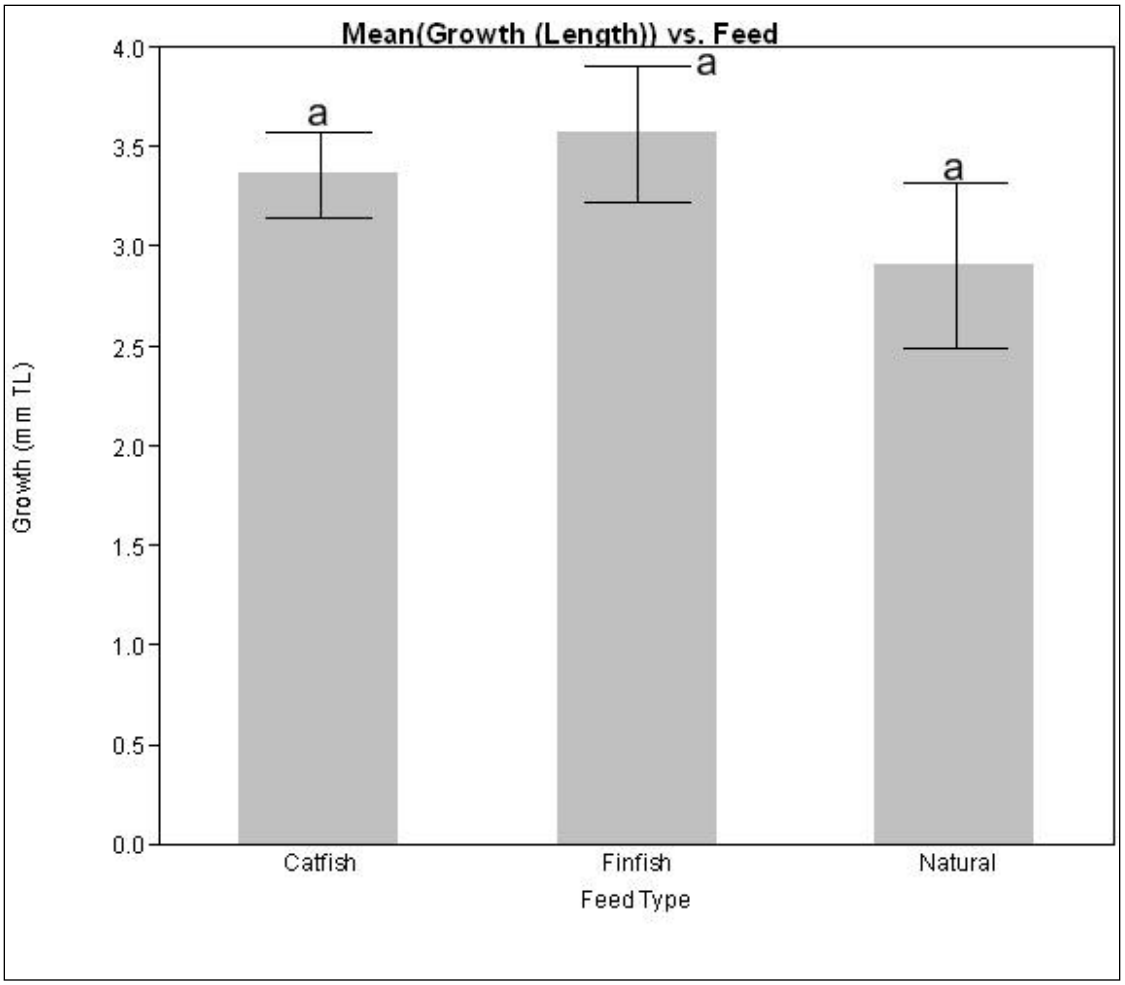

Figure 32. Growth (mm) of small juvenile headwater chub by feed type treatment. Natural feed was Hikari Bio-pure brine shrimp, Spirulina brine shrimp and bloodworms; "Catfish" was fine-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was ground Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

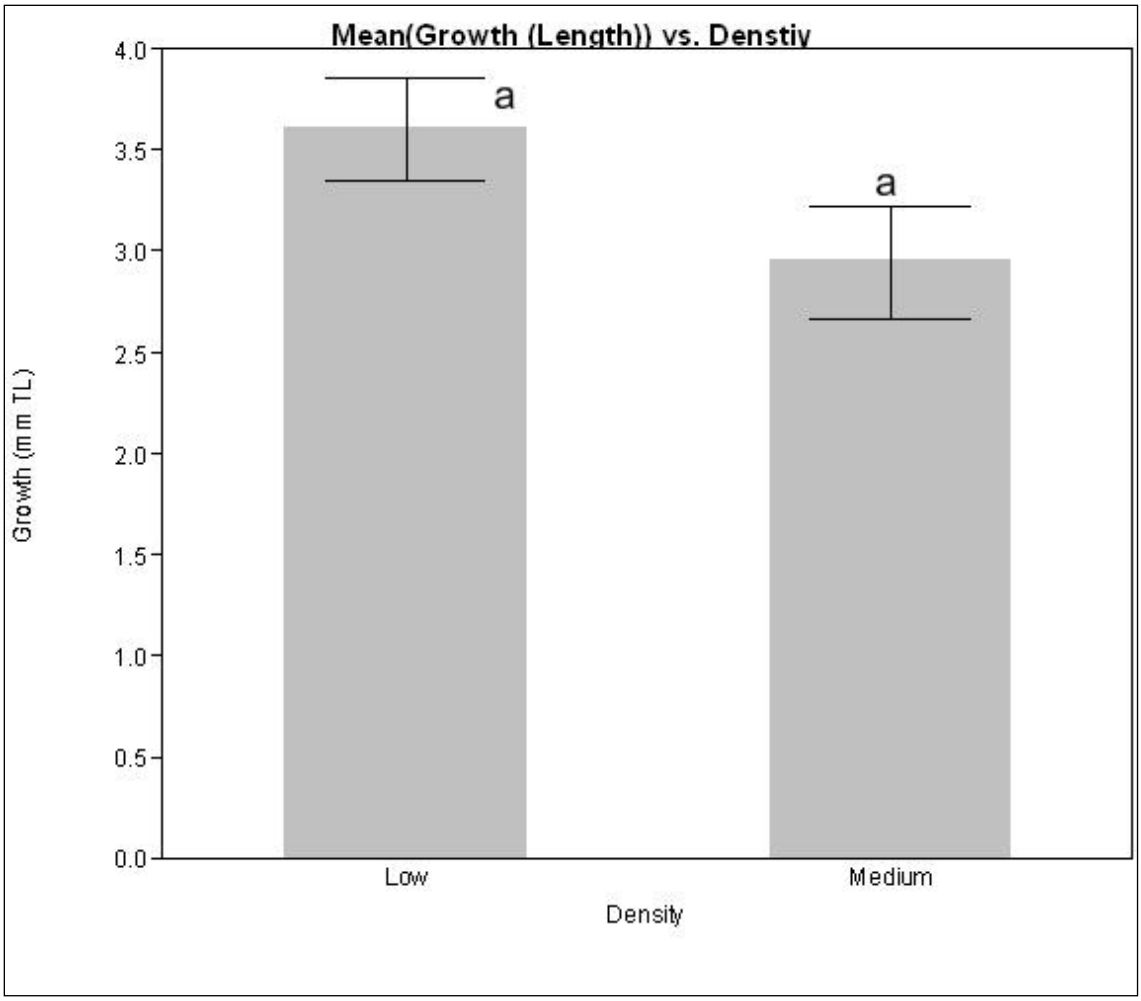

Figure 33. Growth (mm) of small juvenile headwater chub by density treatment. Densities were low (0.03 fish/cm3) and high(0.04 fish/cm3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

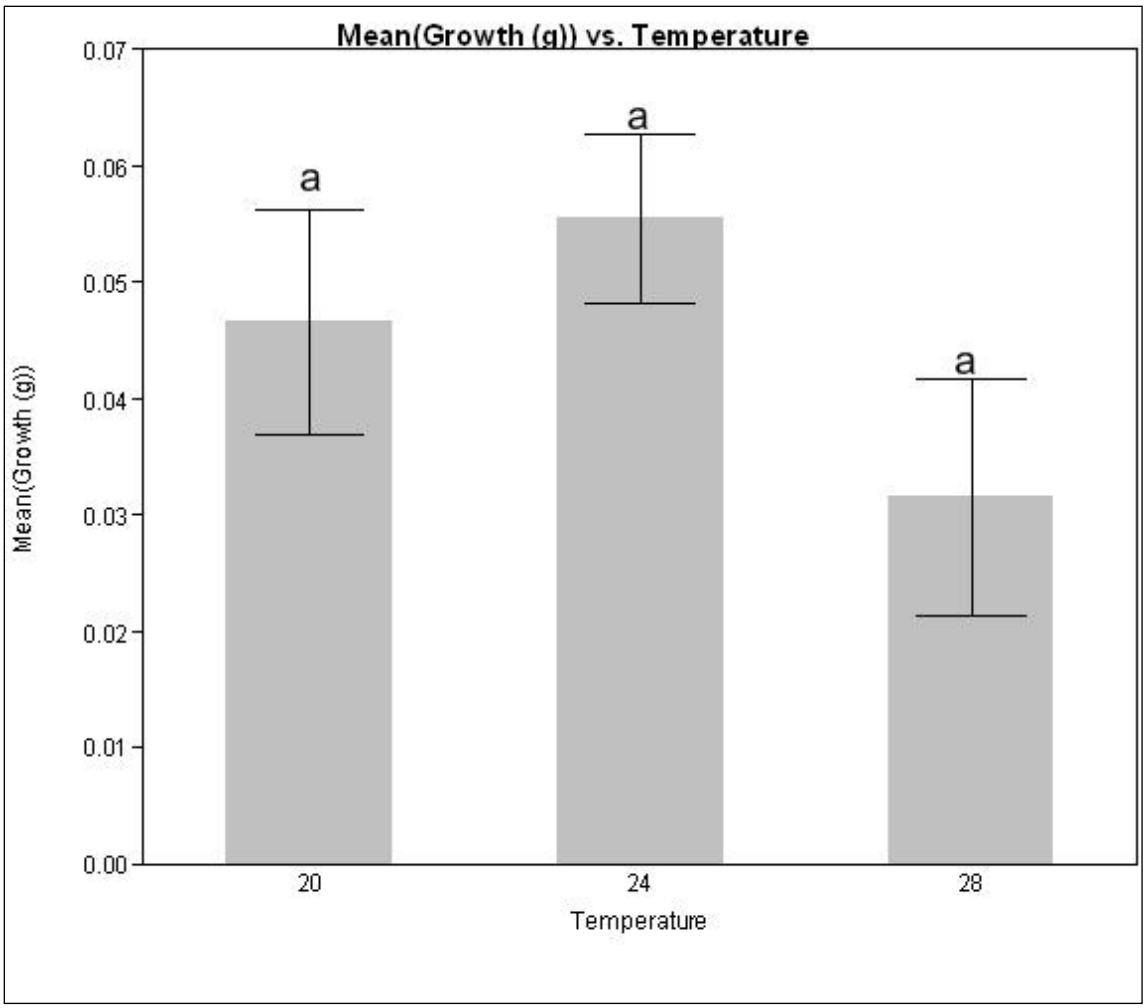

Figure 34. Growth (g) of small juvenile headwater chub by temperature treatment. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

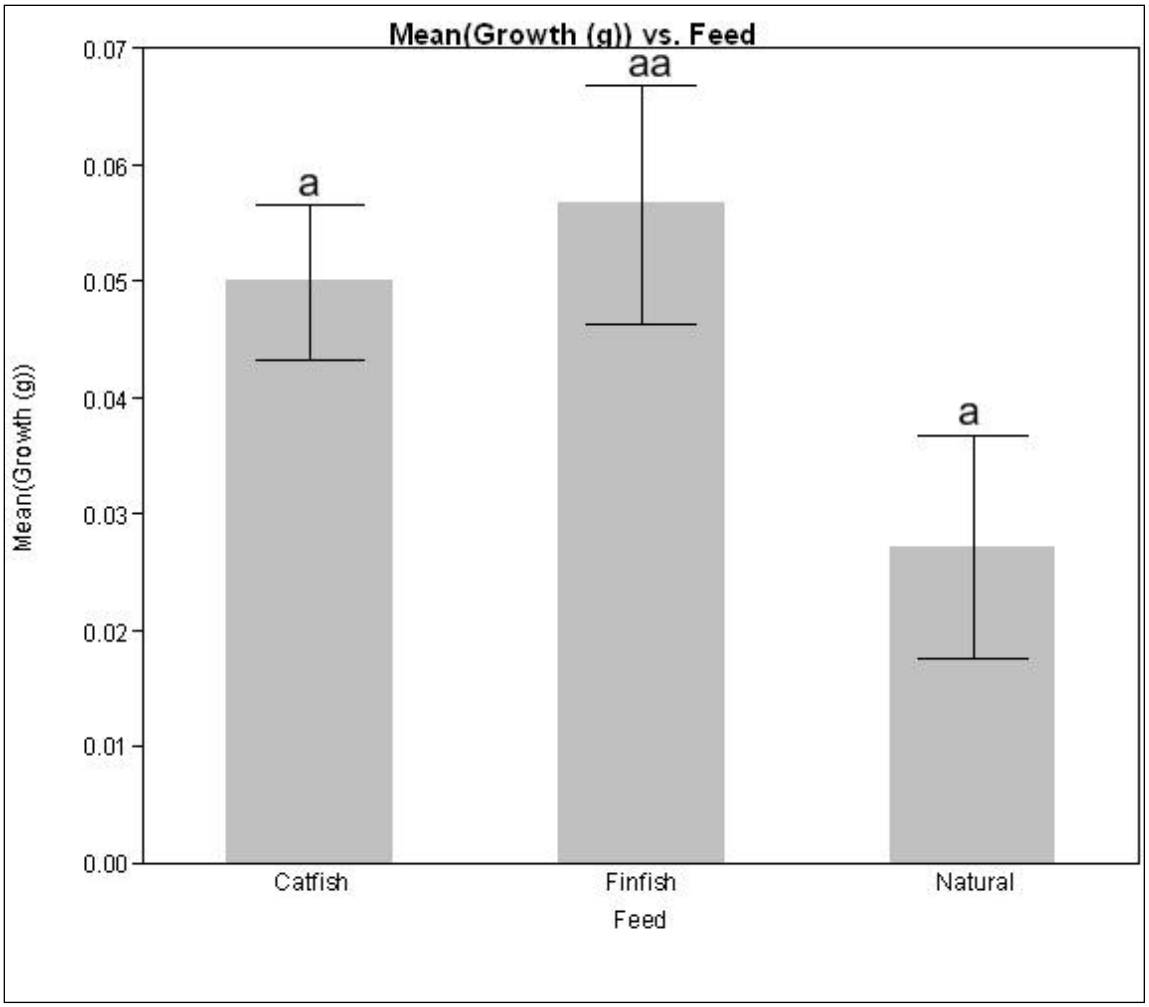

Figure 35. Growth (g) of small juvenile headwater chub by feed type treatment. Natural feed was Hikari Bio-pure brine shrimp, Spirulina brine shrimp and bloodworms; "Catfish" was fine-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was ground Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

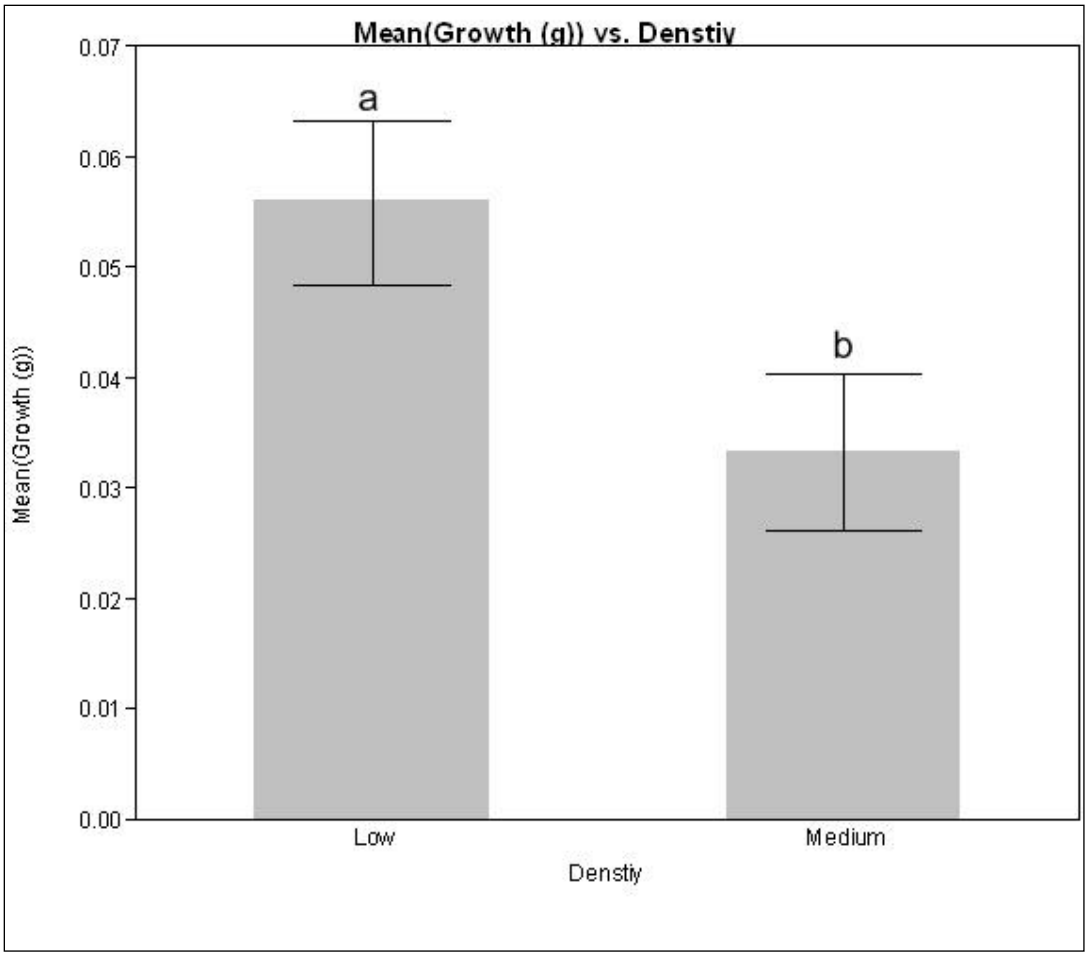

Figure 36. Growth (g) of small juvenile headwater chub by density treatment. Densities were low (0.03 fish/cm^3) and high(0.04 fish/cm^3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

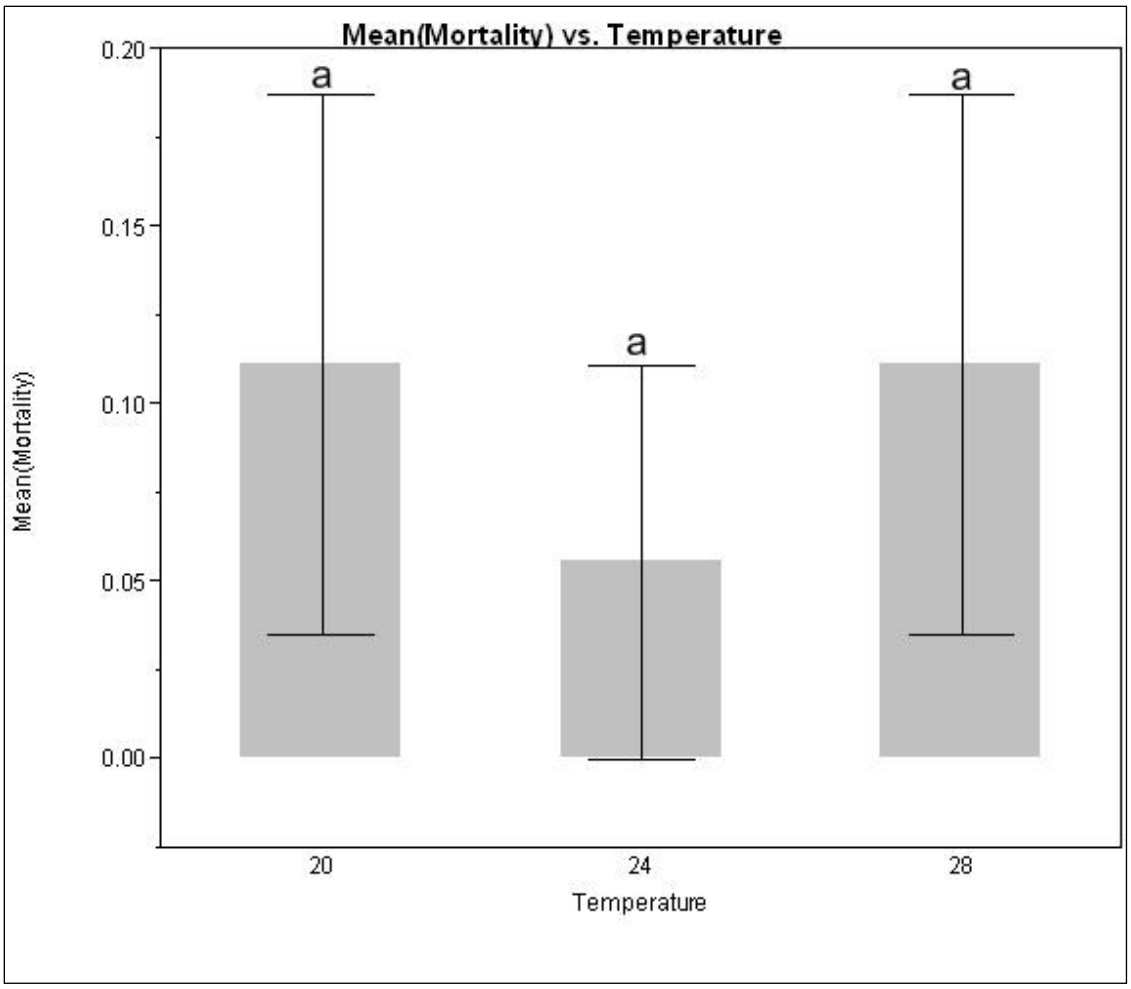

Figure 37. Mortality of small juvenile headwater chub by temperature treatment. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

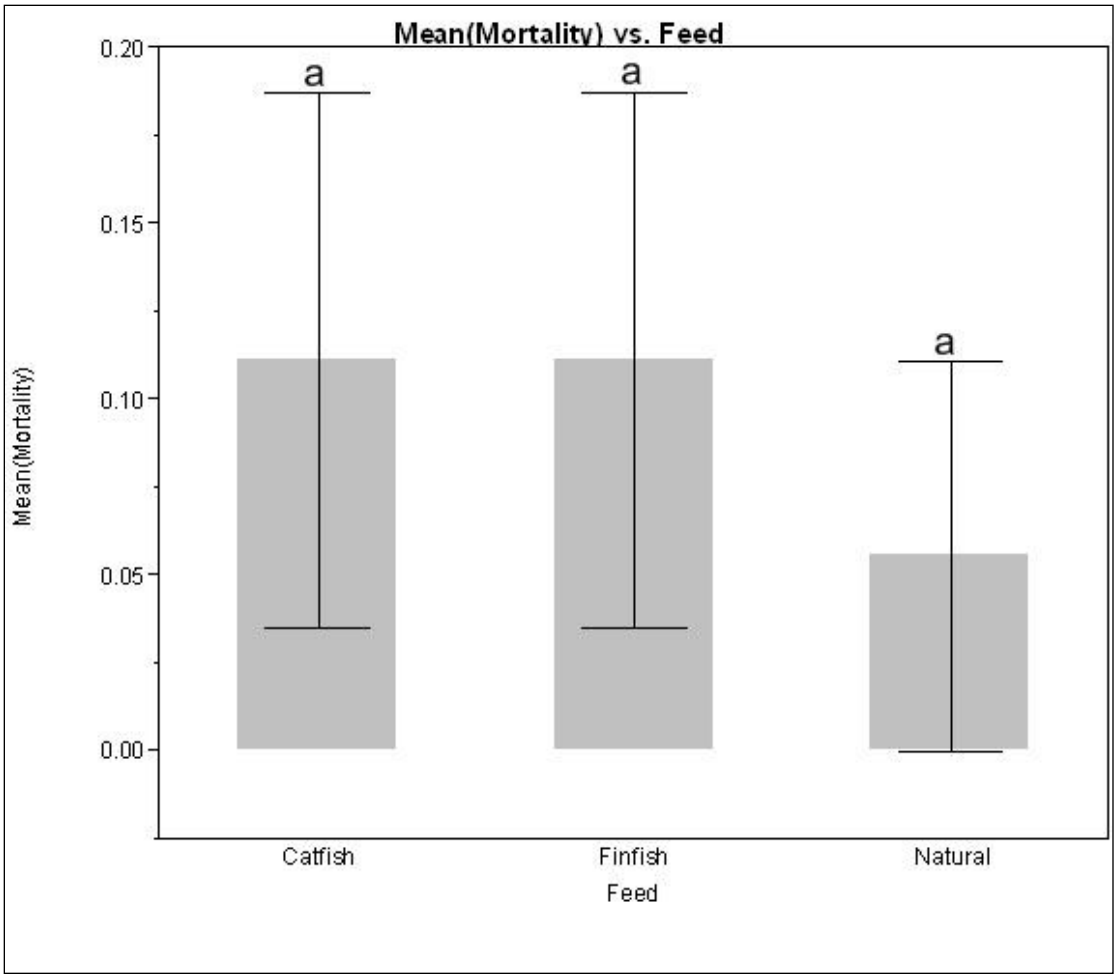

Figure 38. Mortality of small juvenile headwater chub by feed type treatment. Natural feed was Hikari Bio-pure brine shrimp, Spirulina brine shrimp and bloodworms; "Catfish" was fine-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was ground Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

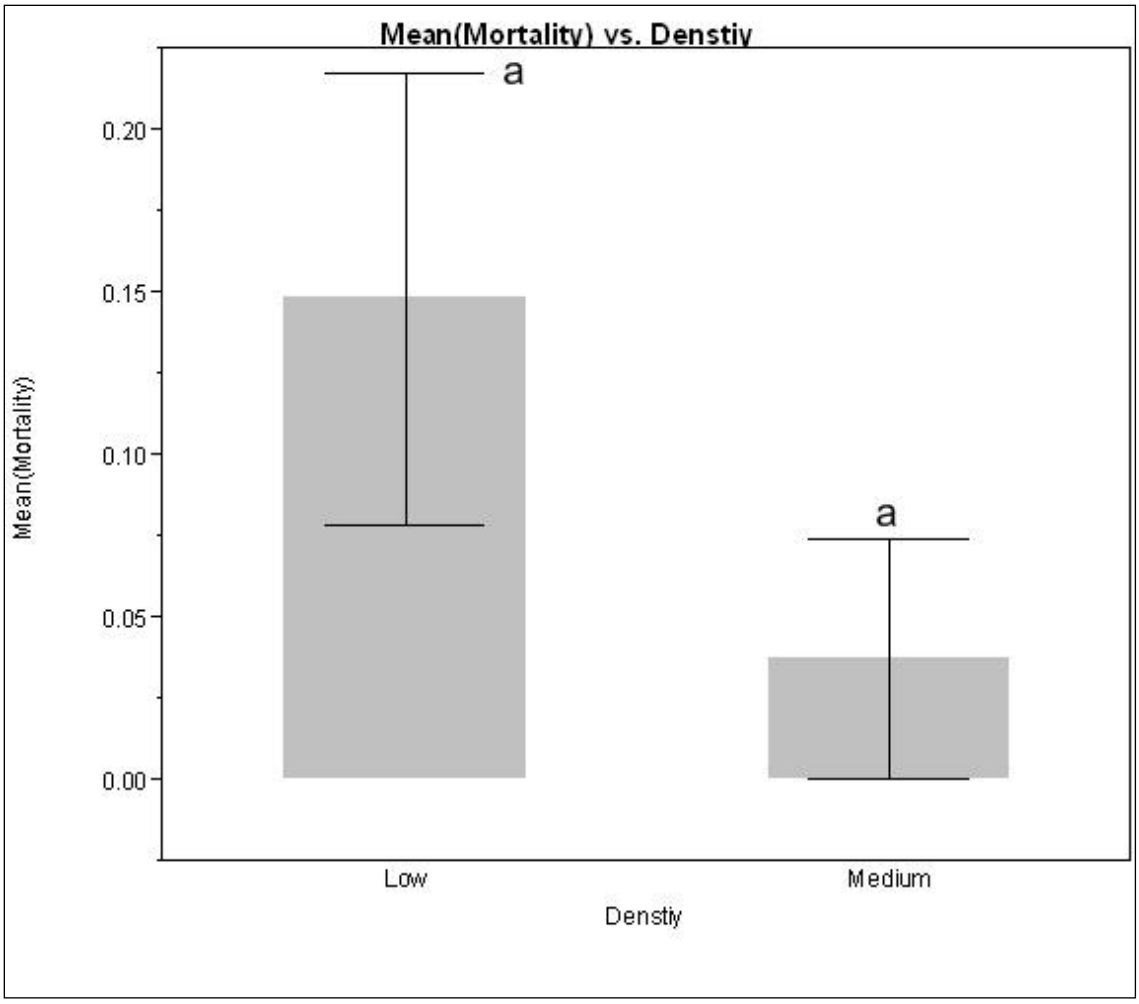

Figure 39. Mortality of small juvenile headwater chub by density treatment. Densities were low (0.03 fish/cm^3) and high(0.04 fish/cm^3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

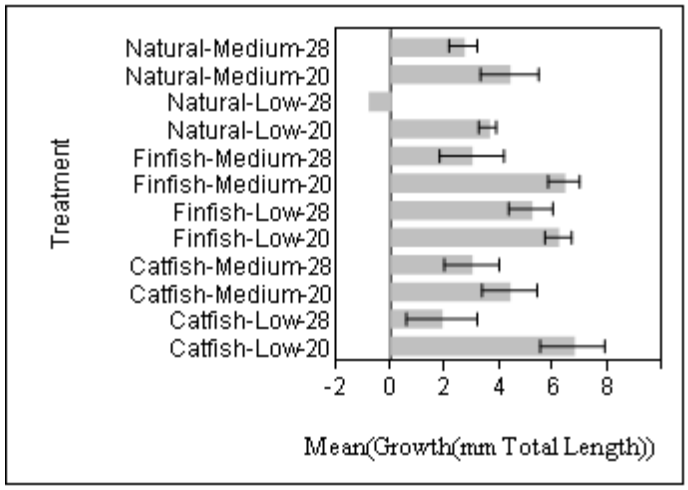

Figure 40. Mean growth (mm) ± SE of large juvenile headwater chub by treatment type. Natural feed was Hikari Bio-pure bloodworms; "Catfish" was coarse-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Temperatures were 20 and 28°C and densities were low (0.006 fish/cm^3) and high (0.012 fish/cm^3).

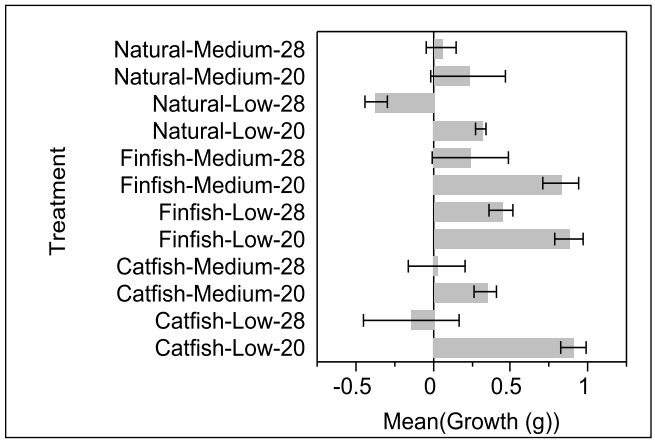

Figure 41. Mean growth (g) ± SE of large juvenile headwater chub by treatment type. Natural feed was Hikari Bio-pure bloodworms; "Catfish" was coarse-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Temperatures were 20 and 28°C and densities were low (0.006 fish/cm^3) and high (0.012 fish/cm^3).

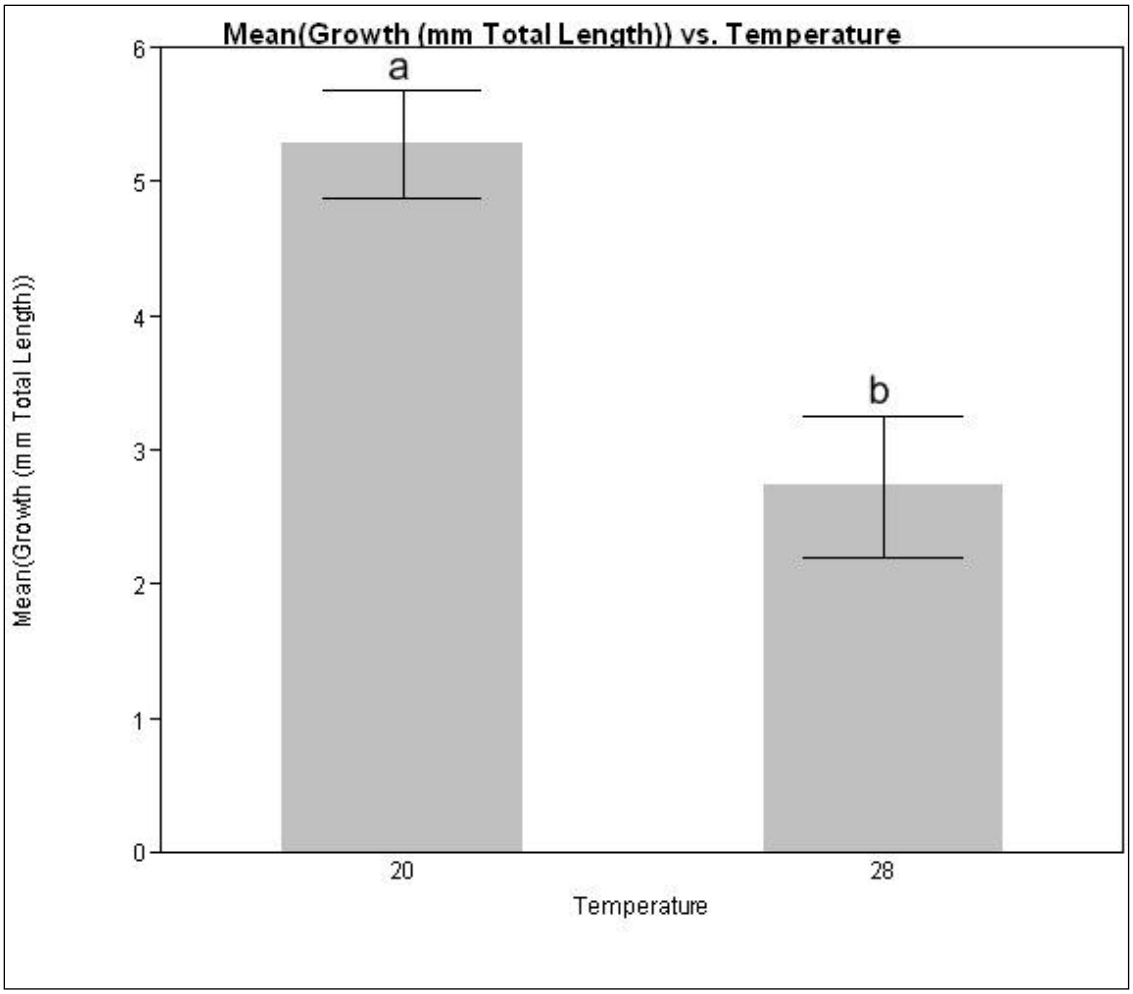

Figure 42. Growth (mm) of large juvenile headwater chub by temperature treatment. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

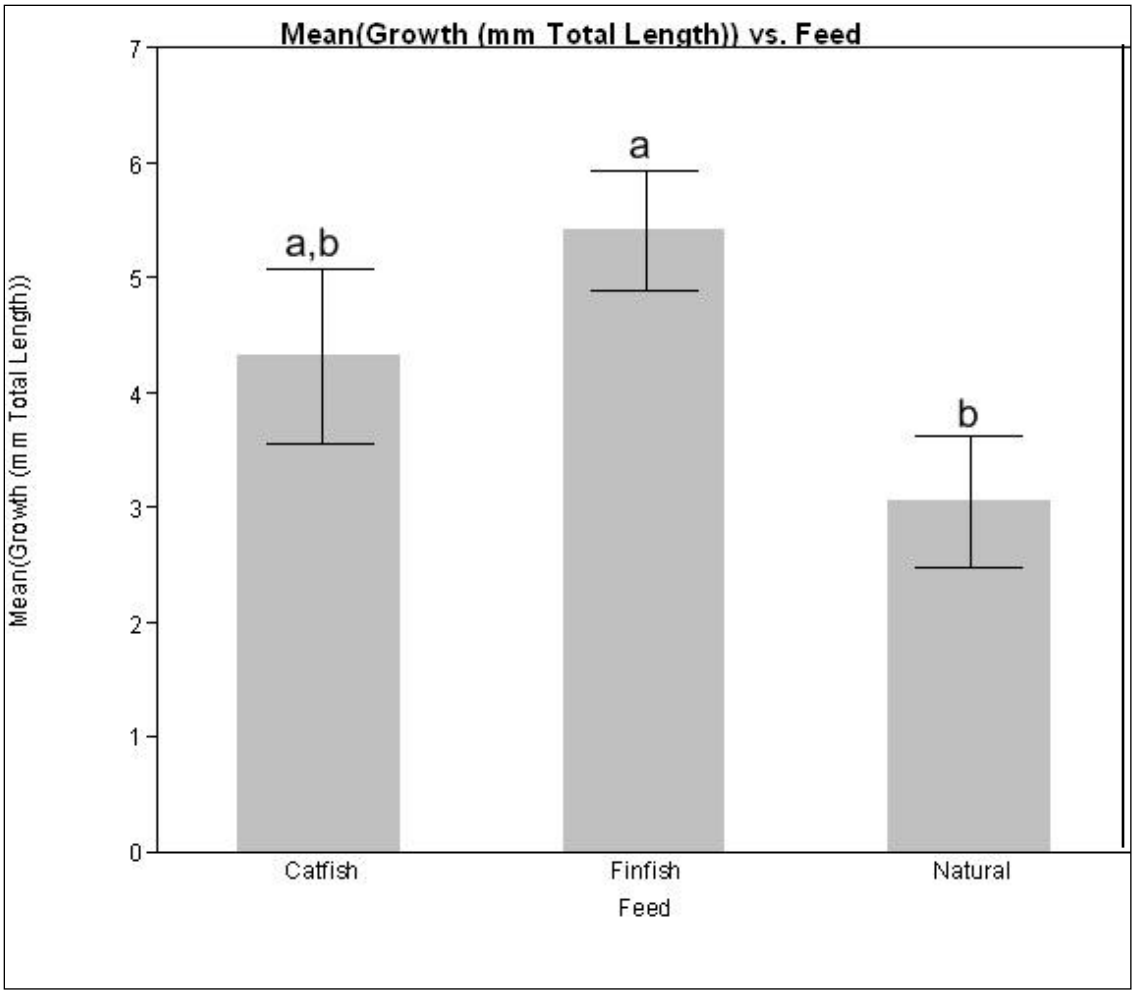

Figure 43. Growth (mm) of large juvenile headwater chub by feed type treatment. Natural feed was Hikari Bio-pure bloodworms; "Catfish" was coarse-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

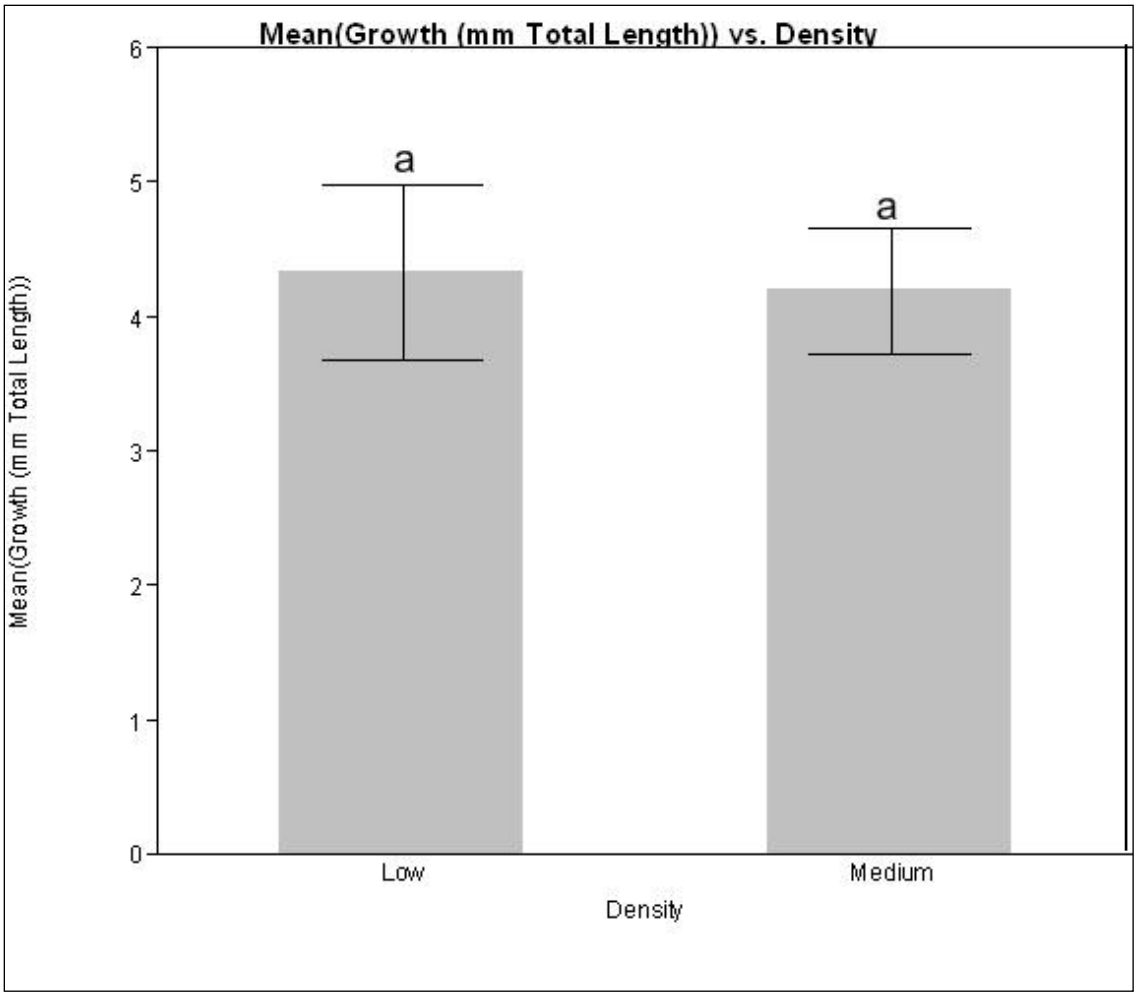

Figure 44. Growth (mm) of large juvenile headwater chub by density treatment. Densities were low (0.006 fish/cm^3) and high (0.012 fish/cm^3). Error bars represent one standard error from the mean. Bars represented by different letters are significantly different from each other.

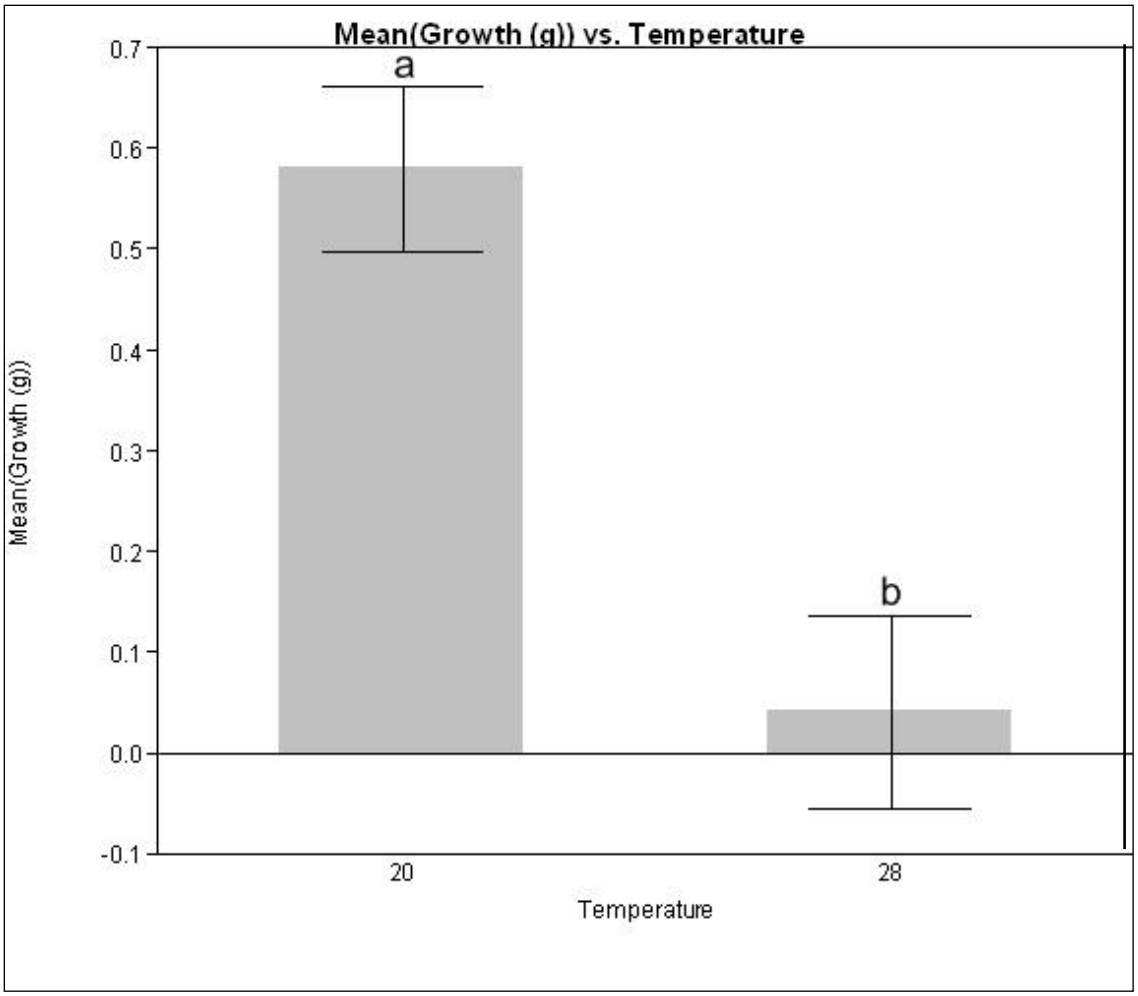

Figure 45. Growth (g) of large juvenile headwater chub by temperature treatment.
Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

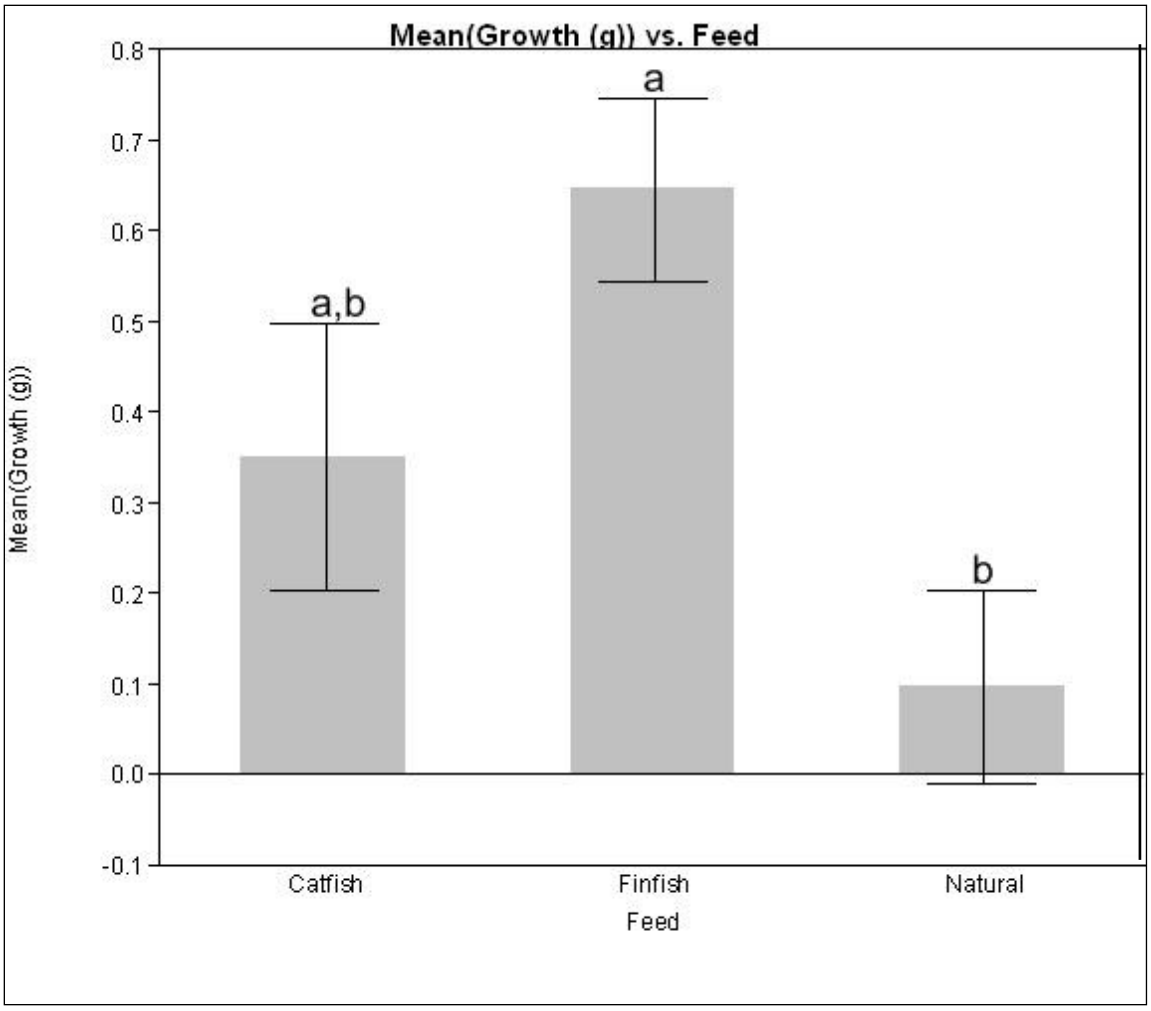

Figure 46. Growth (g) of large juvenile headwater chub by feed type treatment. Natural feed was Hikari Bio-pure bloodworms; "Catfish" was coarse-ground catfish pellets (Rangen Catfish EXTR 350, 35% protein); "Finfish" was Aquatic Ecosystems Dense Culture Feed, 43% protein starter pellets. Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

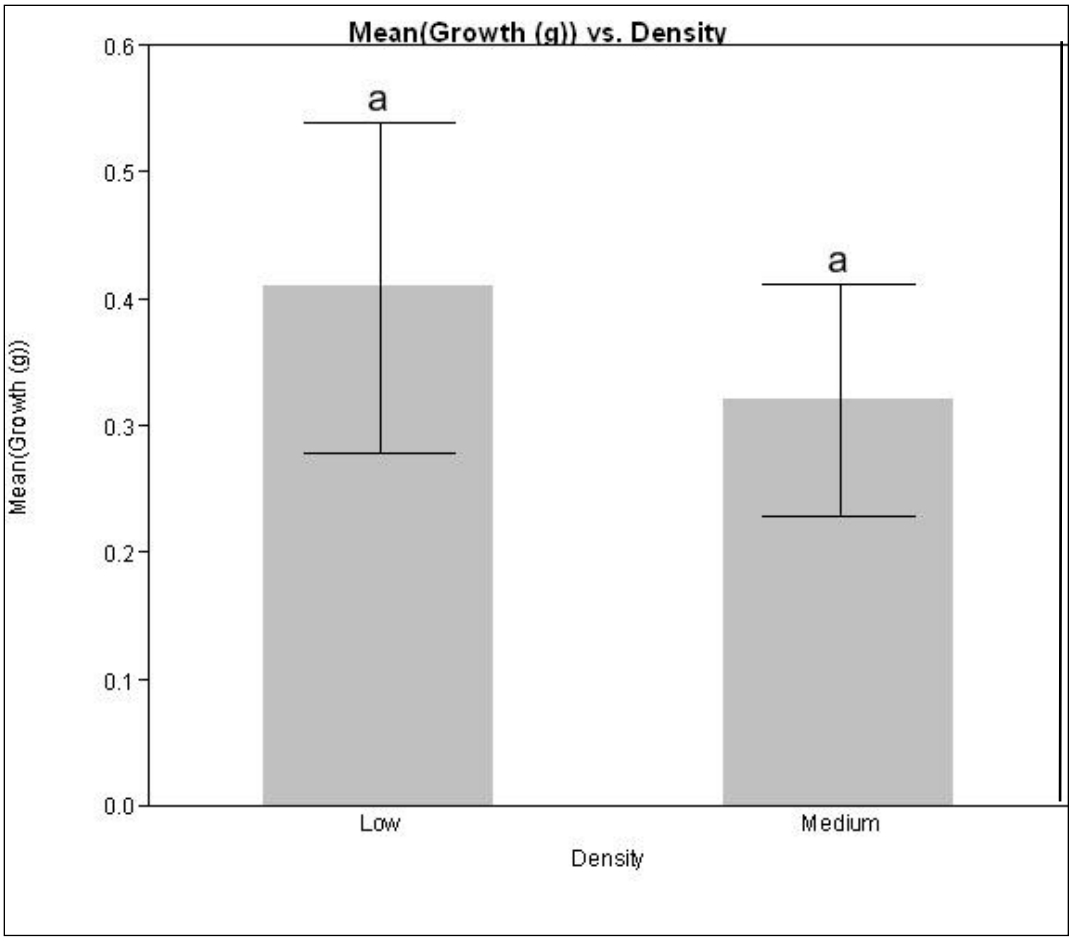

Figure 47. Growth (g) of large juvenile headwater chub by density treatment. Densities were low (0.006 fish/cm^3) and high (0.012 fish/cm^3). Error bars represent one standard error from the mean. Bars not connected by the same letter are significantly different ($P \leq 0.05$).

Table 1. Results of regression analysis of factors affecting headwater chub larval growth (mm TL) for the first larval experiment.

Source	DF	Sum of Squares	F Ratio	Prob > F
Model	56	566.5334	7.8671	<.0001*
Tank	9	111.5281	9.6365	<.0001*[a]
Feed	0	1.3965e-12	.	.[a]
Density	0	-6.519e-12	.	.[a]
Temperature	0	0.0000	.	.[a]
Feed*Density	1	1.2233	0.9513	0.3297
Feed*Temperature	0	5.364e-13	.	.[a]
Density*Temperature	0	-9.972e-12	.	.[a]
Feed*Density*Temperature	13	11.1615	0.6677	0.7957
Error	716	920.7354		
Total	772	1487.2688		

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.

Table 2. Results of regression analysis of factors affecting headwater chub larval growth (g) for the first larval experiment.

Source	DF	Sum of Squares	F Ratio	Prob > F
Model	56	0.04854705	14.1779	<.0001*
Tank	9	0.0145138	26.3740	<.0001*
Feed	0	-3.895e-17	.	.[a]
Density	0	-9.568e-17	.	.[a]
Temperature	0	0.0000000	.	.[a]
Feed*Density	1	0.0000401	0.6562	0.4182
Feed*Temperature	0	1.5921e-17	.	.[a]
Density*Temperature	0	-2.759e-17	.	.[a]
Feed*Density*Temperature	13	0.0007614	0.9578	0.4920
Error	716	0.04378004		
Total	772	0.09232709		

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.

Table 3. Results of regression analysis of factors affecting headwater chub larval mortality for the first larval experiment.

Source	DF	L-R ChiSquare	Prob>ChiSq
Model	324	295.7386	0.8682
Tank	84	97.9277095	0.1421
Temperature	0	0	.
Density	12	0.00022212	1.0000
Feed	12	9.26555e-5	1.0000
Temperature*Density	48	0.00020242	1.0000
Temperature*Feed	48	0.00021617	1.0000
Density *Feed	24	0.00024952	1.0000
Temperature*Density *Feed	96	5.88156859	1.0000

Table 4. Results of regression analysis of factors affecting headwater chub larval growth. (mm TL) for the second larval experiment.

Source	DF	Sum of Squares	F Ratio	Prob (1) > F	Prob (2) > F[b]
Model	39	135.72661	8.8776	<.0001 *	
Tank	10	30.404787	7.7560	<.0001*[a]	
Temp	0	0.000000	.	[a]	0.0618
Density	1	0.410852	1.0480	0.3117	0.3501
Feed*Density	2	5.708155	7.2805	0.0000	0.0000
Feed*Temp	8	4.185654	1.3347	0.2528	0.2775
Density*Temp	4	4.025492	2.5672	0.0515	0.0582
Feed*Density*Temperature	8	1.723766	0.5496	0.8123	0.7737
Error	43	16.85668			
Total	82	152.58329			

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.
[b]Prob (2) > F are estimates using random effects on the "tank" variable. This method allowed estimation of some parameters that could not be estimated without the random effect (Prob [1]) and produced roughly equivalent probabilities in other parameters. However, it did not allow for model and error probabilities and so is presented alongside the non-random effects probabilities.

Table 5. Results of regression analysis of factors affecting headwater chub larval growth. (g).

Source	DF	Sum of Squares	F Ratio	Prob (1) > F	Prob (2) > F[b]
Model	39	0.00330151	5.5992	0.0000	
Tank	10	0.00076339	5.0493	<.0001*[a]	
Feed	2	0.00126475	41.8271	0.0000	<.0001*
Density	1	0.00004233	2.8000	0.1015	0.1273
Temperature	0	0.00000000	.	[a].	0.0773
Feed*Density	2	0.00018618	6.1574	0.0000	0.0051*
Feed*Temperature	8	0.00006510	0.5382	0.8211	0.8597
Density*Temperature	4	0.00005321	0.8799	0.4839	0.5131
Feed*Density*Temperature	8	0.00004828	0.3992	0.9148	0.8761
Error	43	0.00065011			
Total	82	0.00395162			

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.
[b]Prob (2) > F are estimates using random effects on the "tank" variable. This method allowed estimation of some parameters that could not be estimated without the random effect and produced roughly equivalent probabilities in other parameters. However, it did not allow for model and error probabilities and so is presented alongside the non-random effects probabilities.

Table 6. Results of regression analysis of factors affecting headwater chub larval mortality.

Source	DF	L-R ChiSquare	Prob>ChiSq
Model	234	166.1427	0.9997
Tank	84	68.3652524	0.8922
Feed	12	0.00005549	1.0000
Density	6	7.01023e-5	1.0000
Temperature	0	0	.
Feed*Density	12	1.38671e-5	1.0000
Feed*Temperature	48	8.85571e-6	1.0000
Density*Temperature	24	0.00005158	1.0000
Feed*Density*Temperature	48	1.76942e-5	1.0000

Table 7. Results of regression analysis of factors affecting headwater chub small juvenile growth (mm TL).

Source	DF	Sum of Squares	F Ratio	Prob > F
Model	23	74.48303	2.9773	0.0000
Feed	2	4.123581	1.8955	0.1678
Density	1	5.874002	5.4004	0.0000
Temperature	0	1.5987e-14	[a].	0.4772[b]
Feed*Density	2	1.397026	0.6422	0.5332
Feed*Temperature	4	7.060541	1.6228	0.1943
Density*Temperature	2	18.066759	8.3050	0.0000
Density*Temperature*Feed	4	8.632096	1.9840	0.1224
Tank	6	22.918356	3.5117[a]	0.0094*
Error	30	32.63104		
Total	53	107.11408		

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.
[b]Prob > F in red are estimates using random effects on the "tank" variable. This method allowed estimation of some parameters that could not be estimated without the random effect and produced roughly equivalent probabilities in other parameters. However, it did not allow for model and error probabilities and so is presented alongside the non-random effects probabilities.

Table 8. Results of regression analysis of factors affecting headwater chub small juvenile growth. (g).

Source	DF	Sum of Squares	F Ratio	Prob > F
Model	23	0.04928704	1.9687	0.0411 *
Feed	2	0.0085815	3.9418	0.0302*
Density	1	0.0068907	6.3304	0.0175*
Temperature	0	-1.258e-17	[a].	0.2686 [b]
Feed*Density	2	0.0034481	1.5839	0.2219
Feed*Temperature	4	0.0061296	1.4078	0.2553
Density*Temperature	2	0.0042259	1.9411	0.1612
Density*Temperature*Feed	4	0.0052185	1.1985	0.3318
Tank	6	0.0095444	1.4614[a]	0.2250
Error	30	0.03265556		
Total	53	53		

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.
[b]Prob > F in red are estimates using random effects on the "tank" variable. This method allowed estimation of some parameters that could not be estimated without the random effect and produced roughly equivalent probabilities in other parameters. However, it did not allow for model and error probabilities and so is presented alongside the non-random effects probabilities.

Table 9. Results of regression analysis of factors affecting headwater chub small juvenile mortality.

Source	DF	L-R ChiSquare	Prob>ChiSq
Model	23	27.77231	0.2245
Tank	8	13.5502753	0.0943
Temperature	0	0	.
Density	1	5.337e-6	0.9982
Feed	2	2.17039e-6	1.0000
Temperature*Density	2	0	1.0000
Temperature*Feed	4	0	1.0000
Density *Feed	2	5.4213e-6	1.0000
Temperature*Density*Feed	4	7.06616e-6	1.0000

Table 10. Results of regression analysis of factors affecting headwater chub large juvenile growth. (mm TL).

Source	DF	Sum of Squares	F Ratio	Prob > F
Model	11	95.72000	3.9808	0.0047
Tank	3	4.137778	0.5876	0.6324[a]
Feed	2	27.075111	5.7674	0.0139*
Density	1	0.022222	0.0095	0.9238
Temperature	0	0.000000	[a].	0.0100*[a b]
Feed*Density	2	8.336444	1.7758	0.2032
Feed*Temperature	2	1.160444	0.2472	0.7841
Density*Temperature	1	1.088889	0.4639	0.5062
Feed*Density*Temperature	2	10.453778	2.2268	0.1423
Error	36	3.3333333		
Total	53	4.5370370		

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.
[b]Prob > F in red are estimates using random effects on the "tank" variable. This method allowed estimation of some parameters that could not be estimated without the random effect and produced roughly equivalent probabilities in other parameters. However, it did not allow for model and error probabilities and so is presented alongside the non-random effects probabilities.

Table 11. Results of regression analysis of factors affecting headwater chub large juvenile growth. (mm TL).

Source	DF	Sum of Squares	F Ratio	Prob > F
Model	11	4.4205833	7.4316	0.0001
Tank	3	0.3727778	3.1034	0.0584[a]
Feed	2	1.4089678	17.5948	0.0001*
Density	1	0.0196356	0.4904	0.4945
Temperature	0	0.0000000	.[a]	0.0262*[a b]
Feed*Density	2	0.1857144	2.3192	0.1326
Feed*Temperature	2	0.0810344	1.0119	0.3870
Density*Temperature	1	0.2390756	5.9710	0.0274*
Feed*Density*Temperature	2	0.2500744	3.1229	0.0735
Error	18	0.9733667		
Total	29	5.3939500		

[a]Lost degrees of freedom due to insufficient data to fill out the least-squares combinations that need estimating, or indicates there is some kind of confounding or collinearity of the effects. Note DF = 0.
[b]Prob > F in red are estimates using random effects on the "tank" variable. This method allowed estimation of some parameters that could not be estimated without the random effect and produced roughly equivalent probabilities in other parameters. However, it did not allow for model and error probabilities and so is presented alongside the non-random effects probabilities.

Captive Propagation and Culture of Roundtail Chub *Gila Robusta*

Erica A. Sontz and Scott A. Bonar

The roundtail chub is a stream-dwelling cyprinid that averages 250-350 mm in length, but can grow up to 500 mm or longer in larger streams (USFWS 2009). The smaller fish tend to be less deep-bodied than headwater chub of similar length, giving them a longer, more streamlined appearance (personal observation). They tend to be olive-gray to silvery on the sides with a pale to white belly (USFWS 2009). Larger fish tend to be more mottled and less silvery than the smaller fish, and fish from Aravaipa Creek, AZ tend more towards coppery-brown than olive-gray (Erica Sontz, personal observation).

The roundtail chub occurs throughout the western United States in the Colorado River basin, in small to large streams and tributaries of the Colorado River. Genetic analysis of the species throughout the Colorado River basin points to two historic population centers, one in the upper basin and the other in the lower basin, with limited gene flow between them (Dowling and DeMarais 1993; Minckley and DeMarais 2000; USFWS 2005b). Ongoing analyses indicate a range of genetic diversity along geographic lines within the lower basin (T.E. Dowling, personal communication 21 February 2005). The lower basin population has been accepted as a discrete population segment that warrants listing under the Endangered Species Act. In the lower basin, roundtail chub tend to occupy deep pools and eddies and often associate with cover such as boulders, undercut banks and vegetation. Roundail chub are omnivorous and tend to consume food as it is available, including detritus, invertebrates, plant material, fish and other vertebrates. Aquatic plants and invertebrates may be major portions of the diet (USFWS 2009).

While the roundtail chub has lost a large percentage of its historic habitat, the lower basin population is still relatively large, even while local populations may be unstable and threatened (Voeltz 2002). Roundtail chub is thus a prime candidate for genetic mismanagement through incorrect application of supportive breeding techniques.

Methods

Collection And Housing Of Broodstock

We attempted to collect adult roundtail chub from Central Arizona Project (CAP) and Salt River Project (SRP) canal draw-downs in Phoenix, AZ and from Bartlett Reservoir, Phoenix, AZ. Fish were collected in seine nets and moved to holding tanks before transport back to Tucson, AZ. During the draw-downs in November 2006, six, large, adult roundtail chub were removed from the canals. Only one adult roundtail chub was captured in Bartlett Reservoir. Only one fish from the CAP collection survived due

to infection by *Ichthyopthirius*. The single fish from Bartlett Reservoir did not survive transport to Tucson, AZ and was suspected of incurring injury during capture.

I amended our permit after consultation with Arizona Game and Fish Department (AZGFD) to allow collection of a limited number of adults from Aravaipa Creek, Safford, AZ using a combination of seines and backpack electroshockers. Ten adult roundtail chub of similar size to the headwater chub of Fossil Creek were collected from the east end of Aravaipa Creek on the Nature Conservancy preserve in the area around the guest house, outside of the canyon proper during the spring fish survey; half of those were lost just outside of Tucson in a freeway motor vehicle collision (9 March 2006). The remainder of the fish were brought alive to the Fish Propagation Laboratory at the University of Arizona, and moved to glass aquarium tanks under standard protocol (Widmer et al. 2005) on 10 March 2006. We followed standard protocol in acclimating fish to tanks and used prophylactic treatment for disease.

The fish from the first Aravaipa collection remained *Ichthyophthirius*-free, but had a persistent *Lernaea* infestation that proved difficult to eradicate. We tried several chemical treatments and repeated manual removal of the parasite under anesthetic and spot treatment using potassium permanganate and eventually eliminated the *Lernaea*. Fish were also tested for Asian tapeworm, with negative results.

An additional 10 adult chub were collected from Aravaipa Creek during the spawning season on 23 April 2006, and these fish were successfully transported back to the University. Because of the *Lernaea*, fish from the two different collecting trips were housed separately.

Spawning

Roundtail chub tanks were exposed to the same conditions of temperature decrease and photoperiod manipulation as described above for headwater chub. One tank was chilled to 17^{o}C over the course of 4 weeks (the tank reached 15^{o}C at 2 weeks and fluctuated between 15-17^{o}C). At four weeks, the water return hose was found to have fallen out of the tank and drained ~90% of the water from the tank. The tank was refilled and the hose was anchored to the side of the tank. Temperatures were slowly raised after that, reaching 17^{o}C one week later and 20^{o}C 16 days after the tank was drained and refilled. The tank was chilled using the same chiller used for the headwater chub (which proceeded without incident).

A second tank of roundtail chub cooled under natural, ambient conditions (roundtail chub were housed in the same facility as headwater chub and were exposed to the same ambient laboratory conditions).

Several attempts were made to induce spawning through injection of the hormone Ovaprim (Syndel Laboratories Ltd., Qualicum Beach, British Columbia, Canada). The first attempt to induce spawning occurred on 28 January 2008. Materials for the injections were set up in advance (hormone, syringe, anesthetic bath, etc). Fish were captured from their tank and moved to a holding tank to consolidate them. The holding tank was filled with the same water used for water changes in the main tanks and some

additional water from the main tanks. Air was supplied from pumps via air stones to ensure appropriate aeration. Fish were captured by nets from the tanks and moved to the

holding tank where they were left undisturbed while the tank was cleaned and final preparation for injection was made.

We returned to Aravaipa and collected an additional 10 fish on 3 March 2008 using standard seining and shocking techniques. We collected the first 10 fish encountered and transported them successfully to the Fish Propagation Laboratory. The fish were added to the tank with the one remaining fish from the previous batch. Three days later, on 6 March, one fish was found dead in the tank. It is likely that this was that one last fish from the previous collection, though we could not be certain. Fish were fed the same feeds and on the same schedule as the headwater chub and were allowed a period of acclimation before we began any temperature manipulations to induce spawning. Fish were chilled naturally and with the chiller in an attempt to spawn, but spawning never occurred. We also made another attempt at hormone injection with the same negative results as previously.

Results

Collection And Housing Of Broodstock

Fish were placed into tanks and acclimated to feed as described for headwater chub in the previous chapter. Housing of the large adult fish from the Verde River and Reservoir system proved impractical given the available facilities. The smaller fish from Aravaipa Creek were much easier to house and care for, as they were of similar size to the headwater chub.

Spawning

Roundtail chub never spawned in the laboratory no matter the wide variety of techniques used to induce this behavior. We tried virtually the same methodology that was successful for headwater chub and a number of other species, including other captive roundtail chub (Doug Sweet, personal communication).

Trips to Aravaipa Creek occurred during the roundtail chub spawning season. Fish showed obvious spawning coloration and milt had been extruded from some of the males in the field. We did not handle any ripe females, but selected fish such that we hoped to have a mix of males and females, based on size, appearance and extent of breeding coloration and appearance of the vent. None of our attempts to induce spawning in roundtail chub were successful; though, fish showed visible readiness in the form of breeding coloration and behaviors similar to that described in the literature and observed in the headwater chub. The one, large, Verde River fish that we maintained at the laboratory also developed spawning coloration during the same time periods as other roundtail chub and the headwater chub. One tank of roundtail chub underwent

temperature manipulations to induce spawning; however, the tank was inadvertently drained of water. The fish did not spawn during this time, and we suspect that the stress of water loss may have overridden any spawning tendencies. One tank of roundtail chub was experiencing the same non-manipulative conditions at the same time, but no spawning occurred.

Our attempts to use hormones to induce spawning also failed, and we remain uncertain whether Ovaprim can induce spawning in roundtail chub. Moving the fish prior to hormone-injection resulted in near 100% mortality of the fish before hormones could be injected (that is, we were never able to proceed to injection due to rapid mortality of the fish). After fish had been moved to the holding tank, we allowed them to acclimate while preparing the hormone treatments.

Upon returning to the holding tank some 30 minutes later, we found all fish moribund, either settled listlessly on the bottom or floating at the surface with rapid, shallow respiration. In our first attempt, no fish were injected with hormone; they were returned to the main tank in the hope that normal surroundings might resuscitate them. By the end of the night, four fish had died. Necropsy showed brown and deteriorated gills with no sign of parasites, though it is uncertain whether this was a preexisting condition or one induced by something about the holding tank or the process of moving the fish. Necropsy also showed that all of the fish collected (not including the 5 survivors of the vehicle accident – these fish had not been moved) were all males, eliminating any possibility for successful spawning. All but one fish died over the next few days, and by 31 January, only the one fish remained.

We attempted to induce spawning via hormones a second time, using the second batch of Aravaipa roundtail chub. We only moved half of the fish to the holding tank, but all of these fish died as a result of some part of the procedure, as with the last attempt. Again, no fish actually received hormone injection at this time. The remaining half of the broodstock was left undisturbed in the tank so as not to induce 100% mortality.

Discussion

We were unable to successfully spawn roundtail chub; however, these fish have been successfully and inadvertently spawned in captive settings previous to our attempts (Muth et al. 1985; Doug Sweet, personal communication). We were never able to try the hormone Ovaprim because fish reacted poorly to some part of the preparation procedure. There was speculation that the fish died as a result of stress from handling, but fish had been previously handled during collection in the field, transport to the lab and had been handled and anesthetized for the manual removal of *Lernaea*. None of the fish showed any adverse reaction to that treatment, which involved much the same procedure. Several individual headwater chub had also been handled extensively to treat wounds incurred within the tank. These fish never showed any adverse reactions to being handled, even while being restrained in a net without anesthetic. Additional suggestions included that there might have been a chemical contaminant on the holding tank itself, but we used the same large cooler that we used to transport the fish from the field to the laboratory and

every time, the fish survived the transport without incident. Further speculation included some persistent, low-level stressor in the tank itself that weakened the fish enough that additional stress from handling resulted in mortality. Tanks were treated in the same manner as the headwater chub tanks, so we are uncertain what effect, or suite of effects, might have proved detrimental to only the roundtail chubs.

In addition, roundtail chub developed spawning coloration and tubercle growth when subjected to the changing temperature regimen that eventually induced spawning in headwater chub. This, combined with the high levels of similarity between the two species (even experts cannot reliably distinguish individuals in the wild) and other documented instances of captive spawning, lead us to believe that not only is it highly likely that this species can be spawned, but that conditions similar to what were used to spawn headwater chub will produce results in roundtail chub. All of the fish that were necropsied in the lab were male, and we suspect that we never collected any female roundtail chub. Larger sample sizes or a better ability to distinguish between male and female fish in the field (such as only collecting ripe individuals during the breeding season) may have allowed us to collect a successful broodstock population. Additionally, it would be interesting to investigate whether or not there is any population segregation between male and female roundtail chub in Aravaipa Creek, since we apparently did not encounter any females on at least one collecting trip (we collected the first and only ten fish encountered; all were male).

The larger, Verde River roundtail chub require larger facilities than we had available to us to house and spawn in captivity. While the Aravaipa Creek roundtail chub were of a size similar to the headwater chub collected at Fossil Creek, and were often collected from the same or smaller-sized stretches of stream (all of the Fossil Creek headwater chub were collected from a large impoundment while the roundtail chub were often collected from much smaller pools that we could access with a seine and backpack shocker) they may also require larger facilities than we were able to provide in the lab during this study period. One inadvertent spawning of fish occurred in large, public aquarium holding tanks of much larger volume than what we had available (Doug Sweet, personal communication). Further investigation with larger tanks or ponds or even with renovated stream systems may prove successful where our small-scale operation was not.

Captive breeding of Gila chub *Gila intermedia*

Andrew A. Schultz and Scott A. Bonar

The requirements necessary to culture the Southwest's threatened native fishes for recovery efforts are unknown for certain species, yet may prove critical for conservation. Gila chub *Gila intermedia* are one of seven chub species of the genus Gila inhabiting the Colorado River Basin. All are threatened by non-native species, habitat loss, and other factors within the basin. Roundtail chub *Gila robusta* remains the only species not listed or proposed for listing as endangered. Published accounts of captive spawning/culture efforts for these chubs are few. Hamman (1982a; 1982b; 1985) reported on the spawning and reproductive biology of humpback chub *Gila cypha* and bonytail *Gila elegans* in captivity. Muth et al. (1985) did the same for roundtail chub. Current research on spawning/culture techniques and requirements for both headwater chub *Gila nigra* and roundtail chub is reported in the two previous chapters. Previous observations (Ken Wintin, Arizona-Sonora Desert Museum, personal communication; Jeanette Carpenter, U.S. Geological Survey, personal communication; and Andrew Schultz, personal observation) confirm that Gila chub have the ability to spawn and be maintained in captivity but spawning/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. The objectives of this study were to establish a group 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.

Methods

In March 2003 we collected Gila chub from Sabino Creek, Arizona to serve as broodstock. Fish were 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 in rectangular glass tanks with water capacities of about 280 and 330 L. After their first spawn (at 14.9°C), we varied temperatures to estimate the range of temperatures at which Gila chub would spawn. Most spawning trials were conducted between 18-24°C with temperatures held relatively static. 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 dechlorinated 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 with a combined filtering capacity of about 3784 L/h for spawning tanks and 1135 L/h for egg-incubation tanks. The returned water from the bio-filters created a surface disturbance and slight flow within the spawning tanks. The main diet of adults consisted of thawed natural feeds, mainly chironomid larvae (Hikari Bio-Pure Bloodworms, Hikari, Inc., Hayward, CA). We fed adult Gila chub in slight excess twice during each day 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 using a siphon hose, which resulted in a water exchange of about 5-20% daily. Water quality (i.e., pH, ammonia, nitrite, and temperature) 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 rigid 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. Following spawning, tiles were removed from spawning tanks, tiles with eggs were gently rinsed clean of debris by dunking in water from which they originated, and the number of eggs present on the tiles was recorded. Tiles with eggs were then placed vertically in vinyl covered metal dish racks submersed in 57-L aquaria. We counted larval Gila chub following hatch, which usually occurred within 24 h.

We used an ocular micrometer to measure diameter of spawned eggs and total length (to nearest 0.1 mm) of larval Gila chub. We measured wet-weight (to nearest 0.0001 g) of Gila chub larvae 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.

Results

Gila chub taken from Sabino Creek, Arizona in March at a temperature of 12.3°C spawned at 14.9°C within 10 days of initial introduction into the lab. Gila chub consistently spawned in the laboratory thereafter without hormonal, chemical, photoperiod, temperature or 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 spawned less frequently at temperatures above 24°C. Most trials were conducted between 18-24°C and groups of Gila chub would usually spawn within 14 d of tanks being set up for spawning within this temperature 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. Spawning colors were present to varying degrees near ventral and pectoral fin bases, ventral body areas, opercle, and mouth, with strong, dark-colored horizontal banding noted on the most active fish.

Nudging and 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, perhaps including 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 our visual observations. 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 from each spawn was 6.36 % (SD = 8.8). Eggs of Gila chub were demersal, adhesive, ovoid, and translucent with the inner 80-90% of the egg a light yellow cream color and the remainder 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 48). The regression equation for this relationship was:

$$\text{Time to Hatch (d)} = 21.77 - 0.72 \text{ Mean Incubation Temperature } (C^o)$$

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 h. Larval Gila chub accepted several types of natural and prepared/commercial feeds upon exogenous feeding.

Discussion

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

The highly adhesive nature of Gila chub eggs created challenges when first trying to efficiently count, aerate, and rear 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 protected the eggs from adult

predation and the tiles provided an easily cleaned, efficient system for transfer and counting. Some eggs were cannibalized prior to falling through the grating.

Cannibalization of eggs might 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 tile 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. 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 between adult fish and tiles is unnecessary, the glazed tiles are more easily cleaned, and we found 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. However, construction, maintenance, and monitoring of our spawning system did require considerable labor.

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 also likely given our observations. It is unknown 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 14.9°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* (Kline and Bonar 2009), Mohave tui chub *Siphateles bicolor mohavensis* (Archdeacon and Bonar 2009) and headwaters chub (this report).

Minckley (1973) noted Gila chub had an extended spawning regime in a relatively constant temperature and water-level 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 stable environments having moderate, steady temperatures, consistent high-quality food resources, consistent access to mates, and/or reduced predator threats.

Gila chub often exhibit brilliant orange/red colors when in a heightened reproductive state. A previous field study described reproductive colors and a subsequent rating system for Gila chub (Schultz and Bonar 2006). We found that spawning color of Gila chub that released gametes when collected in the field ranged from moderate to very strong. The most intensely colored Gila chub (≥ strong spawning colors) were captured where daytime water temperatures ranged from 12-28 °C. 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 slower 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 suggest that 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 underestimates due to cannibalization of eggs prior to falling through the protection grid, and any loss of eggs from tiles during transfer. In addition, unavoidable disturbance of tanks (e.g., cleaning activity) may have arrested spawning activity, accounting for occasional spawns of low magnitude. The 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) could not be explained by size differences in Gila chub 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 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 studies should be possible to obtain.

The ability to domesticate and spawn adult fish of a species without inducement may 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 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.

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).

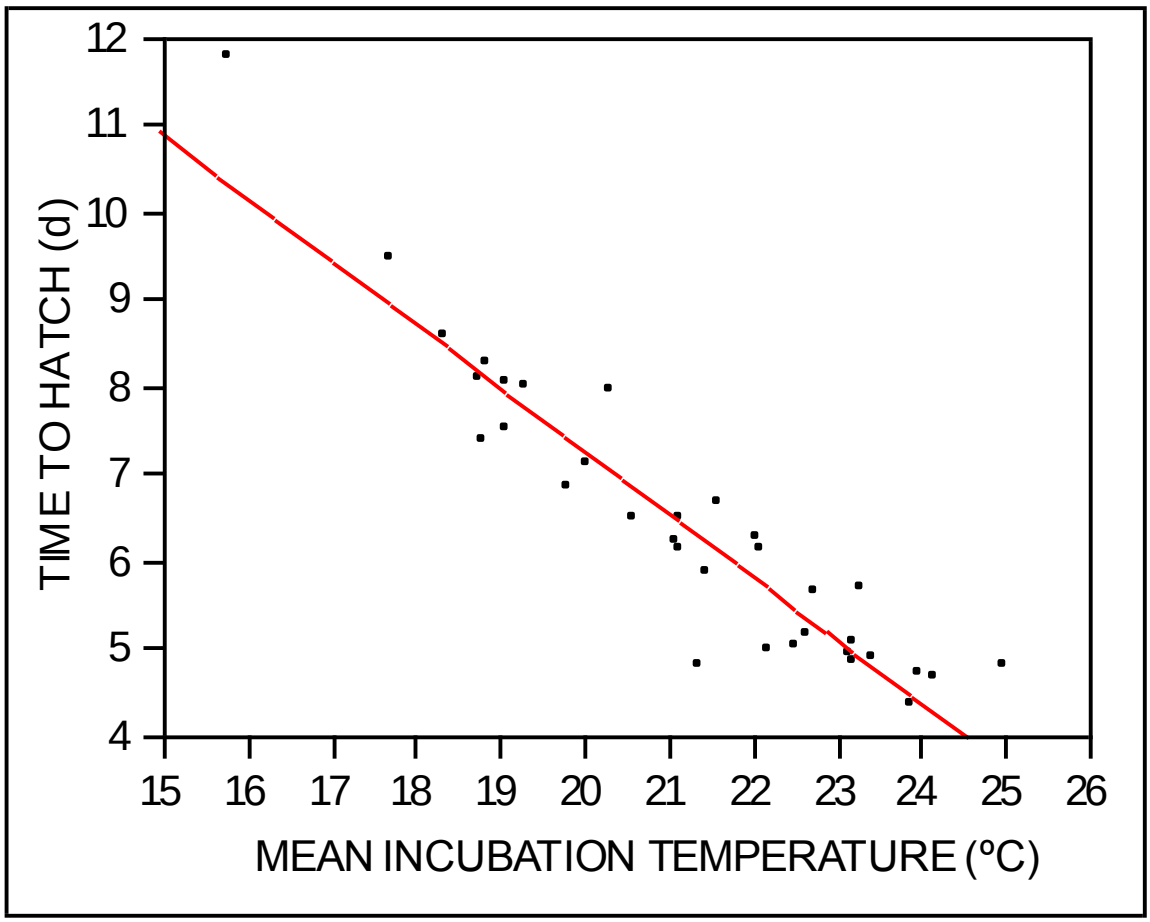

Figure 48.- Relationship between hatching time and mean incubation temperature (with linear regression fit) for larval Gila chub (*Gila intermedia*).

Culture of Gila Chub: Effect of Feed Type, Water Temperature, and Rearing Density on Growth and Survival

Andrew A. Schultz and Scott A. Bonar

Gila chub have been maintained and grown in captivity (Ken Wintin, Arizona-Sonora Desert Museum, personal communication ; Jeanette Carpenter, U.S. Geological Survey personal communication; and Andrew Schultz, personal observation) but the environmental requirements necessary to efficiently culture the endangered Gila chub *Gila intermedia* are unknown at this time. Gila chub appear to eat a variety of artificial and natural feeds in captivity (Andrew Schultz, personal observation). It has been demonstrated that feed characteristics (Bardi et al. 1998; Mohler et al. 2000; Barrows and Hardy 2001; Mischke et al. 2001), water temperature (Harrelson et al. 1988; Abdel et al. 2005; Fitzsimmons and Perutz 2006), and rearing density (Irwin et al. 1999; Alvarez-Gonzalez et al. 2001; Anderson et al. 2002; Jodun et al. 2002; Sahoo et al. 2004; Rahman et al. 2005) have significant impacts on growth, survival, and health of fishes in captivity. The purpose of our study was to identify the effects of different water temperatures, feed types, and rearing densities, on growth, survival, and overt health/appearance of Gila chub larvae and juveniles under laboratory conditions.

Methods

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 (thawed *Artemia* sp. nauplii, Hikari Bio-Pure Baby Brine Shrimp, Hikari, Inc., Hayward, CA), a prepared feed (chicken *Gallus domesticus* egg-yolk powder, John Oleksy, Inc., Schaumburg, IL), 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 (thawed *chironomid* sp. 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., Secaucus, NJ; Golden Pearls Weaning and Juvenile Diet, Brine Shrimp Direct, Inc., Ogden, UT; Silver Cup, Nelson and Sons, Inc., Murray, UT), respectively, fed to excess three times daily (Table 12). 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-h of hatching. Final larval length and weight measurements were derived from a random subsample (n = 10) of survivors from 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 mean weight of all individuals per tank for the analysis.

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 feed types. 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 randomly assigned Gila chub to each of four different treatment levels (test temperatures) with three replications (tanks) per treatment level for each size class tested. Each 38-L rectangular glass tank was fitted with a recirculating filter system with a stocking density of 40 larval chub (6.0-7.5 mm TL), 7 small juveniles (32-49 mm TL), or 5 large juveniles (52-72 mm TL) for a mean initial biomass of 0.004 g/L, 0.19 g/L, and 0.49 g/L, respectively. Gila chub were acclimated by increasing water temperature in equally divided intervals over a five-day period until the desired test temperature was reached. 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 = 18) of all fish acquired within 24-h 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., Hayward, CA) 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.) for small juveniles or Silver Cup (Nelson and Sons, Inc., Murray, UT) for large juveniles.

We used one-way analysis of variance (ANOVA) or Welch's ANOVA test (when group variances were significantly different, $P \leq 0.05$) 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 differed among test temperatures.

Density

We randomly assigned Gila chub to each of three different treatment densities and four replications (tanks) per treatment density. Mean initial density (low, moderate, and high, respectively) of Gila chub was 0.065 g/L (38.9 fish/L), 0.540 g/L (319.5 fish/L), and 1.343 g/L (795 fish/L) for larval chub (6.3-6.8 mm TL); 3.618 g/L (4.0 fish/L), 16.986 g/L (20.1 fish/L), and 60.145 g/L (68.3 fish/L) for small juveniles (36-47 mm TL); and 1.681 g/L (0.4 fish/L), 14.346 g/L (2.7 fish/L), and 53.942 g/L (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 ($n = 20$) of all fish 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 by blotting and air drying fish prior to measurement. We used an ocular micrometer to measure initial total length (to nearest 0.1 mm) of larval Gila chub and calipers to measure final total length (to nearest 0.1 mm) of larval Gila chub. We measured total 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., Hayward, CA) 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.) for small juveniles or Silver Cup (Nelson and Sons, Inc., Murray, UT) for 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

Feed Type

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

Mean length gain of small juvenile Gila chub differed ($F = 9.096$; df = 4, 5; $P = 0.016$) among feed types with chironomid larvae strongly outperforming the remaining commercial feeds (Table 2). 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 statistically significant ($F = 3.011$; df = 4, 5; $P = 0.128$) (Table 2).

Mean length and weight gain of large juvenile Gila chub was significantly different ($F = 7.076$ 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 (Table 13). 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 or appearance were noted during either experiment.

Temperature

Mean weight and length gains of larval Gila chub were significantly different (F = 6.87 and 11.05; df = 3, 8; P = 0.05 and 0.03, respectively) among test temperatures. Growth of larval chub was greatest at 28ºC but decreased markedly at 32ºC (Table 14). Mean weight gain of larval Gila chub was significantly greater at 28ºC than 20ºC and 32ºC. Mean weight and length gain of small (F = 0.17 and 1.80; df = 3, 8; P = 0.91 and 0.22, respectively) or large (F = 0.47 and 0.67; df = 3, 8; P = 0.70 and 0.59, respectively) juvenile Gila chub did not differ significantly among test temperatures (Table 14).

Mean percent survival appeared highest for larval chub reared at 24ºC but there was no statistical evidence (F = 2.76; df = 3, 8; P = 0.11) of a difference in survival among test temperatures (Table 14). Mortalities were all but non-existent (one accidental) for either juvenile size-class. 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 for larval Gila chub. 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 differed (F = 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 (Table 15). There was also convincing evidence that mean percent survival of larval Gila chub differed (F = 25.258; df = 2, 9; P < 0.001) among rearing densities with consistently higher survival for those groups reared at a low density (Table 15). Few oddities in overt fish health or appearance were noted during the experiment and physical development largely followed growth rates.

Mean length gain of small juvenile Gila chub differed (F = 5.025; df = 2, 9; P = 0.034) among rearing densities and appeared least for those reared at a high density. However, the multiple comparisons procedure used was unable to identify which treatments differed statistically (Table 15).

Mean weight gain of small juvenile Gila chub differed (F = 7.418; df = 2, 9; P = 0.012) among rearing densities, and was greatest for those reared at a moderate density (Table 15). Survival was 100% for all density treatments with small juvenile Gila chub and no oddities in overt fish health or appearance were noted. Mean length and weight gain of large juvenile Gila chub differed (F = 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 (Table 15). 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 one high density treatment tank.

Discussion

Although maximizing production is likely not the 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 large individuals may be necessary to lower their loss due to predation (Marsh and Brooks 1989).

Feed Type

Natural feeds often 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 (Bardi et al. 1998; Mohler et al. 2000; Mischke et al. 2001). While survival of larval Gila chub fed a natural feed was greater, growth of those fed the commercial diet was equal or slightly better. Mischke et al. (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 this feed 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 (i.e., feeding more than one feed type/size, 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 this value may be even more pronounced for imperiled species such as Gila chub. While growth was equal or slightly less for larval Gila chub fed

Artemia than a commercial larval fish diet, survival was significantly higher for larval chub fed the natural diet. Both growth and survival of larval Gila chub may have been increased if a smaller natural feed had been given for the first few days of exogenous feeding or if a co-feeding strategy where both live and inert feed was given was employed. Rosenlund et al. (1997) found combining live feed and manufactured diets improved growth and survival of marine fish larvae compared to the use of live feed only. Co-feeding was found to serve two purposes by improving and stabilizing the nutritional condition of the larvae and pre-conditioning larvae to accept the manufactured diet when live feed is withdrawn, resulting in a shorter weaning period.

We did not compare growth in Gila chub with respect to nutritive differences among feed types (e.g., protein). Our study provides initial guidelines for the feeding of larval and juvenile Gila chub and 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 of smaller natural feeds for larval Gila chub 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 or appearance 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 growth of larval Gila chub was 28ºC and the growth rate markedly decreased somewhere between 28-32ºC. The survival and health of larval Gila chub appeared better at 24ºC than at other temperatures tested. Although a positive trend with increasing temperatures was sometimes apparent and juvenile Gila chub seemed to grow best between 26–29ºC, statistical differences in growth among rearing temperatures were not found. A statistically significant difference in growth among test temperatures for juveniles may have been revealed by 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 et al. 1988). However, further insight as to the relationship between optimal growth and factors independent of growth can shape criteria when determining optimal culture temperature. Disease susceptibility can vary with temperature (Harrelson et al. 1988) and is always a concern. In addition, rearing

temperature can contribute to development of deformations (Abdel et al. 2005; Fitzsimmons and Perutz 2006). In general a higher incidence of malformations has been found in cultured rather than wild fishes (Komada 1980 and citations therein) and such malformations are considered an important problem in intensive aquaculture (Aritaki et al. 1996; Fraser et al. 2004). While we found the incidence of spinal deformities for larval Gila chub was much higher at 32ºC, any trend in occurrence of spinal deformities was unclear at the other temperatures tested. It is generally considered prudent for culturists to produce fishes that are similar in morphological, physiological, behavioral, and biochemical characteristics to their wild counterparts. We recorded overt signs of deformation, but investigation into unseen affects of various culture conditions upon Gila chub may be warranted. Matsouka (2003) found reared fishes with abnormalities often showed no obvious external signs of deformation.

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 rather than a static temperature regime (Harrelson et al. 1988).

Based on the parameters and results of our study water temperatures from 20-28ºC appear suitable for rearing larval Gila chub, with temperatures from 24-28ºC recommended for faster growth. Water temperatures from 20-29ºC appear suitable for rearing juvenile Gila chub.

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 weight did not show the same relationship. An inverse relationship between rearing density and growth of larvae and juveniles has been noted for other species of fishes as well (Irwin et al. 1999; Anderson et al. 2002; Jodun et al. 2002; Sahoo et al. 2004; Rahman et al. 2005).

Similar to other fishes (Alvarez-Gonzalez et al. 2001; Sahoo et al. 2004), larval Gila chub survived better at low rearing densities. We found little effect of the rearing densities we tested on survival of either small or large juvenile Gila chub during our experiment. Anderson et al. (2002) found no effect of rearing density (up to 667 fish/m3; mean fish weight = 1.76 g) on the survival of juvenile bluegill *Lepomis macrochirus* in a longer study. The few mortalities of juvenile Gila chub we noted took place in high-density treatments. In addition, high density treatments for large juveniles resulted in weight loss over a 45-d period. Thus, high density treatments may have eventually led to a significant increase in mortality rates during a longer experiment.

Irwin et al. (1999) stated relationships between density and growth may not always be 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 affects 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 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, and in other types of systems, rearing density may affect growth patterns differently. Given the increasing limitations on space, water use, and funding often encountered by hatchery managers, recirculating systems may become more prevalent in the future.

Our results provide the first published data on the effects of specific rearing densities upon growth and survival of Gila chub. These results may assist in developing guidelines for initial rearing densities for Gila chub in recirculating systems, with possible relevance to other similar species. Recommended initial rearing densities for Gila chub are dependent upon management objectives and the culture system used. Based on our tests, we recommend initial stocking densities near 39 fish/L if growth and/or survival of larval Gila chub in aquaria are primary considerations. For juvenile Gila chub all densities tested gave acceptable survival, at least in the short term. If maximizing growth rate of juvenile Gila chub is important, we recommend fish be raised at approximately 16.986 g/L for small juveniles and approximately 1.681 g/L for large juveniles. Further research is needed to further define the relationship(s) and any thresholds between rearing density and growth and survival for early life stages of Gila chub. We recommend further research for closed recirculating systems concentrate on testing densities within the range of the low to moderate treatment levels we employed.

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, captive breeding and rearing 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).

Table 12.- Nutrient analysis of diets fed to Gila chub *Gila intermedia*.
Nutrient analysis (percent by weight from data supplied by feed manufacturers) of 2 natural diets (enriched and processed by manufacturers; 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. Values for protein and fat represent minimum guarantee levels; and fiber, phosphorus, and moisture represent a range of minimum of maximum and typical guaranteed levels. Values in parentheses are for a dried version of the feed type.

Diet	Protein	Fat	Fiber	Ash	Phosphorus	Moisture	Size Class Fed
Artemia sp. nauplii	6.8 (47)	1.5 (5.5)	1.2 (0.5)		(0.1)	86 (6)	Larval
Chironomid larvae	6 (65)	0.5 (5)	0.9 (3.5)		(0.1)	89 (6.5)	Sm. & Lg. Juvenile
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. & 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	Lg. Juvenile
Silver Cup	48-51	14-16	3-1	12-9		<10	Lg. Juvenile

Table 13.- Mean weight and length gains (with standard errors of the means) per feed type for larval, small juvenile and large juvenile Gila chub *Gila intermedia*. Feed types for larval Gila chub 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 for small juvenile and large juvenile Gila chub 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]). Values with different lowercase letters are significantly different ($P \leq 0.05$).

Feed Type	Mean Weight Gain (mg/g)[a]	SE	Mean Length Gain (mm TL)	SE	Mean % Survival	SE
Larval Gila Chub						
Artemia Nauplii	3.55	1.22	2.8 xy	0.7	76%	13
Egg Yolk Powder	2.72	0.78	2.3 x	0.3	47%	16
Commerical Feed	3.78	1.94	3.4 y	0.5	49%	23
Small Juvenile Gila Chub						
Chironomid Larvae	0.231	0.024	6.4 x	0.2		
Feed 1	0.093	0.100	2.9 y	0.5		
Feed 2	0.076	0.014	3.2 y	1.0		
Feed 3	0.135	0.080	2.3 y	0.6		
Feed 4	0.031	0.043	2.4 y	1.2		
Large Juvenile Gila Chub						
Chironomid Larvae	1.465	0.185	6.9 x	1.5		
Feed 1	0.838	0.189	3.6 xy	0.2		
Feed 2	0.567	0.054	3.6 xy	1.1		
Feed 3	0.640	0.188	4.1 xy	0.4		
Feed 4	0.765	0.022	2.4 y	0.6		

[a]Data for larval Gila chub are reported in milligrams (mg) and juvenile Gila chub in grams (g).

Table 14.-Mean weight and length gains (with standard errors of the means) per test temperature for larval, small juvenile and large juvenile Gila chub *Gila intermedia*. Values with different lowercase letters are significantly different ($P \leq 0.05$).

ºC	Mean Weight Gain (mg/g)[a]	SE	Mean Length Gain (mm TL)	SE	Mean % Survival	SE
			Larval Gila Chub			
20	26 x	3.7	8.3 xy	0.7	70%	8
24	56 xy	10.3	11.4 yz	1.1	89%	7
28	67 y	12.0	12.1 z	0.8	83%	2
32	2 x	1.7	7.6 x	0.1	73%	4
			Small Juvenile Gila Chub			
20	0.713	0.121	11.6	0.8		
23	0.767	0.270	13.5	1.6		
26	0.857	0.063	12.8	2.2		
29	0.880	0.222	14.5	0.7		
			Large Juvenile Gila Chub			
20	2.122	0.395	9.3	1.5		
23	2.439	0.373	11.9	1.6		
26	3.151	1.101	14.1	0.9		
29	2.437	0.292	13.7	2.1		

[a]Data for larval Gila chub are reported in milligrams (mg) and juvenile Gila chub in grams (g).

Table 15.- Mean weight and length gains (with standard errors of the means) per rearing density for larval, small juvenile and large juvenile Gila chub *Gila intermedia*. Values with different lowercase letters are significantly different ($P \leq 0.05$).

Density	Mean Weight Gain (mg/g)[a]	SE	Mean Length Gain (mm TL)	SE	Mean % Survival	SE
			Larval Gila Chub			
Low	33 x	2	9.3 x	0.0	93%	5
Moderate	23 y	2	8.5 y	0.1	49%	3
High	16 y	2	6.9 z	0.2	51%	6
			Small Juvenile Gila Chub			
Low	0.920 x	0.104	16.5	0.9		
Moderate	1.505 y	0.154	16.5	1.0		
High	1.079 xy	0.049	13.4	0.5		
			Large Juvenile Gila Chub			
Low	3.215 x	0.288	10.0 x	2.0		
Moderate	0.580 y	0.150	1.4 y	0.2		
High	-0.331 z	0.099	-0.1 y	0.1		

[a]Data for larval Gila chub are reported in milligrams (mg) and juvenile Gila chub in grams (g).

Management Implications of Captively Breeding Imperiled Chub Species

Erica A. Sontz

Native fish hatcheries usually operate under conditions that combine a conservation agenda with aquaculture technology. The two approaches are not necessarily compatible because they work towards different goals, i.e. recovery vs. production, or quality vs. quantity (Fiumera, et al. 2004). Because fish fecundity is very high – a single, large female chub is capable of producing tens of thousands of eggs (Hamman 1981, Muth et al. 1985) – the natural inclination is to produce as many offspring as possible. However, in the past, genetic consequences of such actions were not carefully considered, and a number of species have suffered.

Many Southwestern native fishes have suffered losses of genetic diversity as a result of captive breeding efforts intended to maintain the species. Razorback suckers, *Xyrauchen texanus,* (Dowling et al 1996), bonytail chub, *G. elegans,* (Hedrick, et al 2000), Virgin River chub, *G. seminuda,* (DeMarais et al 1993) and the Rio Grande silvery minnow, *Hybognathus amarus,* (Turner and Osborne 2004) have all shown negative effects from captive breeding efforts: reduced heterozygosity, loss of alleles, alterations of gene frequencies and founder effects in broodstocks. A captive breeding program for roundtail or headwater chub would need to take these past efforts and their consequences into consideration to avoid having similar effects.

Management Implications

The establishment of captive breeding programs at state and federal hatchery facilities has become a common conservation practice in native fisheries work. This practice provides refugia for imperiled species and creates fish that can supplement wild populations. Species preservation and recovery efforts have focused on restocking fishes from hatchery-reared offspring of wild-caught broodstocks (Johnson and Jensen 1991) and many native fish species have been collected into hatcheries. Generally, fish are held in these facilities and spawned in artificial habitats, i.e. tanks, pools, raceways and ponds. The young are grown out in captivity to a minimum stocking size and then released into the wild in accordance with species recovery plans (USFWS 2002a, USFWS 2002b).

A number of critically endangered Colorado River basin fishes have been taken into captivity for captive propagation and repatriation. The subject of this our work was, likewise, to develop captive propagation and grow-out techniques for imperiled chub. Fish conservation takes place under a mixture of guidelines that stem from two very different branches of science: aquaculture, which is focused on rapid and efficient production of a homogenized final product at low cost, and conservation biology, which seeks to maintain diversity and consistency with natural, evolutionary and ecological conditions while preserving species through natural or induced population declines. Managers need to be very fully aware of how these two different approaches differ: the

goals they address and the "product" they return, when selecting captive breeding strategies to preserve native fish populations.

Captive Breeding: When To Use It

The captive breeding timeline can be subdivided into three distinct points: pre-hatchery, hatchery and post-hatchery, and the further along a timeline a species progresses, the more selection pressures, stressors and impacts it will experience. The goal of any captive breeding program should be to minimize hatchery impacts and minimize time (both absolute and relative to the species life cycle) in the hatchery.

Pre-Hatchery

By definition, conservation means to preserve something without injury or loss; therefore, it is especially challenging to incorporate hatcheries into native fish conservation because many hatchery programs have resulted in injury or loss to native fish populations in the past. If fish are never subjected to hatchery impacts, they will never face selection pressures that direct them towards hatchery adaptations. With few exceptions, the causes for wild fish declines are environmentally driven, although often anthropogenic in origin, and hatchery-derived fish that are stocked into affected habitats will undergo the same challenges as the original, wild populations (AZGFD 2003, USFWS 2006, Schooley and Marsh 2007). Water use, non-native species impacts, diseases and parasites, stream-side vegetation and riparian community changes all cause native fish declines and often still exist when hatchery-spawned and -reared fish are released.

Overriding causes of species declines must first be addressed or captive and supportive breeding programs will continue to suffer from limited success. Recovery efforts targeted at the causes of decline have been much more effective than hatchery support alone. In their review of published animal relocation studies, Fischer and Lindenmayer (2000) report that of studies where an underlying cause was given for a decline of an animal population (51% of total surveyed), none of those that failed to address the cause (13 studies, 11%) were successful. Of those studies that explicitly stated the factor(s) causing the decline and then eliminated the factor(s), success occurred three times more often than failure (22% success and 7% failure of 41 studies), though the overwhelming outcome (71% of 41 studies) was uncertain. Restocking efforts that have identified but failed to reduce factors causing declines in wild fish populations have generally been ineffective in establishing wild populations. For example, hatchery stocking alone has been unsuccessful, to date, in maintaining self-sustaining populations of bonytail chub and razorback sucker in the dramatically altered mainstem Colorado River, despite of tens of thousands of fish being stocked over several decades (Schooley and Marsh 2007; USFWS 2007; Bestgen et al 2008; Karam et al. 2008; Karam and Marsh 2010). In particular, Schooley and Marsh (2007) estimate that 14.6 million razorback suckers have been returned to Lake Mojave over a 30-year period with a survival rate of

0.27% in the first year post-restocking. In the meantime, the population has dwindled to below 500 fish in that same 30-year window because the factors limiting survival and recruitment in the wild have not been addressed. There is further evidence from laboratory and aquarium studies that managers are only recently becoming aware of the full array of challenges that face recovery efforts based around captive breeding and release of hatchery fish as the major method of conservation (Mueller et al 2006; Bestgen et al 2008, Carpenter and Mueller 2008).

Hatcheries

There are two main scenarios where hatchery propagation has been most effective, given its constraints: a species faces imminent extinction in its natural habitat and hatchery production may be the only way for the species to survive (this includes scenarios where a long-lived population persists but natural recruitment may have failed; although, targeting the cause of the recruitment failure would provide a more effective, long-term solution), or when managers want to produce large quantities of fish quickly with the very clear understanding that these fish may not respond and interact with their wild environment appropriately, possibly producing "phenotypically more uniform and behaviorally predictable" fish (Vrijenhoek 1998). Short of these two scenarios, eliminating or minimizing hatchery time would more effectively maintain healthy, wild stocks of fish.

If hatchery time is deemed necessary, adequate numbers of broodstock are needed to maintain genetic diversity, and maintain future diversity if multiple generations in the hatchery are required (Amos and Balmford 2001, USFWS 2002a, USFWS 2002b, DeSalle 2005, Rees et al. 2005). A single generation is enough to alter fish behaviors and introduce hatchery selection pressures into hatchery-spawned fish (Kostow 2004). Often, grow-out of wild-caught eggs or YOY fish provides fish more suited to the wild than captive spawning, as it reduces the amount of hatchery selection to which the fish are subjected and better preserves the genetic integrity of the species (Turner and Osborne 2004).

Reisenbichler and Rubin (1999), in a review on salmonid propagation, point out that hatchery conditions, and thus the selective pressures they exert, are different than those in a natural habitat. Food and feeding regimes; cover, substrate, water depth and velocity; density of conspecifics and density or presence of predators, competitors and diseases; even the water chemistry itself can all vary from natural conditions and shape fish in the hatchery so that they are better adapted to the hatchery than to the repatriation site. Logically, removing, minimizing or mitigating these differences would also eliminate the selection pressures that drive domestication. In cases where it is feasible, backwater coves, outdoor ponds and cleared streams may serve as better hatcheries and nursery sites than concrete raceways, glass aquaria and production facilities (Minckley et al. 2003). Trout Unlimited published an online document detailing a landscape approach to hatchery redesign (Williams et al. 2003). All of these modifications can be tailored to reduce hatchery impacts by providing a more natural development process for the captive

fish that will minimize the imposition of artificial and domesticating selection pressures (Gilligan and Frankham 2002).

Post-Hatchery

When hatcheries are inevitable, fish must eventually be released back into their native habitats. There is some evidence that short periods of acclimation in backwaters or other on-site, soft-release, such as in-stream net pens, may have better results than simply stocking fish in an unfamiliar stream. A hands-off approach to post-stocking population assessment might also aid in repatriation, as repeated handling and netting may inadvertently decrease the growth in repatriated fishes, though it does not seem to increase mortality (Paukert et al. 2005).

In addition to fish response to natural habitat, reintroducing fish from a hatchery into the wild can impact wild populations. Many studies have shown that fish raised in a hatchery do not interact with a natural environment in a similar manner to fish native to the environment (Jonsson and Jonsson 2006; Kelley et. al. 2006; Salonen and Peuhkuri 2006). Hatchery fish are often more aggressive and may differ in spawning timing or behaviors. They may compete with the wild population for food. Releasing hatchery-raised fish on top of wild populations can alter wild populations through their behaviors and genetic swamping (Ryman and Laikre 1991, Ryman et al. 1995, Tringali and Bert 1998, Ford 2002, Fiumera et al. 2004). To minimize these effects, managers must treat captive-bred or -raised fish as part of the larger population unless they can safely assure that the captive fish will not interact with wild fish, either accidentally (through flood events or other inadvertent fish relocations) or intentionally (through future management actions). Failure to treat captive-bred fish as part of a larger population may inadvertently break up naturally occurring behavioral syndromes or genetic complexes or skew sex ratios and effective population sizes with detrimental effects on the wild population (Oota and Matsuishi 2005).

Because hatchery time can have serious, detrimental consequences on dwindling wild populations, a cost-benefit analysis of a captive breeding program is often useful to weigh impacts against the possibilities of success. A study conducted on razorback suckers *Xyrauchen texanus* comparing mtDNA diversity in wild caught individuals from Lake Mohave with three different year classes produced at Dexter National Fish Hatchery (DNFH) showed maintenance of a relatively high level of heterozygosity and haplotypes in the first two hatchery year classes, but a sharp decline in the third year class, indicating a reduced number of female breeders (Dowling et al 1996). Bonytail *G. elegans* broodstock at DNFH was revealed to be much lower than the presumed broodstock, which was already much lower than recommended for captive breeding purposes. Records from the spawning indicate that the 1981 F_1 progeny were produced by 8.5 wild fish; however, both allozyme and mtDNA data suggest that the number of founders was a much smaller 3.5 individuals (Hedrick, et al 2000). Virgin River chub *G. seminuda* hatchery F_1 juveniles showed alterations of gene frequencies, loss of heterozygosity and loss of alleles, indicating founder effect in the broodstock. The hatchery stock was

deemed sufficiently deviated from the original population that it should not be used for recovery except as a last resort (DeMarais et al 1993). Similar reductions in genetic diversity were reported for Rio Grande silvery minnow *Hybognathus amarus* between wild populations and both captive spawned populations and those grown from wild-caught eggs. In the second group, the erosion of heterozygosity was less pronounced than in the supportive breeding program, suggesting that grow-out of wild-caught eggs or larvae might be a better management practice for native fish recovery (Turner and Osborne 2004). Managers must consider hatchery impacts on genetic diversity of both the captive and wild populations, potential selection pressures under hatchery conditions leading to possible domestication and future impacts of releasing large numbers of hatchery fish into the wild when they choose to begin a captive breeding program. Efforts *in situ,* such as habitat restoration and renovation and the removal of non-native predators, must continue during the period of captive breeding and can only serve to strengthen management efforts. When managed carefully, supplemental breeding can complement, but does not substitute, for effective habitat and species restoration in the field.

Past efforts to use captive breeding technologies for native Southwestern fishes have had mixed results, and in some cases, decades of supplemental stocking have met with extremely low success rates. In the area of captive breeding for imperiled species management, terrestrial conservation biologists seem to be ahead of aquatic biologists in incorporating new developments and theories. Aquatic conservation biologists and resource managers have been holding on to hatchery production technology and techniques that may impede rather than help the ultimate goal of species conservation. Incorporating ideas from behavioral ecology, conservation genetics and captive breeding habitat design could prove very costly for species that do not capture the public eye the way charismatic terrestrial species do. Using the ideas of habitat restoration, clean water preservation for human and wildlife use, multispecies conservation plans and the preservation of wild and scenic rivers may prove beneficial in drawing support for species that might otherwise fly under the public radar. Conservation biologists and resource managers working with endemic, imperiled fish species should be ready to enter discussion on whether the savings garnered by continuing to follow a hatchery production mindset that is likely to do harm to imperiled species outweigh the costs of modifying hatchery programs to meet species conservation needs to preserve these species to the best of our current ability.

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Appendix A. Headwaters Chub Experiments Larval Cage Construction

To construct the cages, strips of screening were cut roughly 20 cm x 6 cm from 600 micron nylon screening, 0.6 mm, 51% open area (Aquatic Ecosystems, Apopka, Florida). Circles of screening were cut 6 cm in diameter. Sheets of craft foam 2 mm thick were cut into squares roughly 7.5 cm per side, and a 6-cm diameter hole was cut in each square to match the bottoms cut for the cages. The screen strips were formed into cylinders and the holes in the foam squares were used as a guide for correct cylinder diameter. Silicone aquarium sealant was used to glue cylinder in shape and to glue the bottoms to the cylinder to make a cup.

Intervals of 2 cm were marked upward from the bottom of the cylinder to designate the heights at which the floats would be attached to hold larvae at different densities. Floats were affixed at one of these three heights: low float height for high larval density (same number of fish in less water volume, 56.5 cm^3), middle float height for middle larval density (113.1 cm^3), and high float height for low larval density (same number of fish in a greater volume of water, 169.6 cm^3). Floats were attached at the correct height to the cages with nylon fishing line tied through the mesh or with beads of aquarium sealant.

Schultz (2009) used larger cages of different construction and larger numbers of fish for his density experiments. He also tested feed, temperature and density separately. Due to the smaller number of headwater chub larvae available, and the design of the split-plot experiments to test all three factors at once, we opted for smaller cages that would both take up less room in the tank and require smaller numbers of fish to "fill" each one. We also chose to use full screen cages, rather than solid walls with screen bottoms. This provided better opportunity for flow-through without having to set up a drip system (see Shultz 2009) over every cage. Such a drip system would have been impractical with the number of cages needed for the experimental design and in some of the laboratory spaces used.