Sustainable Feed and Improved Stocking Densities for Gar (*Atractosteus spp*.) Culture

Sustainable Feed Technology/Experiment/09SFT07UM

Solomon David and James S. Diana
University of Michigan
Ann Arbor, Michigan, USA

Wilfrido M. Contreras-Sanchez
Universidad Juarez Autonoma de Tabasco
Villahermosa, Tabasco, Mexico

Two experiments were proposed for this study. The first experiment investigates multiple treatments of fish-meal substitution (using animal by-products in place of fish meal at 0, 25, 50, 75, 100% substitution) in feed for two species of *Atractosteus* gars, the Cuban gar (*A. tristoechus*) and tropical gar (*A. tropicus*). The experiment using Cuban gars would take place at the University of Michigan (U-M), United States, and the experiment using tropical gars at Universidad Juarez Autonoma de Tabasco (UJAT) in Tabasco, Mexico. The second experiment investigates improved stocking densities of tropical gars and would take place in Tabasco only. Stocking density treatments will be 25, 50, and 100 fish/m^3.

Aquacultured tropical gars were acquired by UJAT and pellet trained for feeding experiments. We are currently awaiting materials for production of the appropriate feed to be used in both Cuban gar and tropical gar experiments. Upon receipt of materials, feeding trials will commence on both species at their respective locations. Improved stocking density experiments will also begin at UJAT upon receipt of funds.

CUBAN GAR EXPERIMENTS

Cuban gars (~13-15 cm) were acquired through multiple shipments during March-June 2011 and were pellet-trained for experimental trials from June-August 2011.

An initial feeding trial using live feed (fathead minnows *Pimephales promelas*) was run for 52 days to establish a baseline for growth rate at 0% fishmeal substitution. The pilot study used 4 replicates each with 3 gars in experimental aquaria. Individuals fed ad libitum on live fish had a mean increase in weight of 395% over the experimental period. Cuban gars (N = 45) are currently prepared for experimental trials at U-M that will begin in 1-2 weeks upon arrival of appropriate experimental feed from UJAT.

A second pilot experiment was carried out to investigate growth rates of Cuban gars on different feed types. Experimental feed consisted of frozen fish (anchovies, used as a proxy for 100% fish meal), freeze dried krill (high-protein non-fish-meal feed), and a high-quality pellet (New Life Spectrum Pellet; primary ingredients were fish meal and krill meal). Initial feed amount was based on 10% of mean fish body weight for each tank. Fish were allowed to feed ad libitum for 30 min, at which point uneaten feed was removed. Amount of feed consumed was also monitored by subtracting weight of remaining feed from amount initially fed. Wet weights and dry weights of each feed type were calculated in order to accurately compare consumption across feed types. Water chemistry (pH, ammonia, nitrite, and nitrate) was also monitored weekly to test for localized effects of feed type on water chemistry. We expected insignificant differences in water chemistry among treatments due to the recirculating aspects of the system. Duration of experiment was 21 days (November 8 – November 29, 2011).

Cuban gars were divided into 3 groups of 3 replicates per group. Group 1 (FISH feed) consisted of 3 replicates, n = 4 fish per replicate; Group 2 (KRILL feed) consisted of 3 replicates, n = 5 fish per replicate; Group 3 (PELLET feed) consisted of 3 replicates, n = 5 fish per replicate. Total N = 42 fish. Individuals were maintained in 170 L fiberglass tanks (1 replicate group per tank) which were connected to a closed recirculating filtration system. Water temperature was maintained at approximately 27 °C using heaters in each tank for the duration of the experiment.

Length and weight were measured 3 times over the course of the experiment (days 1, 14, 21) to determine percent growth and growth rates. Descriptive statistics and ANOVA were used to analyze growth and consumption data. After experiment concluded fish were removed from tanks and placed into a single round 1,000 L tank with high-capacity canister filtration. Fish are currently being fed a combination of pellet and krill feed in preparation for arrival of experimental feed from Mexico (expected in mid-late February 2012).

Mean length did not significantly increase over time in any of the treatments, but this was expected due to the short duration of the experiment. Mean weight increased significantly in fish feed treatment, but not significantly in krill or pellet feed treatments. ANOVA tests indicated that both fish and krill feed treatments experienced significantly higher growth than pellet treatment, however, fish and krill feed treatments were not significantly different from each other. Consumption rates were significantly different among all treatments. Consumption was highest with fish feed (23.97 g/feeding), followed by pellet (15.26 g/feeding), and finally krill (11.48 g/feeding). Krill feed had the highest body mass gained per gram of feed consumed compared to other feed types. As expected, water chemistry did not vary significantly among any treatments likely due high water turnover rate by the recirculating system. Descriptive statistics for the experiment are found in Table C1.

Table C1. Descriptive statistics for second pilot study of Cuban gar growth (weight) for 3 different feed types. All values are in grams unless otherwise noted. Day 1 and Day 21 are mean weight ± 1 standard error. Fish feed type was the only type to experience significant increase in mass compared to starting mass. Final mass of fish and krill feed treatments were significantly greater than pellet feed treatment. Mean consumption was significantly different among all feed types.

Feed Type	Day 1	Range	Std. Dev.	Day 21	Range	Std. Dev.	Percent Growth	Mean Consumption (g/feeding)
Fish	44.60 ± 4.31	30.80-88.20	15.55	59.27 ± 5.50	42.50-109.60	19.04	31.78*	23.97*
Krill	43.93 ± 4.75	20.20-98.20	18.41	54.87 ± 5.43	28.60-114.50	21.01	24.92	11.48*
Pellet	54.53 ± 9.08	18.10-122.40	35.15	59.03 ± 9.30	22.40-128.40	36.02	8.25	15.26*

*indicates significant difference based on ANOVA tests

We believe the fish and krill feed treatments experienced greater growth for several possible reasons. Although all fish were trained to consume all three types of feed prior to the experiment, certain feed types seemed to hold greater appeal to the gars and therefore may have played a role in consumption and therefore growth. Fish feed is the most natural feed type to gars; even though feed was non-live chopped fish, the gars readily accepted this feed type and are naturally able to metabolize the food. Krill feed has a bright color and strong odor, both of which attracted the gars. Krill has also been shown to be highly digestible, and gars of all species have been shown to consume invertebrates such as insects and crustaceans. The pellet feed was not brightly colored, and did not have as strong an odor as krill. Both of these differences may have made the feed less appealing to the gars. Additionally, the pellet feed consisted of additional materials (wheat meal, brewer's yeast, spirulina) which may not have been as

digestible to the gars compared to fish and krill meal, therefore growth may not have been optimal on pellet feed.

These issues will all play a role in the full experiment, and we will adjust accordingly. All feed in the primary experiment will be pellet feed, so "appeal" of feed should not be as large a factor. Digestibility of non-fishmeal components will likely be the largest factor. Given these results, we hypothesize that the feed type with the highest amount of fishmeal will experience the highest consumption and the highest growth.

TROPICAL GAR EXPERIMENTS

Gars are top-level predators in their native ecosystems and are characterized by their elongated jaws, cylindrical bodies, and diamond-shaped ganoid scales. Their maximum size and age varies with species from approximately 80 cm and 10 years (shortnose gar) to 300 cm and over 70 years (alligator gar). Gars are generally polyandrous in reproductive strategy, with multiple male individuals spawning with 1-2 females. Gars spawn during late spring and early summer in temperate regions and during the rainy season in tropical regions. Growth is extremely rapid, with all species capable of reaching 30 cm or more in their first growing season (young–of-the-year alligator gar can reach over 30 cm, 250 g in 3 months).

Gars are excellent candidates for aquaculture as they exhibit rapid growth to large sizes, are highly resistant to disease, can be maintained at high densities, readily adapt to artificial feed at early life stages, and are highly tolerant to low water quality conditions due to their air-breathing abilities (Alfaro et al. 2008). Their tolerance of low water quality via aerial respiration also allows for a less complicated technological system for aquaculture, as opposed to other fishes which may require considerable aeration and water turnover. Gars are therefore well-suited for culture in developing regions.

Much progress has already been made in the aquaculture of *Atractosteus* gars (tropical, Cuban, alligator), primarily in regions of Mexico, Cuba, and the southern United States. Broodstock for all three species have been established and are currently maintained in their native regions, and juveniles have been released to help restock diminishing wild populations. Further efforts are being made in the southern US to protect alligator gar populations and manage them as a viable sport fishery, as well as increase its potential as a food fish. Gars are already popular food fish in various regions of Mexico and Cuba.

Due to their unique appearance and predatory nature, gars are becoming increasingly popular in the ornamental fish trade. Gars have been sought-after aquarium fish in Southeast Asia for many years, and are growing in popularity in the United States and other countries. The Florida gar, native to only a small portion of the southeastern United States, is the most popular aquarium species of gar in the US (usually wild-caught) and most readily available abroad. Prices in the United States range from $15-$40 USD for 20-35 cm individuals. Other gar species at similar sizes command a much higher price largely due to their rarity in the aquarium hobby, such as $200 USD for an individual tropical gar and over $300 USD for a Cuban gar (in the United States). Tropical and Cuban gars are also highly valued overseas; in Singapore 15 cm tropical gars average $150 USD and Cuban gars $ 400 USD. Ironically, tropical and Cuban gars are among the most commonly cultured gar species. Specimens exhibiting genetic mutations in pattern or coloration (i.e. melanistic, xanthochroic, leucistic) command an even higher price, ranging from $1000 to over $5000 USD. Hybrid gars, although rare in the trade, are also much sought-after.

In this study we aim to determine optimal stocking densities for grow-out and the possibility to substitute fishmeal, using by-products.

Experiment 1. Determine Optimal Densities for Rearing Tropical Gars

METHODS AND MATERIALS

This study started using tropical gars averaging 13.00 g in weight and 15.66 cm in length. Fish were randomly allocated in a recirculation system composed of $1m^3$-tanks in three treatments (25, 50 and 100 fish/m^3) run with three replicates. Every 15 days we registered growth in total weight and length. During the experiment we registered temperature, dissolved oxygen and pH. Tanks are cleaned by siphoning the bottom to eliminate wastes from fishes and remaining feed. All fish were fed *ad libitum* three times a day and total food consumed was recorded daily. Mortality was observed daily in every tank.

Results were analyzed using a one way ANOVA to determined if there were significant differences ($p<0.05$) between treatments before, during and after the experiment. The package STATGRAPICHS 5.0® was used for statistical analysis and SIGMA PLOT 11® for graphical representation.

RESULTS

This experiment was conducted using 3-month old tropical gars. Average weight and length were 13.00 g and 15.66 cm, respectively. After 15 days of experimentation, no significant differences were observed between treatments for length or weight (ANOVA; $P>0.05$). At this time, fish stocked at 100 fish/m^3 had a mean weight (SD) of 14.94 g (1.13), with 15.56 g (1.22) for 50 fish/m^3 and 17.42 g (0.98) for 25 fish/m^3. Average length ranged from 15.98 to 16.54 cm. Despite no indication of significant differences, the fish with lower density had a slight tendency for better growth. This pattern was confirmed at 30 days of experimentation when statistical differences between densities were detected (ANOVA; $P<0.05$). Highest growth was detected at 25 fish/m^3 having 27.00 g (± 3.23) in weight (Fig. 1A), and 19.08 cm (± 0.58) in length (Fig. 1B). At this time, differences between the lowest and the highest growth groups averaged seven grams.

After 45 days, differences were larger between treatments with 25 and 100 fish/m^3. Highly significant differences were found between treatments for average weight (ANOVA; $P<0.01$). The treatment with 25 fish reached 36.19 g ± 2.82; while treatments with 50 and 100 fish/m^3 reached 31.47 (2.62) and 22.88 (2.64) respectively (Fig. 2). Significant differences were also found in length, being higher for the 25 fish/m^3 (20.36 cm ± 0.53) while the other two treatments reached 20.05 (0.77) and 18.37 (0.69), respectively.

There were no significant differences in survival between treatments (X^2; $p>0.05$), with survival of 100%, 98.6%, and 98.6% for 25, 50 and 100 fish/m^3, respectively.

DISCUSSION

Stocking density is a very important aspect in fish culture systems because it has a significant impact in growth. In our study, the best results were found in the lowest density (25 fish/m^3) compared to densities of 50 and 100 fish/m^3 after 45 days. Similar results were found by Ramon (2003), who found best growth at densities of 10 and 15 fish/m^3 in comparison with 5 fish/m^3. He also mentioned a tendency to gain more weight when tropical gars were stocked at higher density, however, information was not provided. Rivera and Márquez (2001) evaluated the effect of stocking density on growth and survival in gar larvae in captivity, and found no differences in weight or length between 1 and 40 larvae/L.

Experiment 2. Determine the Degree to Which Fishmeal Can Be Replaced Using By-Products in Gar Feeds

In this case we used fish weighing 14.40 g with an average length of 15.79 cm. Four treatments were evaluated (25, 50, 75 and 100% fishmeal substitution) run in three replicates. The fishes were placed in a recirculation system (every tank stocked with 10 fish). Fish length and weight will be measured every 15 days to determine growth over the course of the experiment. From these growth data we will determine optimal feed types and stocking densities. From these trials we hope to develop low-cost and environmentally friendly methods (such as using lower-fishmeal content feeds) for culture of the tropical gar.

This experiment was recently started. Fish averaged 14.40 g in weight and 15.79 cm in length. No significant differences were detected among treatments at the beginning of the experiment (ANOVA; P> 0.05). Fish will be sampled every 15 days to determine significant differences using a one-way ANOVA.

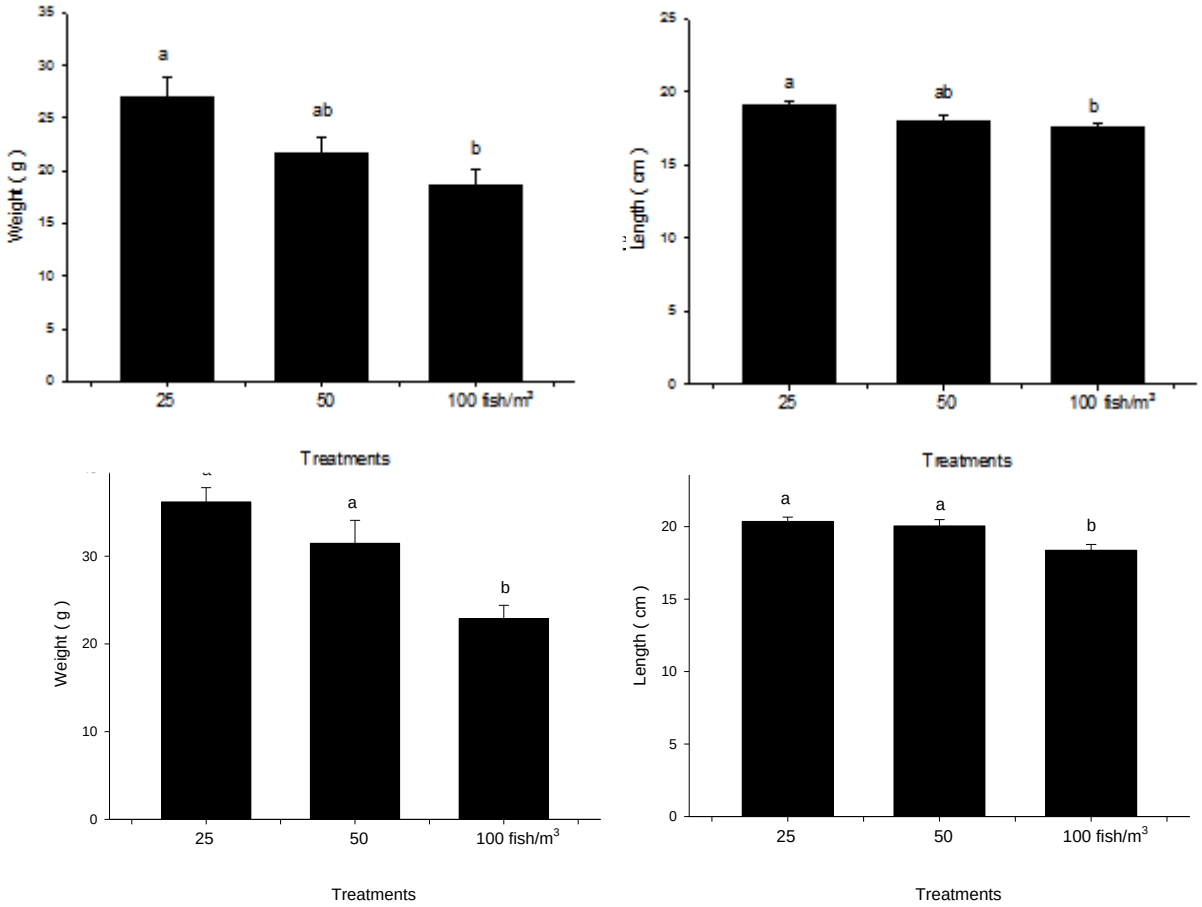

Figure 2. Average weight and length for the three densities evaluated in this study after 45 days of experiment.

SPOTTED GAR EXPERIMENTS

In addition to growth experiments for Cuban gars, we conducted growth experiments comparing two populations ("peripheral" from the Great Lakes region and "core" from the Mississippi River basin) of spotted gars (*Lepisosteus oculatus*) to test for effects of latitudinal and countergradient variation in growth rates between populations (Figure 1). Identifying populations of fishes with higher growth rates at different temperatures can be beneficial in aquaculture by increasing production due to optimal growth. These "strains" of fishes would be the ideal choice for optimizing production (as seen in other species such as salmonids and centrarchids).

Several northern populations of fishes have been shown to have faster growth rates than conspecifics from southern latitudes when reared in common garden environments. We tested for this variation in growth of young of the year (YOY) spotted gars in the following experiments. Based on our findings, other aquacultured lepisosteids, such as the much larger alligator gar (*A. spatula*) or more commonly cultured tropical gar, may exhibit similar interpopulation variation in growth. We propose to expand our research to other gar species and air-breathing fishes as well.

Countergradient variation, or more generally, latitudinal variation, has not been studied in gars; the disjunct distribution and primitive ancestry of the spotted gar makes it a unique model species for investigation of this phenomenon. To explore potential differences in core and peripheral gar populations in the context of countergradient variation theory, we compared growth rates for the first growing season between core and peripheral populations of the spotted gar. Our primary objective was to investigate differences in life history patterns, specifically growth rate in the first growing season, between the Great Lakes (peripheral) and southern United States populations (core) of spotted gars using common garden experiments. Our second objective was to determine whether any potential variation in growth rate might be explained by countergradient variation theory. We hypothesized that spotted gars from the peripheral population would exhibit a faster growth rate and higher capacity for growth at all temperatures than spotted gars from the core population. And further, we hypothesized that this variation in growth rate between populations is evidence of countergradient variation in growth of spotted gars.

METHODS

Spotted gars were acquired from two major sources to represent the core and peripheral populations. Core population representatives were collected via colleagues at Nicholls State University (Thibodaux, LA) in late spring 2009 from several localities in southwestern Louisiana using experimental gill nets, and peripheral population representatives were acquired from several inland lakes in southern Michigan. Fish from Louisiana were the progeny of wild-caught individuals from 2 localities in the Barataria estuary system (Bayou Chevreuil and Golden Ranch) and 1 locality in the Terrebone estuary system (Chacahoula Swamp) collected in March-April 2009. Individuals from the core populations were intermixed in order to reduce potential genetic bias from a single locality, and the same was done for individuals from peripheral populations. Adult fish from all core populations were maintained together in an indoor tank, and spawning was induced at 21°C using Ovaprim™ (Syndel Laboratories) injections at a concentration of 2.0 mL/kg body weight. Ovaprim™ was introduced via intramuscular injection near the anterior base of the dorsal fin, and spawning occurred within 24-48 hrs of injection. Viable embryos from this spawning event were then collected from the tank and approximately 150 specimens were shipped overnight to the University of Michigan.

Adult peripheral population representatives were collected in late spring (May) 2009 from five different inland lake localities in southern Michigan using a boom electrofishing boat. Marble and East Long lakes are part of the St. Joseph River watershed, and Round, Carpenter, and Sugarloaf lakes are part of the Grand River watershed. Adults from peripheral populations were maintained together in an indoor tank similar to that of core population fish. Spawning was similarly induced using Ovaprim™ but was not as successful, therefore several adult fish were stripped of milt and eggs to create embryos (approximately

200 specimens). Core population gars will be referred to as LA fish and peripheral population gars as MI fish from henceforth.

Embryos from both populations were raised in separate 38 L aquaria using aeration and daily 50% water changes to maintain water quality. A 25-watt heater was used to maintain consistent temperature (21-23 °C) during the incubation period as well as post-hatch. Sac-fry and free-swimming larvae were maintained in multiple aquaria separated into core or peripheral populations. Once larvae were zooplanktivorous, they were further separated into 3 aquaria per population to better maintain water quality. Zooplanktivorous larvae were first fed small *Daphnia sp*, and then larger *Artemia* adults. Larvae were fed 2-3 times daily to maintain a constant supply of food. Larvae from both populations were fed small (3.0 cm) fathead minnows *Pimephales promelas* upon converting to piscivory. Larvae were further separated roughly based on size into 3 aquaria per population to reduce cannibalism. To estimate early life growth rates during the period from 1-100 days after hatch (DAH) preceding experiment 1, 30 individuals from each population were randomly selected weekly for measurements of length (0.1 cm) and weight (0.1 g). Mean growth rates (cm•d-1 and g•d-1) were then calculated for each population. Once juvenile gars were regularly feeding on medium-sized (4.5-6.0 cm, size range used in experiments) fathead minnows, individuals were randomly selected from each population and placed into experimental aquariums. All selected individuals were acclimated to experimental aquariums for 4-5 days prior to the start of experiment 1. Excess individuals were maintained in separate aquaria (based on population) as replacements if needed and for experiment 2.

Experiment 1

Twenty 75 L aquaria were used for housing YOY spotted gars from both populations (N = 30 fish from each population). Each aquarium was divided equally into three compartments using thin fiberglass screening, which allowed passage of water, but not other gars or feeder minnows. Each individual compartment housed one gar (3 gars per aquarium, total of 60 gars). Each aquarium also contained an air pump-operated sponge filter to maintain water quality and a 50-watt heater to maintain consistent temperature of 22-24 °C. Temperature range was selected based on mean temperatures experienced during the growing season by both populations (Redmond 1964, Echelle and Riggs 1972, Simon and Wallus 1989, Simon and Tyberghein 1991, personal observation). To further maintain water quality, 50% of the water was changed weekly for each tank, with waste material removed via siphon. Overhead fluorescent lights on electronic timers were used to maintain a consistent 12-hour photoperiod during the experiment. Individual spotted gars were fed fathead minnows ad libitum for the duration of the experiment, 62 days for LA fish and 63 days for MI fish. To accomplish ad libitum feeding, a small group of minnows (approximately 5.0-7.0 g total mass) was consistently maintained in each experimental compartment; consumed minnows were replaced and dead minnows were removed to prevent deterioration of water quality.

Individual gars were removed from compartments to measure length and weight weekly as well as at the beginning and end of the experimental period. Mean length and weight were used to determine increase in growth and growth rate (cm•d-1 and g•d-1) over the experimental period. One-way analysis of variance (ANOVA) was used to test for significant differences in initial and end mean length and weight for both populations. Analysis of covariance (ANCOVA), with population and DAH as fixed factors, was used to determine significant differences in growth rates between populations, if any. I assumed a linear model for growth during the experimental period of development for both populations of spotted gars. Increase in length and weight for each population was plotted versus time (DAH or days of experiment) and analyzed using linear regression to generate growth models. Length-weight relationships were also analyzed with ANOVA and used as a proxy for comparing energy storage between populations.

Experiment 2

To investigate potential differences in growth rate between populations at different temperatures, spotted gars from both populations were divided into three temperature groups; 16 °C, 23 °C, and 30 °C, for a total of six groups (one peripheral group and one core group per temperature treatment). Each group was

comprised of six spotted gars for a total of 36 gars in the experiment. Fish were randomly selected from both experiment 1 as well as excess individuals, and were all reared under the same temperature (23 °C) and feeding (ad libitum) regime for at least 30 days prior to beginning the experiment.

Each group of gars was placed in a 190 L fiberglass tank containing a stand pipe connected to a large recirculating system for constant water filtration. Temperature was maintained using 75-watt heaters in the control and warm treatment group tanks, and was monitored daily. All groups were acclimated to respective temperature treatments for at least 7 days prior to beginning the experiment. Spotted gars in all tanks were given unlimited ration of fathead minnows, and photoperiod was maintained at 12 hours light/dark. Within each tank individual fish were identified by a single fin clip from the right/left pectoral fin, right/left pelvic fin, anal fin, or no fin clip. Marked fins were re-clipped as necessary (due to fin regeneration) on measurement days over the course of the experiment. Length and weight of all fish were measured at the beginning of the experiment as well as weekly for five weeks. Total duration of the experiment was 42 days.

Mean length and weight were determined for both populations in each treatment weekly, and growth rate was calculated as in experiment 1. Length-weight relationships were also calculated and analyzed for each temperature treatment and used as a proxy for energy storage similarly to experiment 1. Due to limitations in replication because of low numbers of available fish and tanks (only 1 replicate of 6 fish for each population per temperature treatment), primarily descriptive statistics were used to analyze experiment 2.

In addition to descriptive statistics, ANOVA tests were run using each fish as a replicate (N = 6 replicates per population in each treatment) to further investigate differences in growth rate and length-weight relationships between populations at each temperature. ANCOVA with temperature and population as fixed factors was performed for analysis of growth rate. All statistical analyses were carried out using JMP SAS (2001) software with significance levels set at $\alpha = 0.05$.

RESULTS

Eggs from both populations hatched 6-7 days after fertilization. Hatching success was 70-80% for both populations, and newly hatched larvae were approximately 1.0 cm in length and weighed approximately 0.5 g. Larval gars consumed their yolk sacs 6-7 DAH and began feeding on Daphnia and Artemia. Juveniles from both populations began eating small fathead minnows 35-40 DAH; 30 fish from each population were then randomly selected and moved into experimental tanks for acclimation.

Growth rates in length and weight during early life were significantly higher (ANCOVA, $p < 0.05$) for LA spotted gars than MI spotted gars held at 23 ºC (Figures 2 and 3). Length and weight regression models explained 96-99% of variation in the data. Although both groups of fish were of similar age when switching to piscivory and acclimating to experimental aquaria, 1-way ANOVA tests indicated MI fish were significantly smaller than LA fish at the beginning of experiment 1 (Table 1). One-way ANOVA tests indicated that end length and weight of MI fish, however, were significantly higher than end length and weight of LA fish. ANCOVA tests also indicated that growth rates of MI gars were significantly greater than those of LA gars. Linear regression analyses generated models of growth rates for both populations and explained 97-99% of variation in the data (Figures 4 and 5).

Length-weight relationships were compared using one-way ANOVA at the beginning and end of experiment 1; ANCOVA was used to compare rate of change in length-weight relationships during the course of experiment 1. At the beginning of experiment 1, MI fish had a significantly lower weight at a given length than LA fish. By the end of experiment 1, however, MI fish had a significantly higher weight at length than LA fish. Linear regression analysis and ANCOVA indicated that change in weight-length ratios were significantly different between MI (higher rate) and LA fish (lower rate) over the course of experiment 1 (Figure 6).

In experiment 2, both populations responded differently to temperature treatments (Table 2, Figure 7). Fish from both populations at 16 °C exhibited very low increases in length (MI fish = 0.02 cm, LA fish = 0.10 cm) and decreased in weight (MI fish = -1.18 g, LA fish = -0.38 g) during the 42-day period. Clipped fins (used to identify individual fish) did not regenerate on any individuals in either cool treatment, and consumption of fathead minnows was very low compared to other temperature treatments. Fish in the 23 °C and 30 °C treatments frequently required re-clipping of marked fins, as well as much more frequent replacement of fathead minnows. MI fish at 23 °C and 30 °C experienced larger mean increase in growth and growth rate (weight) compared to LA fish. One-way ANOVA tests comparing growth rates among all temperature treatments indicated that both populations experienced lowest growth rates at 16 °C, higher growth rates at 23 °C, and highest growth rates at 30 °C (Figure 8). Comparing growth rates within populations at different temperatures, MI fish experienced significantly higher growth in length from 16 °C to 23 °C, but not from 23 °C to 30 °C. LA fish experienced significantly higher growth in length among all three temperature treatments. MI fish experienced significantly higher growth in weight across all temperature treatments, while LA fish experienced significantly higher growth in weight from 16 °C to 23 °C, but not from 23 °C to 30 °C. The 16 °C treatment may have been near the point at which growth ceases in both populations of spotted gars.

DISCUSSION

We hypothesized that spotted gars from two disjunct population segments would exhibit latitudinal compensation in growth similar to several other fish species (Conover et al. 2009), and that under common environment conditions, fish from higher latitude would grow faster than those from lower latitude. Our experiments showed that in a common environment simulating periods within the first growing season (experiment 1: T = 23 ºC, duration approximately 60 days, 95-155 DAH; experiment 2: T = 16, 23, or 30 ºC, duration = 42 days), peripheral population spotted gars had a significantly higher growth rate than core population spotted gars, suggesting that important genetic and physiological differences exist between the two major population segments. Although lack of replication limited the extent of our statistical analyses in experiment 2, results clearly suggest that MI spotted gars maintained a higher growth rate than core population spotted gars even at warmer temperatures, and that both populations had similar thermal minima for growth. These results strongly support evidence for CnGV in growth rate in spotted gars.

As in Atlantic silversides, the model species used to investigate CnGV in growth by Conover and Present (1990) (see also Conover 1992, Present and Conover 1992, Munch and Conover 2002), spotted gars begin spawning at approximately the same temperature (23 ºC) but later in the year with increasing latitude (Redmond 1964, Holt 1973, Trautman 1981, Becker 1983, Snedden 1999). Conover et al. (1990) also noted that later initiation of spawning and earlier onset of winter resulted in a much shorter growing season at higher latitudes. Although the length of growing season decreases as latitude increases, mean size at the end of first growing season does not decrease for several populations of fish species with increasing latitude (Conover et al. 2009). Therefore populations of these species at higher latitudes are able to compensate for shorter growing seasons by evolving faster growth rates than lower-latitude populations (Conover 1992).

These differences in growth rate may be indicative of other potentially interesting eco-evolutionary dynamics between core and peripheral populations of spotted gars (explored in chapter 2) such as differences in life history patterns, as well as morphological and genetic variation. From an evolutionary ecology perspective, my results suggest that a rapid adaptation in growth rate has occurred even in relatively slowly-evolving fishes such as gars (Wiley 1976, Conover et al. 2009, Grande 2010, Carlson et al. 2011). The spotted gar, a warmwater species, entered the Great Lakes region via connections to the Mississippi River drainage (southern refugium) following the last glaciation no more than 8,000 years ago (Bailey and Smith 1981, Hocutt and Wiley 1986). Therefore adaptation of growth rate to length of growing season was relatively recent. Similarly, Mach et al. (2011) showed that in Atlantic silversides, another species expanding northward from a single southern refugium post-glaciation, regional adaptation

(e.g. CnGV) and phenotypic patterns developed relatively recently. Using Pacific salmonids, Carlson et al. (2011) showed that shifts in body size due to selection over even a single generation can have large and lasting evolutionary impacts on both species and ecosystems.

The scope of our study was limited to two major populations (core and peripheral) of spotted gars; including more populations in future experiments may provide a better picture of gradient in growth rate with increasing latitude. Despite this limitation, our study populations did represent a natural break in the distribution of spotted gars, in that the species is completely disjunct between the Great Lakes and Mississippi River basins (Page and Burr 1991), therefore our population comparisons are realistic if not comprehensive. The core population does span a greater latitudinal range than the peripheral population (approximately 1550 km compared to 220 km), therefore growth rate comparisons among fish from multiple core populations are recommended.

Although CnGV has been observed in a diversity of ectotherms, most frequently in fishes, it has not been previously observed in gars. Furthermore, our study is the first to use common garden experiments to test for latitudinal variation in a non-teleost fish; an under-studied group in such investigations, because of their typically late maturation and long generation time (Ferrara 2001), as well as high energy requirements (Alfaro et al. 2008) compared to teleosts in similar studies (Conover and Present 1990, Schultz et al. 1996, Arendt and Wilson 1997, Power and McKinley 1997, Conover et al. 2009, Baumann and Conover 2010). Our results suggest that CnGV may exist in other evolutionarily and economically significant non-teleost species (i.e. lungfishes, sturgeons, alligator gar).

Countergradient variation in growth of spotted gars may also have implications in the context of climate change and range expansion. Using the weak latitudinal temperature gradient of the Pacific silversides *Atherinops affinis* as a proxy for the gradual effects of climate change, Baumann and Conover (2010) showed that two species, Atlantic and Pacific silversides, each experiencing very different latitudinal temperature gradients, still exhibited CnGV in growth. Their study indicated that ectotherms have evolved growth adaptations to even weak climate gradients, and that a pole-ward migration of genotypes will be a likely result of an increasingly warmer climate. As a warmwater species exhibiting CnGV, spotted gars would likely successfully increase their range northward even with gradual increases in temperature.

Previous studies have shown that in aquatic systems, species at higher trophic levels are at higher risk and are more frequently lost than those at lower trophic levels, in part because of their relatively small population sizes (Lande 1993, Petchey et al. 2004). Piscivorous fishes, therefore, may be particularly vulnerable amidst the ongoing biodiversity crisis. Furthermore, non-game piscivorous species (e.g. gars, Lepisosteidae; bowfin, Amia calva) may be even more at risk due to their poorly-studied ecology, perceived low economic value, and the higher priority given to propagation and management of game species (centrarchids, percids, esocids); the latter often leading to the destruction of both non-game individuals and habitat (Scarnecchia 1992). Our study provides evidence of unique characteristics of the peripheral population of spotted gars, and provides more evidence for the general argument that understanding and protecting peripheral populations should be a key component of our programs to conserve natural biodiversity.

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Table 1. Mean length (cm) and weight (g) at initiation and completion of experiment 1, along with total growth (Final-Initial), growth rate ($cm \cdot day^{-1}$, $g \cdot day^{-1}$), and descriptive statistics for LA and MI populations of spotted gars (N=30 fish per population). Experimental durations were 62 (LA) and 63 (MI) days.

Population	**Michigan**			**Louisiana**		
	Mean	Variance	St Dev	Mean	Variance	St Dev
Initial Length	14.29	3.41	1.85	15.56	2.40	1.55
Final Length	20.06	2.70	1.64	18.24	2.11	1.45
Total Growth	5.77			2.68		
Growth Rate	0.09			0.04		
Initial Weight	7.50	9.07	3.01	10.74	10.33	3.21
Final Weight	24.09	46.80	6.84	17.53	16.93	4.12
Total Growth	16.59			6.79		
Growth Rate	0.26			0.11		

Table 2. Mean length (cm) and weight (g) at initiation and completion of experiment 2, along with total growth (Final-Initial), growth rate ($cm \cdot day^{-1}$, $g \cdot day^{-1}$), and descriptive statistics for LA and MI populations of spotted gars at 3 different temperature treatments (N = 6 fish per population in each treatment). Experimental duration was 42 days.

Experimental Temperature (°C)		Michigan			Louisiana		
			Length				
		Mean	Variance	St Dev	Mean	Variance	St Dev
16	Initial Length	21.07	1.05	1.03	19.17	1.59	1.26
	Final Length	21.08	0.95	0.97	19.27	1.87	1.37
	Total Growth	0.02			0.10		
	Growth Rate	< 0.01			< 0.01		
23	Initial Length	20.75	0.67	0.82	19.85	7.30	2.70
	Final Length	23.52	1.26	1.12	21.33	7.37	2.72
	Total Growth	2.77			1.48		
	Growth Rate	0.07			0.04		
30	Initial Length	22.60	6.86	2.62	20.72	0.90	0.95
	Final Length	25.50	2.96	1.72	23.15	1.24	1.11
	Total Growth	2.90			2.43		
	Growth Rate	0.07			0.06		
			Weight				
		Mean	Variance	St Dev	Mean	Variance	St Dev
16	Initial Weight	27.73	22.41	4.73	21.77	24.25	4.92
	Final Weight	26.55	18.58	4.31	21.38	25.16	5.02
	Total Growth	-1.18			-0.38		
	Growth Rate	-0.03			-0.01		
23	Initial Weight	24.63	10.61	3.26	23.73	96.48	9.82
	Final Weight	37.12	44.87	6.70	30.05	195.19	13.97
	Total Growth	12.48			6.32		
	Growth Rate	0.30			0.15		
30	Initial Weight	32.50	134.49	11.60	25.27	20.06	4.48
	Final Weight	51.97	108.97	10.44	36.32	41.93	6.48
	Total Growth	19.47			11.05		
	Growth Rate	0.46			0.26		

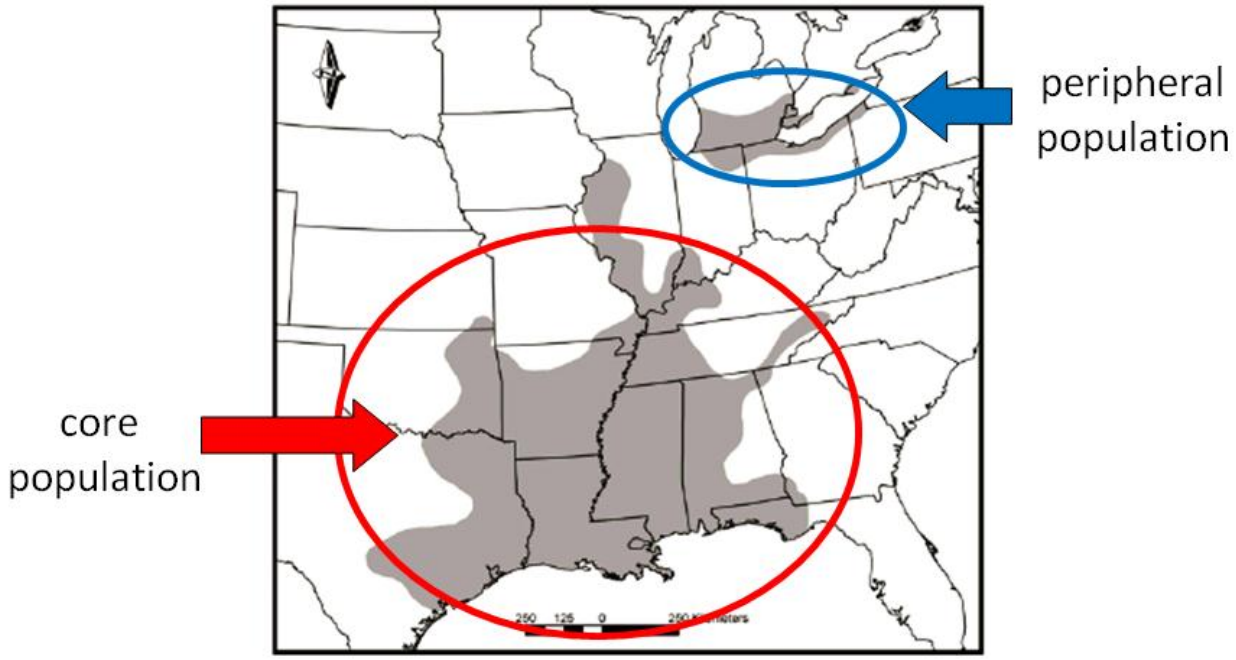


Figure 1. Distribution of core and peripheral populations of the spotted gar *Lepisosteus oculatus*. Note disjunction between populations. Modified from Page and Burr (1991).

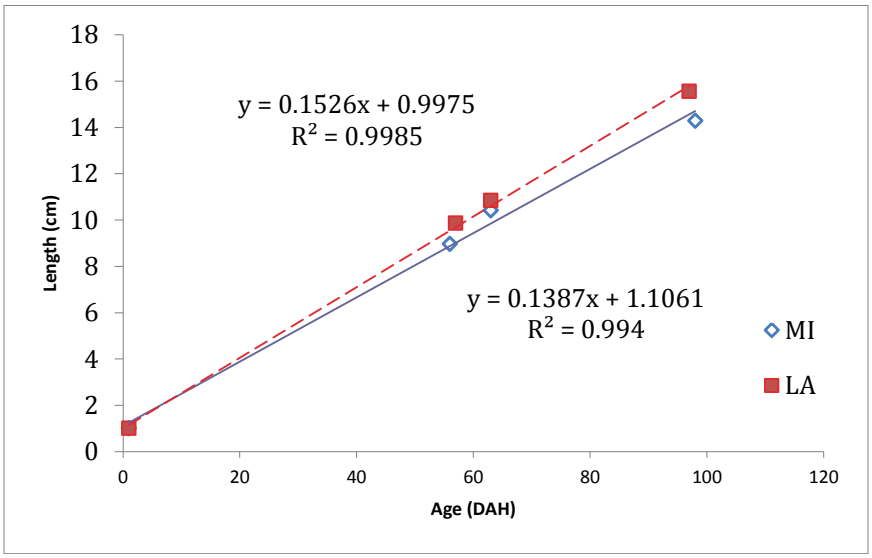


Figure 2. Comparison of early life stage length at age (period prior to start of experiment 1) of LA and MI populations of spotted gars held at 23 ºC (N = 30 fish per population). Larval fish from both populations hatched at approximately 1.0 cm. Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

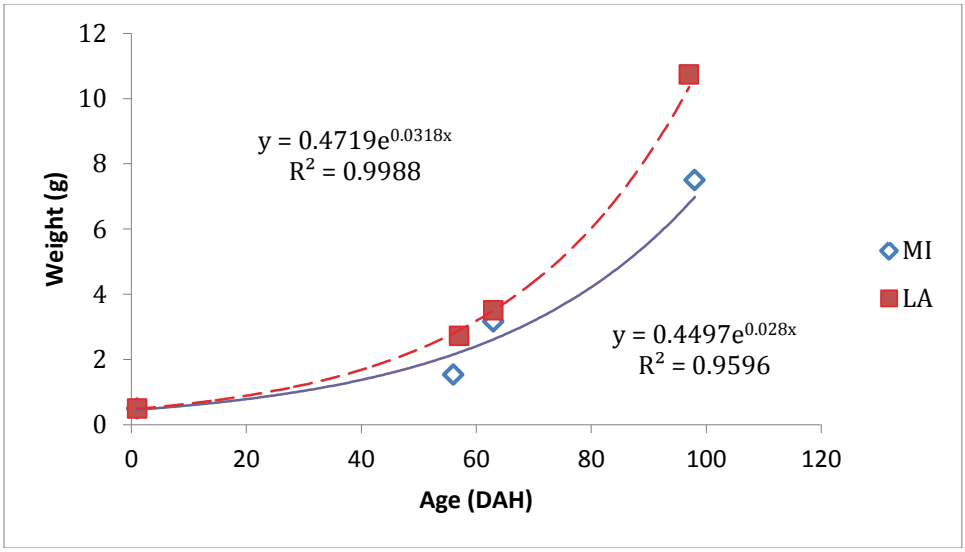


Figure 3. Comparison of early life stage weight at age (period prior to start of experiment 1) of LA and MI populations of spotted gars held at 23 ºC (N = 30 fish per population). Larval fish from both populations hatched at approximately 0.5 g. Exponential regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

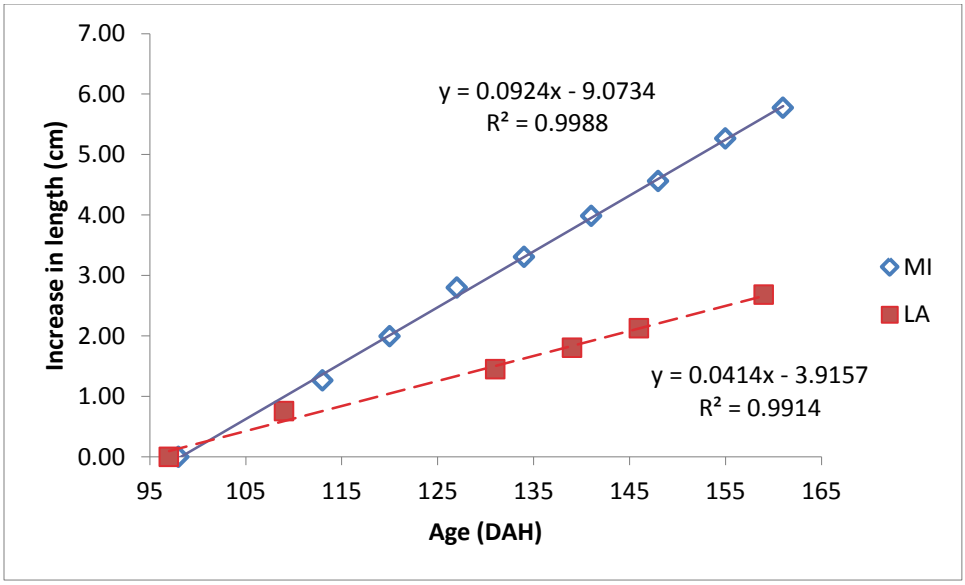


Figure 4. Increase in length over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

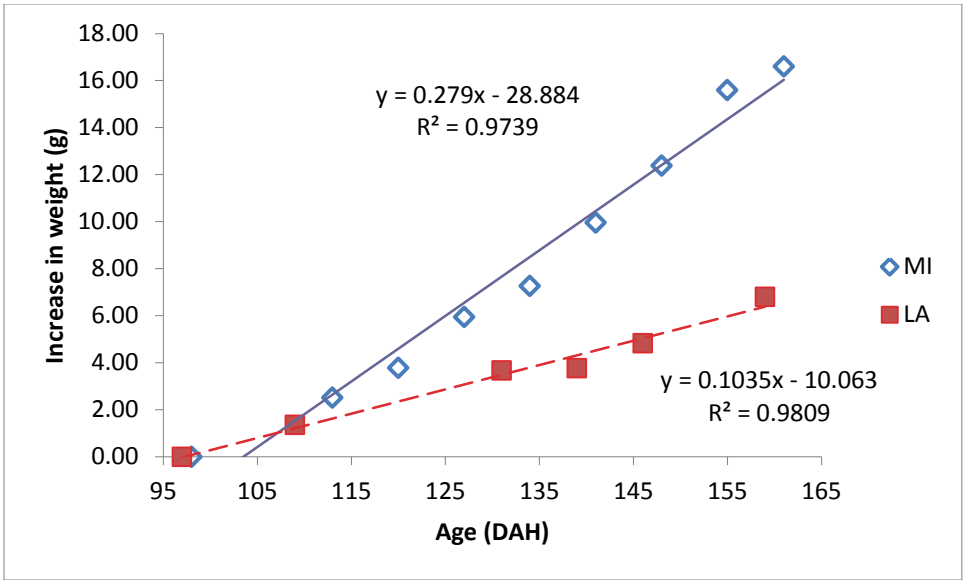


Figure 5. Increase in weight over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

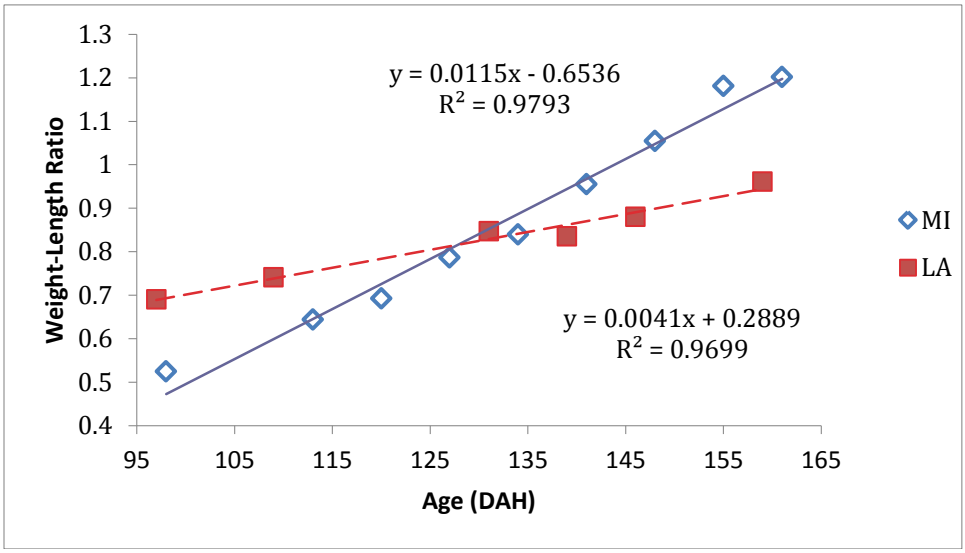


Figure 6. Mean weight-length ratios over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

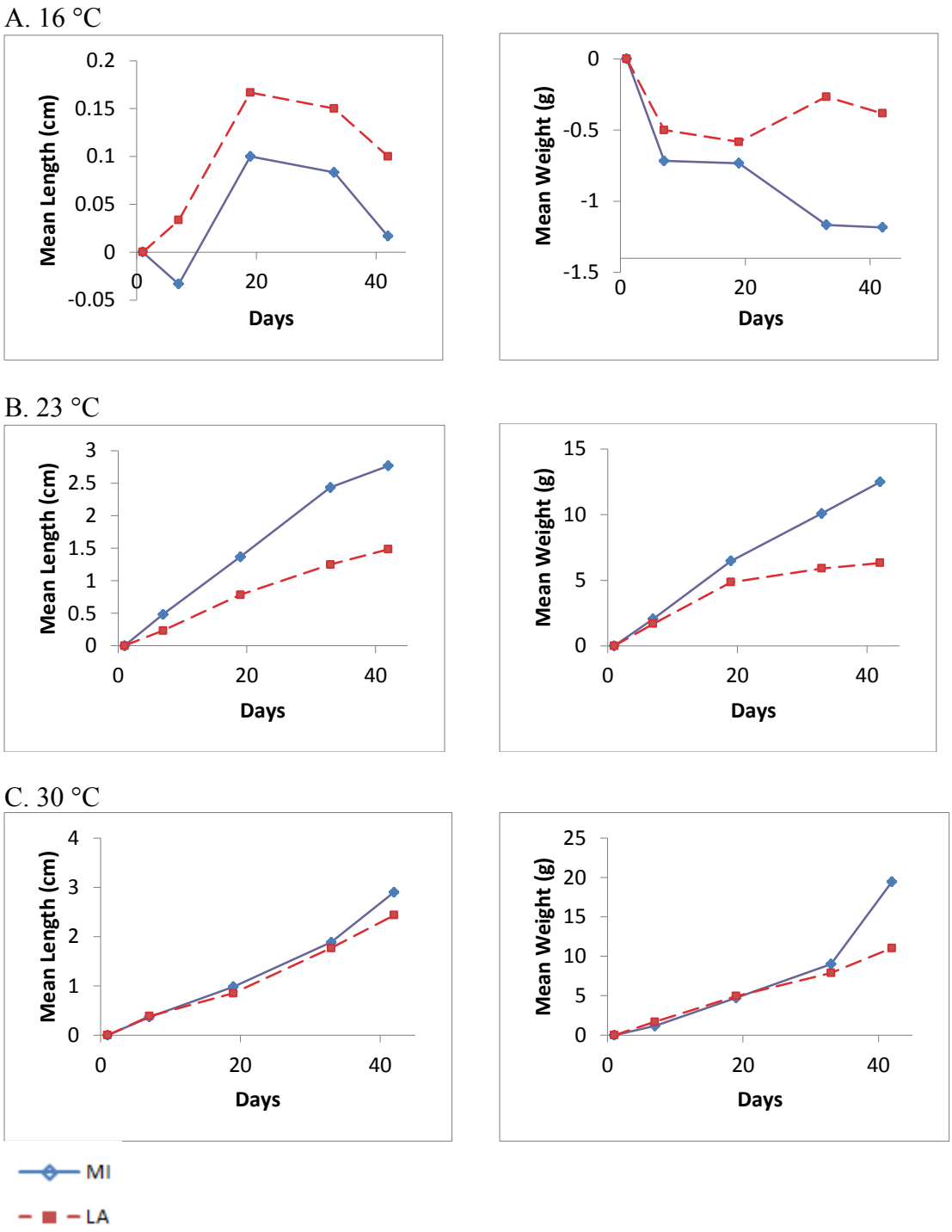
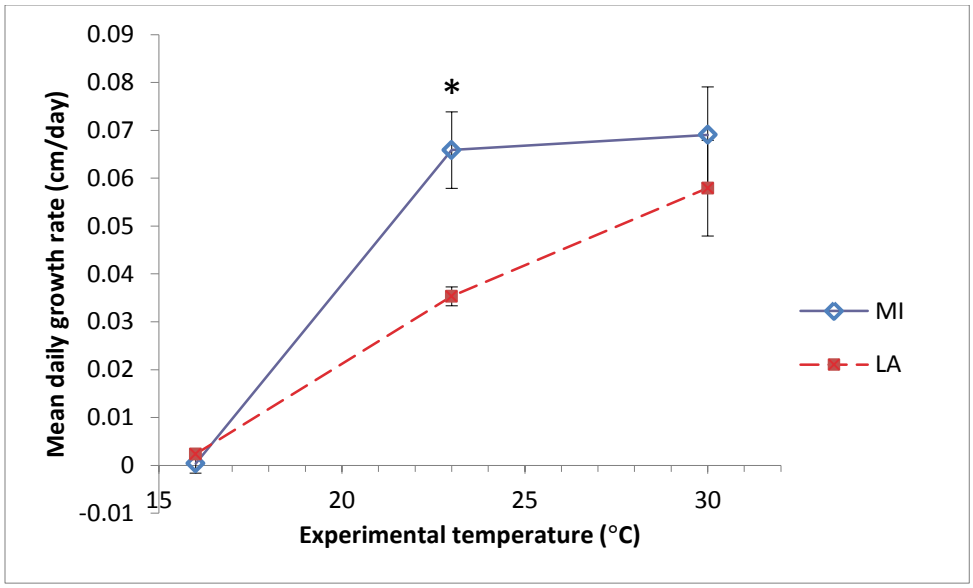


Figure 7. Changes in mean length and weight for MI (solid line) and LA (dashed line) populations of spotted gars at 3 temperature treatments (A = 16 °C, B = 23 °C, C = 30 °C; N = 6 fish per population in each treatment) in experiment 2 (experimental duration = 42 days).



B.

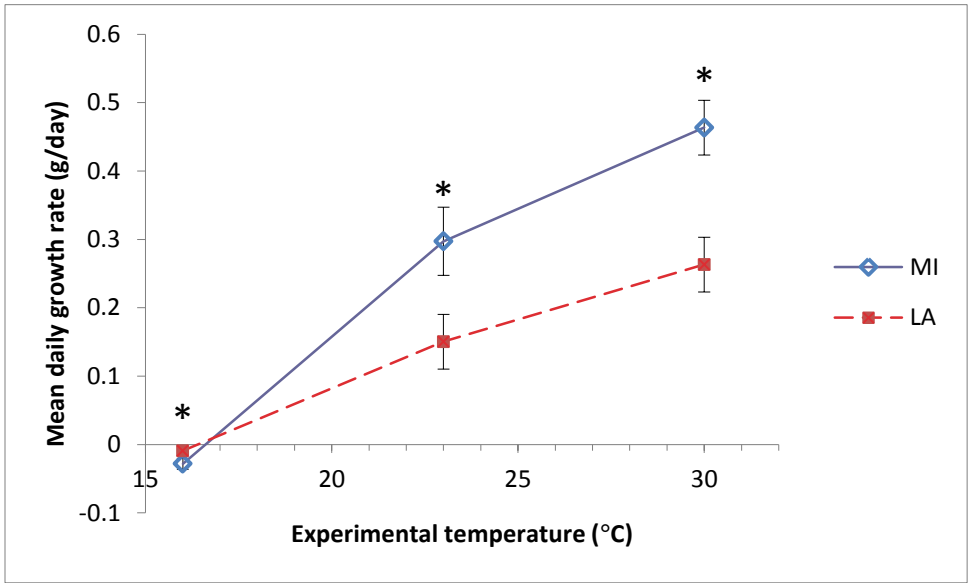


Figure 8. Mean daily growth rates for length (A) and weight (B) of LA and MI populations of spotted gars at three temperature treatments (16 °C, 23 °C, 30 °C; N = 6 fish per population in each treatment) in experiment 2 (experimental duration = 42 days). Error bars indicate ±1 standard error, * indicates significant difference between populations at temperature treatment.