The effects of temperature and density on the growth of golden shiners (*Notemigonus crysoleucas*).

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Aquaculture/Fisheries

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Thesis Proposal

For

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MASTER OF SCIENCE IN AQUACULTURE/FISHERIES

Title: The effects of temperature and density on the growth of golden shiners (*Notemigonus crysoleucas*).

Objectives:

- To determine the effect of water temperature on the growth (average growth, feed consumption and survival) of golden shiners.
- The effect of density on the growth (average weight, feed consumption and survival) of golden shiners.

APPROVAL:

Major Professor **(Date)**

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1. Introduction

Baitfish is a general term used to refer to any fish species used to catch fish. It is a term that is also used for feeder fish used as live food for ornamental fish species (Neils 1979). The three main fish species raised for bait in Arkansas are golden shiners (*Notemigonus crysoleucas*), fathead minnows (*Pimephales promelas*) and goldfish (*Carassius auratus*) (Stone et al. 2004). At the end of the 1940's, with the increase of the popularity of sport fishing, the demand for baitfish also increased (Stone et al. 2004). Natural sources of wild bait could not supply enough baitfish to meet the demand and baitfish farming started to become a viable alternative (Wasko and Clark 1948).

Currently the baitfish industry is the fourth largest aquaculture industry in the United States (Engle and Stone 1996). According to the National Agricultural Statistic Service in 2000, a total of $37.5 million worth of baitfish were produced in the United States. Baitfish are raised in 36 states (NASS 2000), but Arkansas is the state that produces most of the baitfish sold (NASS 2000). Apart from its economic value, the baitfish industry reduces demand for wild-caught bait and the potential negative environmental effects from harvest of wild fish to sell as bait. Before the beginning of baitfish farming, baitfish were only available from the wild, from local lakes and streams (Dobie 1948) and, in some states, wild bait still dominates the market (Stone et al. 2004). Harvesting baitfish from the wild might have a direct effect on wild baitfish populations, and also affect other species that feed upon them. However, the few studies that have been conducted to investigate on the impact of removal on densities of bait and game fish (Brandt and Shreck 1975; Duffy 1996) did not find any significant impact. Another environmental problem caused by harvesting baitfish from natural water is that when bait is caught in the wild, it is not often possible to capture only one species and, wild-caught bait often consists of many different species (Stone et al. 2004). Miller (1952) reported more than 32 species used as bait and Litvak and Mandrak (1993) found 28 different species of baitfish sold in baitshops in Toronto. This can contribute to the spread of diseases or infections among water bodies (Ludwig and Leicht 1996; Gunderson and Kinunnen 2001; Stone et al. 2004). Farmed baitfish are raised in a controlled environment with limited exposure to diseases. Therefore, baitfish farming not only offers a valid alternative to the wild harvest of golden shiners but also helps prevent the spread of infections.

Anglers require different sizes of baitfish according to different kinds of fishing (Stone et al. 1997). Ice or crappie fishing require small baitfish (also called crappie minnows or crappie-size) while anglers prefer medium or large baitfish, to catch other species of fish such as hybrid striped bass, largemouth bass or catfish (Stone et al. 2004). In order to have a successful business producers must be able to meet market requirements for fish of different sizes at different times of the year (Stone et al. 2004). Since there is little variability in the product sold, producers must compete on the quality of the fish sold, on the type of customer service offered, and on the availability of fish of the desirable size (Stone et al. 2004). This explains why size is an important characteristic for baitfish marketing. Because anglers' preferences are highly dependent on the type of fishing, the season and the weather (Stone et al. 2004) they are not easy to predict. In addition, the annual cycles of production and availability of baitfish, make it even more difficult to meet angler's demand. Baitfish farmers target specific market sizes (Stone et al. 2004) and if fish outgrow a particular size range, they can lose some or all of their value. Producers must be able to meet market requirements for fish of different sizes at different times of the year to be successful in the baitfish business. Thus many producers are interested in new techniques to either stimulate or stop baitfish growth. Being able to effectively grow baitfish quickly if a sudden demand for larger fish arises or keep them small during seasonal lapses in demand would greatly help producers to meet the demand of anglers and improve their business.

Although most farmers raise baitfish in earthen ponds, producers have shown interest in trying to hold broodstock in indoor tanks and off-season spawning in order to be able to supply baitfish whenever it is needed. Raising baitfish in earthen ponds does not allow for great control over the production system. In fact, although water quality can be controlled to some degree, by providing aeration, and food abundance can be increased by providing additional feeding, density is the main factors that can be controlled by adjusting stocking rates. For example, when baitfish for the crappie fishing market (small size) must be held for the follwing year, producers stock them at high densities with low feeding rates to restrict their growth and maintain them at a marketable size (Stone personal communication). The application of indoor tank culture could allow producers to develop early season fry production to extend growing seasons. It could also be combined with pond culture to supply golden shiners to the market in shorter time to supply enough quantities of different sized fish during peak shortages increasing profits without further investments.

Although a lot of research has been carried out to investigate fish growth for many species, there is a gap in the fundamental knowledge on baitfish. In particular, there is a lack of information on the basic relationships that link temperature and density to growth in golden shiners. The few studies that have looked at the relationship between growth and temperature in golden shiner (Thomas 1952; Kilambi and Hickman 1973) and on growth and production and different stocking densities

(Roseberg and Kilambi 1975) are of difficult interpretation because of the high variability of the samples, lack of information on water quality, poor survival and lack of food consumption and efficiency data. Greater knowledge of the basic relationships between temperature and growth and density and growth, would allow for a better understanding of results from previous production trials, and would lead to an improvement of baitfish farming techniques.

This study will look at the effects of temperature and density (crowding) on the growth of golden shiners to determine the relationship that links these two parameters to growth. A better understanding of these relationships will be fundamental to allow producers that raise baitfish in ponds, to manipulate stocking rates, stocking times and feeding schedule to obtain the size and number of fish desired. Having greater control over fish size while maintaining fish health and vigor, will help producers to maximize profits.

Objectives of the study

The objectives of this study are:

- To determine the effect of water temperature on the growth of golden shiners.
- To determine the effect of fish density on the growth of golden shiners.

2.Literature Review

2.1 Golden shiner

The golden shiner is native to the Atlantic and Gulf Slope drainages from Nova Scotia to southern Texas, Great Lakes, Hudson Bay (Red River), and Mississippi River basins west to Alberta, Montana, Wyoming, and western Oklahoma (Page and Burr 1991) but is now spread widely in the rest of the country (with the exception of Idaho and Washington) as they were introduced as baitfish, or stocked for forage (Stone and Thomforde 2001).

Golden shiners belong to the family *Cyprinidae*, and they are silvery deep-bodied fish with an olive-colored back and yellowish fins. Their body shape is compressed fusiform, with a long and slender caudal peduncule and a deeply forked caudal fin with pointed tips (Keast and Webb 1966). They are normally found in rivers, lakes, marshes and streams (Scott and Crossman 1973). The adults tend to be near the vegetation during the day and move to more open waters after sunset (Hall et al. 1979), while larvae tend to stay in the nearshore littoral zone (Hatch 1988). Golden shiners usually live in small shoals and also spawn in small groups. Larval golden shiners are 3-6mm long (Snyder et al. 1977; Buynak and Mohr 1980) grow fast and can reach 102 mm during their first year of life (Becker 1983). Golden shiners reached an average length of 76 mm in two summers, based on data collected from 20 sites in Michigan (Cooper 1935). However golden shiners in the Huron River, Michigan, which was eutrophic due to sewage input, reached 76 mm in the first year of life (Cooper 1935). Adults commonly range from 75 to 175 mm (Hall et al. 1979) but they can grow up to 300 mm (Cooper 1935). There is a difference in size between males and females once they reach maturity, with females growing faster and larger than males (Cooper 1935). Golden shiner's spawning temperature ranges from 20 to 27°C (De Vlaming 1975). When water temperature exceeds 27°C there is gonadal regression and spawning stops (De Vlaming 1975). Golden shiners are omnivorous and their diet is mostly composed of cladocerans, other zooplankters, insects and algae (Carter 1947; Keast 1968; Hall et al. 1979). Occasionally, they also eat mollusks and other small fish. Golden shiners are crepuscular feeders (maximum feeding rate in the hours before sunset) and display several different feeding methods (Ehlinger 1989).

Adult golden shiners are capable of feeding throughout the water column (surface, mid water and bottom) and can perform both active searches on individual zooplankton or switch to a filter-feeding mode if zooplankters are abundant (Ehlinger 1989). Larval golden shiners feed mostly on epiphytic rotifers and algae, then switch to epiphytic and planktonic cladocera while juveniles feed mostly on zooplankton (Hatch 1988). Among baitfish, golden shiners are the favorite species because they are hardy, reach a usable size in one season, spawn readily in ponds and do not die after the first spawning season (Forney 1957). However, golden shiners are often infested with a parasite (*Pleistophora ovarie*) that attacks the ovaries and prevents their use after the first year (Ruehl-Fehlert et al. 2005).

2.2 Fish growth

Growth is a measure of the change in size of the whole body or body part between two points in time (Diana 2004). Growth is a bioenergetic process and is a measure of seasonal and/or annual increments of gonad and somatic tissues (Mann 1991). Scientists use growth as an indicator of success because a fish in relatively good condition should demonstrate higher growth rates, greater reproductive potential, and higher survival than a fish that is not in good condition in the same environment (Diana 2004). Growth in fish is extremely variable because, when considering the balanced energy equation, it is often the last process to be performed by fish (Weatherley and Gill 1987; Diana 2004). Therefore, intraspecific differences in growth rate between populations can be very large (Weatherley and Gill 1987; Diana 2004). Metabolism, osmoregulation, and activity must all be accomplished first, then any remaining energy is used for growth (Diana 2004). Slight differences in the energy budget can result in large differences in growth. Energy accumulated for growth is the difference between quantity of food eaten and the amount required for catabolism (Weatherley and Gill 1987; Diana 2004). Genetic differences between species play a major role in setting rates of growth, ultimate size and longevity (Beverton 1987), but both abiotic and biotic features of the environment play an important role.

Contrary to mammals and birds, fish exhibit indeterminate growth. This means that fish reach sexual maturity relatively soon and then continue to grow throughout their whole life (Diana 2004). If a fish is given more food it will grow more rapidly. Fish in the Cyprinidae family, in particular, show great variability in growth rates between different species and between sexes. Moreover, evidence from fish transfers suggests that individuals within a population also show a wide phenotypic plasticity (Mann 1991). This gives them the capacity to respond rapidly to changes in environmental conditions that affect their growth rate and development to sexual maturity (Mann 1991). Fish culture practices such as increasing or decreasing stocking density or feeding rate, take advantage of the quick response of fish to manipulations of population density and nutrition that influence individual fish growth rates.

2.3 Growth rate and its measurement

Growth rate is a measure of change in some metric of fish size as a function of time (DeVries and Frie 1996). Growth can be described in terms of fish length and weight. There are several ways to determine fish growth. These include direct observation, observation of changes in length-frequency distributions in time and back-calculation of length and growth from hard parts (DeVries and Frie 1996). Direct observation is the least variable technique for quantifying growth. The only error possible is that made by the person measuring the fish or instruments (i.e. wrong calibration of balance). This technique is rarely used in natural fisheries studies because of difficulties in identifying individual fish and the necessity to recapture them. However direct observation is the main method used to assess growth in aquaculture or laboratory settings (DeVries and Frie 1996). The most common way to measure growth in fish is to examine the length of individuals over time. Fish show regular changes in length, as they grow older, resulting in an asymptotic curve. This means that smaller fish grow faster in length than longer fish of the same species. What differs between species is how quickly they reach their asymptotic length (McKay et al. 2002). The most common model used to describe growth using fish length was developed by Von Bertalanffy:

$$Lt = L\infty \left[1 - e^{-K(t-t0)}\right].$$

L∞ is maximum body size, K is the growth rate towards a maximum, t is time, and t0 is a calculated correction for the time at which fish were 0 in length. The values of asymptotic length and growth rate are not species specific but vary with the environment. In aquaculture generally weight is used more often than length since fish are sold by the pound. The equation used to express growth in terms of weight, is the Von Bertalanffy equation for growth:

$$Wt = W\infty \left[1 - e^{-K(t-t0)}\right]^{b}$$

When considering weight, generally speaking, there is an exponential increase of weight with age, after which the growth rate declines. The growth of larval and juvenile fish changes dramatically with size compared to adult fish.

Length and weight statistics are fundamental in both fisheries management and aquaculture research. The mathematical relationship between weight and length is used to estimate one quantity from the other, or to measure individual variation from an expected weight at a given length as an indicator of condition (Le Cren 1951; Bolger and Connolly 1989). The weight-length relationship:

$$W = aL^{b}$$

generally describes the weight-length relationship of most fish, where *W* is weight, *L* is length, *a* is a constant, and *b* is an exponent ranging between 2.5 and 4.0 (a fish growing isometrically or maintaining the same shape across length categories has an exponent of 3.0). The exponent *b* describes the curve of the relationship, can vary according to biotic and abiotic influences and is different among species. Therefore different values of *b* may be obtained between sexes or locations, even within the same species (Pope and Kruse 2001).

Fish condition factor refers to a mathematical formula to determine the physiological state of a fish, including its reproductive capacity. Three methods are commonly used to analyze fish condition. Lagler (1956) suggested the use of a coefficient of condition referred to as Fulton-type condition factor. Fulton's condition factor is calculated by dividing fish weight by length cubed (W/L^3). The heavier a fish is for a given length, the higher its condition factor (K) (Ricker 1980).

$$KTL = [(100{,}000)\,(W)]\,/L^3$$

W is the weight in grams and L is the total length in millimeters. Le Cren (1951) proposed the use of the relative condition factor (K_n), calculated as follows.

$$Kn = [W\,/\,W'] \text{ X } 100$$

where, W is the observed weight and W' is the length-specific standard for fish in the population under study. Recently, an alternative method for evaluating fish condition involves the calculation of relative weight (*Wr*; Wege and Anderson 1978) and it is calculated:

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$$Wr = [W / Ws] \times 100$$

where, W is the actual weight of the fish being measured and Ws is the standard weight for a fish of that length. Intercept (a) and slope (b) parameters for standard weight (Ws) equations that have been proposed for various fish species and minimum total lengths (mm) recommended for application. The standard equation format is $\log10(Ws) = a + b\log10(TL)$ and values for a, b and TL for golden shiners have been estimated by Liao et al. 1995. The condition factor is specific for each species and the condition factor of one species cannot be compared with that of a different species. In experimental settings it is used to monitor the condition of individual fish. Condition factor is an important indication of baitfish health. Cone (1989) indicated that the relationship between fish weight and length is frequently used to compare the effect of biotic and abiotic factors on the health or well-being of a fish population. Murphy et al. (1990) stated that condition indices provide a comparative measure of fish plumpness. Low Wr values are generally characteristic of fish in poor health, whereas high Wr values indicate fish in excellent health. Coughlan et al. (1996) proposed incorporation of Wr as part of the fish health assessment index. In particular, it is important to determine baitfish condition after harvest, since fish must be able to survive for two or three weeks without food.

2.4 Metabolic processes of growth

The main factors affecting metabolic rate are activity level, temperature, fish mass and feeding level (Diana 2004). Metabolism is the biochemical modification of chemical compounds in living organisms and cells. This includes the biosyntesis of complex organic molecules and their breakdown. Metabolism usually consists in a series of enzymatic steps. Metabolic processes provide energy for vital processes and activities and new material is assimilated. One way of categorizing metabolic processes, whether at the cellular, organ or organism level is as anabolic or catabolic. Anabolic processes use simple molecules to synthesize complex molecules producing growth and differentiation of cells and increase in body size (Weatherley and Gill 1987). Catabolism is the part of metabolism that consists of a series of degradative chemical reactions. Catabolic processes occur during starvation, stress and illness. Examples of catabolic processes include breakdown of muscle protein in order to use amino acids as substrates for gluconeogenesis, and breakdown of fat in adipose to fatty acids (Wheatherley and Gill 1987). The first set of reactions provides the cell with monomers useful to construct new polymeric molecules, while the second set of reactions usually involves the process of oxidation and is accompanied by a release of chemical free energy in the form of heat and adenosine triphosphate (ATP) (Jobling 1994). Because it is counterproductive to have anabolic and catabolic processes occurring in cells simultaneously, there are many different types of hormones that switch on anabolic processes while switching off catabolic processes and vice versa. Classic anabolic hormones include growth hormone and other insulin-like growth factors, such as insulin, testosterone, and estrogen while catabolic hormones include cortisol, glucagon, adrenalin and cytokines. When anabolism exceeds catabolism, growth occurs. When catabolism exceeds anabolism net loss occurs (Diana 2004).

2.5 Nutritional aspects of growth

The most important factor influencing growth is feed ration. If a fish does not eat, it loses weight (negative growth) while, to achieve zero growth, it needs at least some level of ration (Diana 2004). Fish also have an internal limit to ingestion and, as ration increases from zero to that limit, growth increases at a declining rate in an asyptotic fashion (Diana 2004). There are three main levels of ration. The first is a maintenance ration, which is the amount of food required just to maintain body weight. The second significant ration is satiation or maximum ration, which is the most one fish can eat. Generally this causes maximum growth. The last type of ration is the optimum ration, which occurs at the point at which a tangent line that passes through zero, intersects the growth ration curve (Brett 1979; Diana 2004). Feeding fish at an optimum ration is what produces the most efficient growth. Growth ration curves are different for different species (Brett 1979; Henson and Newman 2000). Generally speaking, at a low ration, slight increases in the ration can result in large differences in growth. At high rations the same increase in ration results in smaller differences in growth (Diana 2004).

In addition quantity of food ingested, frequency and pattern of food delivery also have strong effects on consumption and ultimately growth. This is because fish in nature go through periods in which they cannot feed as much or as often as they would need to for optimal growth (food shortage or reproductive weight loss), so they have the capacity to grow at an accelerated rate once food becomes available. This property of fish is known as compensatory growth (Jobling et al. 1993). Jobling et al. (1993) ran a study on Arctic charr in which groups of fish were fed at different feeding rates. One group was fed continuously while the other groups were subjected to different feeding cycles (from one to three weeks of deprivation). At the end of the experimental period, the group that was fed continuously was the one that grew the most while the other groups grew more or less the same (the group subjected to one week deprivation grew slightly more than the others). However, when comparing an equal number of feeding days, all groups that had been deprived of food grew significantly more rapidly than the group fed continuously. This means that either those groups had eaten more food or they had become more efficient at growth. At the end of the study all groups of fish were allowed to feed continuously for six more weeks, and they all reached similar sizes, although they were still smaller than the fish that had been fed continuously. Data from other studies carried out by Jobling (1994) indicate that both processes (eating more and becoming more efficient at growth) are important. A similar experiment by Hayward et al. (1997) reported that sometimes fish under schedules that stimulated compensatory growth grew more than those on continuous feeding regimes. This means that alternating schedules of feeding could prove useful for aquaculture to obtain more rapid growth and higher feed conversion ratio. Another phenomenon that occurs when fish are exposed to controlled feeding experiments is depensatory growth. Fish compete for food and bigger fish get more food than the smaller ones. This results in an increase in the size difference between the largest and the smallest individuals (Brett and Groves, 1979). Depensatory growth is generally observed in experimental or in aquaculture situations and sometimes happens in natural settings where there is strong competition (Buurma and Diana, 1994). Depensatory growth must be avoided in aquaculture businesses and that is why fish are generally graded in aquaculture facilities to obtain fish of the same size range in each tank.

Conversion efficiency is the efficiency with which a fish converts Kilocalories of ration into Kilocalories of growth (Diana 2004). In simple terms, it is the percent of food eaten that becomes body tissue. Maximum conversion efficiency happens when fish are fed at an optimum ration. A larval fish can convert 65 to 80% of each calorie of yolk into tissue and adult fish have a gross conversion ratio of about 20% (Diana 2004). This is very high when compared to birds and mammals (Weatherley and Gill 1987). Conversion efficiency declines with fish mass. Both maintenance rations and maximum rations decline as mass increases. The difference between maximum ration and maintenance ration is the energy available for fish growth (Warren and Davis 1969). Since the maximum ration declines more rapidly than the maintenance ration, as a fish grows older, the energy available for growth diminishes too because a higher portion of total food consumed goes into maintenance costs (Warren and Davis 1969).

Golden shiners can be grown efficiently using a variety of dietary ingredients according to price or availability. Nutrition studies by Lochmann and Phillps (1995) compared growth and survival of juvenile golden shiners fed diets containing 4% lipid from cod liver oil, poultry fat, rice bran oil or equal portions of cod liver oil and poultry fat, and cod liver oil and rice bran oil. Poultry fat and rice bran oil have none or very low concentrations of n-3 highly unsaturated fatty acids (HUFA), which are essential in the diet of many fish. However, they found no significant difference between the diets in terms of growth and health of fish at the end of the study. They also did not find any significant fatty-acid deficiency signs for the fish fed with the diets low in n-3 HUFA.

2.6 Somatic versus gonadal growth

Once fish mature, a large part of their surplus energy is used for reproduction to produce gametes, migrate, care for young and build nests. This causes a greater decline in growth potential as mass increases. The relative cost of reproduction increases with age, and older fish cease growth because of the increasingly high-energy demands for reproduction. Unlike mammals and birds, fish tend to mature at a fairly small size and then continue growing after maturation (Weatherley and Gill 1987). The age and size of fish at maturation are quite different according to the species, as well as the time needed to achieve maximum size. There are several studies that associate the increase of energy allocated for reproduction with a quantitative reduction in somatic biomass and qualitative deterioration of the soma (Shevchenko 1972; Roff 1983) or with a reduction in swimming activity that increases the risk of being preyed on (Koch and Wieser, 1983). Negative correlations have also been recorded between fecundity and survival in a variety of species and it has also been possible to extend the longevity and increase the growth of some fishes by artificially preventing reproduction (Diana 2004).

Many different species of fish show growth differences between sexes. This difference is due to differential costs of reproduction. The two reproductive strategies in fish are semelparity and iteroparity (Shpak 2005). Semelparity is when fish reproduce only once in their life and then die (salmon) while iteroparity is when fish survive and breed more than once in their lifetime (Shpak 2005). The difference between these two strategies is related to the amount of energy used in reproduction and the likelihood of survival after the event. Iteroparous fish generally use 25-60% of stored energy in the body during reproduction, while semelparous fish use 60-85% of stored energy for reproduction (Diana 2004). The main factor that seems to be behind the development of one strategy or the other, is the variance in offspring number and the intrinsic ‘‘risk spreading’’ and ‘‘bet-hedging’’ nature of iteroparity (Stearns and Crandall 1981; Stearns 2000).

An example of the trade off between body growth and gonad growth is the process of stunting. Stunting happens when there is a drastic decline in growth rate and all fish above a certain age are the same size. At advanced ages there is no surplus energy available for growth but fish stay alive and use all the surplus energy they have to reproduce. Overfishing or high densities, poor food resources or lack of a certain size of prey as well as temperature factors are behind stunting (Diana 2004). In farmed baitfish sometimes deliberate crowding is used to induce stunting to prevent growth beyond market size (Adgbani 1988).

The formation of somatic and gonad tissue by a fish population in a specific time frame is called production (Mann 1991). This includes material formed by fish that die or migrate from the areas studied before the end of the time period (Mann 1991). While growth is a measure of the increments of gonad and somatic tissue, production summarizes the biomass level of the population as well as the rates of growth and mortality all in one value (Mann 1991). In some species of cyprinids gonad products can account for 30% of the total weight of the female and therefore are a high proportion of the annual production for some populations (Mann 1991). Although the contribution of gonad products in terms of weight is generally less than their true contribution because the energy content of gonad material is higher than that of somatic tissue (Le Cren 1951).

De Vlaming (1975) and De Vlaming and Paquette (1977) investigated the effect of photoperiod and temperature on gonadal activity of golden shiners. In fish maintained on a long photoperiod-high temperature regime, final gonadal maturation occurred while long photoperiod-low temperature regimes did not stimulate final gonadal maturation. Short photoperiod-high temperature conditions retard gonadal development or cause gonadal regression in golden shiner while short photoperiod-low temperature regimes do not promote the final stages of gonadal maturation. De Vlaming and Vodicnik (1975) also found that light intensities of below 151x were insufficient to induce spawning while light intensities above 151x increased the incidence of spawning. When temperature rises above 27 °C, golden shiners experience gonadal regression and spawning stops (De Vlaming and Paquette 1977).

2.7 Ecological aspects of growth

Water temperature, food, oxygen and production of metabolites, have been identified as the four main ecological factors that control growth in aquatic species. (Hepher 1978). Therefore growth is one of the most important and reliable indicators of fish health, population production and habitat quality. Temperature appears to be an extremely important factor in determining strong year classes. A possible explanation is that because some fish grow faster during warm summers than cool ones, they are able to pass more rapidly through their most vulnerable stage (Coutant and De Angelis 1983). Burrough et al. (1979) showed how strong year classes in *R. rutilus* resulted in a change in growth rate. When a year class was particularly abundant, growth rates in that year class declined due to food shortage.

Johannes et al. (1989) studied golden shiner abundance in lakes, and concluded that predators, rather than food availability is what controlled their abundance in the wild. This suggests that predator avoidance might also influence fish growth. A number of studies done on fish shoals have reported that fish at the front of the shoal have higher foraging success, in terms of quantity and quality of food items gathered, than other fish in the shoal. However there is also a cost associated with occupying front positions. Larger fish in the shoals tend to stay at the front and end up being more vulnerable to predation. Backiel and Le Cren (1978) noticed that schooling fish in the wild occur in dense populations with growth rates much lower than the potential for that species reported in laboratory experiments. Larger fish tend to be at the front but, at the same time, they might not grow as much as they could, to avoid being predated (Reebs 2001; Ward et al. 2002).

It is also possible for parasites to influence fish growth. As parasites exploit the energy of their hosts, the amount of energy available for fish growth is reduced. Ward et al. (2002) investigated the probability of an existing correlation between larger fish and the occurrence of a parasite (*Crassiphiala bulboglossa*) on fish in shoals of banded killifish in lake Morice (Canada). *C. bulboglossa* is a trematode that seems to manipulate host behavior. Because the parasite uses fish energy reserves, it increases the fish motivation to feed which results in the fish occupying positions in the front of the shoal in order to maximize its foraging rate. Ward et al. (2002) hypothesized that, since larger fish tend to be at the front, and parasitized individuals also occupy a more external position within the shoal there might be a positive relationship between body length and the probability of hosting a parasite. The study found that although fish in the front were more likely to host a parasite, abundance of parasites was not greater than on fish in other positions so there was not a relationship with length and parasite abundance.

2.8 Growth and water quality

Water quality also has an important influence on regulating fish growth. Yu and Permutter (1970) found that metabolic wastes are toxic to fish and inhibit their growth. While Doudoroff and Shumway (1970) and Brett (1979) found that low dissolved oxygen levels influence metabolism and growth rate. Algal blooms, high fish densities, poor water circulation or high temperature can cause low oxygen levels in the water. Shireman et al. (1977) reported that *Ctenopharyngodon idella* showed a decrease in growth rate at very high densities if the oxygen level in the tank was below 4 mg/L even if the fish were fed to satiation.

Tsadik and Kutty (1987) studied tilapia (*Oreochromis niloticus*) in static water aquaria at 28 ± 2°C for 35 days at various ambient oxygen concentrations. Tilapia kept below air saturation in static water aquaria were affected in their feeding, assimilation and growth by low oxygen levels. As oxygen saturation levels decreased, food consumption and assimilation decreased. Under a simulated diel flux of oxygen (from about 20 to 200% air saturation) brought about by an induced bloom of plankton, growth rate of tilapia was considerably reduced when compared with those maintained at dissolved oxygen levels near saturation. The study concluded that dissolved oxygen levels below 50% of air saturation and diel flux of oxygen would cause considerable reduction in pond production of tilapia.

Buentello et al. (2000) studied the effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish. Fish were kept for 6 and 8 weeks under three regimes of varying water temperature and three different dissolved oxygen concentrations (100, 70 and 30% air saturation, at each temperature). The two experiments simulated spring and fall temperature and photoperiod patterns. In both experiments higher temperatures and higher DO levels produced increased feed consumption. As DO declined from 100% to 30% there was a progressive reduction in feed intake. In both trials weight gain was higher for fish held 3°C above the mean water temperature at 100% air saturation values of DO. The lowest values of weight gain were obtained for fish held at 30% air saturation and 3°C below the mean water temperature. Weight gain increased with temperature, with maximum rates at 27.1°C with DO equivalent to 100% air saturation. In contrast, when DO was 30% air saturation, growth rates plateaued as ambient temperature exceeded 22.8°C.

2.9 Effect of density on growth

Density-dependent growth is a phenomenon common in aquaculture ponds. As population density increases, competition for food and for living space increases as well, so animals stocked at excessive densities grow less.

Tilapia stocked at low densities achieved high individual growth rates, but as density increased there was a linear decrease in growth (Diana et al. 1991). Other studies have shown that some species of Tilapia (*Tilapia spp*.) produce a component (high molecular weight and electrophoretically migrated as a beta globulin) that induces cutaneous anaphylactic reactions that increases mortality rate, limiting the density at which this species can be stocked (Henderson-Arzapalo et al. 1980).

Feldlite and Milstein (1999) investigated on optimal stocking densities for three different species of cyprinids (goldfish, common carp and koi) in 1m^3 cages in earthen ponds, and concluded that koi could be stocked at densities up to 2 million fry per ha without showing any negative density effect. On the other hand, goldfish needed to be stocked at low density (500,000-1 million fry per ha) in spring and but could be stocked up to 2 million in summer. Of the three species, common carp was the most influenced by stocking density and its growth rate was the most affected by survival. Therefore, to obtain the required fish size at harvest, stocking density needs to be carefully evaluated.

Mills (1982) studied *L. leuciscus* kept in fine and coarse mesh cages and found that both growth rate and mortality rates changed with different densities and with mesh type. Smith et al. (1978) studied growth of fathead minnows fed ad libitum in tanks at different densities and reported that growth ceased at high densities regardless of food abundance. Roseberg and Kilambi (1975) raised golden shiners at different densities in indoor tanks. Their study focused on growth and production of golden shiners under different stocking densities and protein levels. The study concluded that, even though stocking fish at higher densities showed a somewhat higher production, this was due more to the presence of a fewer larger more aggressive fish in the high density tanks, than to a direct relationship with higher densities. According to their study, stocking golden shiners at lower densities (20 fish per 60 gallon) proved to be best to obtain more uniformly market-size fish. However results of this study are difficult to interpret because of a lack of water quality data.

Nelson et al. (1980) demonstrated that an active, highly ephemeral, substance chemically mediated growth retardation in juvenile lobsters (*H. Americanus* and *H. gammarus*). D'Abramo et al. (2000) suggested that a similar substance might effect the growth of freshwater prawns (*Machrobrachium rosenbergii*). Density-dependent growth in the Cyprinidae is generally associated with food availability, although Pfuderer and Francis (1973, 1975) have shown that goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*) both release a hormone that inhibited growth and production and depressed heart rate.

Keeping some species of fish at high densities elicits aggressive behavior that affects their growth. Fenderson and Carpenter (1971) noted that hatchery reared Atlantic salmon showed more aggressive fighting behavior when reared at high densities than lower densities. Ako et al. (2005) also found that fish that are kept at high densities exhibit behavioral limitations that influence their growth. Swordtails reared at high densities (6 fish/L) grew significantly slower than fish kept at a higher density of (1 fish/L and 3 fish/L) (Ako et al 2005). Fish held at higher densities ate less food per individual than fish in the other trials, explaining the slower growth rate. In addition, this slower growth rate per individual led to size differences that produced prey-predator interactions (cannibalism). Dominant animals tended to eat more and to intimidate submissive animals keeping them from feeding. This led to lower survival rate in the higher density trials. This experiment suggested that species that are competitive feeders might experience depressed growth when reared at high densities.

Ako et al. (2005) compiled a list of selected freshwater ornamental fish and labeled them as competitive or non-competitive according to whether their growth rate decreased as density increased. Fish labeled as non-competitive feeders can probably be reared successfully at high densities. Behavioral traits in common between non-competitive species are that these fish do not swim in schools and seem to neither chase nor avoid one another. They also do not seem to interfere with other fish when feeding. Ako et al 2005 concluded that koi were non-competitive feeders. When reared at high densities mortality rates were significant. Half-eaten carcasses without eyes were found in the tanks suggesting that those fish had been attacked. This short experiment suggested that juvenile koi are non-competitive feeders but are very aggressive fish. Other researchers have reported aggressive behavior in ornamental fish such as blue gourami and angelfish (Degani 1991, 1993) and foodfish, particularly in the larval-to-juvenile stage such as larval African catfish (Hecht and Applebaum 1988). The same species experienced higher growth rates during the juvenile stage when grown at high densities (Hecht and Uys 1997; Almazan-Rueda et al. 2001). The authors explained this behavior by saying that higher densities stimulated schooling behavior that repressed cues responsible for initiating aggressive, territorial behaviors. Similar aggressive behavior has been noted in cod (Folkvord 1991) although, in this case, growth rate was not density-dependent.

2.10 Effect of temperature on growth

A considerable amount of research has been conducted on the relationship between temperature and physiological processes of fish. Fish are poikilotherms which means that their body temperature changes as water temperature changes. Different fish species vary in their tolerances to water temperature and each species has an optimum temperature for growth (Brett 1979). Some species tolerate a wider range of water temperatures than others, but all species have an upper and lower lethal temperature. Fish can generally take a 2°C change instantaneously. In experimental settings when fish need to be acclimated to higher or lower temperatures, this is done gradually, raising or lowering water temperature by 1°C every 20 to 30 minutes (Brett 1956).

Temperature is related to fish growth because it influences fish metabolism since water temperature affects the rate of enzyme activity, mobility of gases, diffusion and osmosis (Brett 1979). Therefore, as body temperature changes, reactions also change, influencing metabolic rate (Diana 2004). Variations of temperature have an effect on standard and total metabolism as well as on the quantity of food that can be eaten by fish. Generally there is a positive exponential relationship for many species of fish between standard metabolism and temperature (Brett 1964). As temperature increases, standard metabolism increases (Diana 2004). Total metabolism also increases as temperature increases; it reaches its maximum at an intermediate temperature and then declines as temperature continues to rise (Brett 1969, 1979). The young stages of almost all species show a rapid increase in growth rate as temperature increases. They pass through a peak (optimum temperature) after which growth rate decreases rapidly, as further increases of temperature become adverse (Brett 1979).

There is also a difference in growth between fish kept at constant or fluctuating temperatures. Hokanson et al. (1977) studied growth in different groups of rainbow trout. Some groups were kept at constant temperatures (8,12,16, 20, 24 °C) while the others were kept at cycling temperatures (±4 °C of the mean temperatures). Trout kept at cycling temperatures had a lower optimal temperature for growth than those kept at constant temperatures. Once temperatures went above optimum temperature for growth, metabolism increased dramatically and growth declined. Fish kept at fluctuating temperatures seemed to adapt more to the warmer rather than to the average part of the cycle. Also fish kept at cycling temperatures had a different growth relationship than those kept at constant temperatures. Fish kept in a cycle with an average temperature below their optimum for growth grew more rapidly, while fishes in a cycle with an average temperature above their optimum for growth grew more slowly. Fish grown at constant temperatures tend to have their optimum growth at intermediate temperatures. Biette and Geen (1980) studied growth of young sockeye and found that growth was greater under cyclic rather than constant temperature. Spigarelli et al. (1982) studied trouts kept three different temperature regimes. A diel temperature cycle (9-18 °C) simulating that selected by brown trout residing in thermal effluent areas in spring; a constant 13 °C, which is the optimum constant temperature for brown trout growth; and a naturally fluctuating, arrhythmic temperatures similar to those of inshore Lake Michigan waters (4-11 °C). The study showed that raising brown trout at fluctuating temperatures, increased its growth significantly as well as its feeding and lipid deposition compared to trout raised at a constant temperature obtained as the mean of the fluctuations. Spieler at al. (1977) studied the relationship between fluctuating temperatures and weight gain in goldfish and reported that fluctuating temperatures did not always correspond to an increase in growth. In fact, according to the time of the day in which the thermocycle was initiated, fish growth would be either stimulated inhibited or remain the same as that of fish raised at constant temperatures (warmer or colder).

Golden shiners are tolerant to a wide range of temperatures (Stone et al. 2004). There have been several studies on golden shiners to investigate their reactions to different temperatures. Hart (1952), as cited by Beitinger and Bennett 2000, reported linear temperature tolerances for golden shiners to be from 0 °C to 34.7 °C with an acclimation independent zone that goes from 14 °C to 26 °C. Reutter and Herdendorf (1975) reported that golden shiners in the wild do not avoid thermal discharges in the fall, winter or spring months, and if barricades are not used, they die as a result of thermal shock. Alpaugh (1972) conducted a study on high lethal temperatures for golden shiner, and found that high lethal temperatures vary as the fish are acclimated to higher temperature. This study reported that the high lethal temperature for golden shiners acclimated to 22 °C, was near 40 °C. Coutant (1977) indicated that the general preferred range for the golden shiner was 17-24 °C. A laboratory study conducted by Cincotta and Stauffer (1984), reported that the final temperature preference for golden shiners was 23.8 °C. Upper avoidance temperatures ranged from 15 to 36 °C when acclimated from 6 to 36 °C. Thomas (1958) looked at the relationship between temperature and food consumption in golden shiners. His study was conducted in five, 39-L aquaria at a density of 8 golden shiners per aquaria. These feeding aquaria were alternated with four, 19-L heating aquaria containing water to heat the system. Because of the limitation in the cooling capacities of the equipment, a series of three trials were conducted at three different temperature ranges 4,4-11.1 °C; 11.6-29.4 °C; 16.1-34.4 °C (40 °F-52 °F; 53 °F-85 °F; 61.5 °F-94 °F). In this way, five different constant temperature ranges could be maintained within any of these ranges. This study showed that water temperature had a linear relationship with the amount of food consumed. Thomas also suggested that there might be a curvilinear relationship between water temperature and weight gain or loss, and that golden shiners seemed to have a favorite temperature range for efficiency of food conversion between 12.7 °C (55F) and 18.3 °C (65F). However, data from this study were highly variable, maybe because of the small size of his sample. Kilambi and Hickman (1973) also studied the effects of temperature on golden shiners stocked at different densities (800, 600, 400 lb/acre) and subjected to different feeding rates (1%, 3%, 5%). However, feed was supplied as meal, so that food consumption and efficiency data were not collected.

Poor survival compromised the density trials and the lack of water quality data, make the results difficult to interpret.

2.11 Food abundance and growth

Food consumption, water temperature and growth are closely related. As temperature increases, maintenance ration also increases. Maximum ration follows a similar trend to that of total metabolism, reaching an optimum at intermediate temperatures and then declining (Brett 1979). The mathematical difference between maximum ration and maintenance ration is called the scope for growth and reaches its maximum at intermediate temperatures (Brett 1979; Diana 2004). The optimum temperature for growth is lower than the optimum temperature for consumption. This is due to increasing costs of maintenance at higher temperatures. When a fish is fed at a reduced ration, the optimum temperature for growth is lower than when a fish is fed at maximum ration (Brett et al. 1969).

Several studies have investigated the relationship between quantity of food consumed, growth rate and their relationship to changes in temperature. Early studies by Hathaway (1927) showed that fish consume different rates of food per day at different temperatures, showing a direct relationship between temperature and food consumed at a particular temperature range. Brett (1971) wanted to verify if experimental results obtained with sockeye salmon reflected how those fish were distributed in the environment. He hypothesized that if food was unlimited he should have found sockeye salmon at a water temperature of 12 °C (optimum growth found in the laboratory), while if food was limited, they should have been at cooler temperatures. Contrary to what he had hypothesized, sockeye salmon did not stay in only one layer of the water column, but moved up and down in it. They fed in the warmer surface water (17 °C) in the early morning and late at night, then spent the rest of the time in deeper waters colder than 12 °C. This appeared to reduce metabolic rate and increase growth even more than if the fish had stayed near the surface in warmer temperatures all the time.

Fish seem to be able to use different water temperatures and food to maximize their growth rates (Diana 1984). When food is scarce, fish move to colder waters to lower their maintenance requirement and obtain better growth on that lower ration (Diana 2004). By moving to different temperature zones within a water body, fish can move to the best temperatures for metabolism, consumption, or growth. Studies by Baldwin (1956) on trout, showed that their weekly consumption of minnows doubled for each 4 °C rise in temperature, and 50% of the body weight was consumed weekly at 13 °C. On the other hand, Müller and Meng (1986) found that in lakes, variations in growth for *R. rutilus* due to changes in lake productivity were masked by variations in growth caused by temperature changes. Betsill and Van Den Avyle (1997) investigated the effects of temperature and zooplankton abundance on growth and survival of larval threadfin shad. They found that there was a positive correlation between temperature and growth up to 28 °C, but the situation with prey abundance was more complex. As prey abundance started to increase, growth increased until prey abundance reached a certain density (160-290 organisms/L). After that, growth rate either stopped or decreased. Food abundance seems to have an effect also on the size of fish shoals. Mann (1991) reported higher efficiency of large shoals of goldfish at finding food when it was patchily distributed compared to smaller shoals. Currently there are no data that show if or when this improved foraging efficiency is directly translated into changes in growth rate (Mann 1991). Keast (1968) sampled golden shiners in different locations during low temperatures and found that 63-70% of golden shiners had empty stomach at 4 °C, while golden shiners obtained at 8 °C and

15 °C all had full stomach. Lochmann and Phillips (1997) estimated the use of food by golden shiners looking at the effects of stocking densities, dissolved oxygen, natural productivity and feeding rates. Golden shiners were raised in 0.1-acre ponds for 8 weeks. Fish were stocked at different densities and some were fed a complete diet while some were not fed at all. The study showed that weight gain increased with increasing feed amount up to a maximum value, then declined with additional inputs and in particular at high stocking densities and high feed imputs, decreased golden shiner growth instead of increasing it.

2.12 Effect of stress on growth

Cortisol is the most abundant circulating steroid produced by the adrenal cortex. This hormone is effective in anti-inflammatory activity and blood pressure maintenance as well as gluconeogenesis, calcium absorption and secretion of gastric acid and pepsin. Cortisol has both gluco and mineralcorticoid functions in fish (Flick and Wendelaar Bonga 2001). In fish, as well as in other vertebrates, synthesis and release of cortisol is under primary and acute control of ACTH (adrenocorticotropic hormone), which is produced and released by a cluster of cells in the pituitary gland. However in fish not all rises in plasma cortisol reflect a rise in ACTH (Flick and Wendelaar Bonga 2001). Cortisol is the hormone released when an animal needs a sudden burst of strength or speed to fight off if attacked or escape from a predator. One of its main functions is to increase the flow of glucose, protein and fat out of tissues and into the circulation system to rapidly increase energy levels in response to a physical threat. Several studies have shown that cortisol levels remain high when an animal - or a human - is constantly under stress, and seems to be the trigger for many of the physiological effects linked to long term stress. Therefore blood plasma cortisol is commonly used as an indicator of primary and secondary stress in fishes (Mazeaud and Mazeaud 1981). When animals are exposed to prolonged stress energy that would normally be available for processes like growth, immune response, or reproduction, is used to counteract the stressful event (Consten et al. 2001).

In natural environments high plasma cortisol levels occur in fish of subordinate rank (Gregory and Wood 1999). These fish acquire a smaller share of available food and grow more slowly. Gregory and Wood (1999) studied the effects of chronic plasma cortisol elevation on the growth and feeding behavior of juvenile rainbow trout. They found that chronic plasma cortisol elevation had significant negative effects on individual appetite, growth rate, condition factor, and food conversion efficiency, independent of whether the fish were held under unmixed (alone) or mixed (with other groups) conditions. In mixed conditions, mean share of meal was reduced and fin damage increased in cortisol-treated fish. When food was limited negative growth effects were more severe but even in the absence of other groups, cortisol-treated fish showed more variable feeding patterns. When compared at the same individual ration levels, cortisol-treated fish had lower growth rates, reflecting a higher "cost of living". These results suggest that the structure of the feeding hierarchy may not be determined solely by competitive ability but may also be greatly influenced by differences in the feeding behavior of unstressed fish versus stressed fish caused by cortisol elevation in the latter.

2.13 Interactions among factors affecting growth

While in experimental conditions it is possible to control abiotic and biotic parameters (e.g. light, water quality, food supply, temperature, predation) and study the influence of a specific parameter on different characteristics of an organism (e.g. survival, growth, weight gain, reproductive success), in the wild this is not possible. In fact, abiotic and biotic factors interact and sometimes enhance or mask their influence on a specific parameter.

Railsbach and Rose (1999) investigated bioenergetic modeling of growth to assess the effects of temperature changes on rainbow trout *Oncorhynchus mykiss.* They wanted to determine the relative effect of temperature versus food consumption on model-predicted growth and to identify relationships between model-predicted food consumption and commonly measured environmental variables. A bioenergetic model for rainbow trout was calibrated to apparent age-1 growth in summer and fall–spring periods for 10 years at eight Sierra Nevada, California, study sites. The model showed that the year-to-year variation that had been observed in summer growth was related to food consumption but not to temperature. Temperature was more important, but still of secondary importance to food consumption in observed variation in fall-spring growth. Growth at all sampling sites appeared lower and more variable in summer than in other seasons. The model also showed that the variation among sites and years in food consumption was highly related to environmental variables during fall–spring but not during summer. During fall-spring, most of the variation (80%) in food consumption was explained by a linear regression model that included temperature, flow, and trout density as variables. Summer food consumption values were only weakly related to stream gradient. This study concluded that when not extreme, temperatures in summer may have less effect on growth than during other seasons and that growth is more affected by factors controlling food consumption (including indirect effects of temperature) than by the direct effects of temperature.

Stone and McNulty (2003) investigated the effect that different stocking rates and different feeding rates have on goldfish growth. The study wanted to determine the best stocking densities to keep goldfish small (between 1.1- and 1.8 g) since 90%

of the market demand is for this fish size. They found that by keeping them at densities between 254-508 fish/m^3, and feeding them at a rate of 1% bw/d growers could minimize goldfish growth. This study showed that manipulating stocking rates alone was not effective in keeping goldfish size small. Stocking densities needed to be combined with reduced feeding rates to keep average fish weight within targeted market size.

2.13 Golden shiners culture and growth

Golden shiners are both wild harvested and cultured. When cultured, they are mainly raised in earthen ponds (generally 2-10 ha) (Stone et al. 2004). The production cycle starts in spring (when fish reproduce naturally) and the egg transfer method is used, although sometimes free spawning and fry transfer methods can be used (Giudice et al. 1983). In the egg transfer method, spawning mats are placed in brood ponds where fish lay eggs on them. The mats containing fertilized eggs are transferred to nursery ponds where the eggs hatch. Fry stocking rates vary according to the intended use of the fish (Stone et al. 2004). If fish need to be grown to market size they are stocked more lightly (500,000 to 1.25 million/ha) or at higher densities (1.8 million/ha) if fry are stocked for later use (Stone et al. 2004). The growing season is from 120 to 180 days according to the region in which they are grown (Gunderson and Tucker 2000). Where growing season is shorter, one single growing season is not enough to reach marketable size. In the North Central region spawning usually begins when water temperatures reach 20–21 °C and continues into August (Gunderson and Tucker 2000). In Arkansas the usual spawning season starts in April and ends in mid-June. After eggs hatch, the fry feed on naturally occurring zooplankton or formulated feeds can be provided according to the region and the type of business. In the North Central regions management consists mainly of keeping competing fish from entering the pond and selectively harvesting the golden shiners as they reach market size. Aeration may be used to prevent losses during summer and/or winter. When the fry transfer method is used, ponds can be prepared and fertilized before fry are stocked into the ponds. Ponds with existing plankton blooms might be treated with Dylox to reduce copepod population. Copepods are predacious and can wipe out fry populations (Stone et al. 1998). In Arkansas management also consists of daily feeding, monitoring water quality, maintain levee vegetation and control aquatic weed.

3. Material and Methods

3.1 Experimental setting

The experiments will be carried out at the Aquaculture Research Station, University of Arkansas at Pine Bluff. Three studies will be carried out for a period of 8 weeks, 8 weeks and 12 weeks, respectively. The first study will investigate the effects of density on golden shiner's growth while the second and third studies will investigate on the effects of temperature of golden shiner's growth.

Fish will be maintained in 110-L aquaria in a flow-through system supplied with well water. Water will be pumped into a reservoir inside the laboratory and distributed by gravity to each aquarium. The reservoir will be used to guarantee a constant water supply throughout the study. Water circulation inside the aquaria will be maintained and aeration and bio-wheel filters with activated carbon will also be added to each tank to maintain good water quality throughout the study. The light cycle will be kept on a 12-hour-light, 12-hour-dark cycle with an automatic timer. Changes from light to dark and vice versa will be instantaneous.

3.2 Water quality and waste removal

Total alkalinity, total hardness, total ammonia nitrogen, pH and DO will be measured at the beginning of the experimental period and total ammonia nitrogen, DO and pH will be measured weekly. Temperature will be measured daily. Waste will be removed as needed using a siphon.

3.3 Feeding schedule

Fish will be fed to apparent satiation twice a day with 32% protein minnow feed with 4-6% fat (ARKAT). A pelleted feed will be used to facilitate uneaten food recovery to determine feed efficiency.

3.4 Conditioning period

Preceding the beginning of the experiment, fish will be allowed a conditioning period of one week. Mortalities will be replaced during the first week of each experiment.

3.5 Data collected and parameters calculated

Initial and final total weight and length will be measured. Individual fish weight will be recorded every two weeks. After weighing the fish 3gr/L of sodium chloride will be added to each aquarium to prevent bacterial infections due to handling procedures. Weight of uneaten feed will be collected daily to calculate feed efficiency (g of eaten food/ number of fish). Mortalities will be recorded to measure survival rates. Mortalities will also be taken into consideration for daily food consumption. Growth performance will be assessed determining instantaneous growth rate ($YT= Yte^{g(T-t)}$). This will provide a discrete method to detect shifts in growth performance among treatments (Brown and Smith 2004). Daily growth rate will be also calculated to quantify growth patterns in each treatment. Daily growth rate will be calculated by using total weight or length gained during the experimental trial, divided by the number of days of the trial. Condition factor will be calculated for individuals at the end of the study as well as size variation. Fish will be sexed at the end of the study.

3.6 Study 1 (Crowding)

Fish will be maintained in fifteen 110-L aquaria. Water temperature will be maintained at 28 °C±1 °C using fifteen heaters (Jager TS Automatic Heater 250 watt). Golden shiners (>1.0 g) will be stocked into each aquarium at a density of 15 fish per tank. Mesh tank dividers will be used to confine the fish to all, half or only one quarter of the tank. Ring feeders will be used to avoid dispersion of the food within the tank and facilitate recollection. This study will be conducted for 8 weeks.

3.7 Study I1 and III (Temperature)

Fish will be maintained in twenty 110-L aquaria. Water temperature will be adjusted to four different temperatures: 15 °C-20 °C-25 °C-30 °C. Each temperature will be maintained using Trade Wind Chillers (¼ HP drop-in chillers with digital LCS temperature controller) for the lowest temperatures, and heaters (Jager TS Automatic Heater 250 watt). Each aquarium will be provided with mesh screens on top to prevent fish from jumping out. The first temperature study will be carried out using juvenile golden shiners with average weight of approximately 0.4g stocked at a density of 30 fish per aquarium (based on nutrition studies by Lochmann and Phillips (1995)). For the second temperature study we will use golden shiners with an average weight of 1.0g stocked at a density of 20 fish per aquarium. Satiation is expected to be approximately 7% of body weight for the 0.4g fish, and 3-4% of their body weight for the 1.0g fish. Golden shiners will be allowed to acclimate to different temperatures in tanks (500-L) in the laboratory and then, after a week, they will be moved into each individual aquarium. Data loggers will monitor temperature in one aquarium per temperature, while for the other aquaria, temperature will be monitored and recorded once a day during feeding. Because we expect colder water temperature to slow golden shiner growth, tests will be run until the fish reach a target of 250% increase in body weight, which is expected to be approximately 8 weeks for juvenile shiners (0.4g) and 12 weeks for the shiners in the second trial (1.0g).

3.8 Stress Test: whole body cortisol assay of small fishes

To test for cortisol levels 10g of golden shiners will be netted from each tank and immediately cold shocked, homogenized and extracted with ethyl ether. This procedure will be repeated until a sample of 10g is obtained from each experimental replicate. The solvent will be evaporated from the ethyl extract using a gentle stream or nitrogen. Dried samples will be kept frozen at -70°C. Samples will be first reconstituted and then radioimmunoassay will be performed at Stuttgart National Research Center.

3.9 Statistical Analysis

Growth (weight and length gain), survival, FCR, condition and growth performance obtained will be compared to temperature, density and sex. Data will be subjected to a two-way statistical analysis of Variance (ANOVA). Significance will be assessed at a level of 0.05. When significant differences among treatments will be found, treatment means will be compared using Tukeys HSD test. Non linear regression will be used to determine the relationship between growth rate/temperature. This will allow an extimation of the temperature range at which maximum growth will occurr. Fulton's K values will be also tested among different treatments with Analisys of Variance.

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