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Evaluation Of Hatchery And Growout Factors For The Successful Production And Stocking Of Juvenile Gulf Coast Walleye

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EVALUATION OF HATCHERY AND GROWOUT FACTORS FOR THE SUCCESSFUL PRODUCTION AND STOCKING OF JUVENILE GULF COAST WALLEYE

By

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Temperature-dependent weight gain of Gulf coast and northern strain walleye *Sander vitreus* fingerlings was determined by rearing fish in flow-through aquaria at different water temperatures (range 15-32°C). Maximum growth of Gulf coast and northern strain walleye occurred between 20 and 26°C, and weight gain of northern walleye exceeded that of the Gulf coast strain by 1.5 times. An acclimated chronic exposure method to assess upper thermal limits determined that walleye survived 35, 9, and 1 days at 33, 34, and 35°C, respectively. A post pond-rearing feed-training practice (21 d) at a mean density of 6,290 ± 1,247 fish/m^3 using formulated feeds was successful (32-85% survival), and walleye continued to consume a formulated diet feed after stocked at densities of 12,250 and 24,700/ha into 0.04 ha earthen ponds. After 125 days, survival in ponds was poor (< 30%) and production varied substantially.

DEDICATION

I would like to dedicate this research to my parents Fred and Carolyn, to my brother Jesse, and my sisters Angela and Nikki. Thank you for your support and encouragement.

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CHAPTER I

Introduction

Successful culture of walleye *Sander vitreus* can be attributed to the enormous amount of information collected about the developmental biology of the walleye, principally the northern walleye strain (Ellis and Giles 1965; Collette et al. 1977; Stickney 1986; Summerfelt 1996a). In contrast, knowledge about the southern walleye strains, including the Gulf coast walleye, is limited. The Gulf coast walleye are native to south flowing drainages in northeastern Mississippi, inhabiting the Tombigbee River and its tributaries (Cook 1959; Brown 1962; Hackney and Holbrook 1978). Because this population of walleye has been steadily declining (Kingery and Muncy 1990; Schramm et al. 2001), propagation and stocking of walleye fry and fingerlings have been used to maintain the current population. However, information regarding developmental biology and factors that affect their abundance may not be realized until an evaluation of the remaining population begins.

The need to make informed management decisions about the Gulf coast walleye stimulated the development of a draft conservation and management plan (Schramm and Miranda 2001) that outlined management and research strategies. Evaluation of hatchery production methods is one objective of this plan, and several state hatcheries and one federal hatchery have developed or are interested in a Gulf coast walleye culture program. Therefore, an evaluation of production methods has strategic and widespread value for production and stocking of Gulf coast walleye in their native range.

Due to insufficient recruitment, stocking is required to maintain, enhance, or reintroduce many walleye fisheries, and this practice is steadily increasing throughout the geographic range of the species (Fenton et al. 1996). In addition to the increasing demand for stocking by resource agencies, a commercial industry that produces food-size walleye is being developed and will also require a reliable supply of fingerlings (Malison and Held 1996). Several production practices and descriptive literature have evolved during the past 40 years, including intensive feed training processes (Cheshire and Steel 1972), out of season spawning methods (Malison et al. 1994), and a walleye culture manual (Summerfelt 1996a; Summerfelt 2000). Various methods are used to intensively and extensively culture walleye, and no single method has proven to be the best. Additionally, most methods have been developed for northern walleye.

This study focuses on the progressively developing intensive culture of the Gulf coast walleye. Due to the limited information about performance of this strain in intensive and extensive culture systems a wide array of topics exists. This study focused on the feed training process used to grow advanced fingerlings and temperature-dependent growth responses of fingerlings, two important aspects that will partially define the suitability of the Gulf coast walleye for different aquaculture practices.

This chapter has several purposes. First, specific background information for the study is provided, focusing on a description of culture methods used to feed-train walleye and the relationship between water temperature and growth. Second, the statement of the problem is outlined and the objectives of the study are presented. The justification for the study is presented and followed by descriptions of the scope and limitations of the study. A brief summary concludes the chapter.

Background

In the context of hatchery management, walleye culture includes collection of broodstock, manual spawning, and growing and stocking of fry or fingerlings. Walleye fingerling culture can be divided into two areas. One, extensive culture, lacks any rigorous control and monitoring of the culture environment and may include raising walleye from fry to fingerling in drainable and undrainable ponds that generally results in unpredictable yields (Dobie 1956). The second, intensive culture, requires much more labor and time for monitoring and fish husbandry, and may include feed training of pond-reared walleye fingerlings (Cheshire and Steel 1972). Fish are usually confined in closed systems and many variables relative to production are controlled. A brief review of the feed training process and temperature-dependent growth responses follows.

The Feed Training Process

Advanced (> 100 mm) northern walleye fingerlings have been produced using formulated feed whereby small fingerlings (25-50 mm) are harvested from rearing ponds and then transported to an intensive culture environment in tanks (Cheshire and Steele 1972; Nagel 1974; Colesante et al. 1986). There, they are confined to a small space and trained to consume a formulated diet. Newly hatched fry can also be raised to small fingerlings in an intensive culture environment (Beyerle 1975; Summerfelt 1996b).

Feed training walleye depends principally on the condition of the fish at the onset of feed training and the use of a high quality starter diet (McIntyre et al. 1987; Malison and Held 1996). For the intensive feed training process, survival of wild strain progeny generally ranges from 30 to 50%, but increases to nearly 90% for progeny of domesticated broodstock (Nagel 1996). Mortality often occurs when fish fail to accept feed and die from starvation. Therefore, rapid acceptance of formulated feed is critical (Bristow 1996).

The Relationship between Temperature and Growth

Temperature is an important environmental variable that influences growth rates and survival of walleye (Hokanson and Koenst 1986). Temperature influences growth by affecting oxygen consumption, feed consumption, feed conversion, and the metabolic rate of fish. Fish can be cultured at temperatures that minimize energy losses due to metabolism and thereby allow most energy to be channeled into growth (Colt and Tomasso 2001). Nickum and Stickney (1993) suggest that feed training of northern walleye fingerlings be conducted between 20°C and 25°C to achieve optimal growth. The temperature associated with maximum growth of juvenile northern walleye has been reported to be 22°C (Koenst and Smith 1976) and 26°C (Hokanson and Koenst 1986). The upper lethal temperature for northern and Gulf coast walleye fingerlings is approximately 35°C (Peterson 1993).

Statement of the Problem

Since the 1980s, the Mississippi Department of Wildlife, Fisheries and Parks (MDWFP) has periodically produced walleye fingerlings at hatcheries using extensive culture techniques. The management practice consists of stocking fry (4-7 days post-hatch) into 0.4 ha drainable ponds. At 28 to 45 days post-hatch, fingerlings are harvested and then stocked into the Tombigbee River and its tributaries. The difficulty of maintaining suitable densities and sizes of forage during pond culture has led to variable success in production of small fingerlings (Dobie 1956). Small fingerlings can also be restocked into ponds at lower densities, fed minnows, and grown to advanced sizes for fall stocking. However, MDWFP rarely uses this practice. If stocking more and larger sizes of fingerlings is warranted, rearing Gulf coast walleye fingerlings on a formulated diet rather than minnows would be more efficient and economical (Jorgenson 1996; Raisanen 1996). Although both methods have succeeded, the latter may be cost prohibitive on a production scale and lack of availability of minnows may preclude this approach (Jorgenson 1996; Raisanen 1996). Further, cannibalism will result in lower survival, especially if the stocked forage fish or the natural forage base is depleted (Dobie 1956; Cuff 1977). Whether Gulf coast walleye will successfully accept a formulated diet or tolerate crowding during intensive culture is unknown.

Further, the relationships among temperature, growth, and survival for Gulf coast walleye have yet to be thoroughly investigated. The upper thermal tolerance of Gulf coast strain walleye fingerlings has been documented through observation of their susceptibility to rapid increases in water temperature (Peterson 1993); however, it is not known whether walleye can survive an extended period of time as temperatures approach and remain at their upper tolerance limits. Several published studies have documented northern walleye growth at different temperatures (Kelso 1972; Hokanson and Koenst 1986; Kuipers and Summerfelt 1994).

Objectives

The current study investigates performance of the Gulf coast walleye in intensive culture and also examines growth under controlled food and temperature conditions. Specifically, the feasibility of feed training pond-reared fingerlings as a means of producing advanced fingerlings is evaluated. In addition, the relationship between temperature and growth is developed as a means to understand the physiological response in relation to the culture environment. My investigation was based on the following objectives:

1) Determine the relationship of temperature to weight gain of Gulf coast walleye fingerlings under laboratory conditions.

2) Determine temperature tolerance of Gulf coast walleye fingerlings relative to culture temperature under laboratory conditions.

3) Determine if a successful feed training process can be established for Gulf coast walleye fingerlings.

4) Assess the feasibility of production of advanced sizes of Gulf coast walleye fingerlings raised in earthen ponds at different stocking densities and fed a formulated diet.

Justification

An evaluation of factors influencing growout is a necessary step toward effective culture of any fish species. Knowledge of maximum and optimal growth temperatures can lead to the establishment of successful culture practices where temperature can be controlled (i.e., intensive culture). Temperatures used to intensively culture northern walleye may need to be modified for efficient production of Gulf coast walleye. Knowledge of relationships among temperature, growth, and survival is important in determining success of practices where temperature cannot be controlled (i.e., hatchery ponds or stocking sites). This information is especially important for Gulf coast walleye which are genetically unique from their northern counterparts and occur at the southern limit of the walleye's native range (Wingo 1982; Murphy 1990; Billington and Strange 1995).

Experimental stocking will provide information that is essential to increasing the likelihood of success of recovery activities such as habitat improvement. Stocking different sizes and numbers of marked fish into a system will be necessary to collect information about survival, growth, and their environment. To provide a substantial number of advanced fingerlings for these activities, fish would be reared intensively in tanks where they can be feed-trained. Raising walleye on commercial fish feed that is readily available is less expensive and allows for more control over production than the alternative of using forage fish in a pond (Jorgenson 1996; Raisanen 1996). Fingerlings trained to feed could be restocked into ponds (Coyle et al. 1997) and raised on a formulated diet. Determination of the best methods for raising advanced sizes of fingerlings is important to maximize hatchery production and provide a variety of sizes for stocking and evaluation.

Scope and Limitations

The objectives of this study were to gather basic knowledge about the intensive culture of the small and advanced sizes of fingerlings. Of special interest was a comparison of results with similar studies carried out with northern strain walleye to identify possible strain-specific differences in growth, survival, and fish performance. The lack of information about Gulf coast walleye performance under different hatchery conditions precludes any comparison. However, important publications about the abundance and history of Gulf coast walleye in Mississippi and their genetic distinctiveness do exist (Schultz 1971; Wingo 1982; Murphy 1990; Billington and Strange 1995; Ross 2001). The results of this study will be used along with those found in previous publications to develop management practices and recommend future research needs.

Summary and Plan of Presentation

Numerous walleye strains exist throughout their geographic range. However, none may be as unique as the Gulf coast walleye, purely because this fish was geographically isolated until the completion of the Tennessee-Tombigbee Waterway in 1985. Additionally, although the walleye is considered a cool-water species, it exists in the subtropical climate of Mississippi. These characteristics suggest that Gulf coast walleye may have acquired unique adaptations over time based upon geographical separation from its northern counterparts. This study attempted to learn whether unique characteristics do exist, and how they might be incorporated into management practices for intensive culture.

The remaining thesis consists of three independently constructed chapters. Chapter two reports on objectives one and two; chapters three and four report on objectives three and four; chapter five provides a summary of the results and provides management recommendations, respectively.

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CHAPTER II

Effects of Water Temperature on Growth and Thermal Tolerance of Walleye Fingerlings

Introduction

Effects of water temperature on growth and survival have been well documented for northern strain walleye in the laboratory and in nature (Hokanson and Koenst 1986; Galarowicz and Wahl 2003; Quist et al. 2003). In nature, walleye growth varies geographically because of habitat and regional climatic differences (Quist et al. 2003). In the laboratory, temperature effects are influenced by environmental conditions such as length of time to acclimate, food ration, and light intensity (Brett et al. 1969; Huh et al. 1976; Hokanson and Koenst 1986). Most laboratory studies have focused on the early life history to determine factors that influence growth and survival (Koenst and Smith 1976; Hokanson and Koenst 1986; Clapp et al. 1997). Hokanson (1977) and Armour (1993) have provided reviews of temperature-growth relationships for different life stages of walleye.

Knowledge of temperature requirements, including temperature-dependent growth, has not been well established for southern walleye, particularly the Gulf coast strain (Peterson 1993). This strain of walleye is genetically unique (Wingo 1982;

Murphy 1990; Billington and Strange 1995) and exists at the species southern natural limit (Cook 1959; Brown 1962). Therefore, the Gulf coast walleye may have adaptations that are unique relative to the northern strain because populations occur at the extremes of their natural limits.

Peterson (1993) evaluated tolerance of Mississippi and Iowa walleye fingerlings to high water temperatures by observation of susceptibility to rapid increases in water temperature (1°C/min). He reported that the level of tolerance to high water temperatures did not differ significantly, suggesting that these populations were alike in this temperature response despite genetic differences. He also reported that the Mississippi and Iowa walleye could survive water temperatures to at least 31°C. Although Peterson (1993) documented no difference between these strains in susceptibility to increasing water temperatures, whether Gulf coast strain walleye can uniquely tolerate water temperatures that exceed 31°C for an extended period is yet to be determined. Populations of Gulf coast walleye are subjected to prolonged high summertime air temperatures resulting in a long growing season. Therefore, the Gulf coast strain of walleye would likely be uniquely able to flourish under prolonged periods of warm water conditions.

Knowledge of the magnitude and durations of this potential high temperature tolerance may be an important factor to consider when advanced fingerlings are produced for stocking into natural habitat during the fall. Typically, fish are raised through the summer in outdoor culture areas, such as drainable ponds in southern climates; high summertime air temperatures may result in water temperatures that exceed 31°C for an extended period of time. The objectives of this study were to determine the relationship of temperature to growth of juvenile Gulf coast strain walleye and northern strain walleye reared in aquaria and to determine upper thermal tolerance of juvenile Gulf coast strain walleye and northern strain walleye reared in aquaria.

Methods

Aquaria Facilities

Glass aquaria, each constructed with a 38 cm standpipe to maintain a volume of 85 L, were supplied with city (Starkville, MS) water as part of a flow-through system. Before entering the aquaria, the water passed through three large charcoal filters to remove any chlorine. These filters were back-flushed weekly to ensure high water quality was maintained throughout the duration of the experiments. Each aquarium was supplied with water from one main head tank (city water tower) at a rate of approximately 0.2 L/min, for a turnover rate of approximately 3.5 times per day. Water temperatures were controlled by either individual immersion heaters (Visitherm 300 W, Aquarium Systems, Inc. Mentor, OH) positioned within each aquarium or chillers positioned in series as part of the flow through system. To maintain water temperatures that were less than room temperature, the sides of aquaria were insulated with styrofoam. Water temperatures were recorded daily with an oxygen meter (Model 550A, YSI Incorporated, Yellow Springs, Ohio). Water in each aquarium was aerated using air stones. A day length of 16-h-light and 8-h-dark was provided during both acclimation and testing, and illumination was provided by overhead fluorescent lighting. Tanks were cleaned daily by siphon removal of feces and uneaten food.

Growth-Temperature Experiment

In 2003, pond-reared Gulf coast strain walleye fingerlings were obtained from the Private John Allen National Fish Hatchery (NFH), operated by the U.S. Fish and Wildlife Service, in Tupelo, MS. Captive broodfish used for spawning were originally captured from Luxapallila Creek near Columbus, MS and held in a 0.4 ha pond until spawning. Five broodfish were successfully spawned at the hatchery and 5 d post-hatch fry were stocked into a 0.4 ha drainable earthen pond.

After 31 d (28 March 2003), approximately 4,000 pond-reared fingerlings (total length 37-41 mm) were harvested and transported to the hatchery for the feed training process which produced 300 emaciated fish. On 1 June 2003, the remaining fingerlings were transported in bags filled with oxygenated water to the Fish Nutrition Lab of the Department of Biochemistry and Molecular Biology, Mississippi State University. Walleye were acclimated inside the bags to water conditions in the aquaria (water temperature 24°C) and were then distributed into five aquaria. Originally, the experimental design was based upon an evaluation of the effect of five water temperatures (19, 22, 25, 28 and 31°C) on growth with five replicates per treatment and ten fish per tank. Assuming that the walleye to be used in the experiment had successfully weaned to a formulated diet, a formulated diet was fed; however, within a week, the majority of fingerlings apparently died from starvation leaving approximately 100 fish. These remaining fingerlings were then fed a daily diet of live blackworms *Lumbriculus variegatus* (Aquatic Foods, Fresno, CA) in excess. As a result of the unanticipated mortality, the experimental design was modified to evaluate the effect of four water temperatures (15, 20, 25, 30°C) on growth. These temperatures were chosen based on a similar range of temperatures used in studies with northern walleye so that growth responses at temperatures frequently experienced by walleye throughout their geographic range could be compared.

On 21 June 2003, walleye were individually weighed to the nearest 0.01 g, and distributed into experimental aquaria arranged in rows. Each treatment was replicated five times. Initial mean (± SD) individual weight was 0.63 ± 0.06 g. Fingerlings were stocked at 5 fish/85 L. Water temperatures were either increased or decreased 1°C/day from 24°C until final test temperatures were reached (2-7 days). Then, fish were held an additional 11-16 d after the final treatment temperatures were reached. On 14 July, walleye were removed from each aquarium to determine wet weight by lightly dabbing each fish with a paper towel to remove surface water and then placing the fish into a pre-tared container full of water. Walleye in each aquarium were also bulk weighed to evaluate the accuracy of the weighing procedure. Fish were measured again after 20 d on 3 August and after 42 d on 25 August. Mortalities were recorded during the experiment. The duration of the experiment, including time of acclimation, was 65 d. Water temperatures in the aquaria fluctuated slightly (range ± 0.3 to ± 0.6 SD)

In 2004, pond-reared Gulf coast strain walleye fingerlings were obtained from the Private John Allen NFH and northern strain walleye fingerlings were obtained from the Normandy Fish Hatchery operated by the Tennessee Wildlife Resources Agency. Broodfish captured from Wall Doxey State Park near Holly Springs, MS, were used for spawning at the Private John Allen NFH. One broodfish was successfully spawned at the hatchery and 7 d post-hatch fry were stocked into a 0.4 ha drainable earthen pond. After 57 d, post-hatch walleye fingerlings were harvested. Normandy Fish Hatchery used broodfish captured from a wild population. Broodfish were spawned at the hatchery and 5 d post-hatch fry were stocked into 0.4 ha drainable lined ponds. Fingerlings were harvested 45 d post-hatch and transported to the Private John Allen NFH on 7 May.

Training to a formulated diet was attempted with 336 pond-reared Gulf coast walleye fingerlings, but suspended after 10 d to avoid any starvation-related mortality. These fish were then fed blackworms daily in excess. Because the original experimental northern walleye fingerlings perished for unknown reasons, northern walleye fingerlings used in a feed training experiment (see Chapter III) were obtained for the laboratory experiment. On 16 June, these feed-trained walleye fingerlings were transported from the Private John Allen NFH to the Eastern Unit of the National Warmwater Aquaculture Center located at Mississippi State University. There, they were stocked into six 0.04 ha experimental earthen ponds for growout to advanced fingerlings.

On 25 June 2004, Gulf coast walleye fingerlings were transported in bags filled with oxygenated water to the laboratory. Northern walleye fingerlings were collected by seine from one of the experimental ponds at the Eastern Unit of the National Warmwater Aquaculture Center and transported in a tank to the laboratory. Walleye were acclimated inside the bags and tank to water conditions in the aquaria (water temperature 24°C), individually weighed to the nearest 0.01 g, measured for total length to the nearest millimeter, and distributed into experimental glass aquaria to evaluate the effects of five water temperatures (20, 23, 26, 29, 32°C) on growth. Initial mean (± SD) total length and weight were 77.4 ± 8.2 mm and 3.08 ± 0.93 g for northern strain walleye and 77.6 ± 8.7 mm and 3.24 ± 1.07 g for Gulf coast walleye. Both strains of walleye fingerlings were stocked at 7 fish/85 L and each treatment temperature was replicated four times, and arranged in a completely randomized fashion. Walleye were held at 24°C until 3 July when water temperatures were either increased or decreased 1°C/d until the final treatment temperatures were attained on 10 July and were held for an additional 18 days at the experimental temperatures. On 28 July, walleye were removed from each aquarium and weighed both collectively and individually. Total length to the nearest millimeter was also determined. Fish were measured again on 17 August and 27 August. The duration of the experiment, including time of acclimation, was 63 d.

Growth rates were based upon mean individual weight derived from bulk weight for each replicate of each treatment and expressed as percentage increase in weight and specific growth rate in percent per day (Westers 2001). A one-way analysis of variance (ANOVA) was used to determine if either specific growth rates or percentage increases in weight differed significantly among treatments. The acceptable level for all type-I errors was set at $P < 0.05$. If significance was indicated, then a Fisher's least significant difference (LSD) test ($\alpha = 0.05$) was used to determine where significant differences between treatments existed.

$$\text{Percentage increase in weight} = \frac{FinalWeight - InitialWeight}{InitialWeight} \times 100$$

$$\text{Specific growth rate} = \frac{(\ln FinalWeight - \ln InitialWeight)}{n(days)} \times 100$$

Thermal Tolerance Experiment

In 2004, the acclimated chronic exposure (ACE) method was used to evaluate thermal tolerance. Procedures were adapted from Selong et al. (2001). Survival response of fingerlings held at three water temperatures (33, 34, and 35°C), at one density (7 fish/85 L) in aquaria was evaluated. Each treatment temperature was replicated three times in a completely randomized arrangement. Walleye fingerlings were randomly selected, weighed, and then placed into test aquaria at the start of the trial. Water temperatures were increased 1ºC/d and staggered at 2 d intervals so that all treatments reached their final test temperature on the same day. When the predetermined treatment temperature was reached, it was held constant for either 60 d or until mortality of the entire population in an aquarium occurred. The time period selected was founded upon the possible duration of exposure to water temperatures in ponds during high summertime air temperatures. Water temperatures were recorded with a digital meter daily and at the time of death (Model 550A, YSI Incorporated, Yellow Springs, Ohio). Tanks were checked twice daily, and any mortalities were removed and weighed. Water in each aquarium was aerated with air stones to maintain dissolved oxygen near the temperature-dependent level of saturation.

To estimate thermal tolerance, the time to 50% mortality was plotted against treatment temperatures and used to determine the temperature at which 50% of the population survived during a 60 d experimental period. ANOVA was used to determine whether thermal tolerance was significantly different between strains and among treatments. The acceptable level for all type-I errors was set at $P < 0.05$. If significance was indicated, then a Fisher's least significant difference (LSD) test ($\alpha < 0.05$) was used to determine where significant differences between treatments existed.

Results

In 2003, after 42 d of growth at test temperatures, growth of Gulf coast walleye fingerlings at 15ºC and 20ºC did not differ significantly but was significantly greater than that at 25ºC and 30ºC (Table 2.1). Growth at 25ºC was significantly greater than at 30ºC. At 20ºC the percentage increase in weight and specific growth rate peaked at 125.1% and 1.98%/d, respectively. In contrast, during the 65 d experimental period which includes acclimation, maximum growth at 20 and 25°C did not differ significantly, but was significantly greater than that at both 15 and 30°C (Figure 2.1). However, after acclimation to test temperatures, growth decreased substantially for fish held at 25ºC (Figure 2.2). At termination of the experiment, mean standard length (± SD) was 68.5 (± 0.9) mm and 68.0 (± 0.9) mm for fish held at 15ºC and 30ºC, respectively, and was significantly less than that of each of the other treatment temperatures. Fish held at 20ºC and 25ºC had a mean standard length (± SD) of 79.8 (± 0.7) mm and 79.9 (± 0.8) mm, respectively. Fin erosion was observed on most of the fish in the 25ºC and 30ºC treatments. No fin erosion was observed for fish in the 15ºC treatment and there was minimal infection of fish in the 20ºC treatment.

In 2004, "ich" (*Ichthyopthirius multifiliis*) caused mortality of all the fish in several replicates of treatments, before termination of the experiment. After acclimation to test temperatures, growth of Gulf coast strain walleye fingerlings at each treatment temperature was significantly less than that of northern strain walleye fingerlings (Table 2.2). Growth expressed as either specific growth rate or percentage weight increase did not differ significantly among treatment temperatures for either strain (Figure 2.3). Growth in one aquarium containing northern walleye measured 19 days after acclimation to 32ºC was 0.99%/d, whereas Gulf coast walleye had a negative growth rate. Growth in one aquarium containing Gulf coast walleye at 29ºC during this same period was 0.64%/d.

Thermal tolerance did not differ significantly between Gulf coast and northern strain walleye fingerlings (Figure 2.4). Time to 50% mortality differed significantly among treatments. The mean ± SD time at which 50% of the population died was 1.3 ± 0.8 days for fish grown at 35ºC, 8.6 ± 2.8 days for fish grown at 34ºC, and 35.1 ± 4.8 days for fish grown at 33ºC. Mean ± SD temperature for each treatment was 35.5 ± 0.4ºC, 33.9 ± 0.2ºC, and 33.0 ± 0.3ºC.

Discussion

The results of the 2003 temperature-dependent growth experiment suggest that temperatures between 15 and 20ºC yield maximum growth rates for Gulf coast walleye. These growth data results differ from those obtained from similar temperature-dependent growth studies with northern walleye. Growth of northern walleye at 20ºC was 3.75 X that at 17ºC (Siegwarth and Summerfelt 1990; Iowa walleye; mean total length 146 mm; mean weight 25 g) and growth at 22ºC was one-third X that at 18ºC (Huh et al. 1976; Wisconsin walleye; mean weight 4.1 g). However, overall growth during the 65 d experimental period, which includes the acclimation period, suggests that maximum growth occurs between 20ºC and 25ºC. Although these growth data are different than those obtained for the 42 d period, response to the treatment temperatures is probably better represented. This belief is supported by the results of the 2004 experiment that demonstrated temperatures between 20ºC and 26ºC yielded maximum growth rates for both Gulf coast and northern strains of walleye. Throughout the temperature range evaluated, northern strain walleye grew faster, but both strains evidenced similar growth responses at their respective treatment temperatures, indicating both strains may not differ in this aspect of physiology. Then, these growth data are not unusual when compared to other studies with northern walleye. Under laboratory conditions, Hokanson and Koenst (1986) reported that maximum growth occurs between 22ºC and 28ºC with optimum growth at 26ºC (Minnesota walleye; mean weight 26.6 g) and Huh et al. (1976) reported greatest growth for walleye at 22°C.

In the 2003 experiment, the growth rate during the acclimation period was substantially greater than that during the remainder of the experiment. This rapid growth is probably a response to the emaciated condition of the fish at the beginning of the experiment due to the period when they failed to consume a formulated diet. Following acclimation to test temperatures, a substantial decrease in growth occurred at 25ºC, believed to have been caused by fin erosion a condition that can decrease growth (Clayton et al. 1998). No fin erosion was observed for walleye in the 15ºC treatment, and only a few fish at 20ºC showed signs of an infection. Previous researchers have recommended that water temperatures for feed-training of walleye fingerlings should range from 15ºC to 20ºC to reduce the threat of disease organisms such as columnaris (*Flavobacterium columnare*) which can lead to fin erosion (Nagel 1976; Kuipers and Summerfelt 1994; Clayton et al. 1998). These researchers also noted that growth rates will be substantially less at 15°C than that at 20°C and 25°C for northern strain walleye.

In the 2004 experiment, northern walleye grew nearly three times faster than the Gulf coast walleye suggesting genetic differences might by operative. The Gulf coast walleye fingerlings used in this experiment were spawned from one female and two males, so the reduced genetic variation arising from three broodfish may have resulted in growth rates that are not generally characteristic of this strain. Billington and Strange (1995) showed that 16 adult Gulf coast walleye, obtained from ponds at the University of Mississippi containing captive walleye originating from Luxapalila Creek, were genetically distinct from other walleye but no genetic variation was detected among the 16 fish analyzed. It is worth noting that additional broodfish obtained from these same ponds were stocked into a lake at Wall Doxey State Park, MS. Thus, the few broodfish used in 2004 were likely of the same genetic composition as those used by Billington and Strange (1995). Since the 1980s, this captive broodstock has been used by the Mississippi Department of Wildlife, Fisheries and Parks to augment the walleye population in the Tombigbee River. The lack of genetic variation combined with captivity suggests that individuals in these populations have likely been inadvertently mated with closely related conspecifics. Loss of genetic variation due to inbreeding can adversely affect growth of individuals and populations (Williamson 2001).

Nonetheless, these growth rates may not be an unusual characteristic of the Gulf coast strain, especially when compared to other populations in the southeastern United States. In Alabama, Moss et al. (1985) collected walleye from Hatchet Creek, a tributary of Lake Mitchell and two state owned lakes, and found that growth of 1 year old walleye (174 mm total length) was generally less than that reported in many southeastern reservoirs, most notably reservoirs in Tennessee (range 248 to 264 mm total length). In fact, 63% of the walleye collected by Moss et al. (1985) were from Hatchet Creek which is inhabited by the Gulf coast strain. Growth of age 1 walleye collected from the Tombigbee River in northeastern Mississippi (range 210 to 236 mm total length) are similar to the growth of walleye in Alabama (Schultz 1971; Wingo 1982).

Galarowicz and Wahl (2003) compared growth rates among walleye populations from Arkansas, Missouri, Wisconsin, and Alberta, Canada (mean total length 120 mm; mean weight 12.7 g) over a temperature range of 5 to 25ºC and found no difference in growth at intermediate temperatures (10, 15, and 20ºC). They attributed this response to the fact that most populations of walleye are often exposed to this range of temperatures. We can speculate that the walleye strains used in this study probably are often exposed to a range of temperatures between 20 and 26ºC, so growth responses may not be different unless fish are exposed at temperature extremes. In the 2003 experiment, a positive growth rate at 30ºC was observed for Gulf coast walleye, whereas in the 2004 experiment a negative growth rate was observed at 32ºC. In contrast, northern strain walleye in the 2004 experiment had a positive growth rate at 32ºC. These results suggest that differences in growth between walleye strains at temperature extremes may exist and agree with Hokanson and Koenst (1986) who suggested that walleye are capable of growing at temperatures above 30ºC under laboratory conditions.

The results from the thermal tolerance testing in the 2004 experiment revealed that the level of tolerance of Gulf coast and northern strain walleye fingerlings to high water temperatures did not differ significantly, indicating that there is no genetic and/or environmental differences that might be manifested through differences in thermal tolerance (Peterson 1993). Lack of differences in thermal tolerance between Mississippi and Tennessee walleye populations may be due to comparatively similar local thermal conditions. However, Peterson (1993) found no difference in thermal tolerance between Iowa (121 mm standard length) and Mississippi (107 mm standard length) populations either, providing evidence that thermal tolerance of walleye fingerlings is similar despite latitudinal separation in habitat. These reports support

Hackney and Holbrook (1978) who speculated that factors other than thermal tolerance probably limit the southern distribution of the species.

Walleye fingerlings can apparently survive temperatures up to 33ºC for as long as 35 days. However, survival decreases rapidly when exposed to temperatures at 34ºC and 35ºC. In a similar study, Wrenn and Forsythe (1978) determined upper thermal temperatures that should not be exceeded for fingerling survival in the summer. Their experimental walleye (Ohio walleye; mean total length 42 mm; mean weight 0.5 g) were exposed to four temperature treatments in an experimental ecosystem including ambient (18.5-30°C from late April through August; 118 d) and 2, 4, and 6°C above ambient water temperature. The experimental ecosystem was designed so that cooler refuge areas were not available to the walleye as the water temperature increased. When temperatures did not exceed 30°C, survival was 63%, whereas complete mortality was observed when the temperature reached 34.5°C. Survival was 46% when the temperatures rose to 33.3°C. As the temperatures rose to 33.3°C, an extended period of elevated temperatures ranging from 32-33°C occurred for 75 days. In the present study, fish grown at 32°C and not infected by ich survived the 63-day temperature-dependent growth experiment. The results derived from this study and those of Wrenn and Forsythe (1978) suggest that walleye are capable of surviving for long periods of time (75 d) at 32°C, and even growing, but that survival decreases rapidly during extended exposures to water temperatures of 33, 34, and 35°C. In other studies, minimal mortality has been reported for temperatures up to 31°C, but mortality increased to 100% as water temperature increased (1ºC/min to

1ºC/day) to 35°C in a laboratory setting (Smith and Koenst 1975; Hokanson and Koenst 1986; Peterson 1993).

Conclusion

The results of this study have important implications for walleye culture in Mississippi. Knowledge of these temperature requirements will help establish successful culture practices under controlled temperature conditions, and will guide culture practices under conditions of no temperature control. Although the Gulf coast walleye is genetically distinct, growth and tolerance response to water temperature do not appear to differ from those of the northern strain walleye. Differences in growth rates between strains may also have a genetic foundation because in this study northern strain walleye fingerlings grew faster than Gulf coast walleye fingerlings under the same environmental conditions.

Results from the 2003 temperature-dependent growth experiment showed significantly greater growth at 15 and 20°C, but a conclusion that maximum growth occurs between these temperatures may not be valid. A likely alternative explanation is that walleye fingerlings used for the experiment were emaciated prior to the initiation of the experiment. Thus, walleye grown at temperatures greater than 20°C may have been more susceptible to fin erosion, so the true growth response at the treatment temperature may have been compromised. This shortcoming and potentially confounding factor could not be rectified. However, culture of Gulf coast walleye at lower temperatures (15°C to 20°C) will probably reduce susceptibility to disease substantially (Kuipers and Summerfelt 1994). This information is valuable because it may be applied to the planning of intensive culture operations for Gulf coast walleye. The maximum growth that occurred between 20°C and 26°C for both Gulf coast and northern strains of walleye in the 2004 experiment suggests that a lower growth rate probably should have occurred at 15°C in the 2003 experiment as reported in similar studies using northern strain walleye (Huh et al. 1976; Siegwarth and Summerfelt 1990).

Gulf coast and northern strain walleye are able to tolerate water temperatures greater than 32ºC for a short period of time. These results concur with those of Peterson (1993) who reported that walleye can survive temperatures up to at least 31ºC. This survival response establishes a strong foundation for the successful intensive (tanks) and extensive (ponds) culture of Gulf coast walleye in Mississippi. Water temperatures up to 33ºC can be tolerated with presumed stress for an extended length of time before mortality occurs. However, productivity and growth will most likely be adversely affected by frequent incidence of exposure to water temperatures greater than 32ºC as suggested by this temperature-dependent growth study and supported by the observations of other investigators (Koenst and Smith 1976; Wrenn and Forsythe 1978; Hokanson and Koenst 1986).

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Table 2.1 Mean growth of Gulf coast walleye fingerlings held at different temperatures in flow-through aquaria over a 65 d experimental period in 2003. Day 23 is weight of fish after acclimation to test temperatures. Day 65 includes growth after acclimation to test temperatures to termination of the experiment (42 d).

	At start of test	Day 23	Treatment temperature (23-65 d)			
Temp (C)	Mean wt (g)	Wt (g)	Wt (g)	Specific growth rate (%/day)	% Increase in wt	% Survival
15.4	0.65	1.76	3.85	1.87	119	92
20.0	0.62	2.53	5.70	1.93	125	96
25.2	0.67	3.17	5.54	1.32	75	100
29.7	0.58	2.21	3.41	1.01	54	96

Table 2.2 Mean growth of Gulf coast (G) and northern (N) strain walleye fingerlings held at different temperatures in flow-through aquaria over a 63 d experimental period in 2004. Day 33 is weight of fish after acclimation to test temperatures. Day 63 includes growth after acclimation to test temperatures to termination of the experiment (30 d).

	At start of test	Day 33	Treatment temperature (Day 33-63)			
Temp (C)	Mean wt (g)	Wt (g)	Wt (g)	Specific growth rate (%/day)	% Increase in wt	% Survival
20.0-G	3.24	6.52	9.42	1.23	44	100
23.0-G	3.18	7.54	9.54	0.78	27	100
25.9-G	3.30	6.90	9.05	0.90	31	50
20.1-N	3.03	9.68	18.86	2.22	95	82
23.1-N	3.07	10.20	21.06	2.42	106	50
26.1-N	3.13	10.50	21.30	2.36	103	64

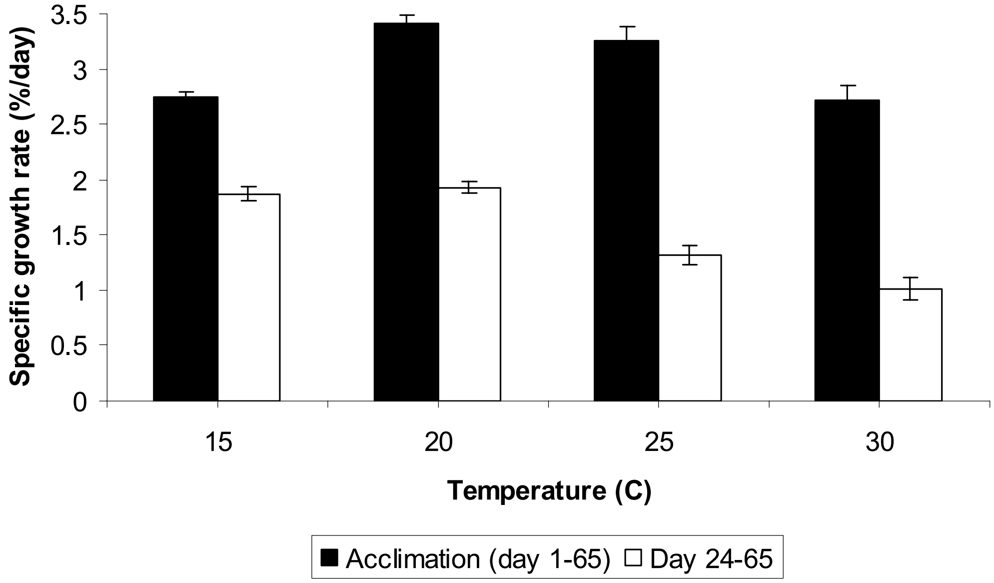

Figure 2.1 Effects of water temperature on the mean (± SE) specific growth rate of Gulf coast walleye fingerlings in 2003. The overall growth including acclimation to test temperatures (1-65 d), and during the test period (24-65 d).

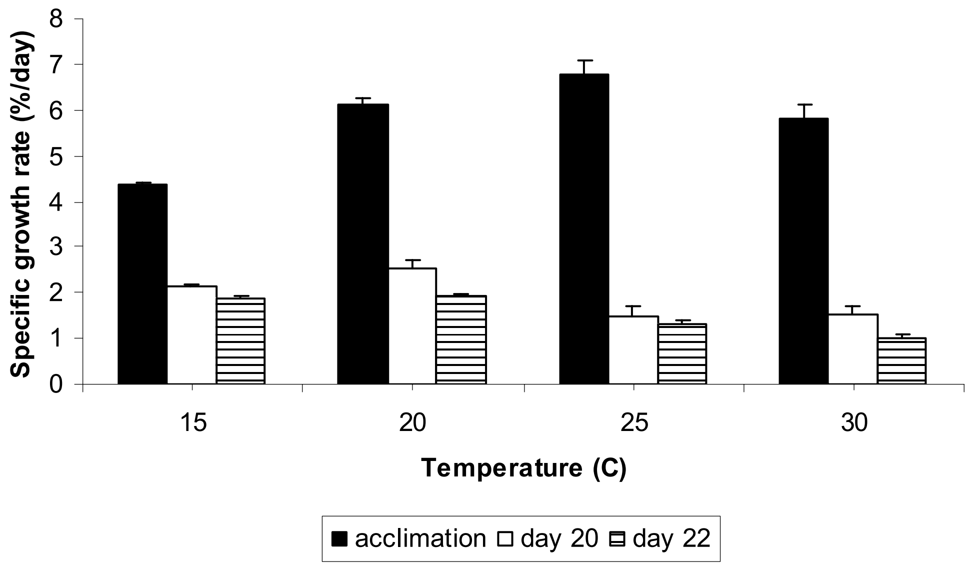

Figure 2.2 Mean (± SE) specific growth rate for Gulf coast walleye fingerlings held at different water temperatures for 65 d in 2003. Growth is reported for intervals during the experiment. Acclimation to test temperatures (23 d); from 14 July to 3 August (20 d); and from 3 August to 25 August (22 d).

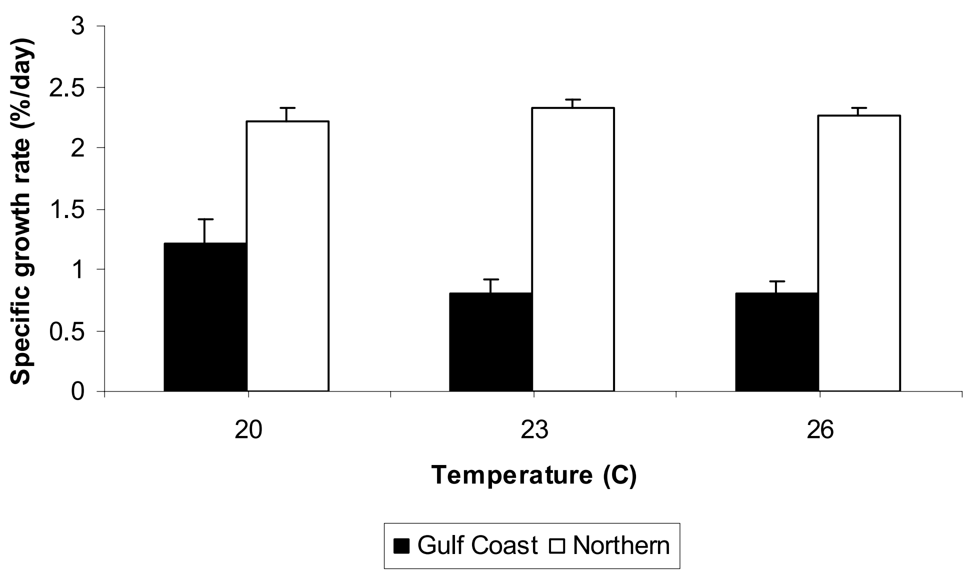

Figure 2.3 Effect of water temperature on the mean (± SE) specific growth rate of Gulf coast and northern strain walleye fingerlings held at different water temperatures for 63 d in 2004.

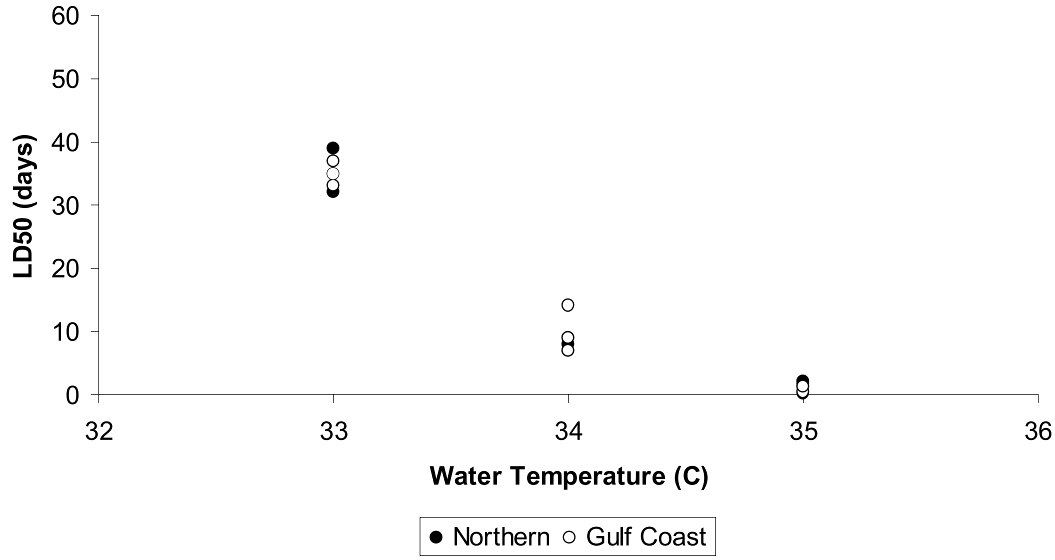

Figure 2.4 Time when 50% of the population of Gulf coast and northern walleye strains died while held at a constant temperature of either 33ºC, 34ºC, or 35ºC for 60 days or until death. ● ○ = LD50 of individual tanks.

CHAPTER III

Survival and Growth of Pond-Reared Walleye Fingerlings Trained to a Formulated Diet

Introduction

Typically, walleye fingerlings are harvested from production ponds when they reach 25 to 50 mm total length. By this time, fingerlings have usually consumed and outgrown the supply of appropriately sized invertebrate forage, and, if not harvested, will subsequently begin to cannibalize one another. The duration of culture in the ponds ranges from 30 to 55 days (Leone 2001). To produce large numbers of advanced fingerlings (> 100 mm), harvested pond-reared fingerlings can be transported to an intensive culture facility where they are trained to consume a formulated diet (Cheshire and Steele 1972; Nagel 1974; Beyerle 1975). Feed acceptance can be poor, resulting in slow growth and low survival (Flowers 1996). Rapid transition to formulated feed reduces loss to cannibalism, starvation, and disease all of which contribute to poor survival (Nagel 1976).

Many formulated diets have been accepted, at least partially, by walleye fingerlings (Beyerle 1975; Kuipers and Summerfelt 1994). These diets usually contain high levels (40-60%) of protein (Malison and Held 1996). The U.S. Fish and Wildlife Service (USFWS) has formulated and tested walleye diets (Barrows 1996).

Because walleye diets still remain in the evaluation stage, hatchery managers use diets formulated for other species while continuing to test experimental diets to feed-train pond-reared fingerlings. The objective of this study was to evaluate a feed training process in conjunction with two formulated diets fed to pond-reared Gulf coast strain and northern strain walleye fingerlings in an intensive culture environment. Response was evaluated through growth and survival.

Methods

Pond-reared Gulf coast strain walleye fingerlings used in this experiment were obtained from the Private John Allen National Fish Hatchery (NFH) operated by the USFWS, Tupelo, MS. Brood fish were captured from Wall Doxey State Park near Holly Springs, MS. One brood fish successfully spawned at the hatchery and 7 d post-hatch fry were stocked into a 0.4 ha drainable earthen pond. At 57 d post-hatch, a total of 336 fingerlings were harvested from the pond. Because these fish were needed for another experiment, the attempt to wean them onto feed was suspended after 10 d to avoid continued mortality. None of these fish was observed to accept feed.

Pond-reared northern strain walleye fingerlings used in this experiment were obtained on 7 May, 2004 from Normandy Fish Hatchery operated by the Tennessee Wildlife Resources Agency. Brood fish were captured from a wild population, spawned at the hatchery and 5 d post-hatch fry were stocked into 0.4 ha drainable lined ponds. At 45 d post-hatch (6 May 2004) fingerlings were harvested. Initial mean ± SD total length and weight were 41.8 ± 3.1 mm and 0.54 ± 0.12 g. The mean condition factor (K= weight in g x 10^5/length in mm^3) of the fingerlings was 0.73 ± 0.06.

On 7 May, the northern strain fish were transported for 4 hours in a 426 L fiberglass tank composed of two 213 L compartments to the Private John Allen NFH. Water in each compartment was aerated with a single mechanical aerator and supplemented with pure oxygen to maintain satisfactory oxygen levels. Prior to transport, a saline solution was added to the water to reduce physiological stress.

Experimental 1.5 m^3 (1,514 L) concrete raceways with light-colored walls and bottoms were used to feed-train walleye fingerlings. Each raceway was covered with a single layer of black landscaping plastic to eliminate detection of natural and fluorescent light and movements by hatchery personnel during the time when specific husbandry activities did not occur (Nagel 1976). Before stocking, the total number of fish/kg ± SD was estimated to be 1,848 ± 49 by weighing four samples of 20 fish. Fingerlings (1.27 kg) were then stocked into each raceway and crowded to one end with a mesh screen to obtain a density of 4.0 kg/m^3 (7,415 fish/m^3; Figure 3.1).

A 5 W submersible light (UL® Underwater Light/ model no ZIA41003U-10) was placed inside a 2.54 cm (diameter) by 15.24 cm (length) polyvinylchloride (PVC) pipe capped at one end. The light was submersed approximately 15 cm into the water and provided a low light intensity in the crowded section of the raceway. A belt feeder (FIAP Belt feeder, 5 kg, 12 hr) was located directly over the submersible light. Well water was supplied to each raceway at 2 Lpm for an exchange rate of 120 Lph. In-tank aeration was supplied by air stones to maintain levels of dissolved oxygen near the temperature-dependent level of saturation. Mean ± SD water temperature during the experiment was 20 ± 0.5°C.

Over a period of 21 d, fish were fed 10% of their body weight/d to provide an adequate amount of food assumed to be sufficient to prevent cannibalism and increase feed acceptance (Cuff 1977). Within this time interval, the majority of fingerling mortality generally results from harvest and transport related stress (Kuipers and Summerfelt 1994). Mortality from starvation will also occur during this time interval if pond-reared fish do not train successfully to the formulated feed. Feed training was initiated 2 h after fish were stocked into the raceway and feeding rates were adjusted weekly to compensate for changes in biomass from the combined effects of fish growth and mortality. Feed was dispensed every several minutes, 22 hours/d using belt feeders. In an attempt to minimize disease-related mortality, a 1 h static bath treatment of 0.5% salt solution was used in the experimental raceways at the time of stocking and after a scheduled sampling.

Six experimental raceways were used to evaluate the effects of two feed types; EPAC pelleted diet (INVE Aquaculture, Inc., Mountain Green, UT) containing 58% crude protein and an initial size of 1.0-1.2 mm, and a krill (*Euphausiid*)-based crumble diet (Rangen Incorporated, Buhl, ID) containing 55% crude protein and an initial size of 0.7-0.9 mm. The result of a proximate analysis of each diet performed by the Mississippi State Chemical Laboratory is provided in Table 3.1. Feces and uneaten food were removed daily by siphoning. Each week, raceways were flushed out by temporary removal of the standpipe to increase water flow. Due to post-transport-related mortality, the final stocking density in all raceways was not equal.

Mean ± SD stocking density was 6,944 ± 1,015 and 5,635 ± 1,255 for fish fed the EPAC and Rangen diet, respectively. Each dietary treatment was replicated three times. Dead fish were removed and counted daily. When a cannibal was visually identified as a fish eating another or a fish with another fish in its mouth, it was removed. Cannibals were counted as 2 individuals.

On days 10 and 21, samples of 70 fish were removed from each raceway and were individually weighed (0.01 g) and measured (total length, to the nearest mm) to determine specific growth rate in percent per day (Westers 2001),

$$\text{Specific growth rate} = \frac{(\ln FinalWeight - \ln InitialWeight)}{n(days)} \times 100$$

and mean condition factors (K= weight in g x 10^5/length in mm^3). This condition factor is used because a standard weight for a fish of a specific length is not needed. However, this condition factor increases with fish length and is not consistent across species so caution should be used when interpreting the results. At the end of the experiment, all fish were individually counted to determine survival. A one-way analysis of variance (ANOVA) indicated that no significant differences in density at the beginning of the experiment existed between dietary treatments. The effect of diet on mean walleye length, weight, growth, and survival on day 10 and 21 of the culture period, was evaluated with ANOVA. The acceptable level for type-I errors was set at $P = 0.05$.

Results

Visual examination of the raceways revealed a portion of the pond-reared fingerlings were accepting both the EPAC and Rangen diets after the first day. However, survival for fingerlings fed the EPAC feed was significantly greater on day 21. Mean survival for fingerlings fed the EPAC feed was 82.4% and ranged from 78.8 to 84.6%. Mean survival for fingerlings fed the Rangen feed was 42.3% and ranged from 31.5 to 51.5%.

On day 10, fingerlings fed the EPAC diet were significantly longer and weighed more. The condition factor was greater for fish fed EPAC than fish fed the Rangen diet (Table 3.2; Figure 3.2). Mean specific growth rate was -2.82% and 1.03% for fish fed the Rangen and EPAC diet, respectively. On day 21, the length and weight of fingerlings fed the EPAC diet were significantly greater than those for fish fed the Rangen diet. The fish condition factor was 0.77 and 0.79 for EPAC and Rangen, respectively. Mean specific growth rates from day 10 to 21 increased to 8.45% and 9.28% for fish fed EPAC and Rangen diets, respectively but were not significantly different. For the 21-day rearing period, mean specific growth rate was significantly greater for fish fed the EPAC diet.

During the first 10 days, survival of fingerlings in both dietary treatments followed similar patterns (Figure 3.3). Mortality of emaciated fish occurred between days 9 and 13. Survival then stabilized for the remainder of the rearing period. Cannibals were observed on day 1 when dead fish were removed, and was assumed to have occurred in the hauling tank during transport. Cannibalism in the raceways was not detected, but may have occurred at a low rate.

Post-transport mortality that occurred in the raceways was probably the result of hauling conditions. Salt was added to the water (0.5%) before hauling to reduce transport stress. However, upon stocking the fingerlings into the raceways, several fish were noted to have cannibalized during transport, suggesting that the fish may have been too crowded in the hauling tank and experienced less than optimal conditions.

Discussion

The northern walleye strain can be successfully feed-trained to EPAC or Rangen feed in an intensive culture environment resulting in comparatively high survival during a 21-day time period. The 78.8 to 84.6% survival recorded for fish fed the EPAC diet was similar to the exceptional 80-90% survival reported by Nagel (1985; 1996). The 31.5 to 51.5% survival recorded for fish fed the Rangen diet was similar to the survival reported by Cheshire and Steel (1972) and Nagel (1976), and is more typical of survival of wild strain progeny (30-50%; Nagel 1996). Based upon growth and survival responses of fish fed the EPAC diet, this diet would be a preferred high quality starter diet for pond-reared fingerlings.

The larger size of the EPAC feed may have played a critical role in its comparative success. Feed sizes of 1 mm or larger are commonly used for fingerlings less than 63.5 mm in total length (Bristow 1996) and fingerlings 998-1,998/kg have been fed a 50:50 blend of 1 and 2 mm feed (Flowers 1996). Our fingerlings were 1,848/kg, so it is possible that the smaller size of the Rangen diet may have reduced its acceptability. Flowers (1996) found that poor feed training resulted from an inappropriately sized 2 mm diet that was too large for small pond fingerlings to consume effectively (fingerling size was not reported). Thus, a diet that is too small could also potentially be a factor in the survival of pond-reared fingerlings as they are introduced to feed. Texture, taste, smell, and color are other variables that affect acceptance but could not be appropriately evaluated.

A portion of the Rangen feed stuck to the belt feeder and formed clumps as the humidity in the culturing room increased, whereas the EPAC feed did not stick to the belt feeder. Routine cleaning procedures took twice as long in raceways containing fish fed the Rangen diet, suggesting lower water stability in comparison to that of the EPAC diet. The majority of EPAC feed slowly fell through the water column after hitting the water surface, whereas a portion of the Rangen feed floated at the water surface until the incoming well water pushed it down through the water column. This difference in physical characteristics of the feeds may have affected access to the diet and the relative success of the feed training process. Nagel (1996) reported that fingerlings from domesticated broodstock swim on their side facing the feeder, whereas fingerlings from a wild broodstock do not exhibit this behavior. Fish swimming on their side would easily locate feed floating on the water surface, whereas wild fish, not engaging in this behavior, may not respond until the feed begins to sink through the water column. Daily observations of feeding behavior revealed that most fish would strike the feed as it slowly fell through the water column. A few fish were observed swimming on their side.

The reported duration of time before mortality of fingerlings first occurs during the feed training process varies. Malison and Held (1996) observed two groups of walleye that were in either poor or good condition after being subject to stressful conditions during transport from the pond to the tank. Most fish in poor condition died during the first 12 days, whereas a small number of fish in good condition died during the first 15 days. Kuipers and Summerfelt (1994) reported that most mortality of fish considered to be in good condition occurred within the first 8 days of training. Similarly, Reinitz and Austin (1980) reported that 71% of the recorded mortality occurred from days 9 to 22 during their intensive culture feeding trial. Walleye in the present study were held inside the hatchery facility at Normandy, TN for a day prior to transport to the Private John Allen NFH. Therefore, most mortality occurred during the first 10 days after fish were harvested from the production pond. Most mortality associated with the feed training process can be expected during the first 8 to 22 days after harvest. Culturists may be able to increase feed acceptance substantially by exercising suitable care to increase the probability that most fish will survive for at least the first 8 days in their new intensive environment.

Condition of the fish prior to harvest and potential stressors such as harvest, transport, and grading are more than likely the cause for variation in time to death among reported mortality rates. Malison and Held (1996) suggested that as a general rule, the better the condition of the fish prior to harvest from the pond, the greater success in adapting them to the new environment and feed. In the present study, the good condition (i.e., not emaciated) of the fingerlings may have been an important factor in achieving a rapid and successful adaptation to formulated feed and the intensive culture conditions.

Most of the mortality for this experiment occurred within the first 10 days and then was minimal for the remainder of the time. Most mortality occurred on either day 1 or day 10. Mortality of fish on day 1 can be attributed to transport conditions. Providing optimal transport conditions should increase survival during the feed training process. Fish that did not accept feed died during the first 10 days from starvation and possibly in conjunction with transport-related stress. Fish that were held for almost 2 days without feed were still successfully feed-trained. Horner (1996) suggested that if feed is not presented to fish immediately, feeding will never commence. The extent of successful feed training after walleye have been held for an extended period of time (> 2 d) in tanks without feed is unknown, but an inverse relationship would probably prevail.

No disease-related mortality was observed during the 21-day rearing period. Horner (1996) suggested that outbreaks of columnaris (*Flavobacterium columnare)* will occur if any delay in feed training occurs. The 0.5% salt baths probably served to prevent any disease-related mortality. During the experiment water temperature was approximately 20°C and results of studies relating growth to temperature during the feed training process have led to recommendations of growing walleye in 20°C water to reduce the incidence of disease even though growth may be greater at a higher temperature (Nickum 1986; Kuipers and Summerfelt 1994). In addition, due to the rapid acceptance of feed, tail biting and other cannibalistic behavior which may increase the incidence of diseases such as columnaris were rarely observed (Horner 1996).

During the experiment, initial growth was relatively slow, and actually negative, for fish fed the Rangen diet. This slow growth is not unusual during the feed training process and was due to poor feed acceptance (Malison and Held 1996). Stocking density in this study (initially 4.0 kg/m^3) was similar to those used by many hatcheries (Bristow 1996; Flowers 1996; Nagel 1996). The substantial increase in growth rates from day 10 to 21 were probably reflective of a decrease in density, due to the death of emaciated fish, combined with successful acceptance of the formulated feed. Although water quality was not monitored, low flush rates (2 Lpm) may have contributed to sub-optimal environmental conditions during the experiment and consequently affected growth. Because oxygen was not injected into the well water prior to entering the hatchery building, aeration of the water by air stones occurred in the raceway. The flush rate could not be increased without causing a corresponding decrease in the levels of dissolved oxygen. In hatcheries where feed-trained fingerlings are regularly produced, a higher flush rate is used to allow complete exchange of the water within an hour or less so that high water quality is assured (Nickum 1986).

Cannibalism was a minor cause of mortality. Kuipers and Summerfelt (1994) reported average cannibalism rates of 4% over a 28-day rearing period. Cheshire and Steel (1972) reported cannibalism rates of 13.2% over a 90-day rearing period. In the present study, cannibals were found when fish were unloaded from the transport tank and were the result of crowded conditions. The exact number of walleye initially stocked into each raceway was not determined, so unaccountable mortality could not be used to estimate cannibalism. However, because of the rapid acceptance of feed, the incidence of cannibalism was probably low. The feeding rate of 10% of the fish's body weight per day probably provided an adequate amount of food that minimized cannibalistic behavior (Cuff 1977). Crowding the fish at one end of the raceway where food was constantly introduced effectively served to quickly train walleye to consume feed.

Conclusion

The results of this study indicate that it is possible to train 45-day-old pond-reared northern walleye fingerlings to a formulated diet even after holding them for nearly 2 days without feed. In the intensive culture environment, the EPAC diet was readily acceptable resulting in rapid feed training and high survival, suggesting that EPAC is a high quality starter diet with high potential for acceptance. Poor diets will extend the period of insufficient feeding from when fish are harvested and then stocked into an intensive environment, and the resulting condition will increase susceptibility to disease (Horner 1996).

Although food is kept abundant during this period of training, training all walleye to accept feed is still difficult. Careful planning and monitoring are needed to ensure success. Identifying variables that influence feed training such as food size and appropriately managing them are also important. Care must be exercised in all phases of walleye production beginning with pond-rearing, a potential first stressor to intensive culture of walleye (Horner 1996). If pond-reared fingerlings are to be transported from one hatchery to another for the feed training process, optimal transport conditions are important to prevent stress that could lead to disease and poor feed acceptance. Additionally, high flush rates during the feed training process should be used to allow complete exchange of the water within an hour or less so that high water quality is assured (Nickum 1986).

Although starter diets are usually more expensive than growout diets, the small amount of food used during the feed training process suggests cost-effectiveness, especially when high feed acceptance is critical to successful production of large numbers of advanced fingerlings. The EPAC diet was composed of 58% crude protein; according to Barrows et al. (1988), only a 51% protein level is needed to produce maximum growth rates in walleye fingerlings. Kuipers and Summerfelt (1994) reported that survival for walleye fingerlings fed diets composed of 41% and 61% protein levels did not differ significantly; however, excess protein was converted to body fat. Protein is the most expensive element of a formulated diet, thus expenses could be reduced by selecting diets with lower percentages of crude protein. An effective formulated diet frees hatcheries from compromising space and spending money for raising baitfish which would be required if fingerlings were not feed-trained (Raisanen 1996). The additional space could be used for stocking more walleye fry. Additionally, feed-trained walleye fingerlings from raceways/tanks can be restocked into a pond, fed a formulated diet, and grown to advanced sizes (> 100 mm) for fall stocking (Nagel 1996; Coyle et al. 1997; see chapter IV).

This successful feed training process establishes practices which could be used to grow advanced sizes of Gulf coast walleye fingerlings. Feed training needs to be tested and developed with Gulf coast walleye fingerlings when sufficient numbers become available to stock at an appropriate density into raceways or smaller experimental tanks. Growth, feed acceptance, and feed conversion may change within a species due to genetic variation among strains (Stinefelt et al. 2004).

These differences may apply to different walleye strains, and would consequently influence diet selection and culture practices.

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Table 3.1 Proximate analyses of the Rangen krill-based and EPAC diets fed to walleye fingerlings during the 21-day time period.

Diet	% Composition (as is)				
	Moisture	Ash	Crude Protein	Acid Hydrolysis Fat	Crude Fiber
Rangen	7.8	10.3	55.2	16.0	1.4
EPAC	6.8	11.1	58.1	13.2	0.4

Table 3.2 Mean production characteristics (± SE) of walleye fingerlings reared in raceways and fed two starter diets for 21 days. Initial mean ± SD total length and weight were 41.8 ± 3.1 mm and 0.54 ± 0.12 g. Within each column, mean values with different superscripts differ significantly.

Diet	10 days post-stocking			21 days post-stocking			Survival (%)
	Length (mm)	Weight (g)	K	Length (mm)	Weight (g)	K	
Rangen	41.0 ± 0.5[a]	0.41 ± 0.02[a]	0.57 ± 0.01	52.1 ± 1.12[a]	1.13 ± 0.07[a]	0.77 ± 0.04	82.4 ± 1.8[a]
EPAC	44.3 ± 0.3[b]	0.60 ± 0.03[b]	0.65 ± 0.03	57.2 ± 0.80[b]	1.52 ± 0.10[b]	0.79 ± 0.02	42.3 ± 5.8[b]

Diet	Growth (%/day)		
	0-10 days	10-21 days	overall
Rangen	-2.82 ± 0.40[a]	9.28 ± 0.33	3.51 ± 0.31[a]
EPAC	1.03 ± 0.72[b]	8.45 ± 0.37	4.92 ± 0.29[b]

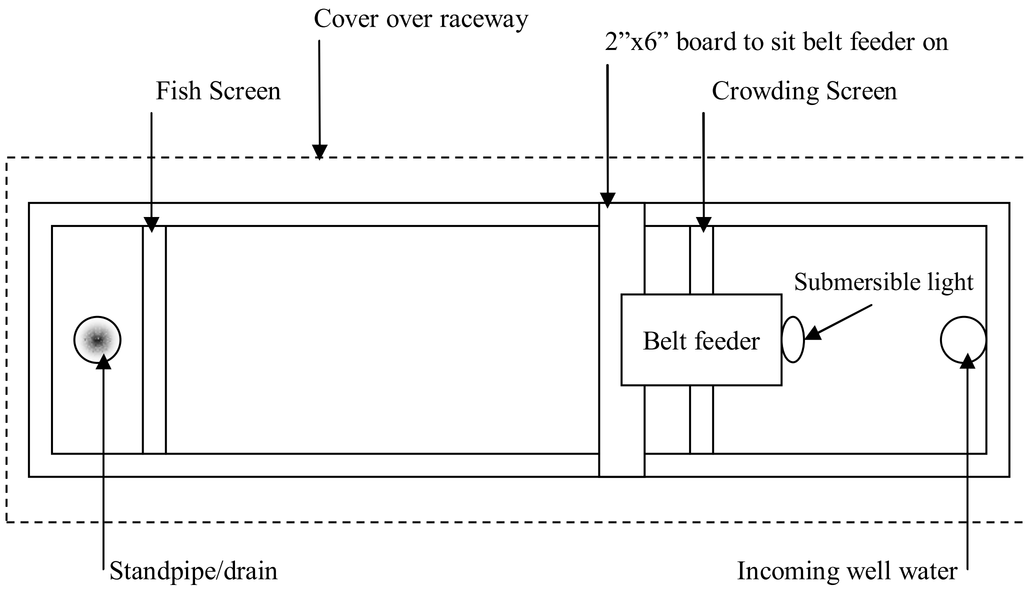

Figure 3.1 Design of raceway used to feed-train pond-reared walleye fingerlings (Top view).

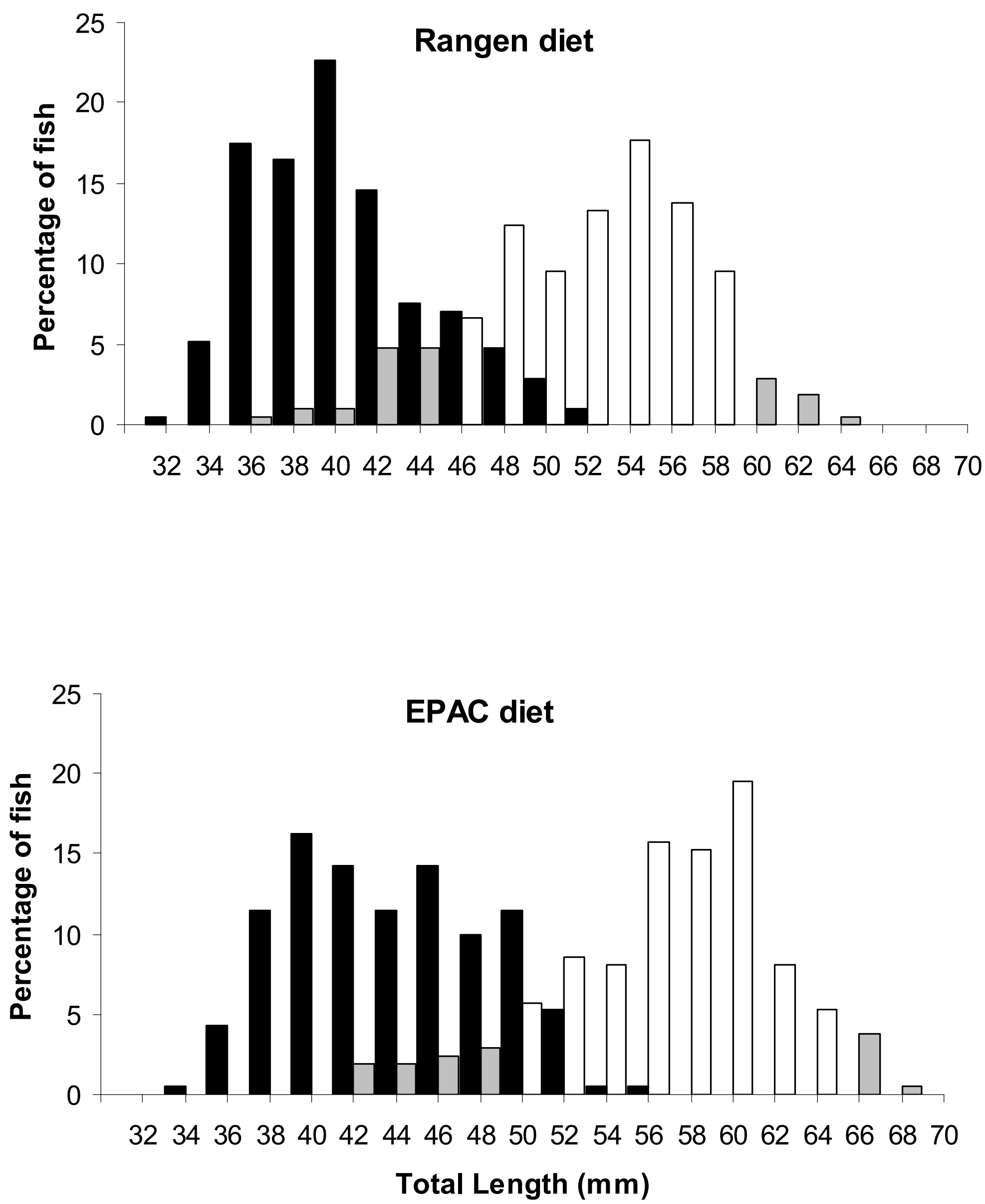

Figure 3.2 Length-frequency distribution of walleye fingerlings fed either a Rangen krill-based diet or an EPAC diet in an intensive culture environment for 21 days. Walleye were sampled 10 (black) and 21 (grey) days post-stocking.

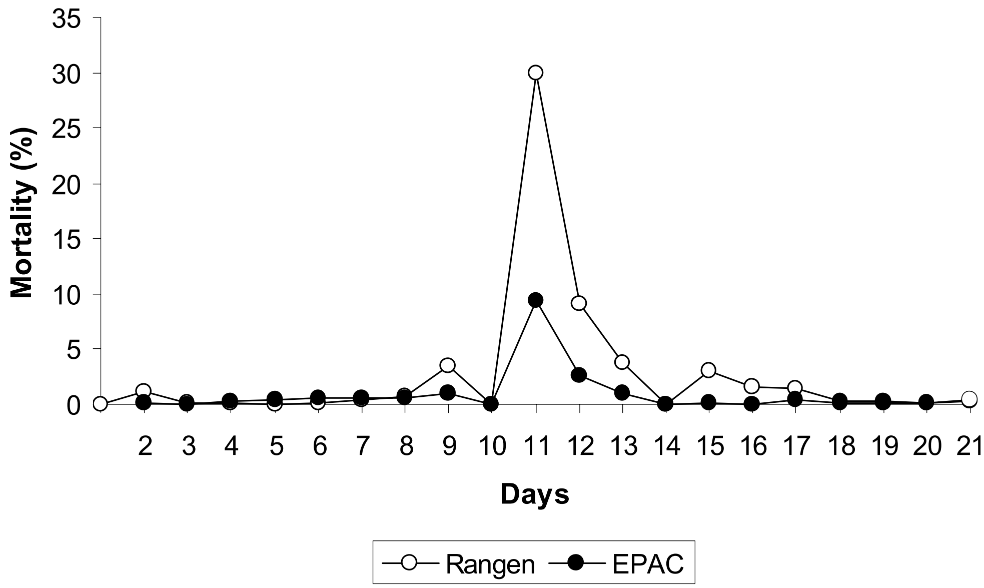

Figure 3.3 Mortality rates of walleye fingerlings in two dietary treatments. Initial mean density was 5,635 fish/m^3 and 6,944 fish/m^3 for fish fed a Rangen and EPAC diet, respectively.

CHAPTER IV

Production of Advanced Walleye Fingerlings Raised in Earthen Ponds on a Formulated Diet

Introduction

To grow advanced walleye fingerlings (> 100 mm), either tank or raceway facilities are used to feed-train fingerlings to a formulated diet. This method involves removal from pond and natural food sources followed by high density confinement where feed is constantly introduced and temperature is controlled (Cheshire and Steele 1972; Nagel 1974; Nagel 1976). This practice usually requires continuous flow of large quantities of water and frequent monitoring of the condition of the walleye. The alternative of producing advanced fingerling sizes of walleye in a pond would be less labor intensive and thereby reduce the cost of production. However, it has been reported that when feed is scattered onto the surface of the pond, walleye fingerlings fail to respond like other cultured species such as channel catfish *Ictalurus punctatus* (Bergerhouse 1996). To compensate for this behavior, pond culture of walleye in cages has proven to be a successful practice to achieve in-pond feed training and growth to advanced fingerling sizes (Bergerhouse 1996; Harder and Summerfelt 1996; Coyle et al. 1997). However, cage culture has shortcomings that are similar to those of tank culture, because localized suboptimal water quality conditions can occur due to high density confinement within the cage area. As a result, production can be less than that achieved in ponds without cages (Mazik and Parker 2001).

Without confinement to a small area in a pond initially, effective in-pond feed training may not be realized. However, if walleye could be attracted and concentrated to an area where feed is being constantly introduced, then feed-trained fingerlings from tanks could be restocked into a pond where feeding on a formulated diet would be resumed and growth to advanced sizes would occur.

Another principal challenge to producing advanced sizes of walleye through pond culture may be chronically high summertime water temperatures that are stressful or lethal. In low latitude temperate climates such as in Mississippi, high summertime temperatures can produce water temperatures that approach the upper thermal tolerance of walleye and therefore negatively impact the potential success of production in ponds. The objective of this study was to assess the feasibility of production of advanced sizes of walleye fingerlings raised in earthen ponds at different stocking densities and fed a formulated diet.

Methods

Due to a lack of sufficient numbers of Gulf coast walleye fingerlings, the management practices to be evaluated were conducted with pond-reared northern strain fingerlings. On 7 May, 2004 fingerlings were obtained from Normandy Fish Hatchery, operated by the Tennessee Wildlife Resources Agency. Broodfish were captured from a wild population, spawned at the hatchery, and 5 d post-hatch fry were then stocked into 0.4 ha drainable lined ponds. At 45 d post-hatch, fingerlings were harvested and transported to the Private John Allen National Fish Hatchery operated by the U. S. Fish and Wildlife Service, Tupelo, MS, to initiate the feed training process. Initial mean (± SD) total length and weight were 41.8 ± 3.1 mm and 0.54 ± 0.12 g.

Fingerlings were feed-trained while held in six 1.5 m^3 (1,514 L) raceways covered with black plastic to eliminate detection of natural light and fluorescent light and movements by hatchery personnel. Fingerlings were stocked into each raceway and confined to one end (4.0 kg/m^3) where feed was constantly introduced by a belt feeder (FIAP Belt feeder, 5 kg, 12 hr). A 5 W submersible light (UL® Underwater Light/ model no. ZIA41003U-10), placed inside a 2.54 cm (diameter) by 15.24 cm (length) polyvinylchloride (PVC) pipe capped at one end, was submersed directly below the belt feeder, approximately 15 cm into the water, and provided a low light intensity in the crowded section of the raceway. Fingerlings were initially fed two different starter diets (krill-based, 55% crude protein, Rangen, Inc., Buhl, ID; EPAC, 58% crude protein, INVE Aquaculture, Inc., Mountain Green, UT) for 21 d, followed by a gradual transition to a growout diet (Aquamax, 45% crude protein, Purina St. Louis, MO) at mixing ratios of 25:75, 50:50, and 75:25 (new:current). Each proportional combination was fed for 2-3 d. Fish were fed 10% of their body weight/d during the feeding of the different treatment diets and the transition period to a growout diet.

On 16 June 2004, after 40 d of feed training, walleye fingerlings were transported from their intensive culture tanks at the hatchery and stocked into five 0.04 ha drainable earthen ponds located at the Eastern Unit of the National Warmwater Aquaculture Center, Mississippi State University. Fish were stocked at two different densities, 12,350/ha and 24,700/ha. There were two ponds (replicates) at 12,350/ha and three ponds (replicates) at 24,700/ha. Before stocking, the mean total number of fish/kg was determined by weighing five samples of 50 fish (332 ± 3 fish/kg). The value obtained was used to determine the appropriate weight of fish to stock to achieve the desired density in each pond. Initial mean ± SD individual total length and weight were 72.5 ± 8.8 mm and 3.0 ± 0.1 g.

Walleye have successfully adapted to consumption of a formulated diet when restricted from eating natural foods. Therefore, to minimize the incidence (or availability) of natural food items, the ponds were not filled until 24 h prior to stocking of the fingerlings. Well water was used to fill the ponds and Aquashade® (Aquashade®, Applied Biochemists, Germantown, WI) was added to the water at the level recommended by the manufacturer to reduce light intensity. To attract fish and concentrate them in an area where feed would be constantly introduced, a 5 W submersible light was placed at a depth of approximately 15 cm directly below a belt feeder to provide a low light intensity at that location in the pond. Use of the light was founded upon the belief that walleye may have been trained or conditioned to associate food with light in the raceway culture and thus would concentrate under the belt feeder in the pond. The belt feeder was positioned at the deep end of the pond and fastened at the end of a platform that extended out over the water approximately

1.52 m perpendicular to the pond levee. During a 20-hour period, the belt feeder constantly introduced food to a small area where walleye would presumably locate the feed easily. In addition to this localized source of food distributed by the belt feeder, feed was scattered onto the surface of the pond.

During the 125 d of pond culture, the fish were fed 5% of their body weight for the first 44 d and then 10% of their body weight for the remainder of the experiment with the intention of providing a higher food density to increase the incidence of encounter. The belt feeder distributed 75% of the feed and the remaining feed was manually distributed onto the surface of the pond. The total amount of manually distributed feed was divided between late afternoon and early morning feedings. For the first three days after stocking of the fingerlings, feed was hand fed exclusively throughout the surface of the pond until belt feeders became operational.

Specific growth rates in percent per day (Westers 2001) were calculated based upon the mean individual wet weight derived from the five separate bulk weights of 50 fish at the beginning and end of a growth period and expressed as percentage per day.

$$\text{Specific growth rate} = \frac{(\ln FinalWeight - \ln InitialWeight)}{n(days)} \times 100$$

On day 40, samples of fish were collected by seine from each pond. Total length (mm) and weight (g) were determined for 25 individual fish and any remaining fish were counted and bulk weighed. At harvest, total individual length and weight were recorded for either 20% of the population or a minimum of 25 fish from each pond.

All fish were counted to determine survival. Also, a sample of 15 fish collected from each pond was immediately placed on ice and later preserved in 10% formalin. These fish were eventually dissected, and stomach contents were removed from the digestive tract by flushing into a petri dish. Identified food items for fish in different length groups were separated into the following categories: zooplankton, macro-invertebrates, crayfish, and walleye. Frequency of occurrence (number of fish having a specific taxon divided by total number of fish) was used to describe composition of the diet.

To verify if the formulated feed was consumed by walleye, an orange fluorescent pigment (Day-Glo Flourescent Pigment, blaze orange, Day-Glo Corporation, Cleveland, Ohio) was used as a marker to detect consumed feed in the digestive tract (Morris and D'Abramo 1990). The pigment and feed were mixed in a 1:10 ratio (weight:weight) for one hour using a motor-driven rotation apparatus to allow for the lipophilic marker to adhere throughout the feed. On day 115, the marked feed was then fed both manually and from a belt feeder onto the water surface in two randomly selected ponds, representing different densities. Within 2 hours after feeding, 10 fish from each pond were collected by seining, placed on ice, and transported to a laboratory. The marked feed was again fed just prior to harvest and a sample of 8 to 11 fish was obtained from each pond for stomach analysis within 2 hours. Digestive tracts were removed from the fish and placed under ultraviolet (366-nm) light to detect whether the fluorescent pigment was present. Stomach contents were then flushed into a petri dish and examined under a dissecting microscope and ultraviolet light.

Aeration was provided by in-pond aerators when dissolved oxygen fell below 5 mg/L. Temperature data loggers (Hobo® H8, Onset Computer Corporation, Bourne, MA), placed approximately 0.3 m from the bottom of each pond, recorded water temperature every 30 min during the experiment. Dissolved oxygen and water temperature were measured approximately 0.15 m below the surface of each pond at 0400, 0700, 1300, and 2200 with a digital oxygen meter (Model 550A, YSI Incorporated, Yellow Springs, Ohio).

The effect of density on mean length, weight, biomass, and percentage survival of walleye at harvest in the five ponds was assessed using one-way analysis of variance (ANOVA). The acceptable level for all type-I errors was set at $P < 0.05$. Data from day 40 and harvest were analyzed using individual measurements to account for differences in sample sizes and unequal variances.

The magnitude of variation in length and weight of fish sampled from each pond on day 40 (N = 25/pond) and at harvest (N = 25 or 20%/pond) was expressed as the coefficient of variation (100 x SD/mean). Coefficient of variation was evaluated in relation to stocking density and final density. Simple linear correlation was used to identify any significant relationships between mean length, mean weight, percentage survival, or production at harvest and stocking density.

Results

Observations during the evening revealed substantial numbers of fish were attracted to the submersible light and were consuming the formulated diet provided via the belt feeders during the initial 40 days. Fish swam in circles below the belt feeder and around the submersible light and struck the feed as it hit the water surface. After 40 days, this feeding behavior was rarely observed so the submersible lights were turned off. Initially, feed that was scattered onto the surface of the pond was not observed to be consumed by walleye. However, later in the study, walleye were observed to consume a portion of the feed several minutes after it was scattered onto the pond surface. Once food was scattered across the pond surface, several minutes passed before the walleye initiated feeding. On windy days, most of the feed scattered across the pond surface was quickly blown against a levee and accumulated there, possibly reducing access. However, the belt feeder frequently introduced feed, so it was assumed that walleye still had the opportunity to consume an adequate amount of feed daily. Feeding activities were rarely observed during mid-day.

Mean daily water temperature for all ponds did not deviate from each other by more than 1°C. The mean daily water temperature during the 125 d grow out period was 25.4°C (range 14.8-32.8°C) near the bottom and 25.5°C (range 15.0-35.0°C) at the surface. Table 4.1 presents the number of days either below or between selected water temperatures. During June, mean daily bottom and surface water temperatures were 27.0°C and 29.1°C, respectively. During July, mean water temperature near the bottom increased to 27.8°C, and by October had decreased to 20.0°C. Mean surface water temperature was 29.1°C in July and by October had decreased to 18.7°C (Figures 4.1 and 4.2).

Mean dissolved oxygen during the experiment was 7.9 mg/L (range 4.9-19.8 mg/L). A tractor-driven paddlewheel was used between 2200 and 0600 hours 60.7%

of the days in June; thereafter, use ranged from 0.5% to 16.6% for each month. The number of times that the paddlewheel was used did not differ significantly between stocking densities.

On day 40, the mean total length, weight, and growth rate of fish in the high density ponds were significantly greater than those of fish from the low density treatments. Mean ± SE individual weight, total length, and specific growth rate of fish stocked at the greater density were 12.6 ± 1.0 g, 112.1 ± 2.8 mm, and 2.86 ± 0.2%, respectively. Mean ± SE individual weight, total length, and specific growth rate of fish stocked at the lesser density was 5.3 ± 0.6 g, 91.1 ± 1.8 mm, and 1.39 ± 0.1%, respectively. The length-frequency distribution was bimodal for fish in both stocking density treatments (Figures 4.3 and 4.4).

At harvest, mean total length ranged from 182.2 to 246.2 mm in the five experimental ponds and length-frequency distributions were bimodal for both stocking densities (Table 4.2; Figures 4.5 and 4.6). Total length, weight, and growth rates did not differ significantly between stocking densities (Figures 4.7 and 4.8). Mean ± SE specific growth rate of fish harvested (125 d) from all five ponds was 2.69 ± 0.05%.

On day 40, the mean coefficients of variation for length and weight were 17.7% (range 6.7-22.9%) and 69.1% (range 24.3-107.8%) for all five ponds. This measure of variation in length and weight was unrelated to stocking density. At harvest, the mean coefficients of variation for length and weight were 21.7% (range 9.0-34.6%) and 48.4% (range 22.9-75.7%) for fish in the five ponds. While unrelated to stocking density, the levels of length (r = -0.94) and weight variation (r = -0.92; Figure 4.9) were related to final density at harvest.

Percentage survival was low in all ponds and ranged from 7.3% to 22.0% (Figure 4.10). Percentage survival did not differ significantly between density treatments. Percentage survival was not correlated with variation in either length or weight at harvest. Mean ± SE production for high and low density ponds was 484.8 ± 183.7 kg/ha and 174.4 ± 30.1 kg/ha, respectively. Although production was greatest in two of the high density replicate ponds, a relationship between production and stocking density was not observed because of the overlap of production values obtained for high and low density ponds (Figure 4.11).

Stomach contents of 162 walleye from all ponds were examined. During the 125 d growout period, macro-invertebrates (46.9%) were found more often than formulated feed (32.7%). Zooplankton and macro-invertebrates were found more often on day 40 than formulated feed, whereas only one cannibal was found (Figure 4.12). On day 125, the proportional composition of the walleye stomachs was similarly divided among formulated feed (38.4%), crayfish (37%), and macro-invertebrates (32.9%). As the total fish length increased, presence of formulated feed in the stomach increased substantially.

On day 40, formulated feed appeared at the greatest frequency in stomachs of fish from the high density treatment ponds (Figure 4.13). Macro-invertebrates occurred more often than formulated feed in stomachs of fish from low density ponds. For both treatment densities, fish < 95 mm total length did not have feed in their stomachs. Macro-invertebrates and zooplankton occurred more often in the stomach of fish < 95 mm total length (Figure 4.14). In contrast, formulated feed was present in 55% of the fish stomachs for all fish > 95 mm total length (Figure 4.15).

Pooling of stomach content data collected for days 115 and 125 revealed that formulated feed occurred most often (53%) in fish from high density treatment ponds, followed by crayfish (50%) and macro-invertebrates (24%) whereas, macro-invertebrates occurred most often (41%) in fish from low density treatment ponds followed by formulated feed (26%). Crayfish (43%) and macro-invertebrates (57%) were detected most frequently in fish < 200 mm. Formulated feed (78%) and crayfish (31%) were present most frequently in fish ≥ 200 mm while macro-invertebrates (8%) were rarely found (Figure 4.16).

Discussion

Feed-trained walleye fingerlings restocked into small earthen ponds in Mississippi grew to advanced sizes and consumed a formulated diet, but survival was poor and production was unreliable. No published results about pond production of feed-trained walleye exist, except for the study of Nagel (1996). During a 20-year domestication process, he restocked feed-trained fingerlings into 0.04 ha ponds and successfully grew them by manually feeding them a floating trout chow diet. In contrast, Coyle et al. (1997) investigated semi-intensive methods for pond culture of feed-trained fingerlings. They stocked pond-reared fingerlings (938/m^3) into cages suspended in 0.04 ha ponds and trained them to a formulated diet. After being feed-trained, fingerlings were restocked (mean total length 140 mm; 18,525/ha) into 0.04

ha ponds. For 2 weeks, the fish were confined to approximately 20% of the pond area with a small mesh net to maintain feeding by crowding the fish while also limiting natural food items. Then, they released the fish to the entire pond. At harvest, mean survival was 67%. Their study demonstrated that an initial high density confinement combined with an overall high density in the pond may improve survival and result in more predictable yields. The acceptance of feed by walleye in a pond, and the apparent enhanced consumption under high density conditions suggest that with some improvements to these management practices, production will greatly exceed that realized from intensive culture in tanks and cages.

The principal challenge to successful walleye pond culture in Mississippi is high summertime air temperatures that may produce water temperatures that are stressful and may lead to mortality. For several days during the culture period, water temperatures near the surface exceeded 32°C and climbed briefly to 35°C, but walleye were believed to have taken refuge near the bottom where temperatures remained well below their upper thermal tolerance of 35°C (Peterson 1993). Whether mortality occurred during the period when surface water temperatures in the ponds were briefly at 35°C is unknown, but no dead fish were observed during this period of time. Peterson (1993) reported that both Iowa and Gulf coast walleye were able to tolerate water temperatures of at least 31°C, and lethal temperatures for both strains were determined to be approximately 35°C under laboratory conditions. Wilkens (see chapter II) used the same strain of northern walleye used in this study and evaluated their thermal tolerance in the laboratory. Walleye were first acclimated from 24°C to test temperatures of 33°C, 34°C, and 35°C (temperature increased at

1°C/day) and held at these temperatures until mortality of the entire population occurred (day 35, 9 and 1, respectively). Based upon these results, the highest maximum pond temperatures that occurred near the pond bottom and surface during this experiment were of insufficient duration to be lethal to walleye. Results from laboratory and field work suggest that northern strain walleye are capable of surviving an extended period of time when water temperatures gradually increase to and remain at thermal tolerance limits during the summer. However, there are some limitations to these laboratory results. Fish in the laboratory were held under constant conditions with little change in temperature (± 0.4°C SD). Fish in the ponds were exposed to temperatures that changed between 2 and 7°C per day. Clapp et al. (1997) assessed the relationship between temperature stress and mortality of walleye fingerlings and reported that large fingerlings (mean total length 95 mm) acclimated to 20°C could survive a temperature increase change of 11°C (temperature increased at 1.5°C/min) but that survival decreased rapidly when increasing temperature changes were greater that 11°C. Fish in the ponds were not exposed to this type of temperature change so it is presumed that the daily changes experienced by the fish may not have been lethal. Whether or not consecutive days of changing water temperatures and the magnitude of those changes have a chronic affect on survival has not been determined.

High water temperatures can result in slower growth and poorer condition (Hokanson and Koenst 1986; Kocovsky and Carline 2001). Growth of northern walleye has been reported to occur at temperatures ranging from approximately 15°C to 30°C with maximum growth occurring at temperatures ranging from 20°C to 28°C under laboratory conditions (Kelso 1972; Hokanson 1977; Hokanson and Koenst 1986). Hokanson and Koenst (1986) reported that growth rates achieved between 22°C and 28°C attained at least 80% of the maximum growth achieved at 26°C for northern strain walleye under laboratory conditions. At temperatures above 28°C, growth is substantially reduced, and may be the result of increasing physiological demands and reduced food consumption associated with higher water temperatures. In this study, water temperatures near the surface and bottom ranged between 22°C and 28°C for 78 and 83 days, respectively. Water temperatures near the surface and bottom exceeded 28°C for 40 and 22 days, respectively and may have negatively impacted growth and overall production. Over 90% of the water temperatures were greater than 20°C suggesting that a growing season could potentially extend from at least June to October.

Stocking density is an important determinant of feeding rates, survival, and growth in ponds and in intensive culture environments (Fox and Flowers 1990; Kuipers and Summerfelt 1994). However, the best stocking densities for feed-trained walleye fingerlings restocked into ponds for growout has yet to be determined. Advanced fingerlings have been successfully raised in ponds through the summer by restocking small fingerlings (< 100 mm) at 24,700/ha to 37,050/ha and feeding them minnows (Raisanen 1996). Culture practices used for striped bass closely follow those of walleye culture up to the point of advanced fingerling production where methods begin to diverge. However, it is useful to compare these two species because striped bass have been successfully trained to accept feed under similar intensive culture conditions, therefore methods used to grow advanced feed-trained striped bass in ponds may provide helpful insight for pond culture of feed-trained walleye. Stocking densities used for production of advanced striped bass (< 100 mm) range from 9,880 to 247,000/ha and averaged 54,340/ha during 1986-1988 (Smith et al. 1990). Jenkins et al. (1989) reported that stocking densities of 24,700-61,750/ha of striped bass resulted in a more uniform size and better survival. Lower stocking densities (12,350/ha) resulted in a greater size variation, cannibalism, and lower survival (Smith et al. 1990). Coyle et al. (1997) stocked walleye fingerlings at one density (18,525/ha) that yielded consistent survival under semi-intensive culture methods.

In the present study, the length, weight, and survival of fish from the high density replicate ponds were greater, but the difference was not significant. The lack of difference between stocking densities may be due to high turbidity in one of the high density replicate ponds that had the lowest survival. Turbid water may not have adversely affected the ability to detect feed, but probably minimized primary production of food, thus limiting energy flow to higher trophic levels and fish production in the pond. Bimodal length distributions were apparent on day 40 and at harvest. The observation that fish > 95 mm in total length were the only fish that had formulated feed in their stomach on day 40 suggests that a portion of the population of fish quickly adapted to consumption of the feed near the submersible light and grew comparatively faster, thereby producing the bimodal size distribution for day 40 and at harvest. Once the size differential was established, larger fish may have cannibalized the smaller fish, resulting in poor survival. In fact, only a small percentage of fish in the small category (< 100 mm) remained at harvest for both densities suggesting that the stocking densities used in this experiment may have been too low (Smith et al. 1990). An inverse relationship between size variation and final density was observed in this study.

The feeding strategy used in this study may have created a level of competition that resulted in the poor survival and variation in size. During the experiment, fish were daily fed 5% and 10% of their body weight, rates that were more than likely in excess (Kuipers and Summerfelt 1994; Nagel 1996) due to the lower than anticipated survival. A high feeding rate strategy in a localized area was designed to provide maximum opportunity for the fish to encounter feed, become accustomed to feed in the pond, and potentially reduce competition. During the first 40 days, walleye were observed at night swimming around the submersible light and consuming feed near the belt feeder; however, no walleye were observed to consume the feed provided manually to other areas of the pond during this time. Because the belt feeder only introduced feed into a localized area, intraspecific competition may have led to dominant fish that consumed most of the feed and, in turn, may have fed on other emaciated fish that failed to consume the feed (Keast and Eadie 1985). Bulkowski and Meade (1983) stated that walleye exhibit negative phototaxis after reaching a total length of 25-40 mm. Therefore, the observation of walleye feeding around the light suggests that a portion of walleye consumed feed in conjunction with the attraction to light. Those fish that made an association of food with light grew faster, became dominant, then ate feed distributed in other areas of the pond and composed the upper end of the bimodal length distribution. This presumed advantage is supported by the higher frequency of formulated feed in the digestive tract of larger fish.

Fish were not observed to consume the manually distributed feed until later in the experiment, suggesting that walleye exclusively obtained feed from the belt feeder during most of the growout period. Nagel (1996) successfully maintained a domesticated walleye broodstock by just manual feeding of trout chow during crepuscular periods. Our fish successfully accepted the manually distributed feed later in the study when they were of larger size (> 200 mm) suggesting that once walleye are trained to accept the diet in the pond they will readily consume a manually distributed feed. Most walleye (140 mm) confined for a short period successfully consumed a formulated feed when fed 2.0% body weight ration at 0700 hours (Coyle et al. 1997). In the present study, it is presumed that the submersible light and belt feeder only served to concentrate a portion of the population. To overcome this drawback, methods that will concentrate all of the population during the initial days after stocking would appear to be an effective strategy to achieve initial feeding and increase survival considerably.

The observation of walleye feeding behavior is supported by Bergerhouse (1996) who reported that walleye do not actively respond like channel catfish when feed is scattered onto the surface of the pond. However, as the duration of the experiment increased, walleye did consume the feed that remained floating. This knowledge may prove beneficial for culturists who desire to estimate feeding rates and monitor fish performance by visual observation of the fish feeding near the surface.

The presence of macro-invertebrates and crayfish in the stomachs of feed-trained fish emphasizes the importance and attractiveness of this food source and suggests that feed served as a supplemental source of nutrition. On day 40, fish that were > 95 mm total length were the only fish consuming feed and proportionately more fish in this size group were consuming feed in the high density ponds. These observations suggest that from day 1 to day 40, walleye in high density treatment ponds encountered the feed more frequently, possibly reflecting a relationship between a higher density of fish and feed encounter. An alternative explanation may be that natural foods became limited sooner in the high density ponds thus fish began accepting the formulated feed.

Conclusion

The climate of Mississippi appears to be suitable for pond culture of walleye fingerlings to advanced sizes. The climate provides acceptable growing conditions throughout most of summer and fall. The majority of water temperatures were between 22°C and 28°C near the pond bottom and surface (62.4 to 66.4%). These temperatures are within a range that produces maximum growth (Hokanson and Koenst 1986). However, temperatures that approach and remain near the thermal tolerance limits of walleye can occur and the effect may be more severe from year to year. Nonetheless, contrary to Peterson (1993), who reported empirical evidence that northern walleye maintained in Mississippi ponds usually do not survive high summer temperatures, northern strain walleye from Tennessee can survive high water temperatures during the summer growout in small earthen ponds. The juvenile phase in the life cycle of percids may be the most thermally tolerant (Hokanson 1977).

Although success in adapting walleye to a formulated diet in ponds was achieved in this experiment, the pond culture methods used did not yield the desired high survival and corresponding predictable production. Based on the results reported by Coyle et al. (1997) and those from this study, the first month (1 to 30 d) of pond feeding probably is the most critical time to achieve feed acceptance by the majority of fish for the remainder of the culture period. This time period is similar to that used to feed train pond-reared walleye fingerlings in tanks. Following the management protocol of the study of Coyle et al. (1997), short-term (1 to 30 d) confinement of the fish to a small percentage of the total pond area to achieve the high density culture used for pond-reared fingerlings in tanks during the feed training process may be critical. Concentration to a small area would include an effort to reduce availability of natural food items to ensure that most fish are accepting the feed and possibly avoid significant differential growth before release from confinement. Additionally, such an approach allows for easier removal of cannibals and grading, if needed. However, an investigation of greater stocking densities without confinement, such as those used for the culture of advanced striped bass fingerlings, may provide evidence that the semi-intensive or intensive culture approach is not required for walleye.

A feeding strategy that maximizes walleye growth through high frequency encounter of the feed needs to be developed. The design of this study included overfeeding in a localized area during the entire experiment, but a high level of acceptance was still not realized suggesting that the submersible light was not effective in attracting all the population to encounter and consume the feed. If fish are confined for a short period of time after restocking, then a belt feeder might be useful to ensure continuous availability of food. If fish are not confined, then a combination of feeding methods may be necessary to maximize the opportunity for fish to consume the feed. An automatic feeder that would spread food over a large area at timed intervals (e.g., every 15 min) combined with manual distribution of a large portion of the daily ration at crepuscular periods might be an effective management strategy. Jenkins et al. (1989) state that high frequency feedings of striped bass increase survival and production, and reduce the size variation among fish. The feeding rate for fish that are confined for a short period of time in the pond may be $< 10\%$ of body weight per day, whereas fish in an open pond may need to be fed at a daily rate of $> 10\%$ body weight, at least temporarily, to encourage encounter and acceptance of the feed.

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Table 4.1 Range of the number of days at different water temperatures measured approximately 1 ft from the bottom and near the surface in all experimental rearing ponds from June 16 to October 18, 2004 (125 d).

	Location of temperature reading	
Measurement	Bottom	Surface
34°C to 35°C	0	1
33°C to 34°C	0	3-4
32°C to 33°C	0-1	5
31°C to 32°C	0-2	5-9
30°C to 31°C	1-4	7-12
25°C to 30°C	72-82	64-68
20°C to 25°C	32-43	30-35
Less than 20°C	4-7	4-6

Table 4.2 Mean production characteristics (ranges in parentheses) for walleye fingerlings harvested from experimental rearing ponds after 125 d.

	Fish/ha (number of ponds)	
Measurement	24,700 (3)	12,350 (2)
Total length (mm)	238 (96-286)	214.1 (89-297)
Weight (g)	117.7 (4.9-184.5)	100.9 (4.6-232.9)
Final density (number/ha)	4,002 (1,803-5,434)	1,952 (1,828-2,075)
Percent survival	16.2 (7.3-22)	15.8 (14.8-16.8)

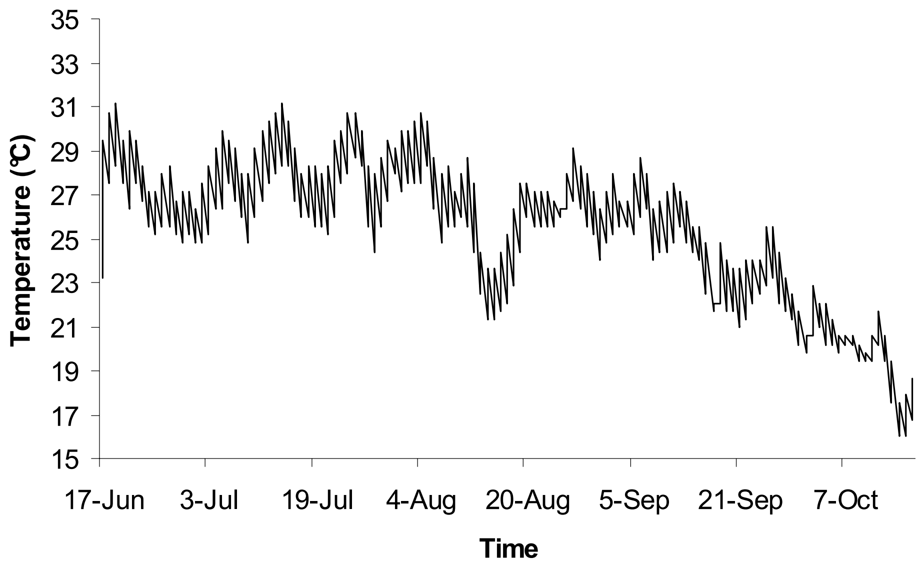

Figure 4.1 Pond temperature recorded near the bottom at 30 min intervals with a Hobo temperature logger during the 125 d growout period in 2004.

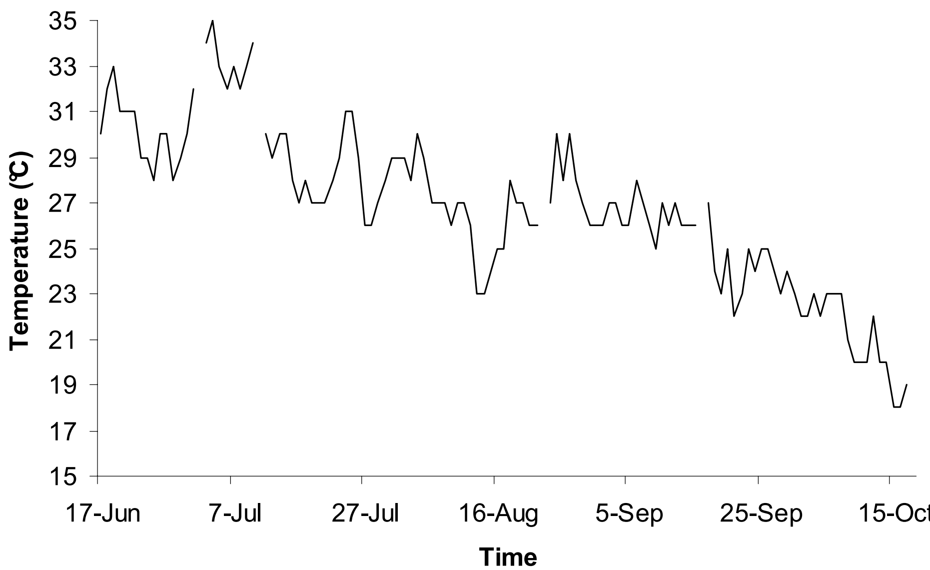

Figure 4.2 Pond temperature recorded near the surface at 1300 hours with a digital temperature meter during the 125 d growout period in 2004.

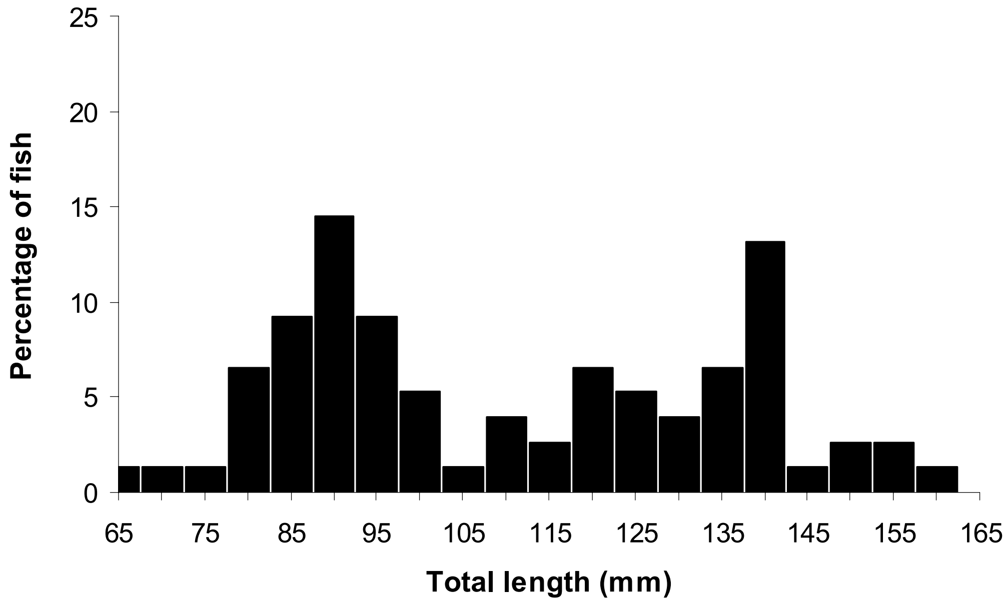

Figure 4.3 Length-frequency distribution of walleye fingerlings sampled on day 40 post stocking from experimental rearing ponds initially stocked at 24,700 fish/ha (N = 76).

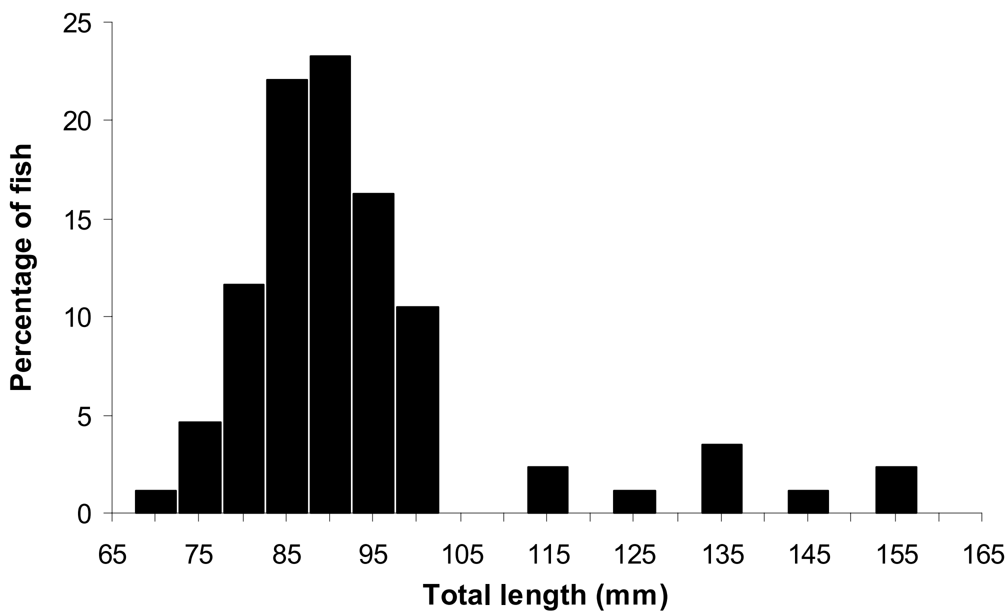

Figure 4.4 Length-frequency distribution of walleye fingerlings sampled on day 40 post stocking from experimental rearing ponds initially stocked at 12,350 fish/ha (N = 86).

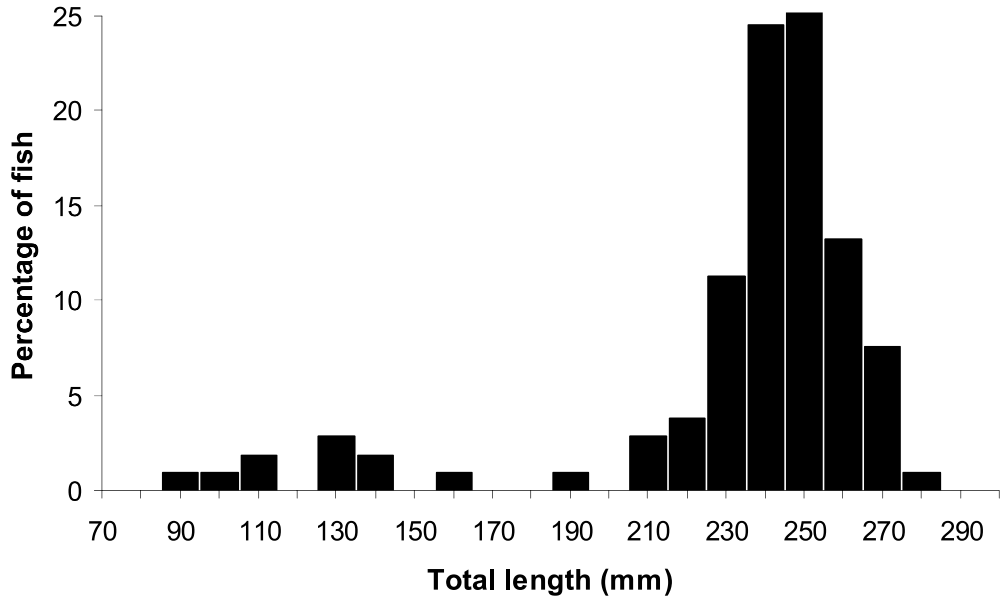

Figure 4.5 Length-frequency distribution of walleye fingerlings sampled on day 125 post stocking from experimental rearing ponds initially stocked at 24,700 fish/ha (N = 106).

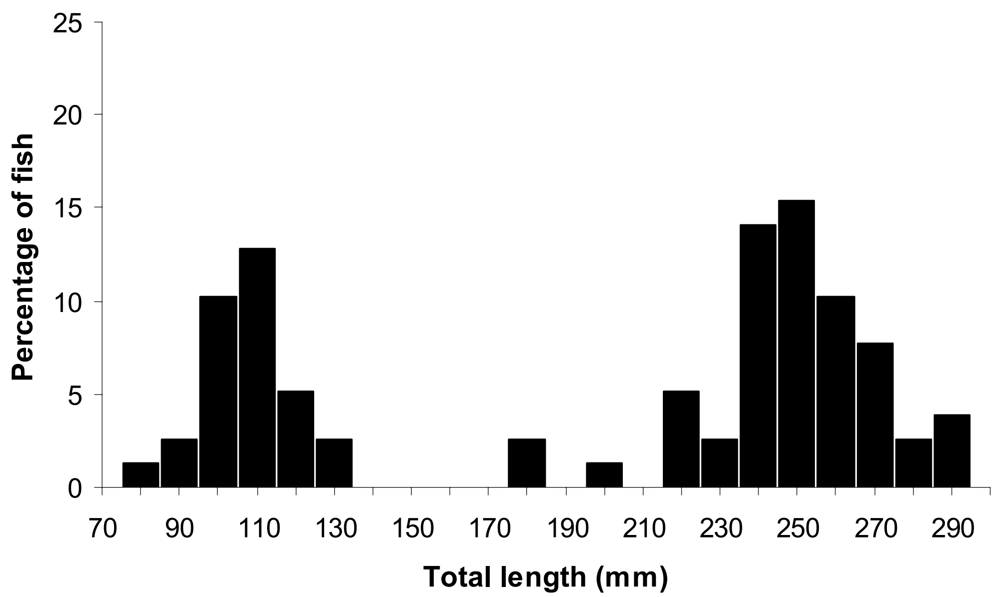

Figure 4.6 Length-frequency distribution of walleye fingerlings sampled on day 125 post stocking from experimental rearing ponds initially stocked at 12,350 fish/ha (N = 78).

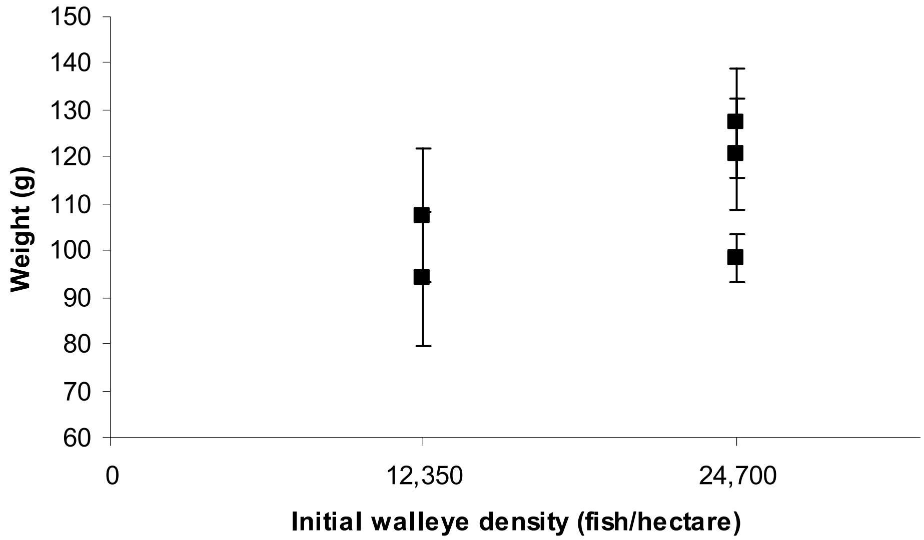

Figure 4.7 Relationship between initial stocking density of walleye fingerlings and mean ± SE weight (standard error y bars) at harvest in experimental rearing ponds.

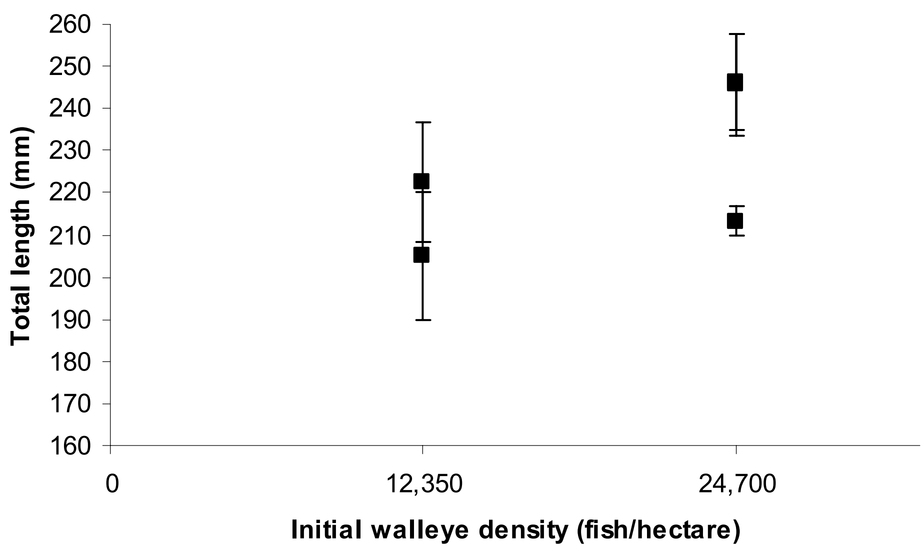

Figure 4.8 Relationship between initial stocking density of walleye fingerlings and mean ± SE total length (standard error y bars) at harvest in experimental rearing ponds.

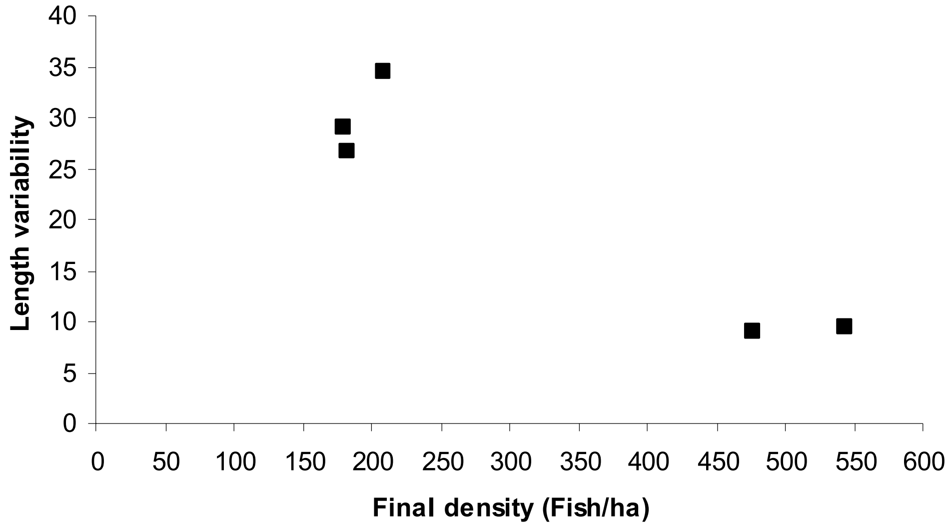

Figure 4.9 Relationship between initial stocking density and the coefficient of variation of length at harvest.

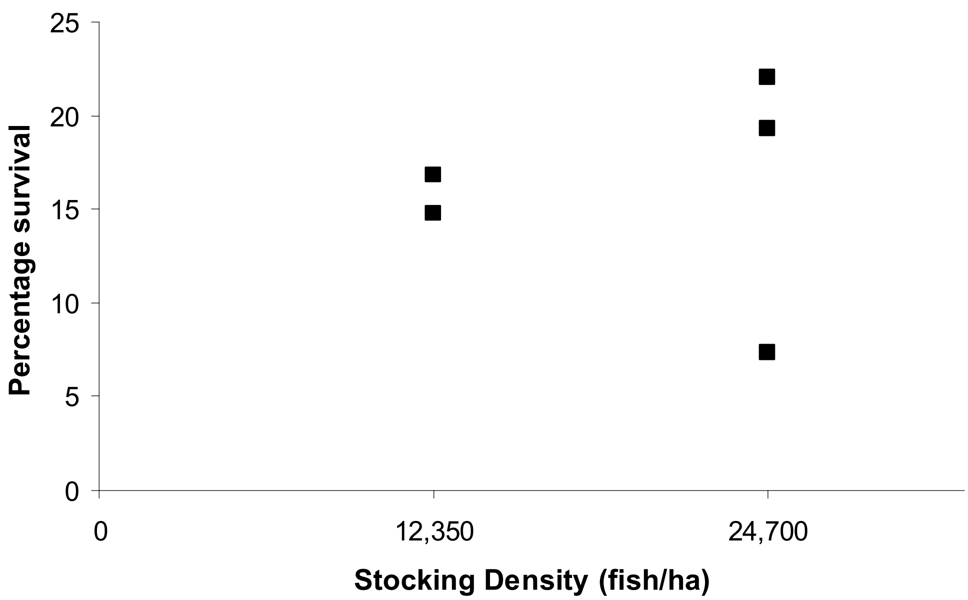

Figure 4.10 Relationship between initial walleye density and percentage survival.

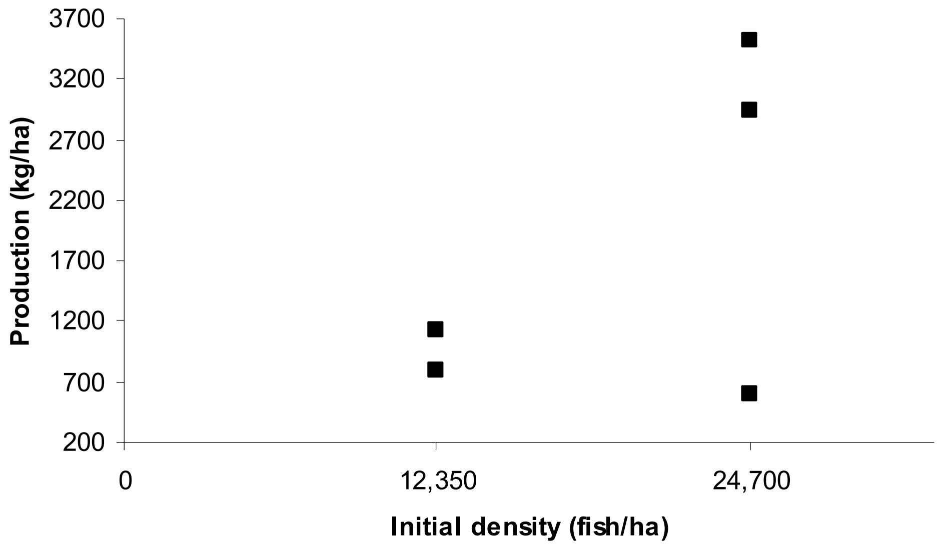

Figure 4.11 Relationship between initial stocking density of walleye and yield at harvest.

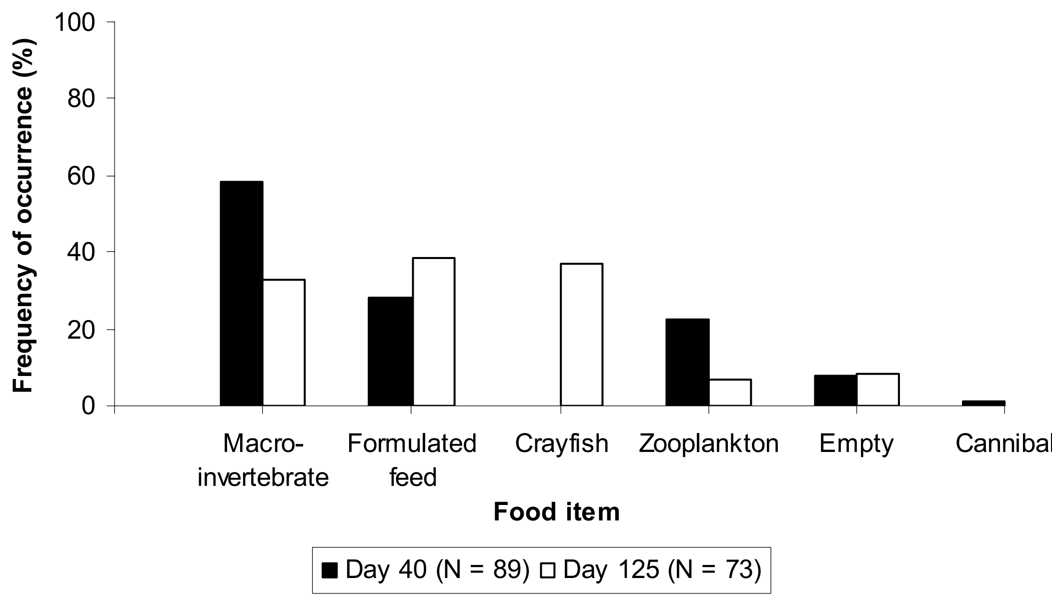

Figure 4.12 Stomach contents of walleye for day 40 and days 115 + 125 post stocking. Fish from both density treatments were pooled.

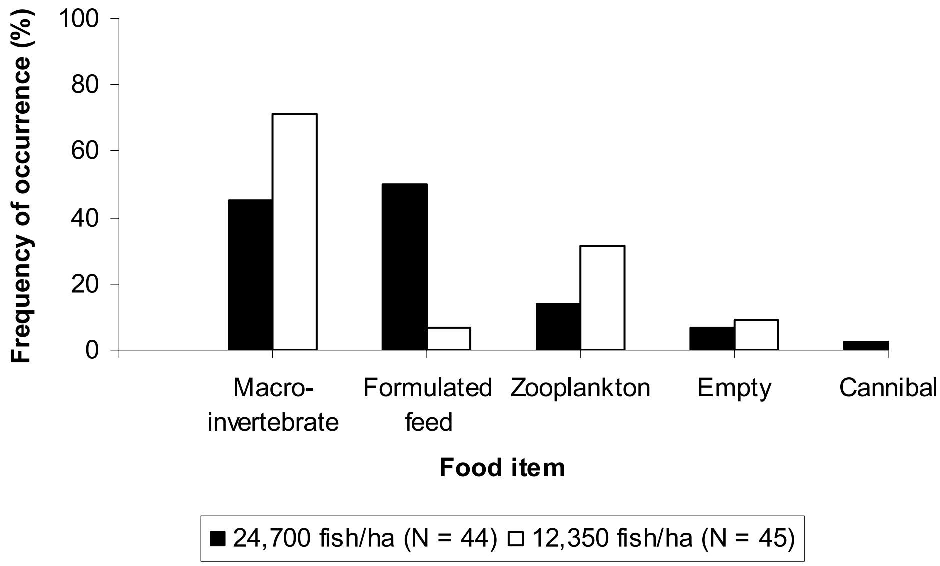

Figure 4.13 Stomach contents of walleye for day 40 post stocking.

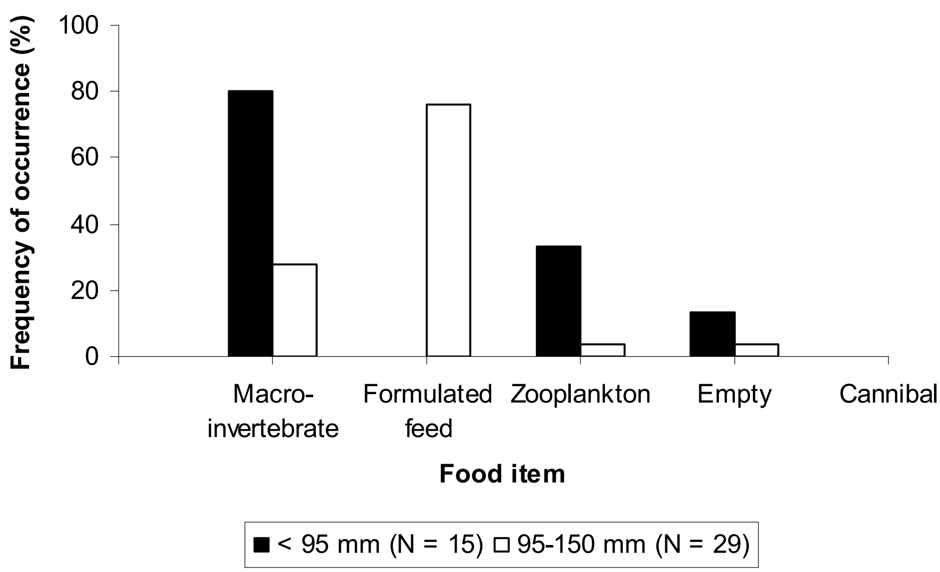

Figure 4.14 Stomach contents of walleye fingerlings sampled day 40 post stocking from experimental rearing ponds initially stocked at 24,700 fish/ha.

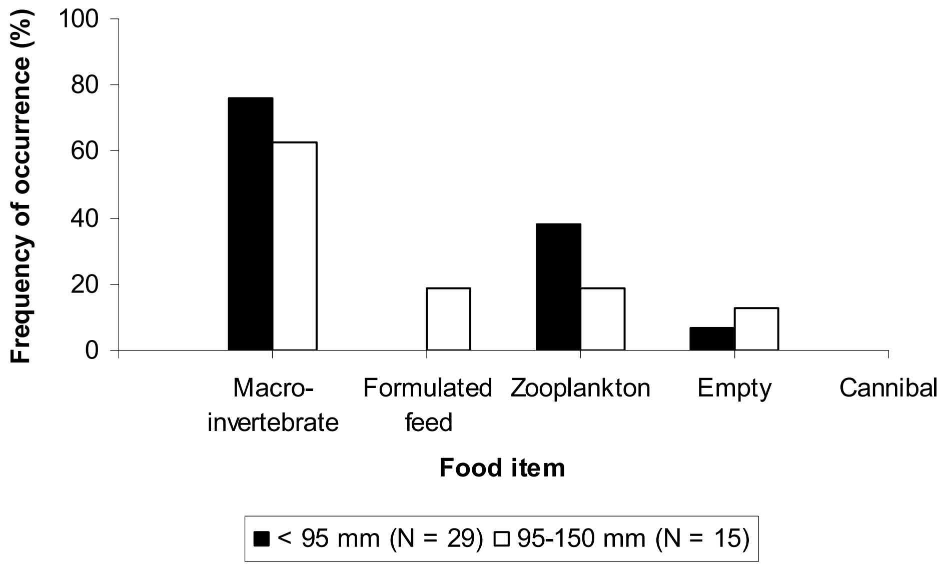

Figure 4.15 Stomach contents of walleye fingerlings sampled day 40 post stocking from experimental rearing ponds initially stocked at 12,350 fish/ha.

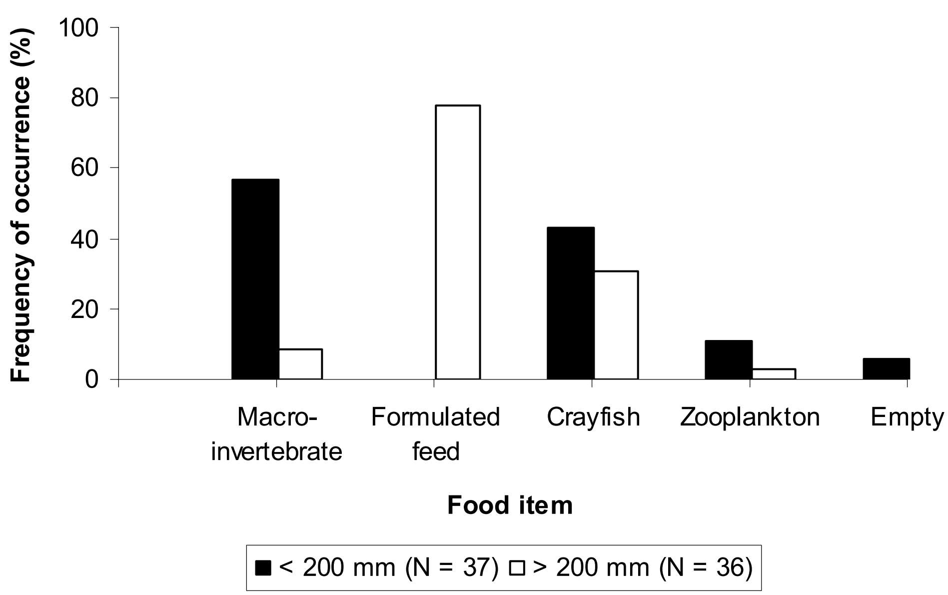

Figure 4.16 Stomach contents of walleye fingerlings of different length categories sampled day 115 and 125 (combined) post stocking. Fish from both density treatments were pooled.

CHAPTER V

Management Implications

The climate of Mississippi appears to be suitable for pond culture of walleye fingerlings to advanced sizes, providing acceptable growing conditions throughout most of summer and fall. During the culture period, over 90% of the water temperatures exceeded 20°C, well above temperatures required for growth of walleye. Walleye fingerlings can apparently survive water temperatures up to 33ºC for as long as 35 days under laboratory conditions. However, survival decreases rapidly when exposed to temperatures at 34ºC and 35ºC. Growth will most likely be adversely affected by frequent incidence of exposure to water temperatures greater than 32ºC. Water temperatures that approach and remain near the thermal tolerance limits of walleye can occur during pond culture and the effect may differ from year to year.

An attempt to feed train Gulf coast walleye was unsuccessful. However, successful feed training of 45-day-old pond-reared northern walleye (42 mm total length) was realized, resulting in high survival (> 80%) for fish fed a particular starter diet. Application of this feed training method to Gulf coast walleye should achieve similar results. The use of formulated feed to grow a large number of advanced size fingerlings is more efficient and economical than raising walleye on minnows. Additionally, the high acceptance and good survival achieved with formulated diets provide for control over quality and quantity of diet fed. Feed training diets remain in the evaluation stages so a confident recommendation about which diet is best for the feed training cannot be offered. To reduce the incidence of disease, feed training of walleye should be conducted at a water temperature between 15 and 20°C, if possible, even though growth between 21 and 26°C may be greater.

Feed-trained fish can be restocked into ponds (0.04 ha) and grown to advanced sizes by feeding them a formulated diet. However, the pond culture methods that were used did not yield the desired high survival and corresponding production. Production characteristics for stocking densities of 24,700/ha and 12,350/ha did not differ; however, during the first 40 days, fish in the high density treatment notably accepted more formulated diet than fish in the low density treatment. Further investigation is needed to determine if the frequency of feed acceptance is related to density. Feeding strategies that will also increase the level of acceptance of feed after fish are restocked back into ponds also need to be investigated. Successful pond culture after restocking may be principally founded upon achieving a high incidence of feed acceptance through greater initial stocking densities and brief confinement of fish to a small area of the pond (< 20%) for an appropriate period of time. With future improvements to pond culture methods, production of advanced sizes of walleye fingerlings will greatly exceed that achieved through intensive tank and cage culture simply because ponds provide more effective space at lesser cost for fish.