ABSTRACT

Title of Thesis: EVALUATION OF OFF-SEASON SPAWNING TECHNIQUES AND LARVAL DIET SUPPLEMENTATION OF YELLOW PERCH (*PERCA FLAVESCENS*)

Mark Allen Rath, Masters of Fisheries Science, 2004

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400 yellow perch (*Perca flavescens*) were divided into four groups, each targeted to spawn in a different season. The photo-thermal conditions of each group were manipulated to mimic seasonal changes that initiate reproductive activity. Spawning occurred in all groups. Using photo-thermal manipulation it is possible to spawn yellow perch at different times of year providing multiple crops of fingerlings.

Eggs and larvae of yellow perch were analyzed for fatty acid content. A feeding trial was conducted comparing experimental diets high in eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) to the traditional larval diet used by perch producers. Enriching live foods with EPA and DHA improved survival. Although it was high in both EPA and DHA, the emulsified diet used resulted in lower survival than the control diet because the larvae did not accept it. However, yellow perch larvae may be able to be weaned onto manufactured diets, further improving survival.

EVALUATION OF OFF SEASON SPAWNING TECHNIQUES AND LARVAL DIET SUPPLEMENTATION OF YELLOW PERCH (*PERCA FLAVESCENS*)

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Masters of Fisheries Science

2004

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Acknowledgements

I would first like to acknowledge my advisor, Dr. Andy Lazur, for all of his help and guidance over the last two years. Thanks also go to the rest of my advisory committee, Dr. Bill Van Heukelem for all of his daily assistance and Dr. Steven Hughes for his long-distance aid and support. I owe enormous gratitude to Jura Jug-Dujakovic, formerly of Atlantis Aquaculture in Emmaus, PA and Dr. Bill Daniels at Delaware State University for providing me with the fish used in this project.

The Maryland Department of Natural Resources provided a great deal of support and guidance, particularly Steve Minkkinen, Dave Seine and Brian Richardson. Thanks also to Erin Ryder at the Horn Point Laboratory for all of the weekends she spent feeding my fish. I would like to recognize the tireless efforts of the Horn Point Laboratory maintenance staff: Blaze Brown, James “Bear” Kampmeyer, Ralph Kimes, Gordy Dawson, Bruce Abbot, and Rusty Kinnamon. I would like to specifically recognize Phil Derry for his help with the control and monitoring systems, as well as Jack Seabrease for his help constructing our larval rearing rack. I would also like to thank the funding agencies who supported this research, the Maryland Agricultural Experiment Station, and the United States Department of Agriculture CREES.

Thank you to my parents for all of their love and encouragement.

Finally, I would like to thank my wife, Jessica, for all of her love and support. I couldn’t have done this without you.

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Chapter 1: Introduction

Yellow Perch (*Perca flavecens*) is a popular game fish species and a traditional food item in the mid-Atlantic and the Midwestern United States, as well as in Canadian provinces adjacent to the Great Lakes. In the northernmost portions of its range, the yellow perch has enjoyed popularity among ice-fishermen. Yellow Perch are freshwater teleosts that are cool to cold water adapted. They are unique to North America, and are found in lakes, impoundments, ponds, and slow moving rivers, and they prefer clear water with moderate vegetation and lots of sand or gravel bottoms (Mansueti 1964). Yellow perch can be found in the Northwest Territories across Canada to Nova Scotia and from the northern US, south to Kansas, Florida, and Georgia. They are especially abundant in Manitoba lakes and the Great Lakes drainage. Yellow perch have also been successfully introduced in the western half of North America including Montana, Idaho, Washington, California, New Mexico, Texas, and British Columbia (Wynne 2002).

Female yellow perch grow faster, reach an overall larger size and live longer on average than do males, but it is common to find both sexes at lengths of 200-290 mm and average weights of 170-300 g (Mansueti 1964). The world record yellow perch was 4 lbs, 3 oz, and was caught in Cross Wicks Creek, New Jersey, 1865. Yellow perch typically live for 7-9 years, and the oldest known age is 13. Larval yellow perch feed on zooplankton, primarily copepods and cladocerans. Juveniles quickly begin to include bigger

food items such as aquatic insect larvae and larval fish. By the end of their first growing season, perch are including small fish, crayfish, leeches, and snails in their diet. Adults continue to eat all of these items, but consume more fish as they grow larger (Brown et al., 1996).

Yellow perch spawn fairly early in the year, beginning in late February or early March and continuing into May. In the northern part of their range, the water temperature only needs to reach 7° C to trigger spawning, but in some southern climates it may need to reach 12-14º C for spawning to begin (Dabrowski et al., 1996; Tidwell et al., 1999; Henderson et al., 2000). It is not the actual water temperature that is the signal to spawn, but the warming of the water as winter ends and spring begins. They build no nest, and there is no parenting of the eggs or young. Just before spawning, adult perch move to shallow, weedy areas of lakes or into slower protected areas of streams. At night females are escorted by two or more males as they move among the vegetation. Females drape their eggs in an accordion-like adhesive matrix over the vegetation and the males fertilize the eggs as they are released. A single female may lay 10,000-200,000 eggs, depending on her size and health (Mansueti 1964). The larvae hatch in about 2 weeks and larvae stay in open water until they are about 25 mm long, when they move into weedy areas near shore. Initially, larvae remain in the tributaries, but will eventually migrate offshore to reduce their risk from predators. When yellow perch reach the juvenile stage (22-25 mm) and predator avoidance has been sufficiently developed, they move back to the shorelines to feed on the richer, nearshore

food sources. Adult yellow perch generally remain in their natal river systems after spawning (Mansueti 1964).

Historically, yellow perch have provided anglers the earliest and easiest opportunity to catch fish following the New Year. Spawning in February and March provided an abundance of fish and, with no restrictions, fishermen harvested all they wanted. Populations in Lake Michigan began to decline in the early 1970's due to unrestrained harvest and habitat deterioration, and in 1989, creel and size limits and area closures were imposed. In Lake Michigan, the infamous zebra mussel may interrupt the rate of transfer of essential fatty acids through the food web, affecting the survival of yellow perch larvae (Malison 2000). The yellow perch commercial fishery became more important in Chesapeake Bay with the imposition of the American shad moratorium and strict striped bass regulations. Commercial fishermen catch yellow perch during their spawning runs in the upper reaches of streams and tributaries (Kelly 2000; Manci 2001). Yellow perch are usually not the sport fish most anglers try for, but they are one that many anglers catch. The nearshore movements of this species make yellow perch available to shoreline as well as boat anglers for the majority of the fishing season (Wynne 2002).

Despite declines in both fish populations and commercial harvests within the Great Lakes, the demand for yellow perch remains strong and commands a relatively high value of $2.30-$3.00/lb for whole fish (Malison 2000). Because of these favorable market characteristics, yellow perch has

been investigated in recent years as an alternative aquaculture species. Many aspects of culture including optimal growth temperature, fingerling and pond growout, and spawning techniques have been studied (Wallat & Tiu, 1999; Wallat et al., 2001). With a preferred market size of about 125 grams for whole fish, successful culture is highly dependent on a consistent supply of lower cost fingerlings (Tompkins & Libey 1999). To date, the majority of fingerlings are produced by stocking hatchery reared fry in nursery ponds managed initially to provide the necessary live foods for larvae, followed by feed training onto commercial diets (Kelly 2000). Although successful in producing fingerlings, this practice allows for only one crop per year. Limited fingerling supply and their relatively high cost (27-40% of market price) is a major challenge to expansion of yellow perch culture as it significantly affects profitability (Manci 2001; Riepe 1997). In contrast, catfish and hybrid striped bass fingerlings cost 6% and 12% of market price, respectively. In addition, inconsistent supply and high price of yellow perch fingerlings was stated as the number one problem currently faced by Maryland foodfish producers in a 2001 roundtable meeting attended by over half of the state's producers (A. M. Lazur, University of Maryland; personal communication, October, 2001).

Using temperature and photoperiod manipulation to control reproduction has been demonstrated with many fish species including as European perch (Migaud et al., 2002), walleye (Malison et al., 1998), channel catfish (Kelly & Kohler 1996), sunfish (Mischke & Morris 1997), hybrid striped bass (Tate & Halfrich 1998), and a variety of salmon species (Macquarrie et

al., 1978, 1979; Johnston et al., 1992). It has also been suggested as a promising technique for allowing the year round spawning of yellow perch (Ciereszko et al.,1997; Kolkovski & Dabrowski 1998).

In an effort to increase the supply of fingerlings, Kolkovski and Dabrowski (1998) spawned yellow perch out of season (late summer) using temperature and photoperiod manipulation. They showed that 50% of females ovulated and larval survival through the eyed stage was 56%. They did, however, observe a relatively high percentage of skeletal abnormalities and poor swim bladder inflation in the larvae. In an additional attempt to control and enhance hatchery production, Dabrowski et al. (2001) successfully raised larvae on live foods in an indoor tank system. Fish were fed live rotifers and *Artemia* nauplii as first foods and then a variety of commercial starter diets. Survival ranged from 35-50% and greatest growth was observed with the *Artemia* fed treatment.

The first phase of this study expands on Kolkovski and Dabrowski's earlier attempts at off-season spawning to provide continuous crops of yellow perch fingerlings. In addition to testing off-season spawning of broodstock batches to produce out-of-phase spawns through photoperiod and temperature control, batches of larvae were fed and maintained in the hatchery to be sure they developed properly to the point of accepting manufactured feeds.

For the second phase of the study, a fatty acid analysis of eggs from both cultured and wild yellow perch was conducted. This analysis was

performed in order to identify differences in fatty acid composition that could have been caused by either the different diets of the parent fish (wild vs. cultured), or by the manipulation of the spawning cycle in the cultured fish. Based on the fatty acid profile of eggs and larvae of both wild and cultured yellow perch, a feeding trial was conducted comparing traditional feeding methods against two experimental diets: one of enriched live foods and one of a manufactured larval feed. The purpose of this portion of the study was to eliminate the need for, or reduce the associated costs of, feeding live rotifers and *Artemia* to yellow perch larvae.

Chapter 2: Materials & Methods

The overall objectives of this project were to improve the ability of hatcheries to supply yellow perch fingerlings and to eliminate or reduce the need for (and associated costs of) feeding live foods to newly hatched yellow perch fry (Brown et al. 1996; Harel et al., 1999). The investigation was comprised of two phases. The first tested off-season spawning of broodstock batches to produce out-of-phase spawns through photoperiod and temperature manipulation. For the second phase of the study, a fatty acid analysis (following the methods of Folch et al., 1957) of eggs and larvae from cultured and wild yellow perch was conducted to determine nutritional differences and potential fatty acid requirements for live and artificial fry diets (Asturiano et al., 2000). Based on the results of those fatty acid profiles, a feeding trial was designed and conducted to compare growth and survival of yellow perch larvae fed diets with different levels of two essential fatty acids: eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA).

Off-Season Spawning

In October of 2002, four hundred yellow perch donated by Atlantis Aquaculture in Emmaus, PA (year class 2001, 18 months old, average length 24.3 cm, average weight 453 grams) were randomly stocked into a recirculating system of sixteen 160 gallon tanks, divided into 4 groups. All tanks in the system shared the same water reservoir and treatment systems. These fish had been raised in an environmentally controlled recirculating

aquaculture facility (16 hour day length and 24°-25° C water temperature) and had not previously spawned. Each group of 4 tanks had independent environmental controls (temperature and photoperiod) and was isolated from the others by black plastic walls and ceilings. Upon stocking, the water temperature in all tanks was 18º C and the day length was 12 hours. Day length was computer controlled and included 30 minutes of dawn and dusk when light intensity was ramped up or down. All groups were acclimated at 22º C and 14 hours of daylight to begin the experiment. Water quality was monitored with NH_4, NO_3, and pH being recorded daily and NO_4, salinity, alkalinity and hardness being recorded weekly. Fish were fed using Sweeny feeders on automatic timers set to deliver approximately 10% of the average body weight per day (Melick Aquafeeds, Catawissa, PA; sinking feed, 45% protein, 16% fat, 4% fiber) and feedings occurred every 90 minutes during daylight hours. Spawning was attempted at quarterly intervals by manipulating the photothermal regime of each group independently. Our protocol was modified from that of Kolkovski and Dabrowski (1998) in that we attempted to shift the photothermal regime out of phase from the natural (spring) yellow perch spawning period at intervals of 3, 6, and 9 months, instead of a single shift of 6 months.

Each group of fish was assigned a target season in which it was anticipated that spawning would occur (Table 1). The first group of fish was scheduled to reach proper spawning conditions in May 2003, and was named Spring Group. This was followed by Summer Group, Fall Group and Winter Group.

Each group was cycled through a series of 3 environmental conditions designed to mimic the seasonal changes that trigger spawning in wild yellow perch populations: Winter Conditions (10º, 10 hours of daylight), Spring Conditions (14º C, photoperiod increasing from 10 to 14 hours of daylight) and Summer Conditions (22º C, 14 hours of daylight). The Winter Conditions acted as a reproductive conditioning period during which vitellogenesis (in females) and spermatogenesis (in males) took place. Winter Conditions were initially scheduled to last for 90 days but were later extended to 120 days after consulting with yellow perch producer Jura Jug Dujakovic of Atlantis Aquaculture Inc. (Emmaus, PA). Previous attempts at off season spawning had used chill periods as short as 60 days (Kolkovski & Dabrowski 1998) and as long as 160 days (Hokanson 1977). Spring Conditions represent the optimal temperature and photoperiod reported to trigger spawning in yellow perch. Previous research indicates that spawning is triggered in yellow perch when water temperatures begin to warm after a prolonged cold period and day length increases (Mansueti 1964). These conditions vary between the northern and southern portions of their range, but in indoor recirculating systems, spawning has occurred when the water temperature was increased from 10º C to 12º or 14º C and day length was increased from 10-12 hours of light towards 14 hours of light (Dabrowski et al., 1996). During this study, Spring Conditions were allowed to persist until all spawning activity had ceased. Summer Conditions acted as a recovery period during which the broodstock fed heavily and re-gained the weight lost during spawning.

Group Name	Winter Conditions	Spring Conditions	Duration of Spawning Activity	Females That Spawned
Spring	January 30, 2003 - April 29, 2003	May 8, 2003 - July 18, 2003	42 Days	67%
Summer	April 7, 2003 - June 27, 2003	July 6, 2003 - September 25, 2003	6 Days	8%
Fall	July 23, 2003 - November 23, 2003	December 2, 2003 - March 21, 2004	54 Days	54%
Winter	October 30, 2003 - March 8, 2004	March 18, 2004 - May 27, 2004	77 Days	80.56%
Spring (2)	October 30, 2003 - March 20, 2004	March 30, 2004 - May 27, 2004	35 Days	30.56%

Table 1: Calendar of Seasonal Manipulations and Spawning Activity for All Four Experimental Groups.

The temperature and day length reductions for Spring Group began on December 5, 2002, and it reached the Winter Conditions required for broodstock conditioning on January 30th, 2003. When water temperatures were lowered, feeders were adjusted to reduce the amount of feed delivered to approximately 5% of the average body weight per day. After a 90 day conditioning period, the water temperature was raised, and day length began increasing. This shift lasted for 10 days, and on May 8th, 2003, Group 1 reached Spring Conditions of 14º C and an increasing photoperiod. After spawning had ceased the temperature and photoperiod were returned to Summer Conditions, and feeders were re-adjusted to deliver 10% of the average body weight per day.

The temperature and day length decline for Summer Group began on March 22nd, 2003, and it reached the Winter Conditions required for broodstock conditioning on April 7th, 2003. After a 90 day conditioning period, the water temperature was raised and day length began to extend. Summer Group reached Spring Conditions on July 6th, 2003. However, their Winter Conditions period had been interrupted when our chilling system failed on June 7th, 2003. The water temperature for this group was elevated from 10º C to 16º C for a period of 5 days. A smaller, portable chiller was used to lower the water temperature of Summer Group to 12º C for 11 days. At that time, the original chiller was repaired, and resumed the normal schedule for temperature change.

On July 16th, 2003 twenty yellow perch (10 males and 10 females) from Summer Group were implanted with 25 mg of mammalian Luteinizing Hormone-Releasing Hormone (LHRH) to stimulate ovulation and spermiation and induce spawning (Rottman, et al., 1991b) (Implants provided courtesy of Maryland Department of Natural Resources). Half of these fish remained in the recirculation system at the Horn Point Laboratory. The remaining fish were moved to the Manning Fish Hatchery in Cedarville State Forest, near Brandywine, MD and were monitored by Maryland DNR hatchery staff. By spawning fish at two separate locations, it was possible to rule out water chemistry as a cause of the egg quality problems experienced during the Spring Group spawn. At the Horn Point Laboratory the temperature and day length were allowed to remain under Spring Conditions until September 25th, 2003 to be certain that spawning was complete. At that point, Summer Group was returned to Summer Conditions and the feeding rate was returned to approximately 10 % of the average body weight per day . After a sufficient period for spawning to occur, the fish that were moved to Manning Fish Hatchery were destroyed on September 25th, 2003 in accordance with Maryland DNR's protocol for using LHRH.

The temperature and day length decline in Fall Group began on July 3rd, 2003, and it reached the Winter Conditions required for broodstock conditioning on July 23, 2003. Based on discussions with Jura Jug-Dujakovic of Atlantis Aquaculture Inc (Emmaus, PA) in September 2003, and Geoff Wallat at the Ohio State University Centers at Piketon "Perch School" (August

2003), some changes were made to the gender ratios in the groups, as well as the method of changing the “seasonal” environmental conditions. First, the male:female ratio of the yellow perch populations in Summer, Fall and Winter Groups was changed from 1:1 to 3:1. Also, the Winter Conditions period was extended from 90 days to 120 days at 10º C and 10 hours of day light. Along with the increased duration of the conditioning period, the rate of temperature change was slowed from 1 degree/day to 1 degree/4 days. The rate of day length change stayed the same at 2-3 minutes per day.

On September 18th, 2003, Hurricane Isabel struck the Maryland coast, and the laboratory temporarily lost power. In addition to a brief temperature spike to 25 º C in Fall Group, the chiller was damaged and was not able to maintain the 10 degree temperature in Fall Group once power was restored. The temperature was elevated to 12° C until the chiller was repaired on October 16, 2003. In an effort to mitigate the elevated temperature, the Winter Conditions period was extended for an additional 30 days. After completing the conditioning period, the water temperature and day length began increasing. On December 2nd, 2003, Fall Group reached Spring Conditions. After spawning, feed delivery was returned to approximately 10% body weight per day and the temperature and photoperiod were returned to Summer Conditions.

The temperature and day length decline for Winter Group and Spring Group began on September 24th, 2003. This cycle was an attempt to initiate

the first spawn from Winter Group and to initiate a second spawn from Spring Group.

Winter Group was scheduled to reach Spring Conditions on March 8th, 2004, but due to a chiller failure on February 20th that condition was delayed until March 18th, 2004. The temperature in this group climbed to 20º C for two days before temporary chillers could be installed to maintain the water temperature at 11º C while the main chiller was repaired.

Spring Group was on the same schedule as Winter Group, but it was impossible to supply the tanks in that group with cold water after the chiller failure. The decision was made to use the limited chilling capability to continue with Winter Group because they had not yet spawned and it was believed they had a greater likelihood of success. The temperature in Spring Group climbed to 22° C and remained between 22° and 23° C until March 1st when the chiller was repaired. In an effort to mitigate the effects of the increased temperature, Spring Group was returned to 10° C and held there until March 30th when Spring Conditions were initiated.

On April 10th, 2004, two females from Winter Group were implanted with 25 mg of LHRH in an effort to induce ovulation. On May 6th, 2004 six females and six males from Spring Group were injected with 400 IU/kg (Rottman et al., 1991b) of Human Chorionic Gonadotropin (HCG) in an effort to initiate ovulation in the females and spermiation in the males (Rottman et al., 1991a).

Larval Nutrition

Within the second phase of the study, a fatty acid analysis of eggs and larvae from cultured and wild yellow perch was conducted to determine nutritional differences and potential fatty acid requirements for live and artificial fry diets (Asturiano et al., 2000). Eurofins Laboratories, in Des Moines, IA was contracted to conduct the analysis. After collecting samples of eggs or larvae, they were preserved by freezing at -80° C prior to shipping. Samples were pooled and coded such that they were unidentifiable by Eurofins staff. The analysis was performed following the methods of Folch et al. (1957), and results were reported as percent by weight of total fatty acid content (Figure 1). Based on the results of the fatty acid profiles, a feeding trial was designed and conducted to compare survival of yellow perch larvae fed diets with different levels of two essential fatty acids: eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). The objective of this phase of the study is to eliminate or reduce the need for (and associated costs of) feeding live foods to newly hatched yellow perch larvae (Brown et al., 1996; Harel et al., 1999).

A 21 day feeding trial was conducted using newly hatched larvae obtained from a strip spawned female in Winter Group and repeated using larvae obtained from a strip spawned female in Spring Group's second spawning period. Four days post hatch, 2700 larvae were counted and divided into 9 four-liter containers. Each container was filled with 3 liters of fresh water and placed into a cool water bath to regulate its temperature.

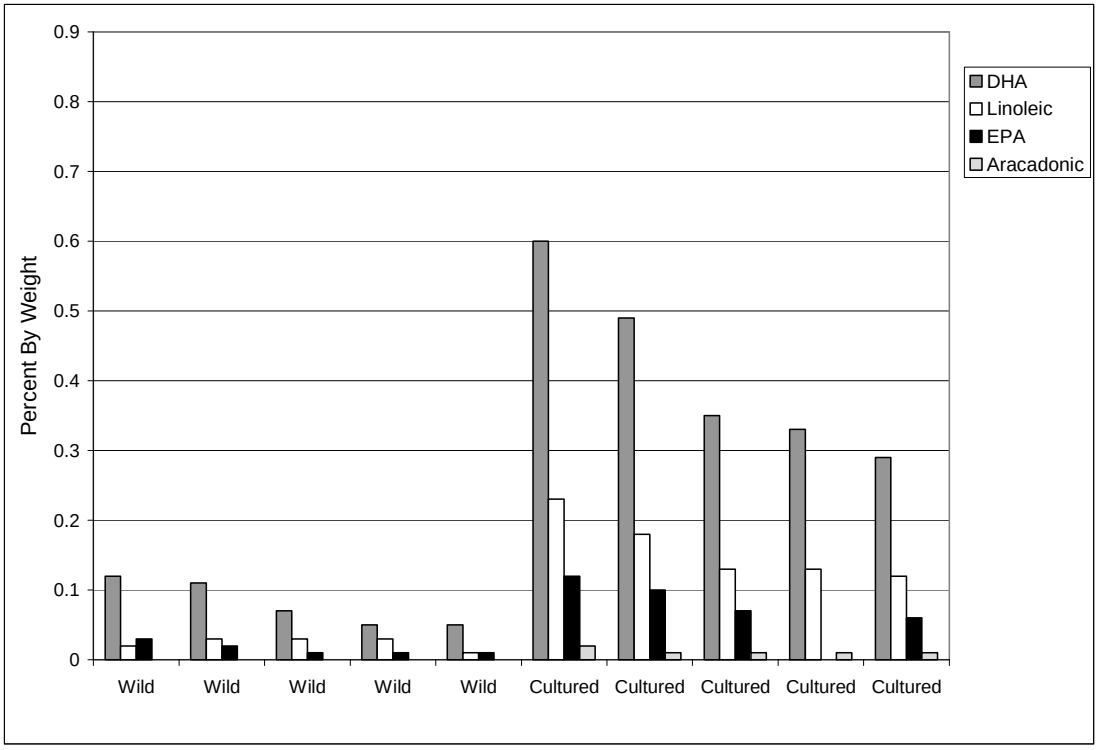

Figure 1: Percentage by weight of 4 fatty acids in samples of yellow perch eggs from wild and cultured sources: Aracadonic Acid, Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA), and Linoleic Acid.

Using this method, the water temperature inside each container was maintained between 14° and 15° C. One-inch air stones were used to provide aeration and water quality was monitored daily with measurements of temperature, dissolved oxygen, pH, and total ammonia. Mortalities and debris were siphoned out daily and 30% of the water volume was exchanged each morning.

The containers were divided into 3 groups of 3 treatments. The first treatment used live foods (rotifers for 6 days and 15 days of *Artemia* nauplii) that are the typical food items offered to captive yellow perch larvae (Harel et al., 1999; Jug-Dujakovic & VanGorder 2002). The second treatment used the same live foods enriched with Aqua-Grow, an enrichment media produced by Advanced Bionutrition that is high in EPA and DHA. Use of Aquagrow has been shown to increase larval survival in other aquacultured species including striped bass (M. Harel, Advanced Bionutrition; personal communication, April 29, 2004). The third treatment was fed a sequence of four artificial diets manufactured by Zeigler marketed as “EZ-Larvae”. This diet comes in emulsions of three different size ranges of particles intended to replace rotifers or copepods: 10-100 µm, 150-250 µm, and 300-600 µm. The fourth is designed to replace *Artemia* nauplii with particle sizes greater than 600 µm.

Beginning one day after stocking, each container was fed 3 times daily at a target feed density of 2 food particles per mL. Each day, small samples of larvae (2-10 individuals per treatment) were examined under a dissecting microscope to determine the presence or absence of food in their gut. Three

times during the study, the total survival was calculated for each container and averaged within treatments to tabulate the total survival at Day 7, Day 14, and Day 21. An analysis of variance (ANOVA) was also conducted on the same data to test for significant differences in survival between treatments.

Chapter 3: Results

Spawning Component

Spring Group

Spring Group reached Spring Conditions on April 22, 2003. The first spawn occurred on the 31st day after reaching Spring Conditions (May 23nd, 2003) and spawning continued until the 76th day (July 3rd, 2003)(Table 1). During this period, 67% of females in this group produced and released egg ribbons and males were spermiating. Despite the high rate of spawning, no fertilization was observed. Egg ribbons were pale white instead of the more typical amber color seen in wild eggs in which good fertilization has been observed. Ribbons spawned by fish in our system also had a looser matrix than wild ribbons and were often found in clumps, rather than in the typical concertina shape produced by yellow perch (Figure 2). Males were found to be spermiating during the entire spawning period. During the experiment, 11 fish from this group died (92% survival).

Summer Group

Summer Group reached Spring Conditions on July 6th, 2003 (Table 1). However, their Winter Conditions period had been interrupted when the chilling system failed. The water temperature for this group was elevated from 10º C to 18º C for a period of five days. We were able to utilize a smaller, temporary chiller to lower the temperature to 13º C for 20 days. At that time, the original chiller was repaired, and the temperature was returned to 10º C.

Figure 2: A yellow perch egg ribbon collected from the Severn River (left) compared to a healthy yellow perch egg ribbon (right). The pale, loose matrix of the Severn River ribbon is similar to the ribbons produced by the fish in this study. (photo courtesy of Steve Minkkinen, Maryland DNR Fisheries Service, 2002)

The Winter Conditions were extended for an extra two weeks in an effort to mitigate the effects of the elevated temperature. Despite this effort, only two females (8%) spawned in this group. The first ribbon was found 55 days after reaching Spring Conditions (August 30th, 2003), and the second ribbon was found 16 days after reaching Spring Conditions (September 5th, 2003). None of the 10 females that received LHRH implants spawned. Males (both implanted and non-implanted) were found to be spermiating during the entire spawning period. Between August 17th and October 2nd, 2003, there were 19 mortalities in this group. During the entire experiment, this group experienced 26 mortalities (74% survival).

Fall Group

This group was also affected by the chiller failure, but it was disturbed earlier in its Winter Conditions, and had a longer period of recovery before spawning was attempted. Fall Group reached Spring Conditions on December 7th, 2003. The first spawn occurred on January 9th, 2004, 33 days after reaching Spring Conditions (Table 1). Spawning continued for 84 days until March 2nd, 2004. Over that period, 54% of females produced and released egg ribbons and males were spermiating. The male:female ratio in this group had been increased to 3:1, as recommended by Jug-Dujakovic at Atlantis Aquaculture and Geoff Wallat at the Ohio State University Centers at Piketon “Perch School”. Despite this, none of the egg ribbons were fertilized and all egg ribbons were pale and had loose matrices. Males were found to

be spermiating during the entire spawning period. During the experiment, 8 fish from this group died (94% survival).

Winter Group

Winter Group was scheduled to reach Spring Conditions on March 8th, 2004, but due to a brief chiller failure on February 20th, this was delayed until March 18th, 2004 (Table 1). The temperature in this group climbed to 20º C for two days before temporary chillers could be installed to keep water temperatures at 11º C until the main chiller was repaired. Despite the use of temporary chillers, the fish in Winter Group began spawning before they reached Spring Conditions. The first spawn took place on February 20th, 2004 when the chiller initially failed. Spawning ceased when the temporary chiller was activated, but began again on March 9th, 2004. Spawning activity continued until May 13th, 2004. Over that period 80.56% of females produced and released egg ribbons and males were found to be spermiating during the entire spawning period. Five females in this group were strip-spawned resulting in two fertilized ribbons. Both egg ribbons developed normally and healthy larvae hatched 8 days post-spawn. This group also produced one fertilized tank-spawned ribbon. This ribbon had improved color and cohesiveness, and after nine days of incubation, healthy larvae hatched. The fertilization rate of all three ribbons was between 80-90%, and the hatching rate was 62% for the two stripped ribbons and 77% for the tank spawned ribbon. The lower hatching rate of the strip-spawned ribbons was due to a fungal infection that appeared on the sixth day of incubation. The infected

ribbons were treated with 600 ppt formalin for 15 minutes to kill the fungus. Winter Group had 14 mortalities over the duration of the experiment (90% survival). Five of the mortalities occurred within 24 hours of the temperature spike in February, 2004. Three more died after that event for a total of 57% of mortalities occurring after the temperature spike.

Spring Group, Second Spawn

Spring Group was scheduled to reach its second period of Spring Conditions on March 8th, 2004, but due to the chiller failure on February 20th, this was delayed until March 30th, 2004 (Table 1). The temperature in this group climbed to 23º C and remained there for 10 days while the chiller was repaired. Once the chiller was repaired, Spring Group was returned to Winter Conditions for 31 days until March 30th, 2004 when the shift to Spring Conditions was initiated. The 14º C water temperature that represented the beginning of Spring Conditions was reached on April 9th, 2004. The first egg ribbon from this groups second spawning cycle appeared on April 8th, 2004 and spawning activity continued for 34 days until the last egg ribbon was released on May 12th, 2004. Over that period 30.56% of females produced and released egg ribbons and males were found to be spermiating during the entire spawning period. Over the course of the entire experiment, 11 fish from this group died (92% survival), but 70% of this mortality occurred within or after the second spawning period.

Larval Nutrition Component

Samples of eggs and larvae, analyzed using the method of Folch et al. (1957) showed differences in fatty acid composition between wild and cultured samples (Figure 1). EPA and DHA decreased over time as eggs and larvae developed, and it was higher overall in the cultured samples (Figure 1). Arachadonic and Linoleic acid were present in much higher concentrations in the cultured samples, probably due to their presence in the broodstock diet being used. Based on EPA and DHA changes, an experiment was designed to test survival of larvae on diets, both live and manufactured. The diets contain different levels of EPA and DHA, and performance of larvae and efficacy of the diets was measured by percent survival.

For the first feeding trial (using larvae from the Winter Group spawn), all treatments were stocked into their containers on April 29th, 2004. Feeding did not begin until May 1st, when it could be determined that the larvae had opened their mouths. By Day 9 of the trial, all larvae in the treatments receiving the EZ-Larvae diet had died. By Day 14 of the trial, all larvae in the treatments receiving the live foods that had not been enhanced had died, and only the treatments receiving enriched live foods remained. At the end of the 21 day trial, only 17 of the initial 900 stocked into the enriched foods treatment survived (0.0189% survival).

For the second feeding trial (using larvae from the Spring Group's second spawn), all treatments were stocked into their containers on May 24th, 2004. Feeding began on May 26th, once it was determined that the larvae had

opened their mouths. By Day 12 of the trial, all larvae in the treatments receiving the EZ-Larvae diet had died. On Day 17 of the trial, all larvae in the treatments receiving the enriched live foods and all larvae in the treatments receiving normal live foods had died.

An analysis of variance (ANOVA) was performed on the survival data from Days 7, 14, and 21 to check for differences between the three treatments (Table 2). There was no difference detected between any of the treatments in the first week of the experiment. It was found that the larvae fed the un-supplemented live foods and the live foods enriched with Aquagrow had significantly higher survival on Day 14 and on Day 21 than the larvae fed the EZ-Larvae diet. It was also found that the larvae fed live foods enriched with Aquagrow, while not showing significantly higher survival during the first two weeks, did have significantly greater survival over the entire 21 day experiment.

	Enriched Food (N=1800)		EZ-Diets (N=1800)	
Day	**F**	**P**	**F**	**P**
7	-	-	-	-
14	-	-	2091.445	.000*
21	1266.232	.000	2086.084	.000*

Table 2: Results of ANOVA Comparing Enriched Foods & EZ-Diets to Natural Live Foods. *Survival of EZ-Diet treatments was statistically significantly less than survival of control treatments.

Chapter 4: Discussion

Despite repeated mechanical problems that interfered with the experimental conditions influencing the reproductive condition and activity of yellow perch, the results of this study show that off-season spawning of yellow perch through temperature and photoperiod manipulation can be used to produce fingerlings year round. If the “seasons” are shifted out of phase, rather than being accelerated or condensed, yellow perch will complete vitellogenesis and are capable of producing viable gametes (Figure 3 and Figure 4).

Kolkovski and Dabrowski (1998) used a similar methodology, but shifted the perch reproductive cycle out of phase by 6 months rather than the 3 month shifts used in this study. Multiple shifts at intervals of 3, 6, and 9 months would be the preferred technique in that it would potentially provide four crops of fingerlings each year compared to the only two crops per year allowed by a 6 month shift. Kolkovski and Dabrowski (1998) did report success with a 60 day “winter” conditioning period compared to the 90 day and then 120 day conditioning periods used here. It may be the case that seasonal shifts of more or less than the 6 month shift performed by Kolkovski and Dabrowski (1998) require conditioning periods of differing lengths to be successful. Further investigations and trials could reveal the optimum conditioning period duration for seasonal shifts of different magnitudes. In addition to the duration of the conditioning period, the rate of temperature change applied when shifting the water temperature from one “season” to

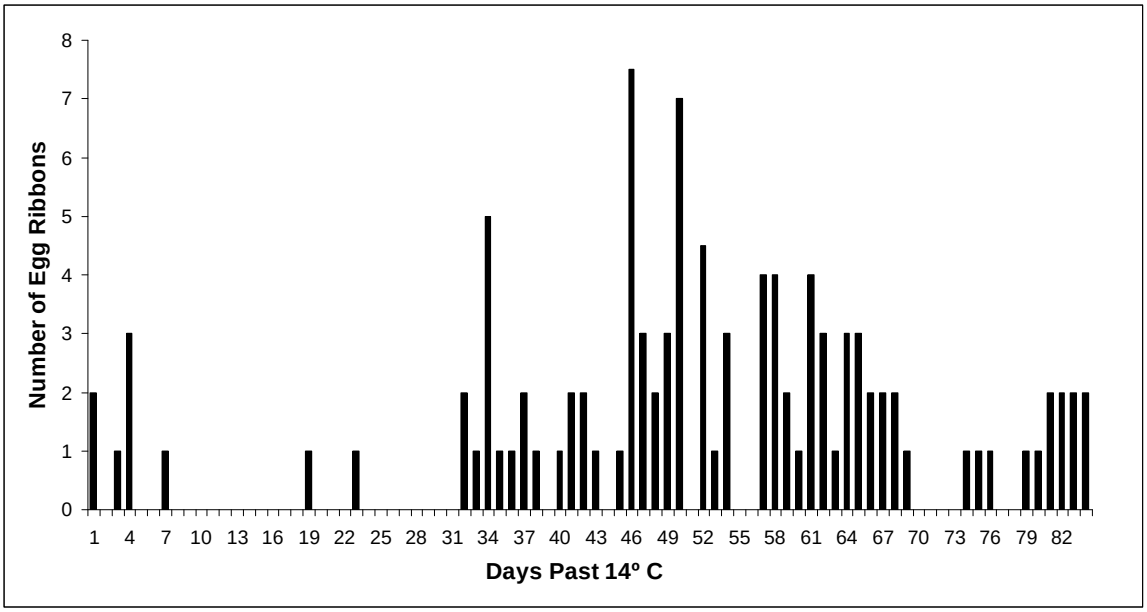

Figure 3: Number of egg ribbons released by females in all spawning groups after the water temperature reached 14º C.

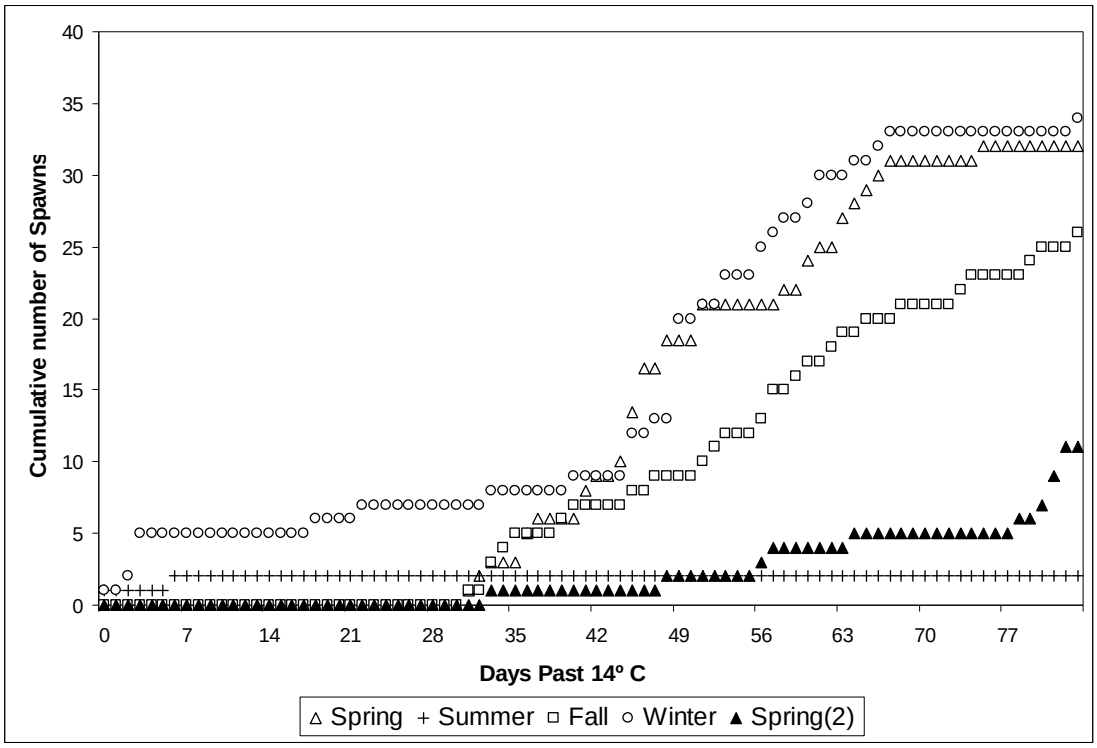

Figure 4: The cumulative number of egg ribbons collected from each of the spawning groups. The groups with the most successful spawning activity (Spring, Fall, and Winter) have very similar lines, indicating they spawned at the same rate, while the groups with poor spawning success (Summer and Spring(2)) have lower profiles.

another is of critical importance. Summer Group, which experienced the poorest spawning activity in this study (Figure 4), had a rate of temperature decline (~1°C per 1.3 days) more rapid than the other spawning groups (~1°C per 5 days). This difference in the rate of cooling may have been responsible for the lack of spawning activity in that group.

Two of the spawning groups, Summer and Spring(2), produced substantially fewer egg ribbons than the rest of the groups. These two groups also experienced the most dramatic disturbances to their chilled periods due to chiller failures. The first chiller failure affected Summer Group early in the winter conditioning period, and while the disturbance only lasted for 5 days, the fish apparently did not recover and spawning activity was severely diminished. The second chiller failure affected both Winter Group and Spring Group(2) and the differences between this event and the first chiller failure were twofold. First, both groups had nearly completed their winter conditioning periods. Dabrowski et al. (1996) stated that female yellow perch in the late stages of oocyte development seem to be resistant to photothermal manipulation. Second, Winter Group was returned to winter conditions after only 2 days while Spring Group(2) was forced to wait 11 days for winter conditions to be restored. The result of these differences was that Winter Group made a complete recovery after the chiller failure and spawned normally. Spring Group(2) experienced a delayed and diminished recovery and while it did produce more egg ribbons than Summer Group, it did not perform as well as Winter Group (Figure 4). It appears from these three cases

that a prolonged interruption of the winter conditioning period, or an interruption early in the winter conditioning period is capable of substantially disrupting the spawning ability of yellow perch under photothermal manipulation.

While the fish in this study did spawn and produce viable eggs, there were problems that would need to be addressed before these techniques could be successfully applied in a commercial aquaculture setting. First, the egg ribbons that were produced by fish in the experimental system were pale in color and were not as strong or elastic as ribbons collected in the wild (Figure 2). They often appeared in clumps rather than a single cohesive strand. While wild egg ribbons are relatively tough and difficult to tear, the ribbons from this study were easily damaged by nets and drain suction. Similar ribbons have been described in the Severn River near Annapolis, MD where perch recruitment has been poor since 1996 (S. Minkkinen, Maryland DNR; personal communication, June 3, 2003). It is possible that these “weak” ribbons are not as likely to be fertilized by male perch and that is having an impact on the ability of the perch population in that river system to recruit. Perch egg ribbons in this condition were also described in a conversation with Dr. Konrad Dabrowksi (February 18, 2004) who has studied yellow perch reproduction and indicated that despite the weak appearance of these ribbons and infrequent “in-tank” fertilization, these eggs were, in fact, viable. He recommended the use of HCG injections to synchronize the reproductive development of males and females, coupled with standard strip spawning

techniques to assure fertilization. While more successful, this technique requires specialized training and is time and labor intensive. Although Dr. Dabrowski and others report increased success using hormone injection, this step is not required for successful fertilization. In fact, most of the females in this study that were injected with HCG or LHRH released their eggs before we attempted to strip spawn them, and the eggs remained unfertilized. By closely observing the reproductive condition of the un-injected females it was possible to strip spawn them and fertilize the eggs manually using milt extracted from the males. In all groups there were always ripe males present during the spawning periods that could be used to perform this type of fertilization.

It is also possible that while viable, embryos from these weakened ribbons could be physiologically deficient and unable to develop into healthy adult fish. So, while off season spawning may appear to remove a bottleneck in commercial yellow perch production by potentially increasing the supply of available fingerlings, poor larvae and fingerling development may present another challenge. While this study could not identify weak egg ribbons as the sole cause of poor fertilization, they may contribute to the problem. Further investigation into the cause of this “weak ribbon syndrome” could help avoid the problem all together. The cause could be related to stress or diet of the broodstock, or some other variable between natural and artificial environments. Any investigation attempting to uncover the cause(s) would need to be able to produce healthy, normal egg ribbons, as well as to

deliberately produce weak, clumpy ribbons so that the precise cause could be identified and corrected.

Brood fish diets play an important role in the reproductive development of the brood fish and the success of their eggs and larvae. The formulations of those diets dictate what fatty acids and other nutrients are present in the ova to be passed into the egg yolk during vitellogenesis. The literature indicates that in other species of coolwater fish, DHA and EPA are the most critical fatty acids for healthy embryonic and larval development (Sargent et al.,1999; Copeman et al., 2002; Harel & Place 2003). The broodstock diets currently in use by yellow perch producers were similar to the diet used in this study. Because the egg samples recovered from the cultured brood fish were higher in essential fatty acids than those collected from the wild, the diet used in this study seems to contain sufficient amounts of these fatty acids to allow for successful spawning and development. Performance of the brood fish (and their eggs and larvae) may be improved by more advanced diet formulations, but that was not a target of this investigation and specific improvements cannot be implied from this data.

The feeding trial conducted on the larvae spawned from Winter Group and Spring Group (2) provided data that led to the following conclusions about larval nutrition in yellow perch. First, some percentage of yellow perch larvae will survive on the commonly used live foods (rotifers and *Artemia* nauplii) but enriching these food items with a product such as AquaGrow has been shown by this experiment to significantly improve survival. While

existing formulations for broodstock diets may enable female yellow perch to provide their eggs with adequate concentrations of DHA and EPA, newly hatched yellow perch larvae need to quickly find another source of these essential fatty acids and un-supplemented live foods may not be sufficient. Further investigation may reveal that AquaGrow or other enrichment products high in DHA could improve larval growth rates.

Second, without a readily consumable food source high in DHA and EPA, yellow perch larvae will starve and suffer high mortality once the yolk sac is consumed. Analysis of the results of this feeding trial indicates that the liquid EZ-Larvae diet, produced by Ziegler Brothers (Garners, PA), resulted in the lowest survival. When examining samples of larvae under a dissecting microscope, there were never any particles of EZ-Larvae present in the gut of fish from that treatment, and heavy mortality occurred in all of the EZ-larvae replicates. While Ziegler Brothers did not provide the precise formulations of the EZ-Diets, they are high in both DHA and EPA. It is possible that the yellow perch larvae did not recognize it as a food item, or that they did not find the product palatable. Because this species of fish is not domesticated, it is also possible that they rely on a predatory instinct that requires they be offered only live and mobile food items. If this were the case it is possible that they could be weaned onto the EZ-Diets, reducing the amount of time they required live food and possibly eliminating the feeding of *Artemia* nauplii. Further investigation is warranted given the practical and nutritional benefits

of using a manufactured diet in both commercial and research aquaculture settings.

In conclusion, the results of this study indicate that off-season spawning of yellow perch is a possible technique that could be implemented by producers to produce multiple batches of larvae. This is despite the increased degree of difficulty compared to off-season spawning techniques of other species such as channel catfish (Kelly & Kohler 1996) and hybrid striped bass (Tate & Halfrich 1998). There should, however, be further study into the issue of poor egg quality that has been described here. Until that issue is resolved, producers would be well advised to continue using pond techniques to spawn yellow perch. Producers that raise larvae in hatcheries can expect to see an increased percent survival by introducing live food enrichment into their larval diets. Liquid formulations and refined techniques for using such diets may be practical in the future but are not yet suitable for newly hatched yellow perch larvae.

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