Chapter One:

Review of Yellow Perch Culture and Related Intensification Techniques

INTRODUCTION

Market aspects

Yellow perch (*Perca flavescens*) is an economically important food fish in the upper Midwest of the United States. Traditionally, wild-caught perch from the Great Lakes have supplied the market. However, the commercial harvest from the Great Lakes has been declining and cannot satisfy the current demand (Calbert and Huh, 1976; Brown et al., 1996). The commercial harvest of yellow perch in North America peaked at 36 million pounds in 1969 with Lake Erie traditionally providing more than 80% of the total catch (Lesser and Vilstrup, 1978). The catch from Lake Erie has dwindled to 4.1 million pounds reported in 1997 (OMNR, 1998). In addition, limits have been placed on recreational and commercial fishing for yellow perch in Lake Michigan as a result of recent declines in wild stocks (Heidinger and Kayes, 1993). This supply reduction has driven the retail price of yellow perch to as much as \$11 lb^{-1} and averaged \$7.45 lb^{-1} from 1996 -1997 (NCRAC, 1996; Riepe, 1998). There is also concern over bioaccumulation of industrial contaminants in Great Lakes fish. There appears to be room for aquaculturally raised yellow perch in the established market of the Great Lakes region.

The market potential for yellow perch in the midwest outside the Great Lakes is strong as well. In a survey of six southern states in the North Central Region, yellow perch was identified as a food fish of high demand with low availability (Hushak et al., 1993). In other words, wholesale and retail buyers would buy perch if it were available. Other authors have also noted the preference for yellow perch within the North Central Region (Brown et al., 1996; Riepe, 1996). As a result of high market demand and declining supply, the Industry Advisory Council of the North Central Regional Aquaculture Center has deemed yellow perch a high priority species for aquaculture research (Malison and Held, 1992).

Current state of production

Pond production is presently the primary method of yellow perch culture in the United States. Brood fish either are allowed to spawn naturally in ponds or are artificially strip-spawned and the resulting eggs incubated in a separate facility (F. Binkowski, University of Wisconsin, Milwaukee, personal communication, 1997). Just prior to hatching, the eggs are stocked into fertilized ponds at densities up to 1,000,000 per hectare, with fry survival proving as high as 32% at that density (Brown and Dabrowski, 1995; Brown et al., 1996). Newly hatched fry depend on zooplankton for initial feeding and can be weaned to artificial feed after several weeks (Brown et al., 1996). Currently, producers must rely on a single, spring spawning season for a supply of juveniles due to the annual egg recrudescence cycle of yellow perch, which requires a prolonged chill period to stimulate gonadal development (Hokanson, 1976).

Extensive culture of yellow perch in ponds requires large amounts of land with a plentiful supply of appropriate quality water. Also, it takes two growing seasons for yellow perch to reach market size of approximately 150 grams when reared in ponds (Calbert and Huh, 1976; Brown et al, 1995). Thus, to reduce these cost prohibitive factors, many producers sell perch at the fingerling stage, production of which demands less time and space. These fingerling producers supply farmers equipped solely for grow-out of the perch, effectively dividing the industry into two distinct segments. Production of fingerlings tends to be highly variable due to the unpredictable nature of zooplankton populations at the time of spawning (NCRAC, 1996). In general, yellow perch production is currently too seasonal and variable to consistently meet the demands of the market.

Intensification of yellow perch culture

Recirculating technology has become increasingly prevalent in commercial aquaculture due to its potential for eliminating the seasonality and variability associated with pond production. As with any new technology, however, commercial application of

recirculating technology is expensive. Therefore, intensification of this sort requires the security of a product with high market demand. As discussed, yellow perch fulfills this condition.

It is important to note that the Eurasian perch (*Perca fluviatilis*) is regarded as biologically equivalent to the yellow perch, sharing similar environmental tolerances, dietary requirements, growth parameters and general behavior patterns (Goubier, 1995). Thus, studies pertaining to either species can be applied interchangeably, with caution regarding size parameters, as Eurasian perch tend to be larger even as embryos (Mansueti, 1964; Thorpe, 1977; Craig, 1987; Goubier, 1995).

Accordingly, both perch species have been the subjects of recent culture intensification efforts. Some aspects of perch reproduction, larval rearing, growth, nutrition, and pathology under intensive conditions have been examined. The following review presents the knowledge to date of reproductive and larval culture intensification of yellow perch and its European congener, the Eurasian perch, as well as relevant information regarding teleosts in general.

LITERATURE REVIEW

Reproduction

Yellow perch are annual spawners which undergo a long recrudescence period of gonadal development during autumn and winter followed by a short burst of spawning activity (2 - 3 weeks) in early spring at temperatures between 8 and 20°C. Female perch produce a cohesive, accordion-shaped hollow egg ribbon, which is dragged through milt released from males and fertilized (Heidinger and Kayes, 1993). Yellow perch also require a chill period of at least 185 days at 6°C or less for 100% spawning participation of females (Hokanson, 1977). Prior to spawning, the gonadosomatic index (GSI), which expresses gonad weight as a percentage of total weight, can range from 20-30% in

females.

As mentioned, this single annual cycle also limits the availability of fingerlings to late summer and autumn. Thus, successful culture intensification of this species will depend on the manipulation of its reproductive cycle to provide fingerlings continuously throughout the year, which requires knowledge of the physiological parameters involved in reproduction (Dabrowski et al., 1994).

Reproductive physiology

The hormonal regulation of most reproductive functions in fish stems from activity along the brain-pituitary-gonadal axis (Sundararaj, 1981; Redding and Patino, 1993; Figure 1.1). In general, internal and external stimuli are transduced by the brain into a series of complex endocrine activities, which control rates of gametogenesis and the time of spawning (Munro, 1990). External stimuli, such as temperature and photoperiod, seem to have the greatest regulatory influence over this process (Sumpter, 1990). However, proper water quality, genetics, nutrition and other modifying factors are necessary for successful reproduction as well (Wooten, 1982).

The control of the reproductive cycle in teleosts starts with the transduction of sensory stimuli by the brain and the subsequent modification of neurohormones and neurotransmitters, which relay this information to the hypothalamus located at the base of the brain (Figure 1). The hypothalamus then secretes gonadotropin-releasing hormone (GnRH) at a rate regulated by the above process. GnRH is transported to the ventrally lying pituitary gland via a specialized portal blood supply (Redding and Patino, 1993). An increase in GnRH correlates with an increase in pituitary gonadotropic hormone (GtH) production. GtH then stimulates gametogenesis and steroidogenesis by the testis or ovary, resulting in sperm and eggs, respectively (Figure 1).

GtH or gonadotropin is found in at least two forms in fish. GtH I is primarily involved in gametogenesis and, in females, GtH II is involved in final maturation and spawning (Redding and Patino, 1993; Peter and Yu, 1997). Dopamine also is produced

by the hypothalamus and inhibits the production of GtH, particularly GtH-II (Peter and Yu, 1997). Thus, final maturation and ovulation correlates with a decrease in dopamine for many species (Peter and Yu, 1997).

Sex steroids include progestins, androgens and estrogens (notably testosterone and estradiol), which are involved in gametogenesis directly and regulate the secretion rates of the hypothalamus and pituitary (Dabrowski et al., 1996). Steroidogenic activity is regulated by GtH; therefore, the counter-control of the steroids on GtH secretion can be viewed as a feedback mechanism, regulating GtH secretion internally (Redding and Patino, 1993).

Prostaglandins (PGs) also appear to be involved in fish reproduction. PGs are stored as precursors in membrane lipids throughout the fish's body, which are mobilized by gonadotropins and steroids and converted to PGs. Once formed, PGs can be found in gonads, semen, ovarian fluid and blood plasma. In teleosts, PG synthesis is positively correlated with ovulation and the onset of female sexual behavior (Stacey and Goetz, 1982).

Environmental cues

Since reproduction is a species' means of perpetuating itself, the timing of reproduction during periods which promote survival of progeny is vital. Thus, fish reproductive cycles tend to reflect the seasonal patterns of their indigenous geographic ranges, in that final maturation and spawning occurs when food abundance and growing conditions are optimal (Lam, 1983).

According to Munro (1990), environmental cues can be categorized by their relative influence during a fish's reproductive cycle. Predictive cues, such as day length and seasonal temperature changes, are involved in gametogenesis and the development of secondary sexual characteristics. These cues are simple, reliable, and indicate suitable spawning conditions are expected. These seasonal changes can act on sensory receptors in the brain and stimulate hormone secretion or can be used to entrain

endogenous reproductive rhythms (Lam, 1983).

Synchronizing cues signal the arrival of proper spawning conditions and serve to coordinate final gonadal maturation with spawning (Munro, 1990). These cues are most important in species which do not ovulate spontaneously and ensure viable gametes are present at the right time. Such cues include photophase (i.e. day or night), specific temperature shifts, rainfall, flooding, presence of mates and/or aquatic vegetation, and lunar cycle (Sundararaj, 1981; Lam, 1983; Hontela and Stacey, 1990). For example, female goldfish (*Carassius auratus*) ovulate with an increase in temperature to 20°C, yet a much greater proportion of female goldfish ovulate when aquatic vegetation is present. In addition, ovulation generally only occurs at night (Hontela and Stacey, 1990).

The goldfish model displays the interactive role cues play in reproduction. As such, many cues are considered modifying factors, in that they serve to enhance or retard the effect of more primary cues (Munro, 1990). Food shortage, for example, which limits the amount of energy available for gonadal growth, can delay spawning time and age in killifish, sticklebacks, and presumably any species that shows size rather than age-dependent reproduction (Wooten, 1982; Hontela and Stacey, 1990).

Terminating cues stimulate gonadal regression after breeding and include the simple loss of predictive cues, the loss of responsiveness to particular cues, and exogenous cues which override otherwise suitable spawning conditions (Munro, 1990). For example, increasing temperature beyond that which is optimal for spawning causes gonadal regression in many spring-spawning teleosts despite optimal water quality, nutrition, etc. (Wooten, 1982; Lam, 1983). Also, as previously mentioned, food shortage can shorten breeding time and induce rapid regression (Wooten, 1982).

Photothermal manipulation

Attempts to manipulate the time of spawning in yellow perch have been made since the late 1970s with limited success. Hokanson (1977) found that a chill period of at least 185 days at or below 6°C was needed for 100% female spawning participation in

Minnesota yellow perch. However, it is not known if yellow perch native to more southern regions require such conditions for recrudescence. Male perch are not as stringently regulated by environmental conditions, as males will produce motile sperm throughout fall and winter (Dabrowski et al., 1996).

Dabrowski et al. (1994b) attempted to condense the recrudescence period of perch by exposing fish in September to decreasing photothermal conditions reaching a winter nadir at the end of October. Then the fish were brought up to spawning conditions (13°C; 14h light) by mid-January. GSI of manipulated females reached a maximum of 14% and egg viabilty was low (36.5%), which seems to confirm the need for a longer chill period suggested by Hokanson (1977).

Kayes and Calbert (1979) were unable to alter the spawning date of perch caught from Lake Mendota, Wisconsin and exposed to a variety of photothermal regimes in February, suggesting that the maturation rate is set once gonadal development reaches later stages. Dabrowski et al. (1996) attempted to shift the entire reproductive cycle of perch by four months and found that fertility of eggs produced from phase-shifted fish was much lower than for eggs from fish kept at ambient conditions (1.6% vs. 78.2%, respectively). Also, maximum GSI and oocyte diameter were reduced in phase-shifted fish compared to control fish. However, these phase-shifted fish were photothermally delayed beginning in October, after ovarian growth and vitellogenesis had begun. Kolkovski and Dabrowski (1998) reported that shifting the reproductive cycle prior to the start of recrudescence yielded higher GSI (26.6%) and egg fertility (56% to eyed stage) than previous studies. Yet, a high incidence of scoliosis and low swim bladder inflation was observed in fry from phase-shifted females. The authors also noted a positive correlation ($r=0.74$) between female body weight and embryo survival to the eyed stage. Given that the maximum weight of females used in the study was 130g, one might expect a higher survival of eggs from larger females.

Hormone-induced spawning

Several exogenous hormones have been used successfully to control reproduction in yellow perch. Kayes (1977) was able to synchronize spawning four days after injecting females with 1 mg/kg of body weight of carp pituitary extract (CPE). Human chorionic gonadotropin (HCG) also has been shown to induce spawning in female yellow perch when given at a rate of 230 IU/kg in two successive injections at 24 hour intervals (Goubier, 1995). Both CPE and HCG, however, are effective only in fish which are already sexually mature (Dabrowski et al., 1996; Figure 1.1).

Luteinizing hormone-releasing hormone analogs (LHRHa) have been used both to stimulate spawning and promote sexual maturation. Dabrowski et al. (1994a) used LHRHa and LHRHa combined with a dopamine inhibitor (pimozide) to synchronize spawning of laboratory-reared yellow perch 4-6 days after injection. Dosages of 100 µg/kg LHRHa in a single injection and 10 µg/kg LHRHa plus 10 mg/kg pimozide followed 48 hours later by 100 µg/kg LHRHa provided normal egg ribbons, while 300µg/kg LHRHa yielded a high percentage of fragmented egg ribbons. Embryo survival percentages, however, were not reported.

Several sex steroids have been shown to stimulate germinal vesicle breakdown (GVBD) and ovulation of yellow perch oocytes *in vitro*. Goetz and Bergman (1978) demonstrated that progestogens and 11-deoxycorticosteroids induced GVBD and ovulation. Goetz and Theofan (1979) also found that 17α-hydroxy-20β-dihydroprogesterone was effective in stimulating GVBD and ovulation. *In vivo* application of these steroids, however, has not been reported.

Embryo development

Hokanson and Kleiner (1974) tested the effects of 24 treatments of constant or rising temperature on survival of yellow perch embryos of known age. The authors also compared the median times to attain 7 morphological stages in living embryos and larvae at 8 constant temperatures. Early embryonic stages tolerated constant temperature exposures from 3.1 to 19.9°C, while the tolerance of older embryonic stages was

between 7.0 and 22.9°C. The optimal range for swim-up larvae was 13.1 to 18.2°C and optimal yields of swim-up larvae (66-85%) occurred when initial incubation temperatures were increased 0.5 to 1.0°C/day. Wang and Eckmann (1994) compared egg development of Eurasian perch over a temperature range of 6 to 22°C and found no significant differences among hatch rates from 12 to 20°C. The best swim-up rates occurred at 16 to 20°C, ranging from 77.5 to 84.2%, while no larvae swam up at 6 and 8°C.

Initial feeding of larvae

Larval perch are relatively small at hatch, from 4-7mm total length (Mansueti, 1964; Ney, 1978). In the wild, larvae initially feed on rotifers and copepod nauplii (<300μm) due to their limited gape size at this stage (Raisanen and Applegate, 1983). Perch larvae, in contrast with many other fishes, begin feeding as soon as they are free-swimming, before the yolk sac is absorbed (Hokanson, 1977). Thus, attempts to propagate larval perch under controlled laboratory conditions have focused on providing zooplankton of the appropriate size immediately after hatch (Hale and Carlson, 1972; Raisanen and Applegate, 1983; Wang and Eckmann, 1994). When initially fed with an artificial diet, larval perch acceptance, growth and survival rates have been highly variable and relatively poor (Brown et al., 1996; Kestemont et al., 1996).

Hale and Carlson (1972) determined that a zooplankton density of at least 250 edible organisms per larval yellow perch (5 fish/liter) fed four times day^{-1} was needed to obtain 50% survival for a period of three weeks post-hatch. Wang and Eckmann (1994) found the best survival (41.7%) and growth of 4-day-old Eurasian perch larvae occurred at a zooplankton density of 120 rotifers per larval perch for a period of 9 days. Both studies utilized zooplankton collected from lakes. In contrast, Awaiss et al. (1992) achieved 83.5% survival of Eurasian perch larvae over 10 days by mass culturing the freshwater rotifer, *Brachionus calyciflorus*, for use as initial food. By the end of the study (10 days), individual larval perch were consuming over 2000 rotifers day^{-1}.

Although generally considered too large (420-480μm) for initial feeding (Mansueti, 1964; Awaiss, 1991), brine shrimp *(Artemia* sp.) nauplii also have been used to rear perch fry successfully. Hinshaw (1985) obtained 44.2% survival of yellow perch after 14 days when fed 80 to 143 *Artemia* sp. nauplii per larval perch three times daily. However, Eurasian perch larvae display higher survival rates when fed a mixed diet of rotifers and *Artemia* sp. (50.19%) compared to a diet of 100% *Artemia* sp. (34.30%) (Kestemont et al., 1996). Thus, it appears a surplus of easily captured (i.e., smaller) prey organisms, such as rotifers, promotes the best survival of perch fry.

Dry diets

As mentioned, attempts to use currently available artificial dry diets as initial food for perch have yielded poor results. Kestemont et al. (1996) reported that while Eurasian perch larvae ingested a particular dry diet from the time of hatching, the maximum survival rate was only 26% after 15 days, and growth was slow (from 0.8 to 2.6 mg). Awais et al. (1992) were even less successful, with only 4% survival after 7 days and almost no growth (from 0.8 to 0.9 mg) using a microencapsulated dry feed. These results support the assertion that dry diet performance remains inferior to that using live food (Table 1.1). Weaning perch fry onto a dry diet after 30-45 days of feeding on zooplankton seems to be the most productive method of rearing (Kestemont et al., 1996).

Tank conditions

Rearing tanks with black backgrounds seem to promote survival of newly hatched fry. Hale and Carlson (1972) reported significantly higher survival in tanks with slate bottoms (63%) than in glass bottomed tanks (10%). Hinshaw (1985) found that fry survival was best in treatments with high prey contrast due to black backgrounds (44.2%) when compared to low contrast systems with white backgrounds (4.6%). There is also evidence that internal illumination of fry tanks improves feeding efficiency,

presumably due to the reduction of shadow related disturbances (Malison and Held, 1992).

Cannibalism

There is some discrepancy among investigations regarding the effect of cannibalism during the larval and post-larval stages of perch. Kestemont et al. (1996) refer to cannibalism as a major factor in the survival of larval Eurasian perch. However, Brown and Dabrowski (1995) discounted cannibalism as a significant influence on survival of yellow perch, except in systems with mixed age classes. Malison and Held (1992) noted an increase in cannibalism amongst yellow perch fingerlings (34-77 days old) in heavily stocked systems (37.4 fish L^{-1}) compared to lightly stocked systems (13.7 fish L^{-1}). Alternatively, Kestemont et al (1995) noted that in heavily stocked systems (4000 eggs m^{-2}) the proportion of cannibals was reduced (2.1%) when compared to a lighter density of 500 eggs m^{-2} (4.2%). Melard et al (1996) also found the proportion of cannibals decreased at higher densities (2000 - 4000 fingerlings m^{-2}) due to a more homogeneous growth pattern when compared to lower densities (500 - 1000 fingerlings m^{-2}). However, the actual number of cannibals at lower densities remained less than 1%. Thus, cannibalism remains a variable factor in the production perch fry.

Dietary influences

There is evidence that perch larvae, as with many other fishes, require high levels of n-3 highly unsaturated fatty acids (HUFA) for proper development, as high levels have been reported in yellow perch larvae and adult Eurasian perch (Dabrowski et al., 1991; Vacha et al, 1993). Abi-ayad et al. (1995) fed Eurasian perch broodstock three diets differing in n-3 fatty acid content and compared the ability of resulting larvae to resist osmotic shock and starvation. The authors found that larvae resulting from parents fed high levels of n-3 fatty acids were comparably resistant to larvae from wild broodstock, while resistance among larvae from parents fed low levels of n-3 fatty acids was

significantly lower than the former two groups. Conversely, Fiogbe et al. (1995) found no difference in survival between groups of Eurasian perch larvae fed with standard *Artemia* metanauplii and those fed *Artemia* enriched with n-3 fatty acids, while the larvae fed standard *Artemia* had a significantly higher mean growth rate. Thus, it seems the endogenous n-3 HUFA level in larvae is more important the exogenous level obtained through food.

OBJECTIVES

Against the background of the advances in perch culture described above, the objectives of this study were to: 1) assess the potential for using intensively reared yellow perch as broodstock through photothermal conditioning and artificial induction of spawning; 2) to evaluate larval rearing techniques for yellow perch derived from reproductively manipulated broodstock; 3) and to estimate the economic potential of commercially applying these intensification techniques by designing and identifying the associated costs of a facility based on such techniques.

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Table 1.1. Survival and growth rates of perch larvae based on initial diet.
[a]SGR = 100 (LnW2-LnW1) T^{-1}; W1 = initial weight, W2 = final weight, T = duration.

Diet	**Duration**	**Survival**	**SGR[a]**	**References**
	days	***%***	***%d^{-1}***	
Rotifer (cultured)	10	83.5	18.7	Awais et al. (1992)
Rotifer (lake)	21	63.0	NA	Hale and Carlson (1972)
Rotifer (lake)	9	41.7	12.6	Wang and Eckmann (1994)
***Artemia* sp.**	14	44.2	10.2	Hinshaw (1985)
Rotifer/*Artemia* sp.	5	50.2	20.7	Kestemont et al. (1996)
Dry diet	7	4.0	1.3	Awais et al. (1992)
Dry diet	15	26.0	7.8	Kestemont (1996)

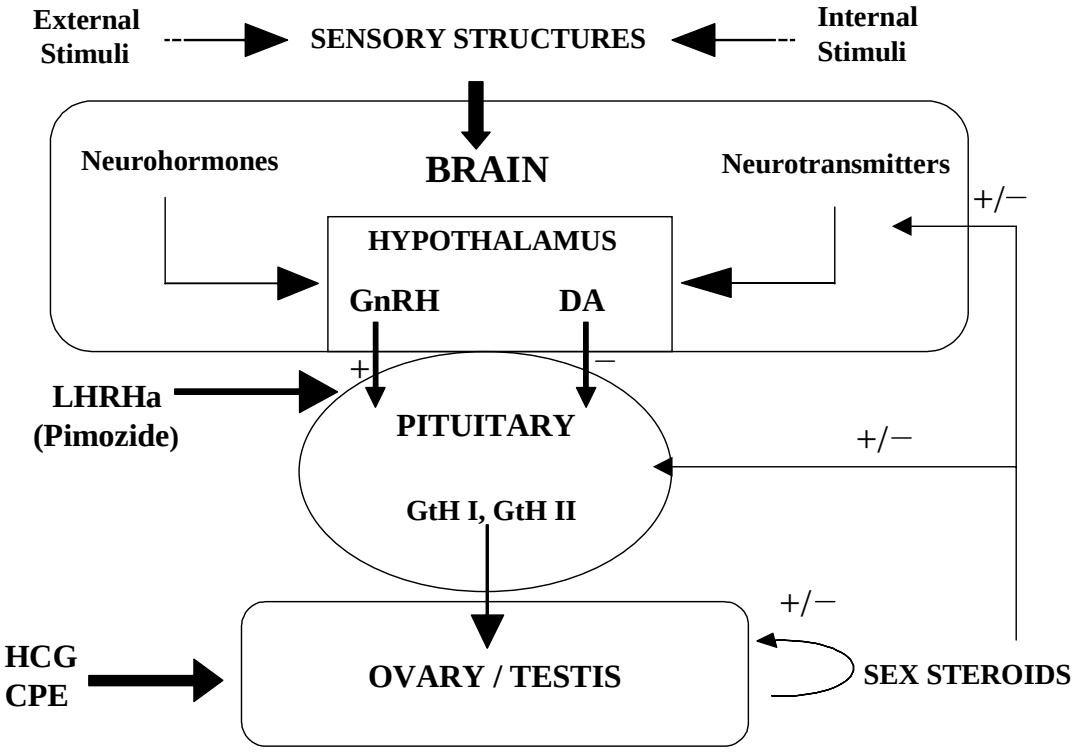

Figure 1.1. Flow diagram of hormonal cascade controlling reproduction in teleosts (from Redding and Patino, 1993).

Chapter Two:

Photothermal Manipulation of Yellow Perch Reproductive Cycle

INTRODUCTION

Yellow perch are annual spawners which undergo a long recrudescence period of gonadal development during autumn and winter followed by a short burst of spawning activity (2 - 3 weeks) in early spring at temperatures between 8 and 20°C. Female perch produce a cohesive, accordion-shaped hollow egg ribbon, which is dragged through milt released from males and fertilized (Heidinger and Kayes, 1993). Prior to spawning, the gonadosomatic index (GSI), which expresses gonad weight as a percentage of total weight, can range from 20-30% in females.

The hormonal regulation of most reproductive functions in fish stems from activity along the brain-pituitary-gonadal axis (Sundararaj, 1981; Redding and Patino, 1993; Figure 1). In general, internal and external stimuli are transduced by the brain into a series of complex endocrine activities, which control rates of gametogenesis and the time of spawning (Munro, 1990). External stimuli, such as temperature and photoperiod, seem to have the greatest regulatory influence over this process. However, proper water quality, genetics, nutrition and other modifying factors are necessary for successful reproduction as well (Wooten, 1982).

Attempts to manipulate the time of spawning in yellow perch have been made since the late 1970s with limited success. Hokanson (1977) found that a chill period of at least 185 days at or below 6°C was needed for 100% female spawning participation in Minnesota yellow perch. However, it is not known if yellow perch native to more southern regions require such conditions for recrudescence. Male perch are not as stringently regulated by environmental conditions, as males will produce motile sperm throughout fall and winter (Dabrowski et al., 1996).

Dabrowski et al. (1994b) attempted to condense the recrudescence period of perch by exposing fish in September to decreasing photothermal conditions reaching a winter nadir at the end of October. Then the fish were brought up to spawning conditions (13°C; 14h light) by mid-January. GSI of manipulated females reached a

maximum of 14% and egg viabilty was low (36.5%), which seems to confirm the need for a longer chill period suggested by Hokanson (1977).

Kayes and Calbert (1979) exposed perch caught from Lake Mendota, Wisconsin to a variety of photothermal regimes in February, but were unable to alter the spawning date suggesting that the maturation rate is set once gonadal development reaches later stages. Dabrowski et al. (1996) attempted to shift the entire reproductive cycle of perch by four months and found that fertility of eggs produced from phase-shifted fish was much lower than for eggs from fish kept at ambient conditions (1.6% vs. 78.2%, respectively). Also, maximum GSI and oocyte diameter were reduced in phase-shifted fish compared to control fish. However, these phase-shifted fish were photothermally delayed beginning in October, after ovarian growth and vitellogenesis had begun. Kolkovski and Dabrowski (1998) reported that shifting the reproductive cycle prior to the start of recrudescence yielded higher GSI (26.6%) and egg fertility (56% to eyed stage) than previous studies. Yet, a high incidence of scoliosis and low swim bladder inflation was observed in fry from phase-shifted females. Also, only 24 of 200 females were strip-spawned. The authors noted a positive correlation (r=0.74) between female body weight and embryo survival to the eyed stage. Given that the maximum weight of females used in the study was 130g, one might expect a higher survival of eggs from larger females.

Several exogenous hormones have been used successfully to control reproduction in yellow perch. Kayes (1977) was able to synchronize spawning four days after injecting females with 1 mg/kg of body weight of carp pituitary extract (CPE). Human chorionic gonadotropin (HCG) also has been shown to induce spawning in female yellow perch when given at a rate of 230 IU/kg in two successive injections at 24 hour intervals (Goubier, 1995). Both CPE and HCG, however, are effective only in fish which are already sexually mature (Dabrowski et al., 1996).

Luteinizing hormone-releasing hormone analogs (LHRHa) have been used both to stimulate spawning and promote sexual maturation. Dabrowski et al. (1994a) used

LHRHa and LHRHa combined with a dopamine inhibitor (pimozide) to synchronize spawning of laboratory-reared yellow perch 4-6 days after injection. Dosages of 100 µg/kg LHRHa in a single injection and 10 µg/kg LHRHa plus 10 mg/kg pimozide followed 48 hours later by 100 µg/kg LHRHa provided normal egg ribbons, while 300µg/kg LHRHa yielded a high percentage of fragmented egg ribbons. Embryo survival percentages, however, were not reported.

Several sex steroids have been shown to stimulate germinal vesicle breakdown (GVBD) and ovulation of yellow perch oocytes *in vitro*. Goetz and Bergman (1978) demonstrated that progestogens and 11-deoxycorticosteroids induced GVBD and ovulation. Goetz and Theofan (1979) also found that 17α-hydroxy-20β-dihydroprogesterone was effective in stimulating GVBD and ovulation. *In vivo* application of these steroids, however, has not been reported.

The successful culture intensification of this species will depend on the manipulation of its reproductive cycle to provide fingerlings continuously throughout the year (Dabrowski et al., 1996). Previous work, as reported, suggests that such manipulation is possible, yet the techniques have not been honed enough for commercial application. This study was conducted to assess the potential of using intensively reared yellow perch as broodstock through photothermal conditioning and artificial induction of spawning.

METHODS

Experimental conditions

Adult (age 1.5 year) yellow perch used in this study were held at the Virginia Tech Aquaculture Center, Blacksburg, VA, where they had been reared in recirculating aquaculture systems and fed artificial diets since 3 months of age. These fish had not been exposed to a natural, annual photothermal cycle and had not reached reproductive maturity. Out of approximately 5,000 fish, 72 female and 144 male perch were selected and transferred to six environmental control chambers in a ratio of 24 males to 12 females (2:1) per chamber. Each chamber consisted of a 2,200 liter, circular culture tank

which drained through a center standpipe and flowed to a 200 liter sump for removal of particulates (Fig. 2.1). Water from the sump was then pumped to the top of a packed tower trickling filter and allowed to gravity flow back into the culture tank. The culture tank was 50% shaded by placing 1/4" PVC sheeting over one-half of the tank to accommodate perch becoming increasingly photonegative with age (Huh, 1975). Fish were fed to satiation daily with a 38:8% (protein:lipid) sinking trout diet (Ziegler Brothers Inc., Gardners, PA). Each female was tagged internally with a passive integrated transponder (PIT) to provide individual identification during spawning.

Temperature within the tank was controlled by an external recirculating water-chiller (Neslab CFT Series, Portsmouth, NH) capable of cooling and heating in the range of 0 to 30°C (Fig. 2.1). Lighting within the chamber was provided by three incandescent lamps (75 watts each) mounted 2.5 meters above the culture tank and manually controlled by an adjustable, digital timer (Fig. 2.1). Each timer was connected to an electronic rheostat providing crepuscular light change (0.5 h) to simulate sunrise and sunset.

Photothermal cycle

After an acclimation period of one month at constant conditions (22°C; 13.5h light), a 12-month cycle simulating the annual light-dark sequence in the north-central region of the United States (origin of broodstock) was used to entrain the reproductive cycle of the perch. The first two experimental chambers were brought to a maximum day length of 12.5 hours of light prior to hormone injection, reflecting the natural day length at the time of spawning among wild yellow perch (Fig. 2.2) . These first two chambers, however, yielded no ovulating females after hormonal injection. Operating under the assumption that an increased day length might help induce ovulation, the remaining four chambers were brought to a maximum of 14 hours of light day^{-1} prior to injection (Fig. 2.3). Temperature was decreased 1°C/day to a chill temperature of 5°C, kept at 5°C for 180 days, and increased 1°C/day to a temperature of 14°C. Chambers were divided into 3 groups of 2 chambers each and photothermal cycles were initiated in each group at one

month intervals. This was to accommodate the logistical impracticalities of spawning all the experimental fish at one time. All maturation parameter estimates reported exclude experimental chambers 1 and 2.

Water quality

Dissolved oxygen and temperature in each chamber was measured daily with a portable oxygen meter (YSI Co., Yellow Springs, OH). Daily measurements of pH and total ammonia nitrogen (TAN) were made with a pH pen and a spectrophotometer, respectively (Hach Co., Loveland, CO). Weekly measurements were made of nitrite and nitrate (spectrophotometer; Hach Co.) and of alkalinity (titration method; Hach Co.).

Induced spawning

After a 180-day photothermal conditioning period, to induce ovulation each female was injected intraperitoneally with LHRHa (Sigma, St. Louis, MO) and pimozide (Sigma, St. Louis, MO) at a rate of 10 µg/kg LHRHa plus 10mg/kg pimozide followed by a second injection 48 hours later of 100 µg/kg LHRHa. At the time of the first injection, between 5 and 20 oocytes were collected by inserting a 100 µl micropipette into the urogenital opening of females and applying a slight vacuum to draw the eggs into the pipette. Eggs were then placed in a clearing fixative (Goetz, 1983) and mean oocyte diameter (nearest 0.1mm) and germinal vesicle position (Goetz, 1983) of each female was measured using a compound scope with an ocular micrometer. All females were anaesthetized with MS-222 (Argent Chemical Labs., Redmond, WA) (150 mg/L) prior to injection procedures.

After injection, females were checked daily for signs of ovulation by collecting oocytes (as previously described) to determine movement of the germinal vesicle (GV). In general, the GV is initially located in the center of the oocyte and migrates toward the periphery as the oocyte sexually matures (Wallace and Selman, 1978). Oocytes in the germinal vesicle breakdown stage (GVBD) were considered near ovulation and females with eggs exhibiting GVBD were checked more frequently (every 1 to 3 hours).

Ovulation was indicated by a swelling of the oocyte chorion and ovulating females were stripped of eggs. Stripped eggs were fertilized with milt from 1 to 2 males by the dry method (Piper et al., 1982). Milt was checked for motility with a compound microscope prior to fertilization. Female weights (g) were measured before and after stripping and the difference was used as the gonad weight in GSI calculations for experimental rooms 3, 4, 5, and 6 (Dabrowski et al., 1994). GSI calculations for rooms 1 and 2 were made by excising ovaries from females and using ovary and body weights directly.

Incubation methods

Fertilized egg ribbons were placed into an incubation system consisting of two, black, polyethylene cattle-feeding troughs (250 liters ea.) (Fig. 3.1). Within each trough, egg ribbons were stretched out and fastened to 1.2m x 40cm screens made of a 1.3cm PVC frame and 0.64cm mesh polyethylene netting (Fig. 2.4). Percent fertility was estimated by taking six samples of at least 20 eggs per sample (24 post-fertilization) from each ribbon and counting the number of living and dead (or unfertilized) eggs in each sample using a dissecting microscope. Living eggs were determined by the presence of a blastodisc at the animal pole of each egg. Individual egg ribbon fertility percentages were then used to calculate an overall mean fertility. Eggs were incubated until the eyed stage, at which time they were either allowed to hatch in the troughs or transported to green-water tanks located in the greenhouse at the Aquaculture Center.

Statistics

Simple linear regression was used to relate the mean oocyte diameter female^{-1} at the time of injection to the time (hrs) to ovulation after injection and percent egg fertility. Simple linear regression was used to relate the gonado-somatic index (GSI) female^{-1} at the time of spawning to the mean oocyte diameter female^{-1}, the time (hrs) to ovulation after injection and percent egg fertility. Simple linear regression was also used to relate pre-spawn weight of females to percent egg fertility. Sample sizes differed for these reproductive parameters and these differences are reflected in Table 2.1.

Analysis of variance was used to analyze the difference between the time (hrs) to ovulation after injection and percent egg fertility by germinal vesicle (GV) position. Tukey-Kramer's multiple comparison analysis was used to establish which GV positions differed in terms of their effects on time to ovulation and percent fertility. Analysis of variance was also used to analyze all maturation parameters by experimental chamber.

RESULTS

Female maturation

Mean GSI for photothermally manipulated females was 24.2% (SE = 0.67; N = 32) at the time of spawning and mean body weight was 374g (SE = 11.7; N = 41). The mean number of eggs g^{-1} of egg ribbon was 179.9 (SE = 14.1; N = 18). At the time of injection, 60% of females exhibited centrally located germinal vesicles, 25% exhibited migrating germinal vesicles and 15% exhibited germinal vesicles in the breakdown stage (N = 20). Mean oocyte diameter at the time of injection was 1.4 mm (SE = 0.01; N = 30) and the mean time to ovulation was 83.1 hours after the first injection (SE = 8.7; N = 25). After injection, 64% of females were artificially spawned and 36% spawned within the tanks (N = 34). GSI differed significantly among experimental chambers ($p < 0.05$; N = 32) (Table 2.1). All other maturation parameters did not differ significantly among environmental chambers ($p > 0.1$).

Mean oocyte diameter and time to ovulation were negatively correlated ($r^2 = 0.46$; $p < 0.01$; N = 25) as represented by the equation: $y = 674.5 - 420.7x$ (Fig. 2.5). No significant relationship existed between mean oocyte diameter and GSI ($r^2 = 0.14$; $p > 0.05$; N = 21) nor between GSI and time to ovulation ($r^2 = 0.03$; $p > 0.1$; N = 20). Mean oocyte diameters by GV position differed significantly ($p < 0.05$) (Table 2.2) and time to ovulation by GV position also differed significantly ($p < 0.01$) (Table 2.3).

Fertility

Overall mean fertility for egg ribbons from photothermally manipulated females

was 26.5% (SE = 6.3; N = 34). Mean fertility for egg ribbons from artificially spawned females was 37.7% (SE = 7.2; N = 22) and mean fertility for egg ribbons from tank spawned females was 6.0% (SE = 9.8; N = 12), which represented a significant difference ($p < 0.05$) (Table 2.4).

No significant relationship was observed between mean oocyte diameter and percent fertility ($r^2 = 0.08$; $p > 0.1$; N = 26). No significant relationship was observed between GSI and percent fertility ($r^2 = 0.03$; $p > 0.1$; N = 24). There was also no significant relationship observed between pre-spawn female weight and percent fertility ($r^2 < 0.01$; $p > 0.1$; N = 29). Percent fertility did not differ significantly by GV position ($p > 0.1$; N = 20) nor by experimental chamber ($p > 0.1$; N = 29).

Water Quality

All water quality parameters were within an acceptable range of values for freshwater temperate fishes (Stoskopf, 1993) (Table 2.5).

DISCUSSION

Female maturation

The results of this study support the assertion of Dabrowski et al. (1996) that shifting the entire reproductive cycle of yellow perch, prior to the start of recrudescence, is a viable way of obtaining fertile eggs at times other than the natural spawning season. Photothermally manipulated female yellow perch exhibited a mean GSI (24.2%) within the range of 20 - 30% reported for wild yellow perch at the time of spawning (Heidinger and Kayes, 1993) and comparable to the mean GSI (26.6%) attained by Kolkovski and Dabrowski (1998) in phase-shifted yellow perch.

Studies attempting to condense or shorten the reproductive cycle of yellow perch have resulted in lower female GSI percentages (12.2 - 14%) than reproductively mature wild yellow perch (Ciereszko et al., 1997; Dabrowski et al., 1994b). Similarly, the mean

GSI of females from experimental chambers 1 and 2, which received a maximum of 12.5 hours of light day^{-1} prior to injection, was 15.1% (N = 4) (Fig. 2.2). The higher GSI percentages reported here were attained by exposing the remaining four chambers to 14 hours of light day^{-1} prior to injection (Fig.2.3). Dabrowski et al. (1996), in an experiment using four different photothermal regimes, suggested that temperature has a greater influence than photoperiod on female maturation. This may be true, however the results of this study indicate that photoperiod, as well, may strongly impact female reproductive performance.

The mean oocyte diameter (1.4mm) of females at the time of spawning in this study was within the range reported for both wild yellow perch (1.0 - 2.1mm) and Eurasian perch (1.0 - 1.9mm) (Thorpe, 1977). The mean time to ovulation was 83.1 hours after the first injection and ranged from 14 to 175 hours post - injection, which is similar to the synchronization period realized by Dabrowski et al. (1994a) of 4 to 6 days post - injection using LHRHa. Kayes (1977) reported that using either carp pituitary extract (CPE) or human chorionic gonadotropin (HCG) synchronized spawning within 2 to 5 days post - injection. Kucharczyk et al. (1996) observed that most Eurasian perch ovulated between 64 and 66 hours after the first (priming) injection of CPE, HCG or a combination of the two. The authors attained such precise synchronization by using only females with migrating germinal vesicles.

The range of ovulation times in females from my study can be explained by the negative correlation between ovulation time and mean oocyte diameter (Fig. 2.4). Oocytes enlarge as they mature toward ovulation (Wallace and Selman, 1978), thus females with larger oocytes at the time of injection were closer to ovulation resulting in shorter ovulation times. Supporting this concept is the fact that the advanced GV positions were associated with larger oocytes and shorter times to ovulation (Tables 2.2; 2.3).

No significant relationship existed between mean oocyte diameter and GSI ($p > 0.05$), which contradicts the positive correlation observed between these two parameters by Ciereszko et al (1997) working with yellow perch. The maximum weight

of females used in that study, however, was 194.3g compared to the mean weight of 374g used in my study. Perhaps such a correlation between oocyte diameter and GSI only exists at smaller body weights.

Fertility

The relatively low fertility rate achieved in this study (overall mean of 26.5%) is partially due to the difference in egg ribbon fertility between artificially (stripped) spawned and tank spawned females. Mean egg ribbon fertility for strip-spawned females (37.7%) was significantly higher ($p < 0.05$) than the mean egg ribbon fertility for tank spawned females (6.0%) (Table 2.4). Kayes (1977) observed that male yellow perch did not always fertilize extruded egg ribbons when kept together with spawning females in tanks and attributed this to factors associated with the artificial environment, such as bright lighting and abrupt noises. He also noted that females would release egg ribbons even if males were not present in the tanks, which may indicate a lack of some exogenous cue(s) in an artificial environment needed by male yellow perch for spawning synchronization.

Another reason for low overall fertility was that the majority of egg ribbons (56%) in this study showed no fertility at all. The remaining egg ribbons (44%), which showed at least some fertility, had a mean fertilization rate of 60.1%. Tank spawned ribbons accounted for only 10 of the 19 unfertilized ribbons, which, as mentioned, may simply be due to the lack of male spawning participation. The nine unfertilized ribbons from artificially spawned females are harder to explain. No correlation was found in this study between fertility and any of the afore mentioned maturation parameters. There was also no significant difference in fertility by either GV position or experimental chamber. Thus, the high percentage of infertile, stripped egg ribbons may be the result of poor spawning technique.

Kolkovski and Dabrowski (1998), in a similar photothermal manipulation experiment with yellow perch, found a positive correlation between female body-weight

and fertilization rate to the eyed stage. No such correlation was found in this study and, again, may be a phenomenon relegated to smaller female body sizes, as the maximum weight used by Kolkovski and Dabrowski (1998) was 130g. The authors did not artificially induce spawning, making other fertility comparisons difficult.

Summary

This study shows that exposing immature yellow perch to an artificial photothermal cycle can yield mature fish and fertile eggs. Shifting the entire reproductive cycle, rather than shortening or condensing it, can be a more effective method of producing yellow perch fry and fingerlings out of season. Hormonal induction following such a photothermal cycle is effective at synchronizing spawning and strip spawning yields the best fertility rates. Also, egg diameter and germinal vesicle position can be used to predict spawning times, which may promote better fertility rates.

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Table 2.1. Sample sizes used to obtain estimates of reproductive parameters.

Parameter	Sample Size (N)
Total number of females spawned	34
Gonadosomatic index	32
Body weight	41
Mean eggs g^{-1} of egg ribbon	18
Germinal vesicle position	20
Mean oocyte diameter	30
Time to ovulation post-injection	25
Fertility	34

Table 2.2. Mean gonadosomatic index (GSI) at spawning time and S.E. by experimental chamber. Means followed by same superscript are not significantly different at the 0.05 experiment wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

Experimental Chamber	Number	Mean	S.E.
3	10	25.6^{ab}	1.0
4	5	21.5^{ab}	1.5
5	8	26.5^{a}	1.2
6	9	21.9^{b}	1.1

Table 2.3. Mean oocyte diameters (mm) and S.E. by germinal vesicle position at the time of injection. Position abbreviations are as follows: CGV = central, GVM = migrating and GVBD = breakdown stage. Means followed by same superscript are not significantly different at the 0.05 experiment wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

GV position	Number	Mean	S.E.
CGV	12	1.38^{a}	0.02
GVM	5	1.45^{ab}	0.03
GVBD	3	1.47^{b}	0.03

Table 2.4. Mean time (hours) to ovulation post-injection and S.E. by germinal vesicle (GV) position at the time of injection. Position abbreviations are as follows: CGV = central, GVM = migrating and GVBD = breakdown stage. Means followed by same superscript are not significantly different at the 0.05 experiment-wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

GV position	Number	Mean	S.E.
CGV	12	109.4[a]	6.3
GVM	5	44.0[b]	9.8
GVBD	3	17.3[b]	12.6

Table 2.5. Mean egg ribbon fertility % and S.E. by spawning method. Means followed by same superscript are not significantly different at the 0.05 experiment wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

Spawn Method	Number	Mean	S.E.
Artificial	22	37.7[a]	7.2
Tank	12	6.0[b]	9.8

Table 2.6. Mean (SE) water quality parameters among experimental chambers. Means in rows followed by the same superscript are not significantly different at the 0.05 experiment wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

	Experimental Chamber					
Parameter	**1**	**2**	**3**	**4**	**5**	**6**
D.O. (mg/L)	**11.9** (0.1)**a**	**11.9** (0.1)**a**	**11.8** (0.1)**ab**	**11.3** (0.1)**c**	**11.4** (0.1)**bc**	**11.3** (0.1)**bc**
pH	**8.4** (0.03)**a**	**8.5** (0.03)**a**	**8.4** (0.03)**a**	**8.2** (0.03)**b**	**8.4** (0.04)**a**	**8.4** (0.04)**a**
TAN (mg/L)	**0.09** (0.01)**ab**	**0.09** (0.01)**ab**	**0.05** (0.01)**a**	**0.19** (0.01)**c**	**0.13** (0.01)**b**	**0.11** (0.01)**b**
NO_2 (mg/L)	**0.008** (0.004)**a**	**0.019** (0.004)**a**	**0.005** (0.004)**a**	**0.006**(0.004)**a**	**0.007** (0.004)**a**	**0.009** (0.004)**a**
NO_3 (mg/L)	**5.4** (1.4)**a**	**4.0** (1.4)**a**	**4.0** (1.4)**a**	**3.3** (1.4)**a**	**3.2** (1.4)**a**	**4.3** (1.4)**a**
Alk. (mg/L)	**271** (13.5)**a**	**287** (13.5)**a**	**280** (13.5)**a**	**306** (13.5)**a**	**324** (13.5)**a**	**317** (13.5)**a**

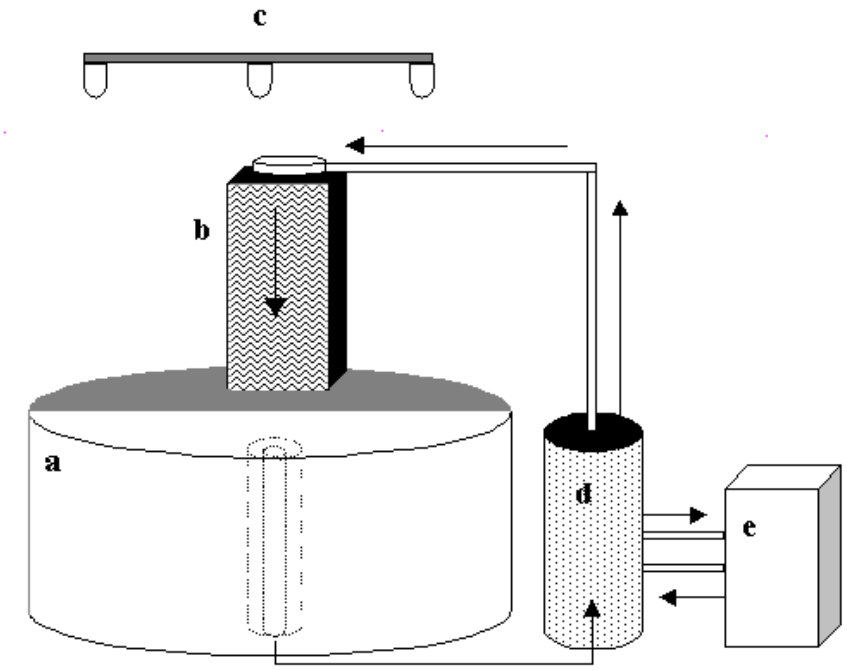

Figure 2.1. Photothermal conditioning chamber: **a-** broodstock tank (2,200 L), **b**- trickling biofilter; **c**- lighting fixture, **d**- solids removal sump, **e**- water chiller.

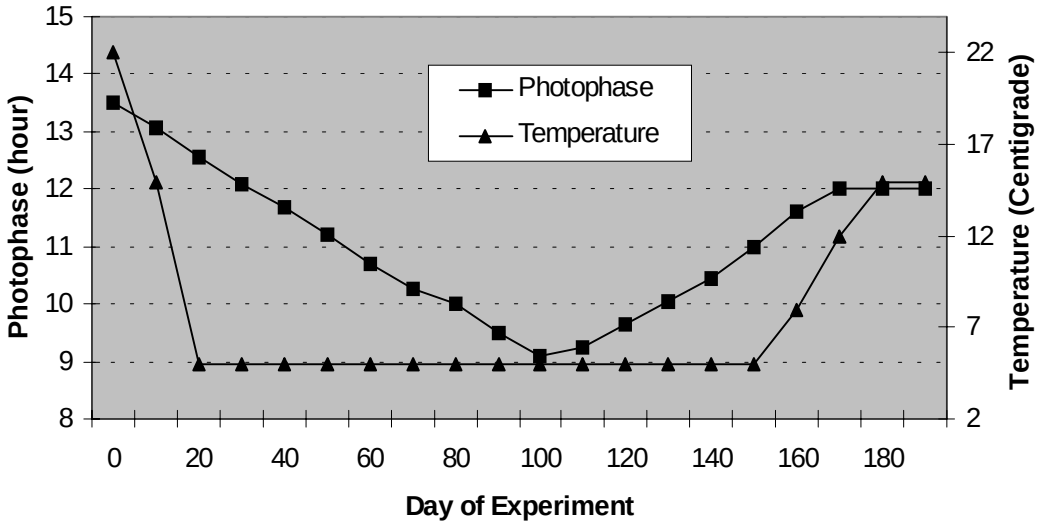

Figure 2.2. Photothermal cycle for experimental chambers 1 and 2. Photophase (day length) at time of injection was 12 hours.

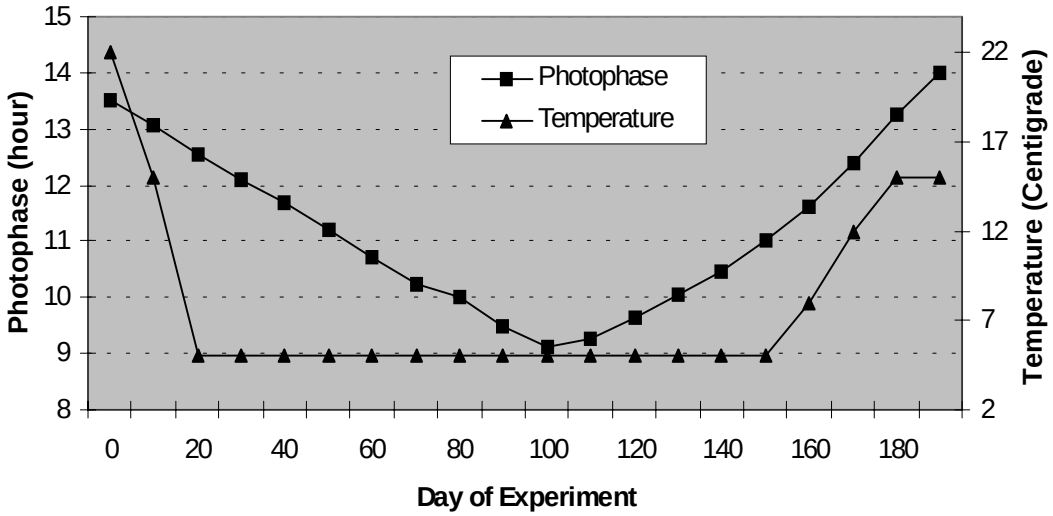

Figure 2.3. Photothermal cycle for experimental chambers 3, 4, 5 and 6. Photophase (day length) at time of injection was 14 hours.

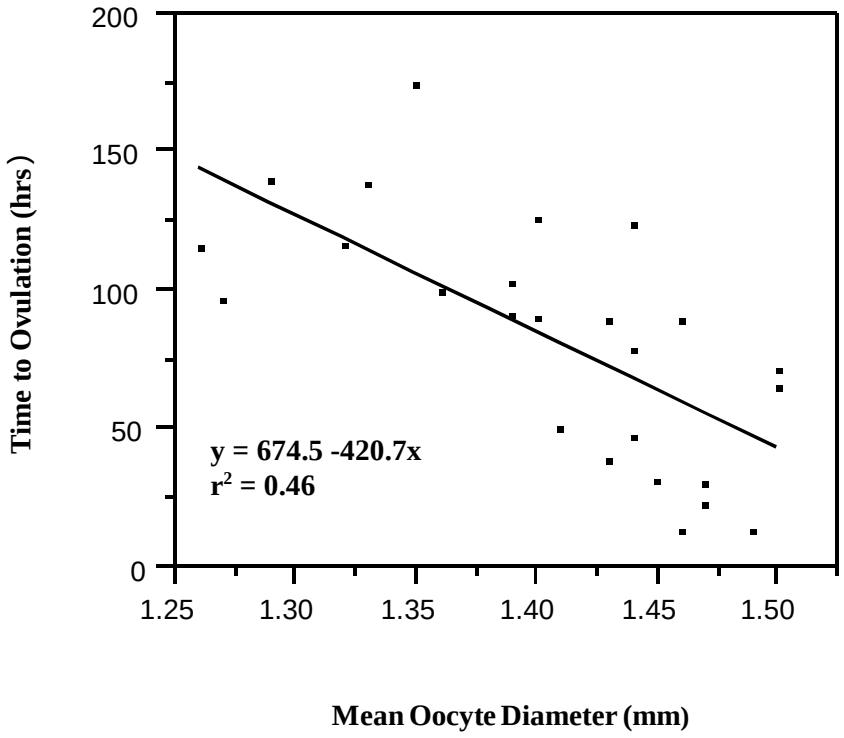

Figure 2.4. Linear relationship between mean oocyte diameters (mm) and time (hrs) to ovulation post-injection in photothermally manipulated female yellow perch ($n = 25$; $p < 0.01$).

Chapter Three:

Larval Rearing of Yellow Perch

INTRODUCTION

The manipulation of the yellow perch reproductive cycle to obtain fry and fingerlings in the off-season leads to the subject of larval rearing techniques. Spawning perch in fall and winter requires alternative methods to pond rearing, since pond conditions are not optimal in the colder months. Techniques employing indoor, controlled conditions will be needed if year-round production of yellow perch is to be realized.

Larval perch are relatively small at hatch, from 4-7mm total length (Mansueti, 1964; Ney, 1978). In the wild, larvae initially feed on rotifers and copepod nauplii (<300μm) due to their limited gape size at this stage (Raisanen and Applegate, 1983). Perch larvae, in contrast with many other fishes, begin feeding as soon as they are free-swimming, before the yolk sac is absorbed (Hokanson, 1977). Thus, attempts to propagate larval perch under controlled laboratory conditions have focused on providing zooplankton of the appropriate size immediately after hatch (Hale and Carlson, 1972; Raisanen and Applegate, 1983; Wang and Eckmann, 1994). When initially fed with an artificial diet, larval perch acceptance, growth and survival rates have been highly variable and relatively poor (Brown et al., 1996; Kestemont et al., 1996).

Hale and Carlson (1972) determined that a zooplankton density of at least 250 edible organisms per larval yellow perch (5 fish/liter) fed four times day^{-1} was needed to obtain 50% survival for a period of three weeks post-hatch. Wang and Eckmann (1994) found the best survival (41.7%) and growth of 4-day-old Eurasian perch (*Perca fluviatilis*) larvae occurred at a zooplankton density of 120 rotifers per larval perch for a period of 9 days. Both studies utilized zooplankton collected from lakes. In contrast, Awaiss et al. (1992) achieved 83.5% survival of Eurasian perch larvae over 10 days by mass culturing the freshwater rotifer, *Brachionus calyciflorus*, for use as initial food. By the end of the study (10 days), individual larval perch were consuming over 2000 rotifers day^{-1}.

Although generally considered too large (420-480μm) for initial feeding (Mansueti, 1964; Awaiss et al., 1991), brine shrimp *(Artemia* sp.) nauplii also have been used to rear perch fry successfully . Hinshaw (1985) obtained 44.2% survival of yellow perch after 14 days when fed 80 to 143 *Artemia* sp. nauplii per larval perch three times daily. However, Eurasian perch larvae display higher survival rates when fed a mixed diet of rotifers and *Artemia* sp. (50.19%) compared to an exclusive diet of *Artemia* sp. (34.30%) (Kestemont et al., 1996). Thus, it appears a surplus of easily captured (i.e., smaller) prey organisms, such as rotifers, promotes the best survival of perch fry.

Attempts to use artificial dry diet as initial food for perch have yielded poor results. Kestemont et al. (1996) reported that while Eurasian perch larvae ingested a particular dry diet from the time of hatching, the maximum survival rate was only 26% after 15 days, and growth was slow (from 0.8 to 2.6 mg). Awais et al. (1992) were even less successful, with only 4% survival after 7 days and almost no growth (from 0.8 to 0.9 mg) using a microencapsulated dry feed. These results support the assertion that dry diet performance remains inferior to that using live food (Table 1.1). Weaning perch fry onto a dry diet after 30-45 days of feeding on zooplankton seems to be the most productive method of rearing (Kestemont et al., 1996).

Rearing tanks with black backgrounds seem to promote survival of newly hatched fry. Hale and Carlson (1972) reported significantly higher survival in tanks with slate bottoms (63%) than in glass bottomed tanks (10%). Hinshaw (1985) found that fry survival was best in treatments with high prey contrast due to black backgrounds (44.2%) when compared to low contrast systems with white backgrounds (4.6%). There is also evidence that internal illumination of fry tanks improves feeding efficiency, presumably due to the reduction of shadow related disturbances (Malison and Held, 1992).

There is some discrepancy among investigations regarding the effect of cannibalism during the larval and post-larval stages of perch. Kestemont et al. (1996)

refer to cannibalism as a major factor in the survival of larval Eurasian perch. However, Brown and Dabrowski (1995) discounted cannibalism as a significant influence on survival of yellow perch, except in systems with mixed age classes. Malison and Held (1992) noted an increase in cannibalism amongst yellow perch fingerlings (34-77 days old) in heavily stocked systems (37.4 fish L^{-1}) compared to lightly stocked systems (13.7 fish L^{-1}). Alternatively, Kestemont et al. (1995) noted that in heavily stocked systems (4000 eggs m^{-2}) the proportion of cannibals was reduced (2.1%) when compared to a lighter density of 500 eggs m^{-2} (4.2%). Melard et al. (1996) also found the proportion of cannibals decreased at higher densities (2000 - 4000 fingerlings m^{-2}) due to a more homogeneous growth pattern when compared to lower densities (500 - 1000 fingerlings m^{-2}). However, the actual number of cannibals at lower densities remained less than 1%. Thus, cannibalism remains a variable factor in the production perch fry.

There is evidence that perch larvae, as with many other fishes, require high levels of *n*-3 highly unsaturated fatty acids (HUFA) for proper development, as high levels have been reported in yellow perch larvae and adult Eurasian perch (Dabrowski et al., 1991; Vacha et al., 1993). Abi-ayad et al. (1995) fed Eurasian perch broodstock three diets differing in *n*-3 fatty acid content and compared the ability of resulting larvae to resist osmotic shock and starvation. The authors found that larvae resulting from parents fed high levels of *n*-3 fatty acids were comparably resistant to larvae from wild broodstock, while resistance among larvae from parents fed low levels of *n*-3 fatty acids was significantly lower than the former two groups. Conversely, Fiogbe et al. (1995) found no difference in survival between groups of Eurasian perch larvae fed with standard *Artemia* metanauplii and those fed *Artemia* enriched with *n*-3 fatty acids, while the larvae fed standard *Artemia* had a significantly higher mean growth rate. Thus, it seems the endogenous *n*-3 HUFA level in larvae has more influence on larval development than the exogenous level obtained through food.

The successful rearing of yellow perch larvae in intensive conditions will

continue to rely on the feeding of live organisms to newly hatched fry until an adequate dry feed is developed, which is probably the reason such intensive culture has not been commercially successful. Live food organisms, such as rotifers, are difficult to raise in the quantity required for large scale production. Also, many studies on larval rearing utilize small rearing systems and it is logistically and economically difficult to scale these small systems up to commercial size.

This study was conducted to evaluate larval rearing techniques for yellow perch derived from reproductively manipulated broodstock. These techniques were designed simulate commercial scale conditions, rather than the often smaller and commercially infeasible laboratory conditions used in other studies.

METHODS

Incubation methods

Fertilized yellow perch egg ribbons were placed into an incubation system consisting of two, black, polyethylene cattle-feeding troughs (250 liters ea.) which were fed water from a 570 liter reservoir by two 1/6 horsepower (HP) submersible pumps at a rate of 4 liters minute^{-1} (Fig. 3.1). Water entered each trough at one end and exited through a 3.8cm diameter PVC drain line at the other end, flowing back to the reservoir. A separate submersible pump delivered water to the top of a trickling biofilter positioned above the reservoir and water was allowed to gravity-flow back into the reservoir. Three light-niches (15 watt incandescent bulbs) equidistant from each other (48cm) provided internal illumination of each trough, which promotes efficient feeding by larvae (Malison and Held, 1992). Light intensity was 180 lux at the water surface directly above the light niches and 2 lux at the surface in the dark regions at either end of the troughs.

An ozone sterilization system drew water from the reservoir through a 5.1cm diameter port via two external, ½ HP centrifugal pumps and returned water back to the

reservoir after sterilization. Ozone concentrations in the reservoir following a 15-minute treatment period per day were kept below 0.01mg/L as yellow perch larvae are sensitive to ozone concentrations as low as 0.06 mg/L (Asbury and Coler, 1980). Temperatures were maintained in the system by recirculating reservoir water through an external chiller (as described in chapter 2).

Prior to placement in the troughs, each fertilized ribbon was weighed (nearest 0.1g). Three samples of each ribbon, consisting of at least 20 eggs per sample (one in the middle and one at either end of the ribbon), were enumerated (eggs/sample) using a dissecting scope and sample weights were estimated by volume displacement within a 10 ml syringe. The syringe was modified to hold water without a vacuum by silicone sealing one end. The sample measurements were extrapolated to estimate the number of eggs/ribbon.

Within each trough, egg ribbons were stretched out and fastened to 1.2m x 40cm screens made of a 1.3cm PVC frame and 0.64cm mesh polyethylene netting. Eggs were incubated until the eyed stage, at which time they were either allowed to hatch in the troughs or transported to green-water tanks located in the greenhouse at the Aquaculture Center. Percent hatch-out was estimated by allowing samples of fertilized egg ribbons (<30 eggs/sample) to hatch in petri dishes and dividing the number of resulting sac fry by the total number of eyed eggs in the samples.

Larval rearing

Larvae were assigned arbitrarily to three rearing treatments. Larvae in the first treatment (T1) were allowed to hatch in the incubation troughs and were fed live rotifers (*Brachionus* sp.) which were cultured extensively in the greenhouse in 1140 liter tanks following guidelines outlined by Hoff and Snell (1997). Rotifers were harvested by pumping culture water through a 53 μm mesh plankton net, effectively concentrating the rotifers to desired densities. Fry were fed at a rate of 250 rotifers larvae^{-1} feeding^{-1} at four feedings day^{-1}. Internal lights were gradually brought to full

intensity with a manual rheostat prior to each feeding. Treatment two (T2) was the same as T1, except T2 larvae were supplementally fed an artificial diet (LiquaLife, Cargil, Inc., Minneapolis, MN) at a rate of 2 ml trough^{-1} feeding^{-1}. Treatments one and two were performed in the same troughs at different times, thus utilizing larvae from different broodstock.

Larvae (T3) were stocked into two greenhouse tanks (2,000 L each) and allowed to forage on naturally occurring zooplankton. Tanks were sloped at each end and lined with black polyethylene (Fig. 3.2). Algal growth in the tanks was stimulated by adding a commercial brand of Guillard's F/2 formulation of inorganic fertilizer (Kent Marine, Inc., Mareitta, GA), which provides a 7:1 nitrogen to phosphorus ratio. Alkalinity was kept above 100 mg/L by adding sodium bicarbonate as needed which helped stabilize the pH. Temperature was kept between 18 and 24°C by a thermostatically controlled heating/cooling system which detects the greenhouse air temperature. Tanks were filled and fertilized close to the date of larval stocking to prevent early peaking of algae and rotifer populations (Culver, 1996). At the time of hatch, fish densities and water chemical parameters were similar among all rearing treatments.

Fry density estimates in each treatment were made by multiplying the estimated number of eyed eggs in each system by the estimated percent hatch-out. Many larvae transported into the greenhouse were stimulated to hatch incidently by stirring, as bucket loads of eyed eggs were stirred and sub-sampled for quantity estimates. Therefore, density estimates of T3 also include a direct estimation of hatched fry transported to the greenhouse. Desired densities in all treatments were 20 larvae L^{-1}.

Growth of fry was estimated by sacrificing 10 to 20 fish from each system daily from the time of hatch and measuring length of each fish to the nearest 0.1mm using a dissecting scope with an ocular micrometer. Frequency of skeletal deformities, food ingestion, yolk-sac absorption and swimbladder inflation was noted from the time of hatch-out by examination under a dissecting scope. All fry were anaesthetized with MS-222 prior to examination and preserved in 5% formalin following examination.

Water Quality and Biotic Conditions

Water quality measurements for all rearing treatments were performed as described in chapter two. Additional daily measurements were made in T3 of rotifers ml^{-1} and algal cells (1×10^6) ml^{-1}. Rotifer counts were made taking 1 liter samples from T3 culture tanks, stirring the sample and dropping 10, 0.1ml sub-samples onto a petri dish. Rotifers within each 0.1ml drop were counted using a dissecting scope. Algal counts and extrapolations were made using a hemacytometer according to supplier's instructions (Aquaculture Supply, Florida Aquafarms, Dade City, FL).

Statistics

Mean comparisons among rearing treatments were made of length (mm), % swimbladder inflation (SBI), % yolk sac absorption (YSA), % of skeletal deformities and % ingestion using one-way ANOVA analysis. All comparisons were made either on or through day 6 post-hatch. Tukey-Kramer's experiment-wise multiple comparison for an honest significant difference (HSD) was used to determine which means differed for each parameter.

Specific growth rate (SGR) was estimated for each treatment with the following formula: $SGR = 100 (\ln L2 - \ln L1) T^{-1}$, where L1 = initial length, L2 = final length and T = time (days). One-way ANOVA was used to compare SGR among treatments.

RESULTS

Survival and Growth of Larvae

Survival in T1 and T3 declined rapidly to 0% on day 6. Survival in T2 was less than 10% after day 11. Initial lengths did not differ significantly among treatments ($p > 0.1$) (Fig. 3.3). Lengths differed significantly among treatments on day 6 ($p < 0.05$), however, specific differences between means were not detected using the Tukey-Kramer HSD experiment-wise method (HSD = 1.77mm) (Fig. 3.3). SGR for the first 6 days of feeding did not differ significantly among treatments ($p > 0.1$) (Tab. 3.1).

SBI and YSA

The percentage of fry with inflated swimbladders did not differ significantly among treatments on day 6 ($p > 0.1$) and was over 89% for all treatments by day 6 (Fig. 3.4). The percentage of yolksac absorption was less than 3% for all treatments and did not differ significantly on day 6 ($p > 0.1$) (Table 3.2).

Ingestion and Deformities

The ingestion of food by larvae reached 84% by day 3 in T3, which was significantly greater than both T1 and T2 (5 and 4%, respectively) ($p < 0.01$). Ingestion rate among treatments, however, did not differ significantly by day 6 ($p > 0.1$) (Fig. 3.5). The total percentage of skeletal deformities (cranial and spinal) during the first 6 days differed significantly by treatment ($p < 0.05$), with T3 (60%) having a significantly greater percentage of deformities than both T1 and T2 (17 and 25%, respectively) (Fig. 3.6).

Water Quality and Biotic Conditions

Dissolved oxygen (D.O.) did not differ significantly among treatments ($p > 0.1$), while all other water quality parameters did differ significantly among treatments (Tab.3.3). The mean number of rotifers ml^{-1} observed in T3 was 30.2 (SE = 10.2) and increased from 5.5 on day 1 to 56.0 on day 6 (Fig.3.7). The mean number of algal cells ml^{-1} was 321×10^3 (SE = 55.4) and increased from 200×10^3 on day 1 to 505×10^3 on day 6 (Fig. 3.7).

DISCUSSION

Survival, Growth and YSA of Larvae

Survival of yellow perch larvae was low in all three treatments with peak mortalities occurring on day 6. Survival estimation for T2 (<10%) is only

observational, as the actual hatch rate in all treatments was visibly much higher than that estimated from egg samples (μ=23%; SE=5.85; N=4). Without an accurate initial density estimate, any survival estimation would be erroneous. Nevertheless, estimated survival was lower than that achieved in comparable studies with perch larvae (Table 1.1).

Starvation may have been a probable cause for low survival, as peak mortality coincided

with the depletion of yolk reserves. Although complete yolk sac absorption had occurred in only a small percentage of fry by day 6 (Table 3.2), larvae had begun to ingest food by day 2 in all treatments and reliance on yolk for energy was probably diminished by day 6. Previous studies have determined that larval yellow perch complete yolk sac absorption at 7mm of length (Mansueti, 1964; Houde, 1969) or, in the case of Kestemont et al. (1996), on day 6 at 21°C. This period of development, between the prolarval and postlarval stages, coincides with high mortality in laboratory reared perch (Mansueti, 1964; Wang and Ekmann, 1994).

The relatively low growth rate observed for all treatments (< 2%; Tab. 3.1) supports the assertion that starvation caused high mortality. The specific growth rate (SGR) of larvae in this study was well below that attained by other studies on perch utilizing live food and was comparable to the growth rate realized by Awais et al. (1992) using a microencapsulated dry feed, which yielded only 4% survival (Tab 1.1).

The lack of an accurate initial density estimate may have led to grossly under-fed larvae in T1 and T2. For example, if 40,000 eyed eggs were in each trough and hatch rate was the estimated 23%, then 9.2 million rotifers day^{-1} would be needed to achieve the desired feeding rate of 1000 rotifers $larvae^{-1}$ day^{-1}. However, if hatch rate was 80%, then 32 million rotifers day^{-1} would be needed. A hatch rate of 80% would have demanded rotifer production in excess of that realized in this study.

Larvae in T3 may have starved due to an inability to capture prey. Mean rotifer count in T3 was 30.2 ml^{-1}, which provided 1500 rotifers $larvae^{-1}$, beyond the desired

1000 rotifers larvae^{-1} feeding rate. However, the number of algal cells ml^{-1} by day 6 was 510 x 10^3, which may have caused the turbidity level to become too high for efficient foraging by larvae. In a study on the effects of larval walleye and fertilization on the plankton community in culture ponds, Qin et al. (1995) found adequate growth and survival of larvae at algal densities under 200 x 10^3 cells ml^{-1}. Similarly, Culver (1996) recommended a fertilization program for larval culture ponds which promoted algal densities that peaked under 400 x 10^3 cells ml^{-1} and had a mean algal density under 300 x 10^3 cells ml^{-1}.

The intestines of T3 larvae which exhibited some ingestion contained copious amounts of algae throughout the first 6 days of feeding. The ingestion of algae by larvae during first feeding has been considered beneficial by some authors (Dabrowski, 1984; Wooton, 1990). This may explain why T3 larvae grew comparably with T2 until day 4, following which T3 larval growth leveled off (Fig. 3.3). Larvae may not have been able to utilize algae for growth after day 4. The ingestion of algae in T3 throughout the experiment suggests that larvae may have passively taken in algae while foraging, since no studies have indicated a positive selection for algae by perch larvae (Siefert, 1972; Guma, 1978; Melbourne et al., 1985).

The mean length among treatments differed significantly on day 6 ($p = 0.03$), but the experiment-wise HSD was 1.77mm, resulting in no significant differences by comparison. However, since there was a weak difference detected overall, the 0.74mm difference between T1 and T2 may be of practical significance, suggesting that the addition of an artificial diet to T2 may have helped generate larger larvae by day 6 (Fig. 3.3). The SGR among treatments did not differ significantly over the first 6 days and was unacceptably low from a practical standpoint (Tab. 3.1). Again, this is probably due to under-feeding in T1 and T2 and inefficient foraging in T3.

SBI

Swim bladder inflation (SBI) was high in all treatments and did not differ

significantly by day 6 (Fig. 3.4). The ability of larvae to inflate their swim bladder through the intake of atmospheric gas has been an obstacle to the intensive culture of many fishes, particularly the closely related walleye (*Stizostedion vitreum*) (Summerfelt, 1991). This problem has been remedied for walleye culture through the use of surface spray to break up oily films on the water surface resulting from artificial feeds (Barrows et al., 1993; Moore et al., 1994). The problem has not been widely reported for yellow or Eurasian perch culture, although Kolkovski and Dabrowski (1998) noted a low incidence of SBI (44%) in larvae derived from off-season spawning. Ribi (1992) also attributed high larval mortality in Eurasian perch to the lack of SBI. The high SBI rate observed in this study suggests that culture conditions were appropriate for initial SBI and may be related to the use of live and sinking artificial diets, which do not create an oily surface film.

Ingestion

Ingestion rate among treatments did not differ significantly by day 6 and was similar to that achieved in other studies on perch larvae. Siefert (1972) and Raisanen and Applegate (1983) found that yellow perch began feeding at a length of 6.0 mm before yolk sac absorption, which is close to the overall mean length of 6.2 mm on day 2 of this study when all three treatments had larvae that exhibited some ingestion. Wang and Ekmann (1994) reported that 50% of larvae exhibited ingestion by day 4 at a length of 6.7 mm and Kestemont et al.(1996) found that larval Eurasian perch ingested *Artemia* nauplii by day 2 post-hatch. Thus, there was no apparent endogenous problem with ingestion ability by larvae, which again lends support to the hypothesis that larvae starved due to under-feeding or inefficient foraging.

Deformities and Water Quality

The percentage of larvae in T3 which exhibited skeletal deformities (either cranial, spinal or both) differed significantly from T1 and T2 (Fig. 3.6). This may have

been due to the handling associated with transferring eggs and larvae from the incubation troughs to the greenhouse. T3 larvae were siphoned into buckets and the buckets were stirred before taking sub-samples. Birchfield (1987) found that vigorously shaking samples of gizzard shad (*Dorosoma cepedianum*) larvae resulted in a significantly higher percentage (86%) of cranial anomalies than treatments in which samples were not shaken.

The percentage of deformities found in T1 and T2, while significantly less than T3, was relatively high at 16.5 and 25%, respectively (Fig. 3.6). This is not uncommon among cultured species of fish and is symptomatic of a variety of maladies. Schaperclaus (1992) identified five major causes of skeletal deformation: genetic incompatibility, physical damage to embryos, injuries, biotic diseases and environmental factors. Environmental factors consist of nutritional deficiencies, metal toxicities, increased water velocity and decreased water hardness (Phromkunthong et al., 1997; Newsome and Piron, 1982; Bengtsson et al., 1985; Divanach et al., 1997; Scarpa and Gatlin, 1993).

The exact cause of deformities cannot be determined in this study, yet undoubtedly contributed to the poor rearing success observed in all three treatments. Andrades et al. (1996) found that 27% of cultured gilthead sea bream (*Sparus aurata*) showed axial deformations and, of these, 82% died soon after hatching. Deformed larvae in the present study, however, exhibited a 51% ingestion rate compared to a 49% ingestion rate of non-deformed larvae. It appears, then, that the deformities observed did not cause mortality due to a reduced ability to ingest food, but may have indirectly contributed to the mortality rate by affecting foraging efficiency and prey capture ability. Vlavonou et al. (1995) attributed low survival rates among Eurasian perch larvae to exhaustion from pursuing prey that were too large or too fast. The deformed larvae may have suffered from a similar disadvantage due to impaired swimming or prey capture ability.

All water quality parameters, with the exception of dissolved oxygen, differed

significantly by treatment (Table 3.3). Ammonia levels, however, were the only parameter which potentially exceeded the tolerance of yellow perch in any of the treatments (Thorpe, 1977; Stoskopf, 1992). The allowable unionized ammonia (NH_3-N) concentration for fish culture is quite debatable and species specific (Meade, 1985). NH_3-N was highest in T3 with a mean of 0.06 mg/L, with T1 and T2 means at 0.01 and 0.03, respectively. These levels unlikely caused acute mortality, despite the varying toxicity reports and are more closely associated with chronic toxicity problems. The NH_3-N levels in this study may have led to inhibited development and growth of the larvae (Burkhalter and Kaya, 1977; Meade, 1985). No histological sampling was performed to determine if larvae suffered the from typical aberrations related to ammonia toxicity.

Summary

The results of this study reflect the conclusions by other authors regarding larval rearing techniques of yellow perch, in that the feeding of such small larvae poses the greatest obstacle to the intensification of this species. The development of deformities by larvae is also a concern and may represent unidentified factors related to such intensification techniques.

The need for an accurate initial larval density estimate is paramount in the success of feeding larvae. Such an estimate can be better attained by allowing fertilized eggs to hatch in a separate container and transferring hatched larvae to the rearing system. Transferring of larvae should be done with great care, however, so as not to damage the fish and induce skeletal deformities.

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Table 3.1. Mean specific growth rate (SGR) and S.E. for first 6 days of feeding by treatment. Means followed by same superscript are not significantly different at the 0.05 experiment wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

Treatment	Number	Mean	S.E.
T1	2	0.9[a]	0.32
T2	2	1.9[a]	0.32
T3	2	1.7[a]	0.32

Table 3.2. Mean yolk sac absorption (YSA) % and S.E. on day 6 by treatment. Means followed by same superscript are not significantly different at the 0.05 experiment wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

Treatment	Number	Mean	S.E.
T1	2	2.8[a]	2.18
T2	2	0.0a	2.18
T3	2	2.6[a]	2.18

Table 3.3. Mean (SE) water quality parameters among rearing treatments. Means in rows followed by the same superscript are not significantly different at the 0.05 experiment-wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

Parameter	T1	T2	T3
D.O. (mg/L)	**8.1** (0.36)**a**	**8.4** (0.36)**a**	**8.3** (0.36)**a**
Temp. (ºC)	**21.6** (0.26)**a**	**19.6** (0.26)**b**	**23.9** (0.26)**c**
pH	**8.7** (0.05)**a**	**8.8** (0.05)**a**	**9.3** (0.05)**b**
TAN (mg/L)	**0.06** (0.03)**a**	**0.16** (0.03)**ab**	**0.18** (0.03)**b**
NO_2 (mg/L)	**0.027** (0.059)**a**	**0.005** (0.059)**a**	**0.414** (0.059)**b**
NO_3 (mg/L)	**2.0** (0.95)**a**	**1.5** (0.059)**a**	**0.25** (0.059)**b**

Insert figure 3.1 here

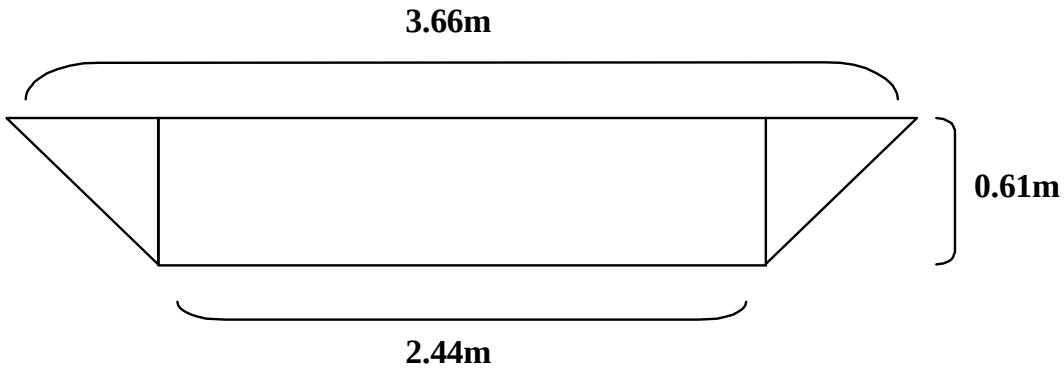

Figure 3.2. Sloped-end fry rearing tank (2,000 L) used in treatment 3.

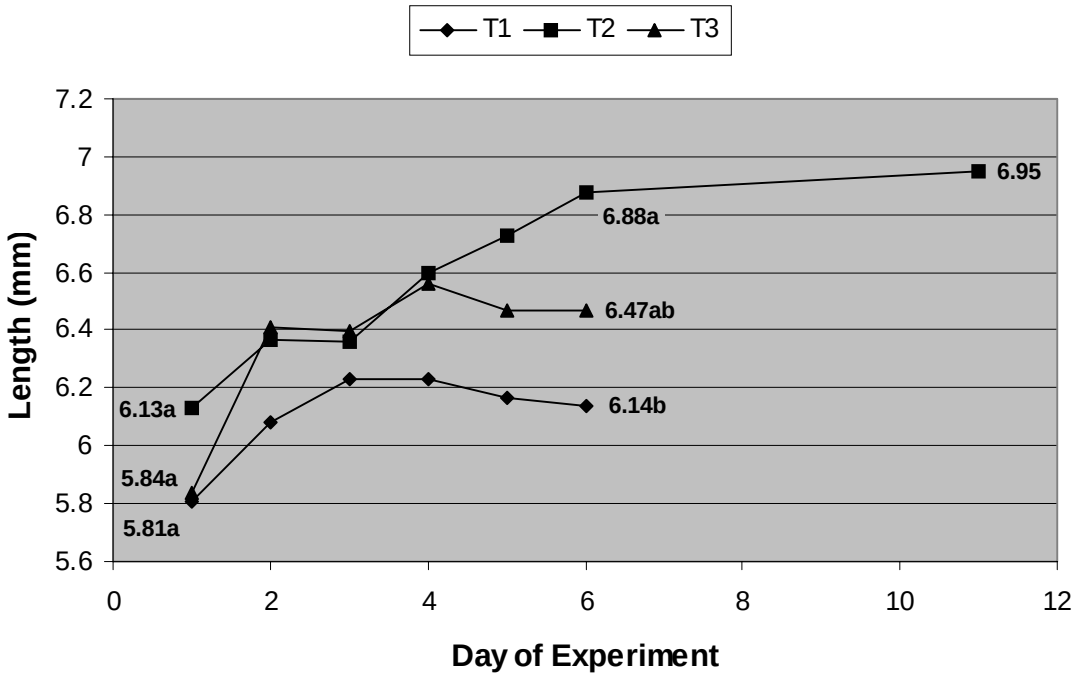

Figure 3.3. Mean daily growth (mm) of larvae among treatments. Means followed by like superscripts on days 1 and 6 are not significantly different at the 0.05 experiment-wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

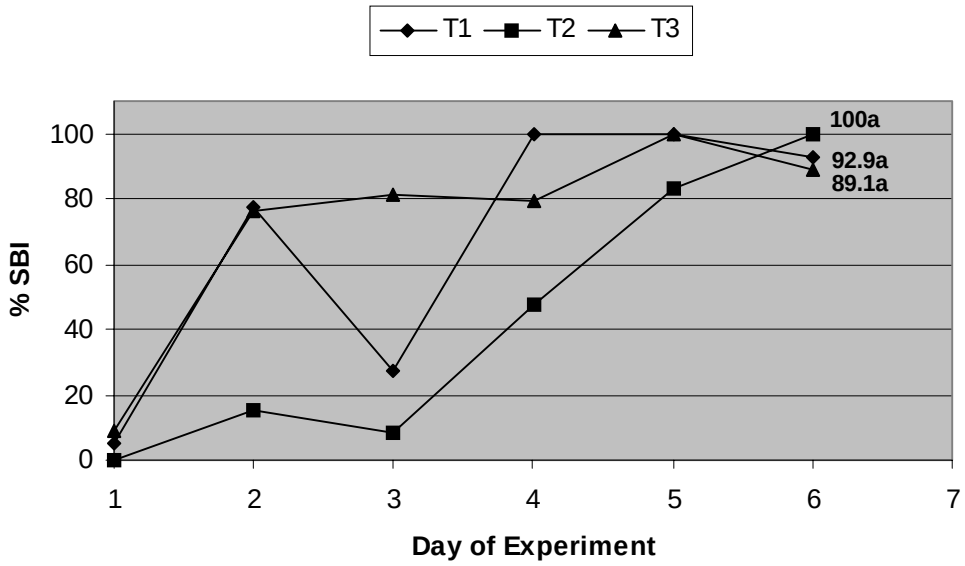

Figure 3.4. Mean % swimbladder inflation (SBI) by day among treatments. Means followed by like superscripts on day 6 are not significantly different at the 0.05 experiment-wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

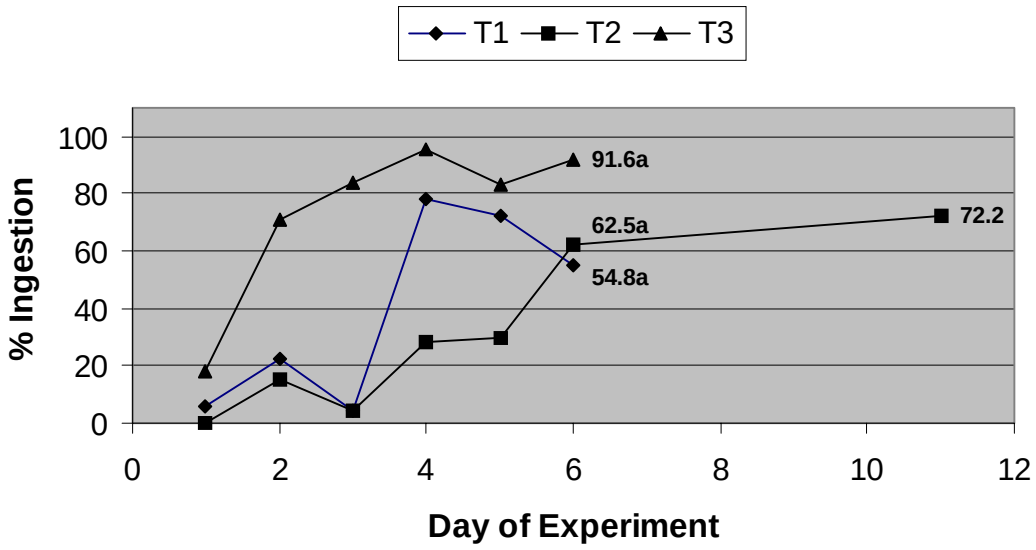

Figure 3.5. Mean % ingestion by day among treatments. Means followed by like superscripts on day 6 are not significantly different at the 0.05 experiment-wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

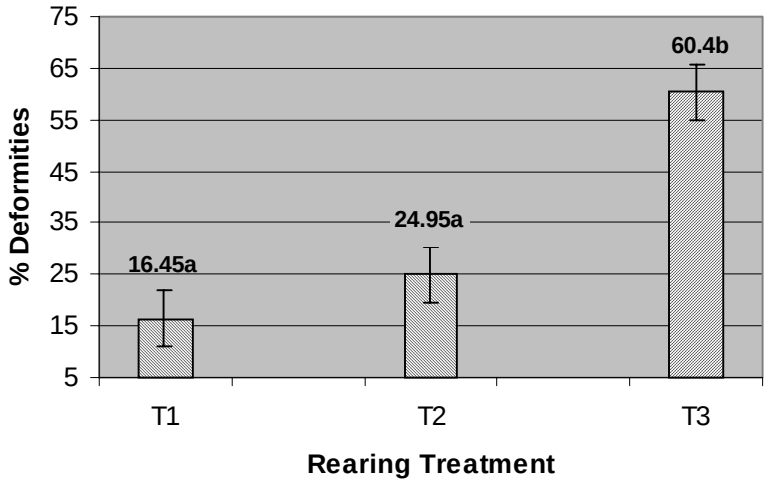

Figure 3.6. Mean % skeletal deformities among treatments over the first 6 days. Means followed by like superscripts are not significantly different at the 0.05 experiment-wise level (Tukey - Kramer HSD, Sall and Lehman, 1996).

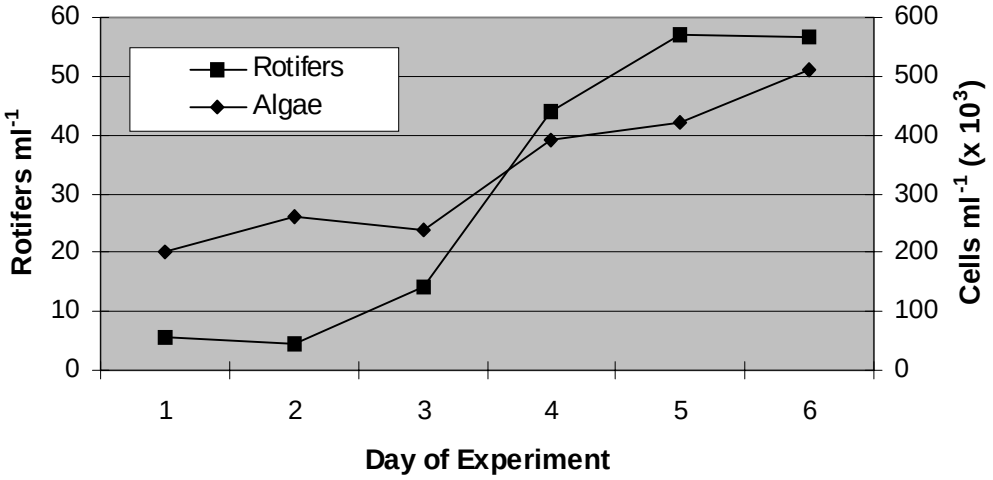

Figure 3.7. Mean number of rotifers ml^{-1} and algal cells ml^{-1} (x 10^3) by day in T3.

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Chapter Four:

Commercial Feasibility of Year-Round Production of Yellow Perch

Introduction

Yellow perch (*Perca flavescens*) is an economically important food fish in the upper Midwest of the United States. Traditionally, wild-caught perch from the Great Lakes have supplied the market. However, the commercial harvest from the Great Lakes has been declining and cannot satisfy the current demand (Calbert and Huh, 1976; Brown et al., 1996). The commercial harvest of yellow perch in North America peaked at 36 million pounds in 1969 with Lake Erie traditionally providing over 80% of the total catch (Lesser and Vilstrup, 1978). The catch from Lake Erie has dwindled to 4.1 million pounds reported in 1997 (OMNR, 1998). In addition, limits have been placed on recreational and commercial fishing for yellow perch in Lake Michigan as a result of recent declines in wild stocks (Heidinger and Kayes, 1993). This supply reduction has driven the retail price of yellow perch to as much as \$11 lb^{-1} and averaged \$7.45 lb^{-1} from 1996 -1997 (NCRAC, 1996; Riepe, 1998). There appears to be room for aquaculturally raised yellow perch in the established market of the Great Lakes region.

Pond production is presently the primary method of yellow perch culture in the United States. Extensive culture of yellow perch in ponds requires large amounts of land with a plentiful supply of appropriate quality water. Also, it takes two growing seasons for yellow perch to reach market size of approximately 150 grams when reared in ponds (Calbert and Huh, 1976). Currently, producers must rely on a single, spring spawning season for a supply of juveniles due to the annual egg recrudescence cycle of yellow perch, which requires a prolonged chill period to stimulate gonadal development.

Recirculating technology has become increasingly prevalent in commercial aquaculture due to its potential for eliminating the seasonality and variability associated with pond production. As with any new technology, however, commercial application of recirculating technology is expensive. Therefore, intensification of this sort requires the security of a product with high market demand, which yellow perch has. The successful culture intensification of this species will depend on the

manipulation of its reproductive cycle to provide fingerlings continuously throughout the year.

The purpose of this paper is to estimate the economic potential of a commercial yellow perch hatchery by identifying the associated costs of production.

General Facility Layout

The following design scheme is based on the assumption that the facility can utilize large quantities (as much as 100 L min^{-1}) of spring, well or clean surface water as a water source. The broodstock and fry rearing segments are combined in one building (referred to as the main building) (Fig. 4.1), while the fingerling production segment is located in a separate building (Fig. 4.2). Included in the main building is a laboratory for testing water chemistry and examining biological specimens (Fig. 4.1), as well as an *Artemia* hatchery for the feeding of fry (Fig. 4.3). The main building also contains a 7,000 gallon (26,600 L) water storage tank for temperature control of replacement water as discussed in following sections (Fig. 4.1).

Broodstock Manipulation

Broodstock are kept in four separate chambers, each with individual temperature and light control (Fig. 4.1 and 4.4). Each room is brought to spawning conditions at a designated annual time such that four separate spawns are obtained each year to facilitate year-round fingerling production.

Each room contains two, 400 gallon (1,520 L) rectangular tanks for holding broodstock, a 55 gallon (208 L) solids removal sump, a submersible pump, an incandescent lighting fixture and a water chiller (Fig. 4.4). Biofiltration is provided by flowing water from the solids removal sump to the top of two packed column trickling filters, each positioned over one of the two broodstock tanks (Fig. 4.4). Water chillers are located outside the main building in insulated chambers located adjacent to each broodstock chamber for efficient heat removal (not graphically depicted). Replacement water is provided from the water storage tank.

Broodstock are exposed to a 12-month cycle simulating the natural, annual light-dark sequence of temperate North America to entrain the reproductive rhythm of the perch (Fig. 4.5). The cycle is offset in each chamber to provide different desired spawning times (Table 4.1). Reproductively mature broodstock are injected with LHRHa (Sigma, St. Louis, MO) and pimozide (Sigma, St. Louis, MO) at a rate of 10 µg/kg LHRHa plus 10mg/kg pimozide followed by a second injection 48 hours later of 100 µg/kg LHRHa. Broodstock are allowed to complete gonadal regression after spawning and are kept at summer-like conditions for the next 5 months until it is time to start the 6 month recrudescence cycle again (Table 4.1).

Egg Incubation

The resulting fertilized egg ribbons are secured to screens within incubation troughs (Fig. 4.6). Incubation troughs are stacked two high and are positioned between the broodstock chambers and the fry production room (Fig. 4.1 and 4.6). The troughs are operated as a flow through system and receive water from the storage tank at a rate of 1 gallon (3.8 L) $minute^{-1}$. The temperature within the storage tank is maintained using an external, in-line water heater which circulates heated water through the storage tank and is thermostatically controlled (Fig. 4.1). Incubation temperatures are increased from spawning temperature (10 -12°C) at a rate of one degree day^{-1} to a maximum temperature of 20°C.

Hatched larvae are harvested by concentrating them beneath a lamp (Fig. 4.6) and collecting them by siphon into a bucket. Each bucket is then sub-sampled to estimate of the number of larvae per bucket and is transferred to the fry rearing tanks. This allows for an accurate estimation of initial fry density.

Fry Rearing

Hatched larvae are transferred to 135 gallon (511 L), rectangular fry rearing tanks (Fig. 4.1). The fry rearing room consists of 30 tanks stacked two high, which contain three internal light niches $tank^{-1}$ and are run as a partial recirculating system

(Fig. 4.1 and 4.7). Exiting water is passed through a rotating drum screen filter for particulates removal and then through a biofiltration sump containing either two or three rotating biological contactors (RBC) (Fig. 4.1 and 4.7). Water is then returned to the rearing tanks via two external pumps at a rate of 1 to 3 GPM (3.8 to 11.4 LPM) through each tank. The flow is increased as the size of the fry increases. The fry rearing system, including sumps, is just over 5,000 gallons (19,000 L) and the entire volume is replaced once day^{-1} from the water storage tank by running fresh water into the drum filter at a rate of 7 gallons per minute (GPM) (27 LPM) (Fig. 4.1). Water is passed through an ultraviolet filter prior to entering the water storage tank for disinfection (Fig. 4.1).

Fry are fed newly hatched *Artemia* nauplii for the first 30 days post-hatch, after which they are weaned to an artificial dry diet. Once fry are weaned, they are transferred to the fingerling production building.

Fingerling Production

Weaned fry are placed into one of four 1,000 gallon (3,800 L) grow-out tanks (Fig. 4.6). The grow-out tanks are paired and run as a recirculating system, with exiting water from the pair of tanks passing through a rotating drum microscreen filter followed by an RBC biofilter (Fig. 4.6). Filtered water then returns to each grow-out tank via two external pumps at a rate of 50 GPM (190 LPM) $tank^{-1}$, providing a turn over time of 20 minutes in each tank (Fig. 4.6).

Juvenile fish are fed progressively larger sized feed as they grow and are size graded twice monthly to reduce cannibalism due to unequal growth rates. Fingerlings are harvested at a length of 3" (76 mm) and sold to grow-out farmers who raise the fingerlings to market size (150 g).

Production Parameters

The fry rearing room contains 30 rearing tanks of 511L (135 gal) each, providing a total rearing volume of 15,330 L (4,000 gal). Fry are stocked at a density

of 30 L^{-1} (115 gal^{-1}), which equals 460,000 fry stocked per spawn. Assuming a 35% survival rate from hatch to fingerling size (Table 1.1), 460,000 fry yields 161,000 fingerlings. This is a quarterly production estimate, since the facility produces 4 spawns $year^{-1}$. Therefore, a total of 644,000 fingerlings are produced $year^{-1}$ at this particular stocking density and survival rate.

The number of broodstock required to produce 460,000 fry is calculated as follows. A mean broodstock weight of 370 g (0.8 lbs), a mean GSI of 24% and a mean of 180 eggs g of egg $ribbon^{-1}$ yields 16,000 eggs $female^{-1.}$ The number of eggs produced is then multiplied by the estimated fertility and hatch-out percentages to obtain the number of fry produced $female^{-1}$. Due to the variety of results obtained in previous studies, a conservative assumption of 50% fertility and 50% hatch-out is used (Hokanson and Kleiner, 1974; Kayes, 1977; West and Leonard, 1978; Dabrowski et al., 1994; Wang and Ekmann, 1994; Kolkovski and Dabrowski, 1998). Each female, therefore, produces 4,000 fry, which means 115 female perch are needed to produce the quarterly fry demand of 460,000. An equal number of males are used for breeding to maintain a high effective breeding number in order to avoid inbreeding problems. Thus, each broodstock room contains 230 fish total in a 1:1 sex ratio and a total of 920 broodstock are needed for annual production.

Feed Estimation

Larvae, from hatching to about 30 days post-hatch, are fed live *Artemia* nauplii at a rate of 500 nauplii $larvae^{-1}$ day^{-1} (Hinshaw, 1985), with the rate decreasing during the weaning period. The quantity of nauplii is adjusted every 10 days to correspond with larval mortality. Assuming 35% survival over the first 30 days (Table 1.1), each 10-day increment realizes 70% survival ($0.7^3 = 0.35$ or 35%). If the survival rate for the first 10 days is 70%, then the feed quantity is reduced from 230 x 10^6 nauplii day-1 (for 460,000 fry) to 160 x 10^6 nauplii day-1 (for 322,000 fry) on day 10. Likewise, assuming 70% survival for the second 10 days, the feed quantity is reduced again to 113 x 10^6 nauplii day-1 (for 225,000 fry) on day 20. Weaning starts at day 20 and lasts

10 to 15 days by progressively decreasing the proportion of *Artemia* fed in relation to artificial feed. Again, a 70% survival rate is assumed during the weaning period (the final 10-day increment). The total estimated quantity of *Artemia* cysts needed year^{-1} is 80 kg (176 lbs).

Weaned fry are fed an artificial dry diet of 55% protein from day 30 to day 75 with feed particle size increasing every 15 days from 600μm to 2mm. Fry are fed a 46% protein diet (2.5mm pellet) for the remaining 15 days of production. The estimated dry diet consumption, at a food conversion ratio of 1.2, is 8,300 lbs (3770 kg) year^{-1}.

Cost Analysis

Start-up cost for this year-round yellow perch fingerling production facility is estimated at just under $137,000 (Table 4.2). First year annual variable costs are estimated at $43,000 (Table 4.3) and annual fixed costs, including amortized loan payments, are estimated at $85,000 (Table 4.4). This cost structure generates a total production cost of $0.20 fingerling^{-1} for the first 5 years (operating loan payback term), $0.16 fingerling^{-1} for the second 5 years (10 year payback on start-up capital) and $0.06 fingerling^{-1} after 10 years.

The selling price of yellow perch fingerlings is currently $0.07 to 0.10 inch^{-1}, which translates to $0.21 to 0.30 fingerling^{-1} produced by this facility. Thus, the economic viability of such a facility is positive, even at a modest selling price.

Summary

The intensification of yellow perch culture through reproductive manipulation and indoor fry rearing techniques can be a commercially feasible way of producing fingerlings throughout the year. The high demand for yellow perch in the Great Lakes

region and the associated high market price of perch makes this species a good candidate for such intensification. The high market price of perch offsets the higher costs inherent to intensive culture relative to pond production.

Potential fingerling producers should be sure that the production parameters discussed herein are attainable at their given site, as many of the biological functions and operating costs relating to the successful culture of this species are site specific. Differing water quality, market value and operating costs, for example, may render such a facility infeasible in certain locations. Prospective producers are advised to contact their local and regional aquaculture extension services for more information. These services can be located through the Aquaculture Network Information Center website at: http://aquanic.org..

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Insert table 4.1 here

Table 4.2. Start-up costs and annual depreciation for year-round yellow perch fingerling production facility.

Item	Cost $	Life	Depreciation
Land (2 acres)	2,000.00		
Buildings			
Egg / fry production facility	34,000.00	20	1,700.00
Fingerling production facility	15,000.00	20	750.00
Waste treatment lagoon (500 cu. yds.)	1,000.00	20	50.00
Emergency generator	20,000.00	20	300.00
Water supply / plumbing / drains	10,000.00	20	750.00
Heating system	4,800.00	20	240.00
Equipment			
Tanks	13,100.00	10	910.00
Filtration	25,000.00	5	3,000.00
Pumps	2,700.00	2	860.00
Pipes / fittings	4,000.00	5	740.00
Water chillers	4,000.00	5	800.00
Laboratory (fully equipped)	5,100.00	10	510.00
Artemia hatchery	1,000.00	10	100.00
In-line water heater	440.00	5	88.00
Blowers	1,600.00	5	320.00
Total	**143,740.00**		**11,118.00**

Table 4.3. Annual variable costs associated with a year-round yellow perch fingerling production facility.

Item	Unit	Rate	Qnty	Total $	Subtotal $
Labor	hours	7.00	3760	26320.00	26320.00
Feed					
Artemia cysts	case	294.00	14	4116.00	
Decapsulation / hatching materials	– –	– –	– –	1070.00	
Dry diet	lb	0.46	14000	3854.00	9040.00
Utilities					
Heat	kw	0.06	83333	5000.00	
Electricity	kw	0.06	16667	1000.00	6000.00
Water Treatment	– –	– –	– –	740.00	740.00
Test Reagents	– –	– –	– –	820.00	820.00
Total				**42,920.00**	**42,920.00**

Table 4.4. Annual fixed costs associated with a year-round yellow perch fingerling production facility.

Item	Amt ($)	%Rate	Term (yrs)	Total $
Manager salary	– –	– –	– –	35,000.00
Loans	– –	– –	– –	
Start-up	140,000.00	9.0(fixed)	10	21,276.00
Operating	100,000.00	8.0(fixed)	5	24,336.00
Insurance	– –	– –	– –	3,000.00
Taxes	– –	– –	– –	1,500.00
Licences / Fees	– –	– –	– –	150.00
Total				**85,262.00**

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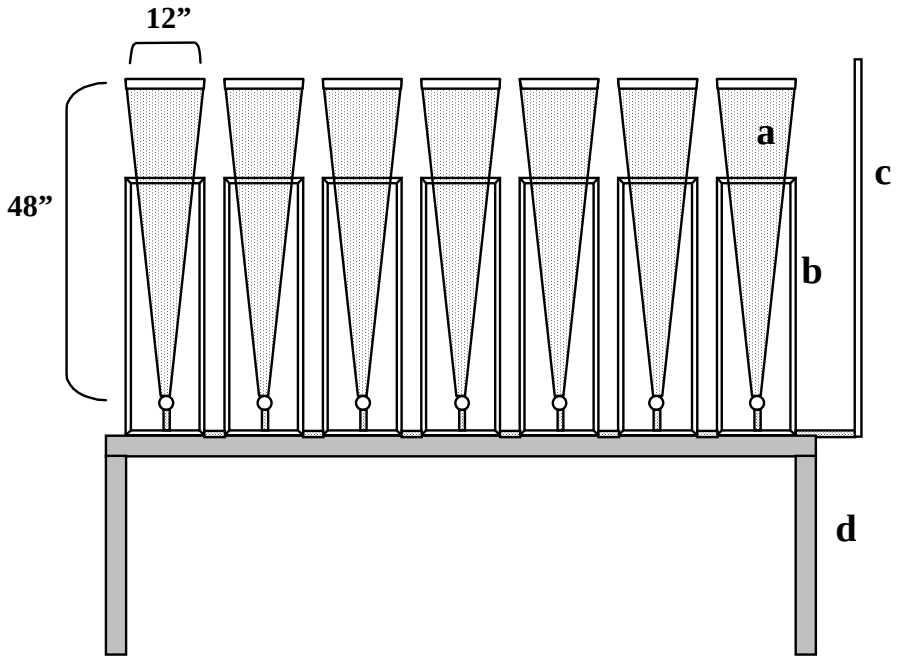

Figure 4.3. *Artemia* hatchery: **a-** hatching cone, **b-** hatching cone stand, **c-** air supply from blower, and **d-** table.

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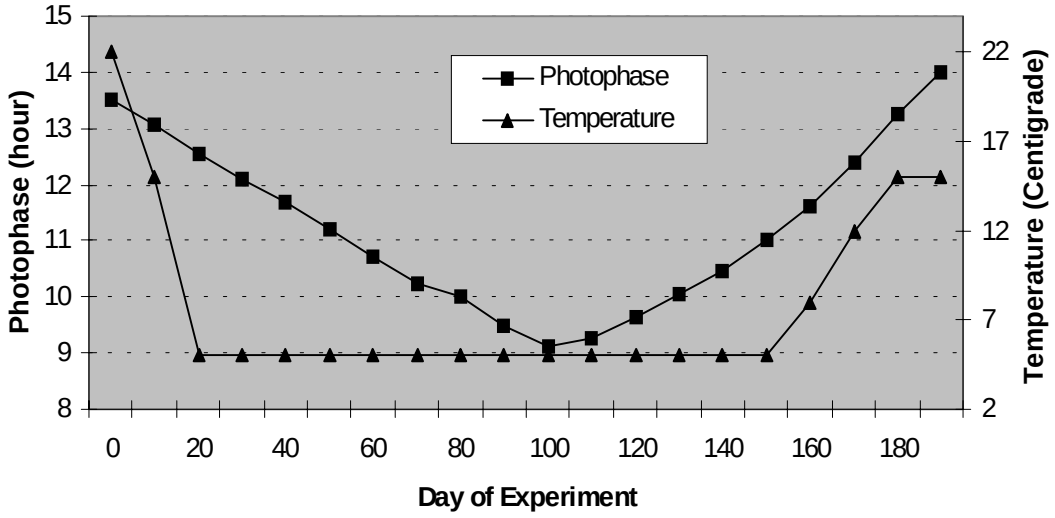

Figure 4.5. Photothermal cycle for broodstock conditioning chambers.

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Appendix A Broodstock Manipulation Start-up Costs.

Equipment	Qty	$ / unit	Total $
Broodstock chambers			
7' x 3' x 2.5' lined tank (400 gal)	8	500.00	4000.00
55 gal drum sump	4	65.00	260.00
Bio Strata corrugated media	96 cu.ft.	16.85	1617.60
Submersible pump (25 gal/min @ 5' head)	6	114.00	684.00
Water chiller	2	2000.00	4000.00
Digital timer	4	20.00	80.00
Plumbing			
6" PVC pipe	25'	1.54	38.50
3" PVC pipe	115'	0.73	83.95
1.5" PVC pipe	80'	0.37	29.60
1" PVC pipe	40'	0.18	7.20
3" elbow	16	7.60	121.60
1" elbow	32	0.75	24.00
3" tee	4	11.28	45.12
1.5" tee	4	1.80	7.20
1" tee	8	0.94	7.52
1" ball valve	8	4.35	34.80
3" gate valve	12	14.50	174.00
3" bulkhead fitting	12	41.80	501.60
3" male adapter	12	5.86	70.32
1.25" x 1.5" reducing adapter	4	2.38	9.52
1.5" coupling	8	0.78	6.24
1.5" x 1" reducer bushing	8	0.94	7.52
Total			**11810.29**

Appendix B Egg Incubation Room Start-up Costs.

Equipment	Qty	$ / unit	Total $
Egg incubation room			
10' x 2' x 10" poly-tank	8	200.00	1600.00
3" PVC pipe	50'	0.73	36.50
2" PVC pipe	40'	0.45	18.00
1.5" PVC pipe	60'	0.37	22.20
3" elbow	2	7.65	15.30
2" elbow	12	2.15	25.80
1.5" elbow	12	1.35	16.20
1" elbow	12	0.75	9.00
3" tee	2	11.28	22.56
1.5" tee	3	1.80	5.40
1.5" x 1.5" x 1" reducing tee	8	2.83	22.64
1" ball valve	12	4.35	52.20
3" cross	4	16.00	64.00
3" x 2" reducer bushing	12	3.75	45.00
2" flexible coupling	12	4.00	48.00
2" bulkhead fitting	12	7.45	89.40
Total			**2092.20**

Appendix C Fry Production Start-up Costs.

Equipment	Qty	$/unit	Total $
Tanks			
Duracast poly-tank (6'x3'x1')	30	60.00	1800.00
Filtration			
4' dia. RBC	5	750.00	3750.00
Rotating drum filter	2	4000.00	8000.00
U.V. Sterilizer	2	472.00	854.00
19" dia. Sand filter w/ pump	1	468.00	468.00
Plumbing			
Delivery line			
In-line water heater	2	450.00	900.00
Pumps (0.13 HP)	4	259.00	1036.00
1.5" PVC pipe	40'	0.37	14.80
1.0" PVC pipe	150'	0.18	27.00
0.5" PVC pipe	45'	0.12	5.40
1.5" tee	3	1.80	5.40
0.5"x1"x1" reducing tee	30	1.00	30.00
1.0" tee	15	0.94	14.00
1.5" elbow	6	1.35	8.10
1.0" elbow	4	0.75	3.00
0.5" elbow	30	0.36	10.80
0.5" ball valve	30	2.45	73.50
Drain line			
4" PVC pipe	90'	1.25	112.50
3" PVC pipe	110'	0.73	65.70
2" PVC pipe	135'	0.45	60.75
4" elbow	4	13.80	55.20
4" tee	3	22.40	67.20

Appendix C Fry Production Start-up Costs (continued).

Equipment	Qty	$/unit	Total $
Drain line			
4" end cap	5	5.20	26.00
3"x4"x4" reducing tee	15	20.45	183.00
3" end cap	15	3.75	56.25
2"x3"x3" reducer tee	45	12.20	549.00
2" elbow	45	2.15	96.75
2" bulkhead fitting	45	7.45	335.25
2" flexible coupling	45	4.00	180.00
Total			**31859.60**

Appendix D Laboratory Start-up Costs.

Equipment	Qty	$/unit	Total $
Dissecting microscope	1	500.00	500.00
Pyrex petri dishes (100x15) 12 pack	1	55.20	55.20
Dissecting kit	2	28.00	56.00
Electronic bench scale (50kg cap)	1	485.00	485.00
Electronic precision balance (600g; 0.1g)	1	222.00	222.00
Freezer / refrigerator	1	400.00	400.00
Sink / work table	1	300.00	300.00
YSI oxygen meter (Y58)	1	1445.00	1445.00
YSI probe w/ 12' cord	1	282.00	282.00
30 repl. membranes + KCL	1	15.50	15.50
Corning pH pen	2	21.00	42.00
Hach colorimeter (DR/890)	1	749.00	749.00
Hach sample cells (6/pack)	2	20.00	40.00
Repipet bottle dispenser	2	73.00	146.00
Hach Digital Titrator	1	98.00	98.00
Magnetic stirrer	1	117.00	117.00
Stir bars (12/pack)	1	22.60	22.60
Erlenmeyer flask (250 ml)	6	2.85	17.10
Graduated cylinder (100ml)	3	4.30	12.90
Sample bottle (250ml)	12	1.40	16.80
Alconox cleaner (4lbs)	1	15.25	15.25
Bottle brush	6	3.60	21.60
Total			**5,058.95**

Appendix E *Artemia* hatchery Start-up Costs.

Equipment	**Qty**	**$/unit**	**Total $**
84 Liter brine shrimp pyramid hatcher	7	275.00	1925.00
Hatcher floor stand	7	170.00	1190.00
8" dia.; 106μm mesh sieve	4	41.50	166.00
Sieve cap	4	11.85	47.40
2' x 7' counter	1	50.00	50.00
Total			**3,378.40**

Appendix F Monthly Labor Costs.

Duty	Hours/Month	$ / Hour	# Months/ Yr	Total $ / Yr
Artemia decapsulation	8	7.00	6	336.00
Artemia harvesting / fry feeding	128	7.00	6	5376.00
Fry tank cleaning / siphoning	104	7.00	8	5824.00
Fingerling feeding	56	7.00	8	3136.00
Water quality monitoring	40	7.00	12	3360.00
Spawning / egg production	84	7.00	4	2352.00
Grading / size sampling	24	7.00	16	2688.00
Shipping	60	7.00	6	2520.00
Tank disinfection	8	7.00	8	448.00
Total				**26,040.00**

Appendix G Yearly Feed Costs.

Item	**Qnty**	**$ / unit**	**Total $**
Artemia cysts	14 cases	294.00 / case	4116.00
Decapsulation / hatching materials			
Liquid bleach (NaOCl)	200 gal	2.00 / gal	400.00
Saltwater mixture (55 lb bag)	20 bags	23.50 / bag	470.00
Sodium hydroxide (NaOH)	10 kg	19.00 / kg	190.00
Sodium hypochlorite ($Na_2S_2O_3$)	5 lbs	2.00 / lb	10.00
Salmon Starter (55% protein; # 2)	1200 lbs	0.57 / lb	684.00
Salmon Starter (55% protein; # 3)	2400 lbs	0.46 / lb	1104.00
Salmon Starter (55% protein; # 4)	3500 lbs	0.46 / lb	1610.00
Trout Grower (46% protein; 2.4mm)	1200 lbs	0.38 / lb	456.00
Total			**9,040.00**

Appendix H Miscellaneous Variable Costs.

Cost	Qnty	$ / unit	Total
Heat			
Water	75000 kw	0.06 / kw	4500.20
Air	8333 kw	0.06 / kw	500.00
Electricity			
Pumps	6000 kw	0.06 / kw	360.00
Lighting	500 kw	0.06 / kw	30.00
Miscellaneous	500 kw	0.06 / kw	30.00
Water Treatment / Anaesthetic			
Sodium bicarbonate ($NaCO_3$)	300 lbs	0.35 / lb	105.00
Salt (NaCl)	30bags (80lbs)	7.99 / bag	239.70
MS-222 (tricane methane sulfunate)	1 kg	389.00 / kg	389.00
Test Reagents			
Nessler reagent	6 bottles	40.00 / bottle	240.00
Rochelle salt-PVA reagent	12 bottles	11.25 / bottle	135.00
NitriVer 3 nitrite pillows	4 packs (100)	19.50 / pack	78.00
NitraVer 5 nitrate pillows	4 packs (100)	23.00 / pack	92.00
Bromcresol green / methyl red	8 packs (100)	9.00 / pack	72.00
1.6N sulfuric acid titration cartridge	6 cartridges	8.75 / cart.	52.50
ManVer 2 hardness pillows	8 packs (100)	8.75 / pack	70.00
EDTA titration cartridge	6 cartridges	8.75 / cart.	52.50
pH buffers (4.0, 7.0, 10.0)	3 bottles	9.25 / bottle	27.75
Total			**6,973.65**

Appendix I Start-Up Loan Amortized Monthly Payments at 9% Interest.

	Amortization in Years				
Amount	**6**	**7**	**8**	**9**	**10**
40,000	701.33	623.45	565.47	541.72	506.71
41,000	718.87	639.04	579.61	555.26	519.38
42,000	736.40	654.63	593.75	568.81	532.04
43,000	753.93	670.21	607.88	582.35	544.71
44,000	771.47	685.80	622.02	595.89	557.38
45,000	789.00	701.38	636.16	609.44	570.05
46,000	806.53	716.97	650.29	622.98	582.71
47,000	824.07	732.56	664.43	636.52	595.38
48,000	841.60	748.14	678.57	650.06	608.05
49,000	859.13	763.73	692.70	663.61	620.72
50,000	876.57	779.32	706.84	677.15	633.38
55,000	964.33	857.25	777.52	744.86	696.72
60,000	1052.00	935.18	848.21	812.58	760.06
65,000	1139.67	1013.11	918.89	880.29	823.40
70,000	1227.33	1091.04	989.57	948.01	886.74
75,000	1315.00	1168.97	1060.26	1015.72	950.07
80,000	1402.66	1246.90	1130.94	1083.44	1013.41
85,000	1490.33	1324.83	1201.62	1151.15	1076.75
90,000	1578.00	1402.76	1272.31	1218.87	1140.09
95,000	1665.66	1480.70	1342.99	1286.58	1203.42
100,000	1753.33	1558.63	1413.67	1354.30	1266.76

* Taken from Loan Payments Handbook, 1974, Computofacts, Willowdale, Ontario.

Appendix J. Operating Loan Amortized Monthly Payments at 8% Interest.

	Amortization in Years				
Amount	**1**	**2**	**3**	**4**	**5**
40,000	3479.54	1809.10	1253.46	976.52	811.06
41,000	3566.53	1854.32	1284.80	1000.93	831.34
42,000	3653.52	1899.55	1316.13	1025.35	851.61
43,000	3740.51	1944.78	1347.47	1049.76	871.89
44,000	3827.50	1990.01	1378.81	1074.17	892.17
45,000	3914.48	2035.23	1410.14	1098.59	912.44
46,000	4001.47	2080.46	1441.48	1123.00	932.72
47,000	4088.46	2125.69	1472.81	1147.41	953.00
48,000	4175.45	2170.91	1502.15	1171.83	973.27
49,000	4262.44	2216.14	1534.49	1196.24	993.55
50,000	4349.43	2261.37	1566.82	1220.65	1013.82
55,000	4784.37	2487.51	1723.51	1342.72	1115.21
60,000	5219.31	2713.64	1880.19	1464.78	1216.59
65,000	5654.25	2939.78	2036.87	1586.84	1317.97
70,000	6089.20	3165.92	2193.55	1708.91	1419.35
75,000	6524.14	3392.05	2350.23	1830.97	1520.73
80,000	6959.08	3618.19	2506.91	1953.04	1622.12
85,000	7394.02	3844.32	2663.60	2075.10	1723.50
90,000	7828.96	4070.46	2820.28	2197.17	1824.88
95,000	8263.91	4296.60	2976.96	2319.23	1926.26
100,000	8698.85	4522.73	3133.64	2441.30	2027.64

* Taken from Loan Payments Handbook, 1974, Computofacts, Willowdale, Ontario.