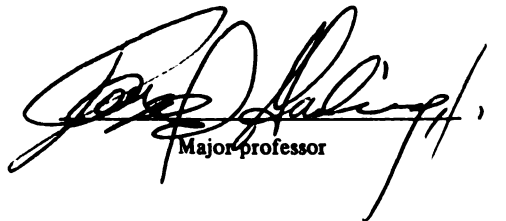This is to certify that the

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EVALUATION OF FEEDING STRATEGIES FOR FIRST-FEEDING YELLOW
PERCH (*PERCA FLAVESCENS*) FRY; GENDER EFFECTS ON
BIOENERGETIC RESPONSES OF YELLOW PERCH FINGERLINGS

presented by

Jon J. Amberg

has been accepted towards fulfillment
of the requirements for

___M.S.___ degree in__Fisheries &__ Wildlife

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AN ABSTRACT OF A THESIS

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Dr. Donald Garling Jr.

ABSTRACT

EVALUATION OF FEEDING STRATEGIES FOR
FIRST FEEDING YELLOW PERCH FRY
AND GENDER EFFECTS ON BIOENERGETIC RESPONSES OF
YELLOW PERCH GREATER THAN 15 CM

By

Jon J. Amberg

First-feeding yellow perch fry were fed *Artemia* nauplii, vinegar eels, Artificial Plankton™ or combinations of the feeds in 15 l green experimental tanks. Mortality rates were 100% due to failure of inflate the swim bladder. However, survival of fry in the holding tank exceeded 75% to 25 mm total length and a high percentage of swim bladder inflation was observed. The 378 l gray holding tank had an increased surface area to volume ratio and a less turbulent flow pattern.

A saturation kinetics model was used to describe the growth rates of all-female (AF) and predominately-male (PM) yellow perch stocks fed varying ration levels (0.5, 1.0, 2.0, or 3.0% wet-weight per day). The growth of the AF stock was significantly greater than the PM stock when fish were fed at 3% body weight per day. At ration levels of 2% body wet weight per day or less, the growth rates of both genders were not significantly different. The proximate analysis of whole fish tissues did not differ between genders or ration levels. The greatest digestion efficiencies were observed for the 1.0-% ration level for both gender stocks.

Dedication

This body of work is dedicated to my family Janet J. Amberg, Edwin E. Amberg, and Laura L. Turley for their guidance in my life. I would also like to dedicate this work in the memory of my late grandparents, Carl and Marie Amberg. Both of who passed away after its inception, but before its completion. Although they were unable to see its culmination, I know they were present with me in spirit. Thank you for the guidance in my life.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

MATERIALS AND METHODS

RESULTS

DISCUSSION

LIST OF ABBREVIATIONS

AA – Amino acids

AF – All female

b - Intercept on the ordinate y-axis

BW – Body weight

CP – Crude protein

CL – Crude lipid

DE – Digestible energy

DM – Dry matter

E – Gross energy

EE – Energy efficiency

EL – Eye diameter/length

FE – Feed efficiency

GPM – Gallons per minute

I - Nutrient intake

K_m - Intake constant for ½ of R_{max}

K_s - Substrate concentration level at which the growth is ½ μ_{max}

LPM – Liters per minute

MH – Mouth height

ML - Maxillary length

MS-222 – Tricainemethane sulfonate

MW – Mouth width

n - Slope factor (compared to K_m, apparent kinetic order of the response with respect to I as I^n becomes negligible)

NCRAC – North Central Regional Aquaculture Center

PM – Predominately male

ppm - Parts per million

r - Physiological observed responses

R_{max} - Maximum response

S - Substrate concentration level

SBI – Swim bladder inflation

SD - Standard deviation

SEM - Standard error of the mean

ST – Standard length

TL – Total length

YPFFF – Yellow perch first-feeding fry

μ - Growth rate (1/time)

μ_{max} - Maximum growth rate (1/time)

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Yellow perch (*Perca flavescens*) are a highly valued commercial food and sport fish in the Great Lakes region. There has been a dramatic decline in wild stocks since the early 1970's that has not been reversed (Riepe 1998). Yellow perch were commercially harvested from the Green Bay (Lake Michigan), Saginaw Bay (Lake Huron), and western Lake Erie. Commercial landings in the Great Lakes reached 16.3 million-kg/yr in 1969, but dropped to 4.7 million-kg/yr in 1976 (Riepe 1998). Data from the Great Lakes Fishery Commission (2000) reported that there was a 14% decline in commercial landings from 1977 to 1990.

The cause of the decline of wild stocks of yellow perch in the Great Lakes is unknown. Researchers have speculated that several factors may have contributed to this decline: introduction of competitive species such as zebra mussel and alewifes, a significant gender shift to a predominantly female population, water quality and habitat degradation, and increased fishing pressure. Fluctuations in the population may also have been a natural cyclic event. The population decline lead to the closure of the commercial yellow perch fishery in Lake Michigan waters in 1996 (David Clapp, Michigan Department of Natural Resources, Charlevoix, MI, personal communication, 1998). Currently, Canadian waters of Lake Erie and Huron are the primary source of commercially harvested yellow perch for the food fish market (Lesser and Vilstrup 1979). Tribal commercial fishermen continue to harvest yellow perch from Michigan waters.

Consumer demand has remained high for yellow perch fillets (Lesser 1978, Lesser and Vilstrup 1979, Riepe 1998). Consumers value yellow perch as a food fish because of their mild taste and firm flesh (Lesser and Vilstrup 1978). Recent price estimates reflect the elasticity of demand for yellow perch. Wholesale prices for perch in the round have ranged from approximately $5.10 to $6.60 per kg (Malison 1999). Wholesale prices for yellow perch fillets have ranged from $13 to $17 per kg (Riepe 1998). Retail prices for yellow perch fillets have reached $24 per kg (North Central Regional Aquaculture Center (NCRAC) 1996). High demand coupled with the decline in commercial harvest has made yellow perch one of the most valuable potential aquaculture species in the Midwest United States (Brown et al. 1996).

Several factors impede the development of a commercially viable, intensive aquaculture industry for yellow perch in the North Central Region (Kelly 2000). In 1997, NCRAC formulated three research objectives to address some of these impediments. This thesis addressed two of the objectives:

1. With the goal of larval intensive yellow perch feeding in tanks from the onset of first feeding, continue to develop methods to produce fingerlings.

2. Increase growth rates of yellow perch greater than 150 mm (6") by evaluating diets, feeding strategies, environmental manipulation, and mono-sex/bi-sex comparisons.

Chapter 1 summarizes experiments designed to improve the survival of yellow perch fry fed in tanks from the onset of first feeding. Chapter 2 summarizes experiments designed to determine the bioenergetic responses of

10 to 17.5-cm male, female, and mixed gender populations of yellow perch using

a saturation kinetic model based on a multiple non-linear regression analysis.

HYPOTHESIS

The specific hypothesis tested for each objective in this study are:

Chapter 1 Objective 1
Null Hypothesis 1.1: Survival rates of first feeding yellow perch will be equal among fry fed vinegar eels, *Artemia* nauplii, artificial plankton, and their combination.

Chapter 2 Objective 2

Null Hypothesis 2.1: The growth rates between male, female and mixed stocks will be equal.

Null Hypothesis 2.2: The percent crude protein on a dry matter basis will be equal between male, female and mixed stocks.

Null Hypothesis 2.3: The percent crude lipids on a dry matter basis will be equal between male, female and mixed stocks.

Null Hypothesis 2.4: The percent energy on a dry matter basis will be equal between male, female and mixed stocks.

Null Hypothesis 2.5: The percent moisture will be equal between male, female and mixed stocks.

Null Hypothesis 2.6: The percent ash will be equal between male, female and mixed stocks.

LITERATURE REVIEW

The market for yellow perch reflects strong consumer demand (Lesser 1978; Lesser and Vilstrup 1979). Riepe (1998) surveyed restaurants that sold yellow perch and found 2/3 of the respondents were located within 50 miles of the Great Lakes. She reported wholesale prices of yellow perch fillets ranged from $13 to $17 per kg. The NCRAC reported perch retailing for $24 per kg (1996). Wholesale for perch in the round changed from approximately $5.10 to $6.60 per kg (Malison 1999). Because of the high demand and limited supply, yellow perch is often replaced with an imported species (i.e. pike-perch, *Stizostendion lucioperca*). These species are often sold as "lake perch", which deceives the public. High demand coupled with the decline in recruitement has made yellow perch one of the most valuable aquaculture species in the Midwest United States (Brown et al. 1996).

The current market size of yellow perch is 115-150-g (Bennett Fish Inc., Loraine, OH, personal communication). Cultured yellow perch can reach market size 2 years earlier than wild fish (Malison 1999). Under optimal conditions of 21°C with 16-h light and 8-h dark, yellow perch fed to satiation have reached a marketable size in 9-11 months (Calbert and Huh 1976). Starr (1991) has shown that yellow perch grown at 20 to 24°C grew 0.8 to 1.1-cm per month with feed conversions (kg feed/kg weight gain) of 1.3 to 3.5. Wild yellow perch are able to reach a market size in 4 years (Carlander 1950).

Yellow perch reared under controlled culture conditions command a higher price than wild caught fish (Chris Bennett, Lake Erie Aquaculture, Lorain, OH, personal communication). Cultured yellow perch have a fillet yield of 45%, 5% greater than wild caught fish (Heidinger and Kayes 1993). Fillets from cultured fish are generally more uniform in size (Lesser and Vilstrip 1979).

Yellow perch has been identified as a suitable candidate for aquaculture within the North Central region. They are able to tolerate high loading and densities, handling, and marginal water quality (Heidinger and Kayes 1986, Glass 1991).

Methods for inducing early or delaying reproduction (Cieszko et al. 1997) and inducing synchronous spawning (Malison et al. 1997) have been developed. Yellow perch are seasonal spawners which require 160-d chill period (temperature less than 10°C) for complete egg development (Hokanson 1977). However, lack of well developed strategies for feeding fry from the onset of first-feeding result in a large variation in survival of fry (see Chapter 1).

Basic nutritional requirements for yellow perch have been determined and practical feeds have been formulated for fingerlings to market size fish (Brown et al. 1996; Brown and Twibell 1997; Ramseyer and Garling 1994, 1998). Ramseyer and Garling (1994) indicated that the protein requirements of yellow perch are more similar to a channel catfish than rainbow trout; but, aquaculturists often feed trout diets to yellow perch.

The optimum environmental parameters for culturing yellow perch have been reported. Since yellow perch are ectothermic, environmental temperature

influences metabolic reactions. Hokanson (1977) demonstrated that yellow perch have a zero net biomass gain at temperatures below 6°C. His study also determined that the optimum physiological temperature is 25.1°C. Huh et al. (1976) established that yellow perch grow best with 16-h of light and 8-h of dark, at a temperature of 20°C. However, other researchers have reported a wide variation in optimal rearing temperature from 20-28°C (Heideinger and Kayes 1993; McCormick 1974, 1976). Some of the differences in reported optimum rearing temperature might be the result of using different geographic stocks. It has been found that the optimal rearing temperature is a function of geographic region. Perch from northern and southern stocks grow better at lower and higher temperatures, respectively. However, optimum growth temperature for all geographic stocks was 20°C (NCRAC 1993).

The dissolved oxygen (DO) level for yellow perch should be maintained at concentrations greater than 3.5-mg/L in ponds (Carlson et al. 1980) and the effluent of flow through systems (Glass 1991). This is similar to the 3.0-mg/L minimum DO requirement for channel catfish (Carter and Allen 1976). Rainbow trout require 5 to 6-mg/L DO in the effluent from raceways (Piper et al. 1982)

Three methods have been used to commercially rear yellow perch: outdoor ponds, raceways, and indoors recirculating systems. All three methods are employed in the North Central Region.

PONDS:

Yellow perch have been spawned in small ¼ hectare ponds. Fish were stocked into the ponds and often allowed to spawn on a substrate (i.e. small pine

tree). Eggs were removed from the substrate and incubated indoors. Shortly after hatch, fry were returned to fertilized outdoor ponds. The fry are feed trained and reared in these ponds until they reach a marketable size.

The advantage of rearing yellow perch in pond was the decreased labor expense. The natural production of food generated within a pond will decrease the feed conversion ratio.

The major disadvantages to raising fish in ponds are the amount of land required, capital cost of building ponds, adequate sources of water, and seasonal changes in temperature and photoperiod.

Pond culture requires a significant investment in land. Riepe (1997) estimated that 20 acres of ponds are required to raise 50,000-lbs/yr (22,675 kg/yr) in central Indiana. She determined that the initial investments cost for 5,000-lb production system were $39,182, and $140,238 for a 50,000-lb production system. The total annual costs are $17,416 for the 5,000-lb facility and $107,079 for the production of 50,000-lbs. The breakeven price (total cost per pound) was $2.14/lb for the 50,000-lb production facility. However, the 5,000-lb breakeven price is $1.34 greater. Wholesale prices ranged from $2.00 to $3.00 per pound. There is a limited profit margin.

Perhaps, the major disadvantage to rearing yellow perch in ponds was the variable climatic conditions and photoperiod in the NCR. The length of time required to grow perch to a market size of 115-150-g or 20-cm TL (Bennett Fish Inc., Loraine, OH) in 8 months when starting with fish of approximately 110-mm TL under natural conditions (Starr 1999, interpersonal communication).

Hokanson (1977) found that yellow perch gained no noticeable weight if temperatures were maintained below 6°C. Pond water temperatures in Michigan remain below 6°C for nearly 6 months. Yellow perch also grow best with 16 hours of light (Calbert and Huh 1976). The photoperiod in the upper Midwest is highly variable. Fish use photoperiod as a cue for hormonal changes and preparation for spawning.

Outdoor ponds may be important to establish broodstock and rear larval fishes (see Chapter 1). Yellow perch reach sexual maturity at 90-120-mm TL (Schott 1980). Reports on perch and other species have shown a correlation between sexual maturation and growth reduction, food consumption, and feed efficiency (Huh 1975, Purdom 1976, Utter *et al.* 1983, Malison 1985).

RACEWAYS:

Yellow perch have been reared in raceways at Bayport Aquaculture, West Olive, MI. Raceway culture enables greater stocking density (kg/l) and loading (kg/lpm) than pond systems. Glass (1991) determined that the maximum loading for yellow perch for commercial raceway production was 0.85 to 1.15-kg/lpm (7.06 to 9.58-lbs/gpm). Fingerlings were reared up to a density of 85-kg/m^3 (0.71-lbs/gallon) without reductions in growth, survival, or performance. This is considerably greater than maximum densities recommended (50-kg/m^3, 0.42-lbs/gallon) for salmonids (Piper 1982).

The primary disadvantage of raceway culture is that the number of appropriate sites able to supply high volumes of water at the appropriate temperature are limited (Glass 1991). Additionally, effluent restrictions on

receiving waters may exclude potential sites with high volumes of water at the appropriate temperature (Gary Boersen, Michigan Department of Environmental Quality, Lansing, MI, personal communication, 1999)

RECIRCULATING SYSTEMS:

Recirculating aquaculture systems (RAS) are comprised of culture tanks, biofiltration systems, solids removal systems, and sterilizers (Lucchetti and Gray 1988). The RAS are designed to provide optimum rearing conditions while minimizing the impact on natural environments. Large areas of land are not needed to house a RAS. Large quantities of fish can be reared within a single tank under adequate conditions. Calbert and Huh (1976) estimated that a 1-1.5-g yellow perch, reared under optimal conditions, could reach a market weight of 140-160-g in 11 months when fed 4% of their body weight per day. Because of these perceived potential advantages, many potential yellow perch producers have opted to use indoor RAS (Kelly 2000).

Timmons (2000a, b, c) has identified various reasons aquculturists have failed in their attempts to establish profitable RAS. An in-depth cost analysis was listed as an important requirement for new aquaculture enterprises; however, many cost analyses of RAS have been superficial. The first general falsehood described by Timmons (2000) was that a current unsuccessful dairy or hog farmer can become a successful fish farmer. Expert and experienced management was identified as a critical component for the success of a recirculating aquaculture system.

Kelly (2000) specifically summarizes characteristics that currently impede the development of commercial yellow perch aquaculture. However, Manci (2000) rebuts many of her assertions by stating current research is focused on advancing the technologies of culturing yellow perch, therefore making yellow perch culture more profitable.

US PRODUCTION OF YELLOW PERCH

Malison (1999) has estimated that yellow perch aquaculture contributed less than 100,000-kg per year to the commercial food market. He reported that existing markets could readily absorb 23-45 million-kg per year. Cost effective culture methods must be developed before aquaculturists can take advantage of the potential food fish market.

CHAPTER 1

EVALUATION OF FEEDING STRATEGIES

FOR FIRST-FEEDING YELLOW PERCH FRY

Introduction

The growth of commercial yellow perch aquaculture in the Great Lakes region is dependent on the development of reliable, cost-effective, culture methods. Methods are needed for each life cycle stage: eggs, first feeding fry, fingerling through market-sized fishes, and broodstock (including out of season spawning).

Currently, the most commonly used brood fish are captured, sexually mature wild yellow perch. Eggs are manually stripped and fertilized using the dry method (summarized in Piper 1982). Yellow perch eggs are deposited as single interconnected, gelatinous ribbon-like mass. A 25-cm total length (TL) female yellow perch can produce up to 30,000 eggs (Malison 2000). The fertilized egg ribbons are incubated in tanks (Malison 2000) or vertical drip incubators (Oekter 1999). The ribbon is stretched over a wire grate within the tank or around pegs placed in the vertical incubator tray to prevent bunching which can smother individual eggs. Water is passed over the egg ribbons.

Once the eggs hatch and the fry are free swimming, they are most often moved to fertilized outdoor ponds. Commercial grow-out of yellow perch first feeding fry (YPFFF) to fingerlings currently relies on intensive pond production methods (Malison and Held 1995). Ponds are drained prior to stocking to remove all remaining fish from the previous year. An organic fertilizer is applied

to promote plankton production and the ponds are refilled. The sac-fry are then transferred from the incubation units to the fertilized pond. The fry consume plankton upon exogenous feeding.

Yellow perch fry are feed trained to formulated feeds once the fry reach 15 – 20-mm TL (Hale and Carlson 1972; Malison 2000). Feed training is changing the diet of the yellow perch fry from a natural diet to a formulated diet. Current methods used to feed train yellow perch fry in ponds employ light to attract the fry to a feeding area (Manci et al. 1983). After a short time delay, feed is dropped into the pond under the light platform. Another method used to feed train fry (called the tandem pond-tank method) relies on harvesting the fry from the pond and moving them into tanks to begin feed training (Malison 2000).

The primary advantage to intensive pond production of yellow perch fingerlings is large numbers of fish can be successfully produced. However, environmental parameters such as temperature, dissolved oxygen, total ammonia, and zooplankton abundance must be consistently monitored. Precipitous changes in any of these parameters can adversely affect the growth and survival of yellow perch fry. Since the aquaculturist has minimal control over these parameters, the entire stock can be lost.

If intensive tank rearing systems can be developed to culture YPFFF, environmental conditions can be controlled to optimize growth and survival of rearing yellow perch larvae to 20-mm. However, high mortality rates ranging from 70 to 100% have occurred when larvae change from their endogenous food source (yolk) to exogenous sources (practical feeds). NCRAC has identified the

following constraints as major causes of the high mortality rates observed in intensive yellow perch larval tank culture systems:

    a. Lack of swim bladder inflation.

    b. Lack of acceptance of a practical feed at first feeding.

A benefit to controlled tank rearing of yellow perch fry is the potential for out-of-season spawning. Researchers are currently establishing protocols for spawning yellow perch out of their normal spawning period (NCRAC 1997) by manipulating photoperiod, temperature, and hormone levels. Out-of-season spawning would enable producers to rear multiple crops per year.

This study was designed to evaluate potential first feeds for the intensive culture of yellow perch that are economically feasible. The culture system was designed to facilitate swim bladder inflation (Barrows 1993). Survival rates of YPFFF fed live *Artemia* nauplii, live vinegar eels, Artificial Plankton™ (a practical larval shrimp diet) produced and marketed by Argent Chemical Laboratories (Redmond, WA), and combinations of three diets were monitored. The live feed items were chosen because they were within the size range of organisms that can be consumed by YPFFF (Oekter 1998), their relative ease of culture, and their established use in aquaculture and the aquarium trade, respectively. Vinegar eels are also very inexpensive to produce. Artificial plankton was also inexpensive, of an appropriate size, had a high-energy content, and a high level of essential fatty acids.

Literature Review

Both pond and tank rearing techniques have been utilized to produce yellow perch larvae. Intensive tank rearing technologies have been inadequate and fail to meet the commercial demands for yellow perch fingerlings.

Commercial yellow perch facilities have reared larval yellow perch in ponds that are fertilized with an organic fertilizer prior to stocking (Manci et al. 1983; Malison and Held 1992). The fertilized ponds provide a natural food source for the fry. Under highly controlled and monitored conditions, production levels of over 570,000 yellow perch fry (25-mm TL) per hectare (1,400,000/acre) have been reported (Manci et. al. 1983; Malison and Held 1992). However, under typical commercial conditions, actual numbers of fry produced have been under 40,000/ha (100,000/acre). Year-to year environmental fluctuations have resulted in large variations in survival. These variations in survival rates could have been caused by changes in the abundance and size distribution of natural food sources (Malison and Held 1995) or stress related to handling during harvesting (Hussain and Summerfelt 1991).

Feed training yellow perch fry in ponds has been moderately successful. Feed was presented to the fish several times per day. Lights have been incorporated to enhance feed training within ponds (Manci et al. 1983). This modification relies on the strong photopositive behavior of yellow perch larvae smaller than 20-mm TL.

Many producers have used the tandem pond-tank rearing method. Once yellow perch fry reach 15 – 20-mm TL, they are moved to indoor tanks to begin

feed training (Malison 1999). Once indoors, the fish would be trained on a commercial starter diet. Culture conditions were monitored and yellow perch fry were consolidated to ensure feed acceptance (Dr. J. Malison, University of Wisconsin, personal communication, 1998). An inverse relationship between fry survival and the length of the fry when formulated feeds are offered (Best 1981). Survival rates have been described as high as 90% from this stage until grow out. However, survival falls to below 50% if the fish is feed trained prior to 16-mm TL (Hale and Carlson 1972).

The principal advantages in tank culturing larval yellow perch are environmental and nutritional variables could be manipulated for maximum growth and survival (Calbert and Huh 1976, Hale and Carlson 1972, Hinshaw 1985). This would be vital for out-of-season spawning of yellow perch (Dabrowski 1998).

Despite advancements in tank culture of yellow perch fry, two factors limit success. YPFFF are inherently small which limits the size of feeds that can be used (Best 1981; Mansueti 1964; Nickum 1978). A large number of yellow perch have failed to inflate their swim bladder when reared in intensive culture systems.

YPFFF have been reported to range from 4.7 to 7.0-mm TL (Heidinger and Kayes 1986; Mansueti 1964; Ney 1978; Whiteside et al. 1985). YPFFF have also been observed to have small mouth gapes which limits the size of prey they can consume (Hansen and Wahl 1981; Wong 1972). Oekter (1998) reported that the yellow perch mouth gape was 318 x 332 µm (Oekter, 1998). Cunha and Planas (1999) estimated the optimal size of prey for turbot larvae was 40%

mouth width and 36% mouth height. The size of the esophagus may also limit

the size of prey consumed (Kestemont et al. 1996; Raisanen and Applegate

1983). Busch (1996) reported a 1:1 ratio in prey width and esophagus diameter

in *Clupea harengus*. Consequently, the size prey consumed by YPFFF may be

significantly smaller than 318-μm.

Historically, tank reared yellow perch fry were feed zooplankton and

phytoplankton that were captured or cultured (Ansari and Quadi 1989; Confer

and Lake 1987; Hale and Carlson 1972; NCRAC 1995; Noble 1973; Raisanen

and Applegate 1983). Zooplankton population structure and size varies during a

growing season and between years due to different environmental conditions

(Houde 1967; Mills et al. 1986). Reports of failures using zooplankton to rear

YPFFF may have resulted from these factors.

The collection of zooplankton from rivers and lakes for feeding larval fish

has been found to be an unreliable source of food for commercial scale

operations (Kamler 1992). Therefore, some researchers have focused on

culturing zooplankton. Live feed production has been successful in Japan

(Kalmer 1992). However, phytoplankton and zooplankton cultures required large

amounts of space and skilled labor.

A "green tank water" method (GW) for culturing multiple zooplankton

species has been developed (Binkowski, see review in NCRAC 1995). The GW

is added to the culture tank as a food for first feeding perch. This method

appears to have had limited acceptance by commercial producers. Many

commercial producers find it difficult to obtain the proper zooplankton species composition and abundance to maintain the fry.

Larval fish growth and survival depend on the chemical properties of the diet (Watanabe et al. 1983). The National Research Council (1993) reported docosahexaenoic acid (DHA, 22:6 omega 3), eicoapentaenoic acid (EPA, 20:5 omega 3), and linolenic acid (18:omega 3) are essential fatty acids for several species of fish. Tissue levels of DHA and EPA were higher in young perch than in the majority of their prey items (Dabrowski et al. 1993). Zooplankton caloric contents are different between species and within a species, seasonally (Schindler et al. 1971). A single species of zooplankton may not be capable of supplying the long-chain fatty acids or energy required for survival and growth of larval fish.

There are alternatives to zooplankton. Newly hatched *Artemia* nauplii could be of the appropriate size for YPFFF (Oekter 1998). There has been some success in feeding *Artemia* nauplii to YPFFF (Hinshaw 1985; Mansueti 1964; McCormick 1976). European perch had a survival rate of 85% at day 14 when fed *Artemia* nauplii (Valvonou et al. 1995). *Artemia* has been used to successfully rear mahimahi and moi, both similar in size to yellow perch, at the Oceanic Institute (Kim et al. 1993). Another possible alternative to zooplankton is vinegar eels. Vinegar eels have been used to successfully rear larval fish (Chen 1981). Vinegar eels may also provide a valuable food source at a lower cost.

Several researchers have been exploring the use of formulated diets as the initial food for first feeding fry (NCRAC 1997). Minimum pellet size exceeds

minimum mouth gape; but, microencapsulated feeds are in the size range that can be accepted by YPFFF. Microencapsulated feeds have been used to successfully rear Dover sole and sea bass larvae (Appelbaum 1985; Walford et al. 1991). Best (1981) reported that YPFFF did not accept or survive on a formulated diet. Researchers have developed a formulated diet for walleye FFF (Barrows et al. 1988).

Physical properties of the feed and the culture system may contribute to feed acceptability, survival, and growth. Ostrowski (1989) found that dolphin larvae reared on a brown feed in black tanks had a greater survival rate than fish reared on the same feed in tan tanks. Martin-Robichaud and Peterson (1998) determined that swim bladder inflation was greater in black tanks compared to white tanks. There were no significant differences in growth of Atlantic salmon parr reared in green or gray tanks (Stefansson and Hansen 1989). Therefore, a culture system of a contrasting color to the feed improved feed acceptance.

Many culturists have believed that the lack of swim bladder inflation has been the main cause of the high mortality of intensively reared YPFFF (C. Starr interpersonal communication 1999). Larval yellow perch fill their swim bladder by swimming to the surface and gulping air. Swim bladder inflation can occur in 11 days at 8°C or less than 2 days at temperatures greater than 15°C (Hokanson 1977). Swim bladder inflation problems have also been reported for walleye (Barrows et al. 1988) and stripped bass (Doroshov and Cornacchia 1979). These species appear to required an unobstructed path to the surface to inflate the swim bladder. Colesante et al. (1986) found that swim bladder inflation rate of

walleye was greater in tanks where fish were fed live feeds when compared to fish fed a formulated feed. Formulated feeds may have caused a surface film on the water. Barrows et al. (1993) reported that a vertical spray at 90° to the water surface would break up the surface film and allow the larvae to fill their swim bladder.

Tank culture technologies must be developed in order to provide a continuous supply of yellow perch to meet commercial food production needs. These techniques will enable commercial producers to rear yellow perch larvae year round when out-of-season spawning techniques are developed.

Materials and Methods

This study was designed to evaluate the potential of *Artemia* nauplii, vinegar eels, Artificial Plankton (Argent), or the combination of all three as a feed for YPFFF. YPFFF survival rates were determined and compared between the diets. Four intensive larval rearing trials were performed. Each successive trial was a modification of the previous trial. All trials were performed at the Michigan State University Aquaculture Research Laboratory.

Intensive Larval Culture: Trial 1

Fish and Holding Tank

Yellow perch sac-fry (fry dependent on endogenous yolk food supply) were obtained from Bay Port Aquaculture in West Olive, Michigan in April 1998. The fry were originally acquired by Bay Port Aquaculture from a commercial grower in Iowa. Sac-fry were held in a 230-L green fiberglass tank for a two-day acclimation period. Heated well water (15°C ±1) was provided at an exchange rate of 0.5 turnovers per hour. The drain of the tank was covered with 300-μm Nitex screening to prevent fry escapement. The photoperiod was maintained at 16-h light and 8-h dark.

Experimental System

Twelve green 5-gallon pales were used to create experimental larval rearing tanks. A tubular center screen was constructed with using a 2" PVC pipe with portions removed. The openings were covered with 150-μm Nitex screen to prevent YPFFF escapement (Figure 1.1). Heated well water (15°C ±1) was
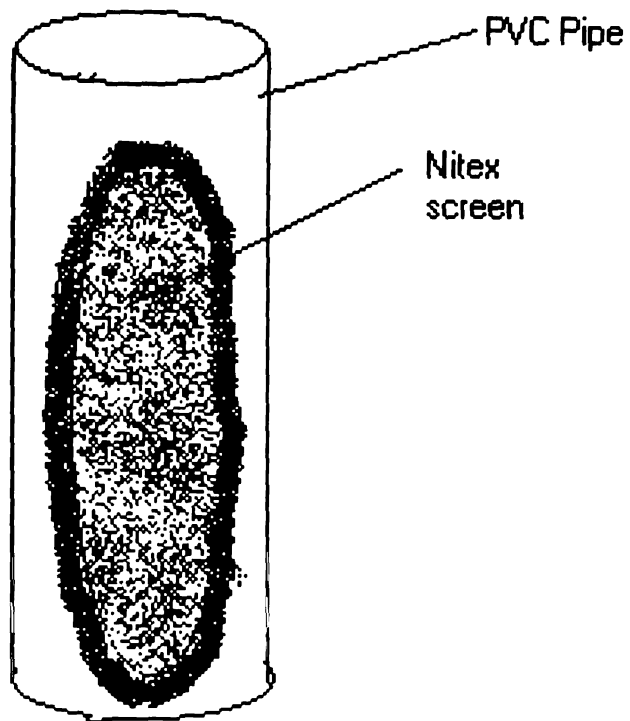
Figure 1.1—— Center drain tube made of 2-inch PVC pipe with the sides cut out and holes covered with 300-μm Nitex screening.

sprayed over the surface at a rate of 0.25-L/min using a system similar to the design described by Barrows et al. (1993). Water levels were maintained in each tank with an external standpipe. Each tank was randomly stocked with 100 YPFFF. The photoperiod was maintained at 16-h light and 8-h dark.

Feeds and Feeding

Four replicate tanks, each, of fry were fed one of three diets: first hatch *Artemia* nauplii, Artificial Plankton™ (both obtained from Argent, Redmond, WA) or vinegar eels. Culture methods for *Artemia* nauplii and vinegar eels are described in Appendix 1 and 2, respectively. Fry were fed 5 times per day at 0800, 1100, 1300 1500, and 1800. *Artemia* was fed at a rate of 15,000 nauplii/tank/feeding. Artificial Plankton was fed at a rate of 1-g/feeding/tank. Vinegar eels were fed at an approximate rate of 15,000 eels/tank/feeding.

Survival and Swim Bladder Inflation

Survival was determined by comparing the number of live fish 28 days after stocking the experimental tanks. Fish were expected to reach 15 – 20-mm under our conditions by the end of the period (Hokanson 1977). Live fish were removed from each tank and observed under 10-power magnification for swim bladder inflation and feed consumption. Moribund and dead fry were collected daily and examined under a dissecting microscope to determine swim bladder inflation rates.

Intensive Larval Culture: Trial 2

Eggs and Incubation

Gravid female and milt producing male yellow perch were collected in May 1998 from southern Lake Michigan, offshore of St. Joseph, Michigan. The parental stock was transported on the day of capture to the Michigan State University Aquaculture Research Laboratory. Eggs were stripped from ripe females into a small dry pale. Milt was collected from males and was directly added to the ripe eggs. Water was added to "activate" the fertilization process. Two minutes after water activation, a gentle flow of fresh water was added to the container for 1 hour to water harden the eggs.

After water hardening, eggs were incubated in a 230-L oval green tank supplied with 15°C ±1 well water. Ribbons were laid out on a screen mat placed 4 inches off the bottom of the tank. Water was supplied to the bottom of the tank to create an upwelling flow. Eggs were incubated for 7 - 11 days. Two days after hatching, fish were stocked into experimental larval rearing units and reared as described in Trial 1.

Intensive Larval Culture: Trial 3

Eggs and Incubation

Freshly spawned eggs were obtained from Bay Port Aquaculture in April 1999. The eggs were transported to the Michigan State University Aquaculture Research Laboratory. The fertilized eggs were incubated in a Heath vertical incubator supplied with 13°C well water at a rate of approximately 2.5-Lpm (0.66

gpm). After fry hatched, they were conveyed to a 378-L (100 gallon) holding

tank. The holding tank dimensions were 122 X 122 x 25.4-cm (48 X 48 X 10-in).

The holding tank was supplied with effluent water from the incubation unit. The

incubation unit water was added at the surface of the holding tank to create a

counter-clockwise vortex to aid in tank cleaning. The incubation and holding

system was maintained under constant light.

Experimental System and Culture

One hundred 1-d post-hatch sac-fry per tank were randomly stocked into

12 square 40-L gray tanks. All tanks were equipped with 2.54-cm (1-inch)

internal drain tubes similar to those described above. Drain tubes were covered

with 150 μm Nitex screening to prevent fry escape. Well water was supplied

using the same method described in Trial 1. Water temperatures were

maintained at 13°C. Photoperiod was maintained at 16-h light and 8-h dark.

Fish reared in the experimental tanks were fed as described in Trail 1.

Fish remaining in the holding tank were fed a combination of all three diets 5

times per day.

Intensive Larval Culture: Trial 4

Fish and System

Approximately 60,000 fry, 1-d post-hatch, were obtained from Bay Port

Aquaculture. The fish were transported to Michigan State University's Upper

River Research Laboratory. Fish were held in a 122 X 122 x 25.4-cm (48 X 48 X

10-in) holding tank at 12.5°C for the duration of the experiment. Well water pumped into a head tank and aeration was supplied to ensure optimum oxygen levels. The water was dropped at near 90° to the surface into the holding tank to create a counter-clockwise vortex to help in keeping the tank clean. The system was maintained under constant light.

YPFFF were fed five times per day beginning one day after stocking. Newly hatched *Artemia* were initially fed at a rate of 75,000 per feeding. However, *Artemia* was not used after day-3 because of a very poor hatch rate. Artificial Plankton was fed at a rate of 3-g per feeding. Vinegar eels were fed at an approximate rate of 200,000 eels per feeding.

Solid wastes were siphoned from the tank each morning after the first feeding. Five fish were removed each day to determine the rate of swim bladder inflation. Each larva was examined under a dissecting microscope under 10-power magnification to determine swim bladder inflation and stomach contents.

Results

In trial 1, feeds were not presented until the oil globule was the only remaining endogenous food source. Experimental tanks were stocked with 100 fry per tank. Feedstuffs were offered immediately after stocking. Total mortality, on day-8 post-stocking, occurred in all tanks (Figure 1.2), including the holding tank. Trial 2 followed a similar pattern. Total mortality occurred within 8-9 days post stocking.

During trial 3, a total of 3 fry per day were removed from the experimental systems and observed under 10-power magnification to determine swim bladder inflation and stomach contents. By day-8, all fry observed had consumed the feeds. Identification of the feed was determined by the color in the digestive tract of the fry. Orange indicated *Artemia* and yellow indicated the Artificial Plankton ™ had been consumed. Vinegar eel body parts were identified within the digestive tract of the YPFFF while the fry were observed under 10X magnification. Swim bladder inflation was not observed in any of the fish sampled from the experimental system. No fish survived in the experimental tanks past day 11 after stocking in trial-3 (Figure 1.3).

During trial 3, fish from the holding tank were sampled to identify swim bladder inflation and feed consumed. Fish had readily consumed all diets by day-8. Swim bladder inflation occurred between day 7 and day 12. Greater than 75% survival was observed through 28 days post-hatch. However, due to increased fungal build up on solids that collected over the effluent screen, the tank overflowed and fish were lost overnight.
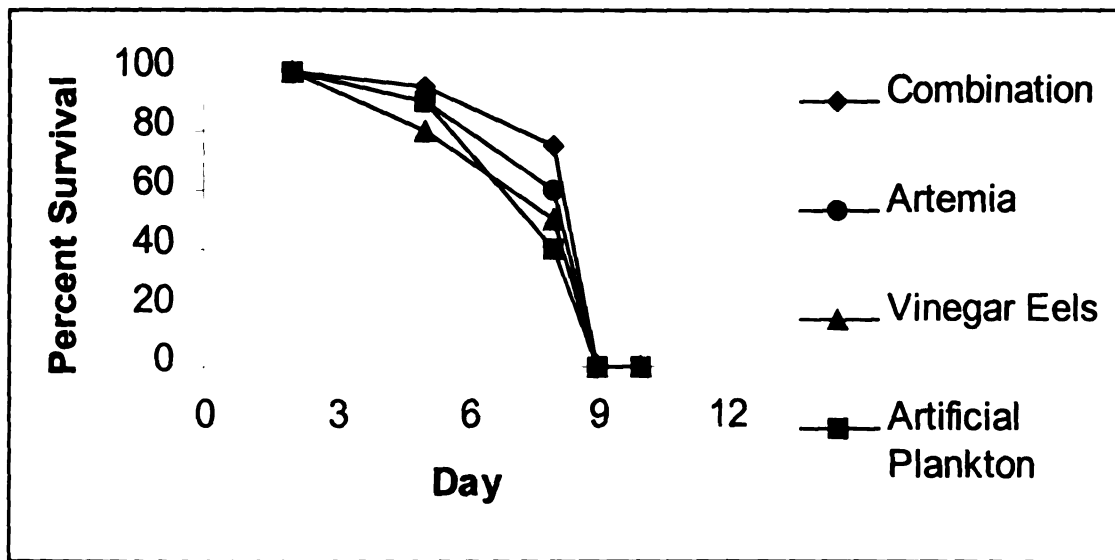
Figure 1.2 ---- Percent survival for first-feeding yellow perch fry of Trial-1 fed *Artemia* nauplii, vinegar eels, Artificial Plankton ™, or combination of all diets reared in an intesive culture system.

Figure 1.3—- Percent survival for first-feeding yellow perch fry of Trial-3 fed *Artemia* nauplii, vinegar eels, Artificial Plankton ™, or combination of all diets reared in an intensive culture system.

Trial 4 replicated the conditions used in the holding tank during Trial 3. Fish were examined for swim bladder inflation and feed consumption daily. Fish survived for 8 days. Mass mortalities were observed on the morning on day 9. No swim bladder inflation or feed consumption was observed.

Discussion

This study was designed to compare the survival and growth rates of first-feeding yellow perch fry using two different live feeds and one artificial feed. Survival rates observed during this study were considerably lower than previously reported (Hinshaw 1985). This could have been due to several factors:

1. Poor water quality

2. Handling stress

3. Improper nutrition

    A. Larval

    B. Parental

        I.   Genetics

        II.   Egg quality

4. Lack of swim bladder inflation

The inherent small size (approximately 7-9-mm) of first feeding yellow perch fry poses another problem. A 150-μm Nitex screening was used over an internal standpipe. The addition of the feeds to the system appeared to inhibit the effluent flow. Increased organic retention may have caused the fungal build up within the tanks. Tanks fed Artificial Plankton™ exhibited the greatest amount of fungal build up. The low effluent flow coupled with the high fungal content contributed to lowering the water quality.

The majority of observed mortalities occurred prior to day-7. Typically, high mortality occurring within 7 days has been attributed to stress. Handling

stress has been identified as a major factor affecting larval yellow perch survival (Malison 2000). No acclimation period was used because of the early stage at which yellow perch fry change from endogenous to exogenous food sources.

Large amounts of fungus on the effluent screens made tank cleaning and maintenance difficult. The increased time siphoning the tank would have contributed to an increase in stress among the fry. The combination of increased stress levels from initial stocking, tank maintenance, and declined water quality may have increased mortality rates above those previously reported.

Temperature affects yolk utilization in larvae (Ware 1975). Newly hatched yellow perch fry use their yolk sac as their primary source of nutrients for 5-7 days post hatch at temperatures of 12.5°C (Hokanson 1977). Therefore, feed was presented to the yellow perch fry immediately after stocking.

Fish were observed feeding directly after feed was presented. This implies that yellow perch begin feeding shortly after hatch. Siefert (1972) had observed that larval perch accepted feed prior to complete yolk absorption. Our study did not determine whether the feed was assimilated. Dabrowski (1997) reported that yellow perch fry have incomplete digestive systems while the oil globule was still present.

Larval fish have a higher energy requirement then adult fish. Therefore, feed must be present within the system at all times. Research has shown fry mortality rates of 100% occur if feed is not present for 12 hours (Jonas and Wahl 1998). It was observed in our study that Artemia settle from the water column within a hour, where as vinegar eels and Artificial Plankton™ remained in the

water column for several hours. In our experimental trials, fry were feed 5 times daily over a 12-h period for culturist convenience. Therefore it can be concluded that in experimental tanks that were fed *Artemia* may not have had feed present for 11 hours, which may attribute to the high mortality rates. Tanks that were fed vinegar eels or Artificial Plankton™ may have been nutritionally deficient.

The yellow perch fry used in this study were offspring of wild caught yellow perch from Lake Huron or Lake Michigan. The nutrition histories of the parents were not monitored. The culturist has no ability to control the nutrition provided to maternal parents to ensure appropriate yolk development. Therefore, the nutritional quality of the yolk could have affected fry survival. The yolk may have been energy deficient.

The nutritional history of the parental stock has been shown to influence the survival rates of their offspring. Thiamin deficiency has been identified as a possible cause of early mortality syndrome in many salmonids from the Great Lakes Region (Brown et al. 1998). In our study and Oekter (1998), yellow perch fry exhibited short vertical swimming bursts followed by a rest period on the tank bottom. This erratic swimming behavior was similar to behavior exhibited by salmonids with thiamine deficiency. Another sign, the failure to inflate the swim bladder, was observed in thiamine deficient salmonid fry and yellow perch fry. Washing the eggs in a thiamine bath or injecting thiamine directly into the gravid female's body cavity alleviated these symptoms (Brown et al. 1998).

The most probable cause of high mortality among the yellow perch fry in our study was the lack of swim bladder inflation. The fry in our study were

observed swimming in vertical bursts of up to 4-inches, followed by a rest period on the tank bottom, it is probable that the yellow perch fry expended the energy reserves of their yolk for maintaining buoyancy, rather than development. Hokanson (1977) demonstrated that at high temperatures the swim bladder was inflated at an earlier stage. Swim bladder inflation can occur within 2 days post hatch at 15°C (Hokanson 1977). Temperatures ranged from 13°C (trial 3 and 4) to 15°C (trial 1 and 2), which is bordering the limit of optimal development. Consequently, factors other than temperature probably caused the larval mortality observed in the majority of our experiments.

A survival rate greater than 75% was observed for perch fry from the holding tank for trial-3, until day-28. The holding tank had a larger surface area and was shallower than the research tanks. The increased surface area to depth ratio (S:D) may increased the chance of the fry filling their swim bladder by allowing the fish to reach the surface with less effort. The increased surface area could have inhibited the accumulation of surface debris, which would allow the fry an increased probability of an unobstructed gulp of air. The holding tank was maintained under constant light, which may have altered the biorhythm of the fry. Yellow perch fry are found to feed at night (Faurot and White 1994). The lack of a photocycle may have increased the chance of the fry to feed while the feed was presented. Also, a combination of Artemia nauplii, vinegar eels, and Artificial Plankton™ were fed to the holding tank. A single feed may not meet the nutritional requirements of the yellow perch fry.

Trial-4 was a replication of the holding tank system used in trial-3, one year later. Total mortality occurred during trial-4. The mortality was attributed to feed. *Artemia* was excluded from the feed because of very low hatch rates (<10%). The previous years Artificial Plankton™ was used because of limited funding. Nutritional degradation may have affected the quality of the Artificial Plankton™. Therefore, trial-4 fry were feed a diet that consisted of vinegar eels, and expired Artificial Plankton™.

Development of cultured brood fish is vital for the expansion of yellow perch aquaculture. The availability of cultured brood fish would enable researchers to develop diets that would ensure the nutritional quality of the yolk which could decrease the variability in larval fish survival rates. If wild brood fish are used as parental stock, research should be done to determine if thiamine injections can increase the percentage of larval fish with inflated gas bladders.

Another possible area of interest for future research is the effect of surface area to depth ratio on larval yellow perch survival. The increased surface area to depth ratio could have had a positive affect on the survival of the fry. The large surface area may have reduced the surface tension created by the meniscus at the top of the tank and may have decreased the amount of surface film from decomposing and uneaten feeds. The decreased depth may have also enhanced the ability of the fry to reach the surface to fill their swim bladder.

Summary and Conclusion

The culture of yellow perch larvae has been identified as a research focus for the North Central Aquaculture Center. This study was designed to compare potential feeds for first feeding yellow perch fry. Fry were either purchased from a local commercial yellow perch producer or where hatched from fertilized egg ribbons of wild caught yellow perch females.

Diets used in this study were *Artemia* nauplii, vinegar eels, and Artificial Plankton™. Fry appeared to consume all diets when the diet was presented directly after hatch. A high percentage of swim bladder inflation was achieved by increasing the surface area to volume ratio in the holding tank. However, this result was not duplicated in experimental tanks. In all experimental trials, mortality rates of 100% were observed. Factors that affected mortality rates may have been:

- Handling stress

- Poor water quality

- Improper nutrition or feed availability

- Lack of swim bladder inflation

- Improper parental nutrition

Handling stress can be minimized by not moving fish from tank to tank. The small sizes of the fry increase their susceptibility to thermal stresses. Water quality could be maintained if Artificial Plankton™ had not been used. However, water quality was not an issue in tanks fed *Artemia* nauplii or vinegar eels.

Improper nutrition and lack of swim bladder inflation are two areas, which require further research. The development of brood stock is vital to the development of proper larval yellow perch nutrition.

Chapter 2

GENDER EFFECTS ON BIOENERGETIC RESPONSES

OF YELLOW PERCH FINGERLINGS

Introduction

Various researchers (Malison and Garcia-Abiado 1996; Malison et al.

1985, 1986; Schott et al. 1978) have reported that under intensive culture

conditions, yellow perch females grow faster than males after achieving a total

length (TL) of 110-mm. Determination of the bioenergetic responses of male,

female, and mixed gender populations of yellow perch may help commercial

farmers develop culture strategies to avoid gender based growth differences.

A kinetic saturation level model (Mercer 1980, 1992; Mercer et al. 1989,

1993, 1996) has been used to determine the basal metabolic energy

requirement, efficiency of diet utilization, and the theoretical maximum response

to feeds of mixed gender populations of *Tilapia zillii* by Annett (1985) and Nile

Tilapia, *Oreochromis nilotica*, Belal et al. (1991). This study was designed to

determine the effects of gender on the bioenergetic response of yellow perch

greater than 110-mm using a saturation level kinetic model. The general model

(Morgan et al. 1975) can be defined by the equation:

$$r = \frac{b(K_m)^n + R_{max}[I]^n}{(K_m)^n + [I]^n}$$

Where:

r   = Physiological observed responses,

b   = Intercept on the ordinate y-axis,

K_m = Intake constant for ½ of R_{max},

R_{max} = Maximum response,

n = Slope factor (compared to K_m, apparent kinetic order of the response with respect to I as I^n becomes negligible),

I = Nutrient intake.

The model was used to calculate the theoretical maintenance level and the maximum and optimal efficiency values for single and mixed gender stocks. Values were determined based on growth (wet weight) and changes in proximate analysis (protein, crude lipids, gross energy, moisture, and ash on a dry matter basis) of whole body tissues. The values generated were used to determine if reported differences in growth of male and female stocks have been caused by differences in maintenance requirements or in the efficiency of feed utilization.

A collateral objective of this project was to identify yellow perch gender by a reliable secondary sex characteristic. The identification of an external secondary sex characteristic would enable researchers to determine sex effects as a factor in future research studies, as well as allowing commercial yellow perch producers to evaluate single gender culture in practical culture operation levels.

Literature Review

Gender Identification

Female yellow perch begin to grow faster than male yellow perch after reaching a size of 110-mm TL and, ultimately, reach a larger TL (Schott et al. 1978; Malison et al. 1985, 1986; Malison and Garcia-Abiado 1996). Wild yellow perch reach this length within the second year of life. Yellow perch also develop gametes during their second year of life. Gonad development often influences the growth rates of fish including yellow perch (Hayes and Taylor 1990).

Yellow perch have an annual reproductive cycle. The development of yellow perch gonads is dependent on temperature and photoperiod. Kayes and Calbert (1979) determined that temperature has a more important role in gonad development than photoperiod. Yellow perch require a minimum "chill" period of 160 days at 10°C or less (Hokanson 1977).

There are internal morphological differences between male and female yellow perch. The ovaries of female yellow perch are a single, fused organ. Eggs develop in a long cylindrical mass that is interconnected by a gelatinous matrix. As the female spawns, the thin membrane covering the genital opening ruptures and the egg ribbon is expelled through the urogenital opening. Male yellow perch produce milt in pared testes. The milt is expelled from the urogenital opening via a seminal duct (Schott et al. 1978).

Kayes and Malison (unpublished data) have described a method of sexing yellow perch greater than 150-mm TL with 70% accuracy. They observed that

the urogenital openings of males were round and females were crescent shaped. When pressure was applied to the abdomen of males, it resulted in the urogenital opening forming a V towards the point of pressure. When pressure was applied to the abdomen of females, it resulted in the urogenital opening retaining its crescent shape. Malison (1999, personal communication) indicated that the accuracy of this method decreased to nearly 50% when fish were sexed at less than 120-mm TL.

A method of creating all female yellow perch has been developed by Malison and Garcia-Abiado (1986). Yellow perch were fed a diet with methyltestosterone (MT) at 20-35-mm TL. The female fish produced ovotestes. The ovotestes produced sperm that contained only X chromosomes. When the fish matured, the ovotestes were removed and the sperm was extracted by pressing it through cheesecloth. When the milt was mixed with the eggs of a nonmasculinized female, all offspring produced were XX females. Recently, the University of Wisconsin-Madison was approved for an INAD (Investigative New Animal Drug) for the use of methyltestosterone in the masculinization of yellow perch.

Vitellogenin, a precursor hormone to yolk development, has been used to successfully determine the sex of stripped bass (Kishida et al. 1992) and carp (Tyler et al. 1999). They produced an Enzyme Linked Immunosorbent Assay (ELISA) to simplify determinations in the field. Vitellogenin is produced by the liver and can be excreted into the slim layer of the fish. A major shortcoming of the use of vitellogenin as a gender identifier is that only females that are in

41

oocyte development produce the hormone. Additionally, vitellogenin-producing male rainbow trout have been observed in the wild (Jobling et al. 1996). Mellanen and co-workers (1996) believe that an increase of phyto-estrogens and other estrogen-mimicking contaminants may be the cause of the increased vitellogenic hormone production in male trout.

Many yellow perch producers harvest marketable size fish from their rearing units that have been stocked with multiple size and age cohorts. By continuing to culture the smaller fish while removing only the large fish, producers create a higher proportion of small fish in their tanks and may inadvertently select for slower growth. Culling smaller, slower growing yellow perch might result in a more uniform size distribution. Uniform size distributions have been shown to increase the growth rate of cultured eels (Wickins 1987) and limit the hierarchical dominance that is exhibited by some fish species (Jobling 1982). It has not been determined if yellow perch exhibit a social hierarchy. These harvest strategies could also lead to unintentional genetic selection for slower growing fishes (Kapuscinski and Jacobson 1984).

Gender-Related Growth Differences

Yellow perch appear to exhibit compensatory growth. Compensatory growth refers to an animal's ability to have a rapid weight gain directly proceeding a food deprevation or reproductive cycle (Broekhuizen et al. 1994, Jobling 1994). Compensatory growth occurs in both invertebrates and vertebrates (Broekhuizen et al. 1994, Russell and Wootton 1992). Hyperphagia

and a possible decline in metabolic rate from fasting may have caused the observed increased weight gain (Ryan et al. 1993).

Estrogenic compounds have been shown to inhibit growth in rainbow trout, coho salmon, and goldfish (Fagerlund and McBride 1995, 1997; Hirose and Hibaya 1968; McBride and Fagerlund 1976). However, estrogenic compounds promote growth in plaice (Cowey et al. 1973) and yellow perch (Malison et al. 1985). When fed to yellow perch, 17β-estrogen appeared to promote while methyl-testosterone appeared to decrease the growth rate by increasing food consumption rates (Malison et al. 1986). The physiological - behavioral mechanism by which estrogens increased food consumption has not been determined.

Saturation Level Kinetics Model

Hill (1911) and Michaelis and Menten (1913) originally developed a model to describe enzyme kinetics. It was based on enzymatic responses to different levels of substrate and to varying reaction environmental components such as pH and temperature. The conceptual model was based on the interaction between a dependent variable, the observed response (r), and an independent variable, substrate intake (I). Morgan et al. (1975) expanded the use of the model to describe physiological responses in animals.

The basic principle behind the model is that for any nutrient (I) given to an animal, there is a certain response (r) created, whether negative or positive. For example, an animal given a nutrient (I) along with its specific receptor (M) will

create the complex (MI), which produces a physiological response (pr) in a

proportional manner to concentration level of (MI), as follows:

$$I+M \xrightleftharpoons[K_2]{K_1} MI \quad \text{------------------------------- (1)}$$

$$pr = K_3[MI] \quad \text{----------------------------- (2)}$$

(Where K_1, K_2, and K_3 are constants and [ ] denotes concentration level).

At equilibrium, the combination of the above equations can be written as:

$$\frac{[M][I]}{[MI]} = \frac{K_2}{K_1} = KI$$

(Where KI is also a constant)

Thus, for the total receptor concentration level (Mt), where [Mt] = [M] + [MI], or

[M] = [Mt] – [MI], then:

$$\frac{[Mt-MI][I]}{[MI]} = KI$$

After rearranging:

$$\frac{[MI]}{[Mt]} = \frac{[I]}{KI +[I]}$$

When all of the receptors are occupied, the maximal physiological response of

the system (PR$_{max}$) is:

$$PR_{max} = K_3 [Mt] \quad \text{----------------------------- (3)}$$

Then:

$$\frac{pr}{PR_{max}} = \frac{[MI]}{[Mt]}$$

$$Pr = \frac{PR_{max} [I]}{K1 + [I]} \qquad \text{------------------------- (4)}$$

The linear model equation of Michealis-Menten (1913) from equation (4):

$$Y = a0 + a1X \qquad \text{---------------------------- (5)}$$

There are two limitations of equation 4 that do not match the observed responses. First, responses are found experimentally to be sigmoidal, but this equation only describes a hyperbola. Adding another parameter (n) to the equation may solve this limitation:

$$pr = \frac{PR_{max} [I]^n}{K_I + [I]^n} \qquad \text{---------------------------- (6)}$$

Where n is the apparent kinetic order.

As n increases above one, the curve of this equation fluctuates from a hyperbola to a sigmoidal (n>1) curve. This equation has been utilized in enzyme kinetics (Hill 1911).

The second limitation of the equation deals with the (X, Y) axis system, which describes a curve that passes through the origin (0, 0) and does not account for energies used in the metabolism of the nutrient. To solve this, it is necessary to add the parameter b (the intercept on the ordinate y-axis) to the previous equation:

$$PR_{max} [I]^n$$

$$pr = \frac{\text{---}}{K_I + [I]^n} + b \qquad\qquad \text{---------------------(7)}$$

Then:

$$pr = \frac{PR_{max}[I]^n + bK_I + bI^n}{K_I + [I]^n} \qquad\qquad \text{------------------(8)}$$

If $(PR_{max} + b) = R_{max}$ and simplify pr to r, the four-parameter mathematical model for physiology responses can be written:

$$r = \frac{bK_I + R_{max}[I]^n}{K_I + [I]^n} \qquad\qquad \text{------------------(9)}$$

This canbe also described as:

$$r = \frac{b(K_m)^n + R_{max}[I]^n}{(K_m)^n + [I]^n} \qquad\qquad \text{------------------(10)}$$

Where:

r = Physiological observed responses in the fish,

b = Intercept on the ordinate y-axis,

K_m = Intake constant for ½ of R_{max},

R_{max} = Maximum response,

n = Slope factor (compared to K_m, apparent kinetic order of the response with respect to I as In becomes negligible),

I = Nutrient intake.

This model has been successfully used to predict weight gain, nutrient deposition, dietary requirements, plasma nutrient levels, net nutrients, tissue enzyme kinetics and other physiological processes in a variety animals (Belal

1987; Belal et al. 1992; Mercer 1980; Mercer 1992; Mercer et al. 1989; Mercer et al. 1993, 1996).

Belal et al. (1992) studied two practical feeds fed to *Oreochromis niloticus* fingerlings by using the saturation level kinetic model. Other models that have been used for the determination of the standard metabolic rate, optimum feed ration level, growth responses, and protein energy requirements for fish fed both practical and natural diets. A similar threshold-corrected hyperbolic model was used to demonstrate the relationship of the specific growth rate of *Tilapia zillii* to three different feeds (Annett 1985). He also used this model to determine the relationship between the growth and the size of the fish to water temperature.

It is important to understand the origin of the model that Annett used. Pierre-Francsois Verhulst (1845) proposed that during the early stages of growth, a population would increase exponentially until such time that the critical resources became limiting. Upon such limits, the rate of growth was retarded in a symmetrical, sigmoidal curve of growth. This curve, labeled the "logistic growth model" can be written as:

dN/dt = rN((K – N)/K)   -------------------------(11)

Where:

K = maximum carrying capacity

N = number of individuals

r = maximum growth rate (1/time)

t = time

Pearl and Reed (1920) used the logistic growth curve to describe the growth rate

of individuals in bacterial and protozoan populations. The logistic growth model

can be used in this manner provided the following criteria are met:

1) both r and K are constant,

2) no time lags exist,

3) all individuals exert an equal effect on the reduction of the growth rate

   as population density changes.

When the logistic formula is rearranged to express the rate of increase per unit

time the following equation results:

$$dN/Ndt = r(1 - (N/K)) \quad\quad\quad \text{-------------------------(12)}$$

Lotka (1925) suggested the reason populations and individuals follow the

same trends was that they had the same basic fundamental mechanisms (e.g.,

individuals are basically large populations of cells).

The logistic growth curve serves the important function of incorporating a

rate dependency concept into growth modeling. Verhulst (1845) claimed that

growth rates would be influenced by the crucial limiting resource's availability.

However, the logistic curve fails to relate changing growth rates directly to

specific environmental resources. The logistic curve describes the growth rate of

a population over time.

In unicellular and multicellular animals, enzymes regulate biochemical

reactions. These reactions contribute to the growth of the organism. Certain

enzymes associate themselves with the substrate to form an intermediate

complex, which act as a catalyst.

There are two factors important to the reaction rate, the relative amounts

of enzyme and substrate. At low substrate concentration levels, the reaction rate

would be proportional to the amount of substrate. At these substrate levels, the

enzyme is able to react with all the substrate forming an enzyme-substrate

complex (ES) helping the reaction. As the concentration level of the substrate

increases, a higher percentage of the enzyme is bound in the ES. There are

fewer enzymes to react with the substrate. Eventually, if the substrate

concentration level were to increase sufficiently, nearly all the enzyme would be

bound in a complex. This enzyme-substrate reaction rate response has been

described by the Michaelis-Menten equation as follows (Engel 1977):

$$V = V_{max}(S/(K_s + S))$$ ----------------------------(13)

V = reaction rate (1/time)

V_{max} = maximum reaction rate (1/time)

S = substrate concentration level

K_s = substrate concentration level at which the reaction rate is ½ V_{max}

When S becomes very large compared to K_s, the reaction rate V

approaches V_{max}. When S is very low relative to Ks, the reaction rate is

proportional to S.

Limiting factors (i.e. nutrients or environmental conditions) restricted

bacterial growth (Monod 1949). When experimental conditions were established

to provide an excess of all but one limiting nutrient, and the concentration level of

that limited nutrient is gradually increased, the growth demonstrated an initial

rapid increase. The growth rate then declined until it leveled off at high substrate

concentration levels. Monod (1949) established that the relationship is similar to that determined for the enzyme-catalyzed reactions and proposed the following equation to relate growth to substrate:

$$\mu = \mu_{max}(S/(K_s + S)) \quad \text{-----------------------------(14)}$$

μ = Growth rate (1/time)

μ_{max} = Maximum growth rate (1/time)

S = Substrate concentration level

K_s = Substrate concentration level at which the growth is ½ μ_{max}

The levels of the constants are an expression of the ability of the enzyme systems to utilize the particular limiting substrate for growth. This model has one flaw. The model does not take in account the maintenance value. Subtracting this value (Sq) will yield the equation:

$$\mu = \mu_{max} \frac{S - Sq}{(K_s - Sq + S - 2Sq))} \quad \text{---------------------------(15)}$$

Dividing both sides by (S – Sq) and inverting yields:

$$\frac{S - Sq}{\mu} = \frac{S - Sq + K_s - Sq}{\mu_{max}} \quad \text{---------------------------(16)}$$

Then rearranging the equation in the linear form Y = a + bX:

$$\frac{S - Sq}{\mu} = \frac{Ks - Sq}{\mu_{max}} + \frac{1}{\mu_{max}} (S - Sq) \quad \text{--------------------------(17)}$$

Using this equation, one is able to determine an estimate for the Y-intercept. In a conversation with Mercer (2000), the model used in this study (equation 10) requires an estimate for the Y-intercept.

The four-parameter saturation level kinetic model for physiological

response (Mercer 1978) was used to analyze the data:

$$r = (bK_m + R_{max}I^n) / (K_m + I^n) \text{ -----------------------------------(1)}$$

Mercer (personal communication) indicated that an estimate of a 0.0 ration level

was necessary to generate equation 1. The 0.0 ration level was estimated using

King's model:

$$\mu = \mu_{max} \frac{S - Sq}{(K_s - Sq + S - 2Sq))} \text{ --------------------------(2)}$$

Equation 2 was rearranged to:

$$\frac{S - Sq}{\mu} = \frac{Ks - Sq}{\mu_{max}} + \frac{1}{\mu_{max}} (S - Sq) \text{ --------------------------(3)}$$

An efficiency parameter that measures the greatest response with the smallest

intake value (Mercer 1982) is described in the equation below:

$$I_{me} = K_m (n - 1)^{1/n} \text{ ---(4)}$$

The basal metabolic requirement was determined by setting the response,

r, in equation 1 to 0.

Materials and Methods

Secondary Sex Characteristics

Fish

Yellow perch were obtained from Bayport Aquaculture, West Olive, Michigan. Approximately 250 fish, 130± 10 mm total length, were transported to the Aquaculture Research Laboratory, Michigan State University, East Lansing, Michigan. All fish were held and acclimated in a 250-gallon flow-through tank. Well water from an aerated head tank was supplied at $11.5 \pm 1°C$ at a rate of 2.25 exchanges per hour.

A random sample of 40 fish was taken from the holding tank for measurements and evaluation of secondary sex characteristics. Fish were anesthetized by placing them in a solution of MS-222 at a concentration level of 0.5-g/L. Multiple observations and measurements were performed on each individual fish.

An otoscope was inserted into the urogenital opening of fish to determine if the seminal duct could be observed. A green food-grade dye was used in an attempt to aid in the identification of the seminal duct.

Eye diameter (ED), maxillary length (ML), mouth width (MW), mouth height (MH), standard length (SL), and total length (TL) were recorded for each fish. Ratios between all variables were determined and compared for gender significance. A comparison of means, variances, and standard deviations were done to identify if further statistical test would be required.

Pressure was applied to the abdomen in an anterior direction to observe differences in the shape of the urogenital opening. The urogenital opening was

also observed under 10X magnification to identify slight differences in the shape of the urogenital opening. A food-grade die was used to aid in clarifying any of the subtle changes in shape.

After observations and measurements were completed, all fish were euthanized by placing them in a solution of MS-222 at a concentration level of 1.0-g/L for 10 minutes. The gender of the individual fish was verified visually by dissection and examination of the gonadsThe measurements and observations were tabulated by gender for analysis of mean, variation, and standard deviation.

Saturation Level Kinetics

This study was designed to determine the effects of gender on growth and performance of yellow perch greater than 110-mm TL using a saturation level-kinetics model. All feeding trials were conducted after an initial acclimation period of two weeks at the Upper River Aquaculture Research Laboratory, Michigan State University. Fish were acclimated to water and tank conditions and fed a commercial trout diet, Purina Aquamax Grower 400 from lot A-5D04, containing at least 45% crude protein and at least 16% crude lipids. Each feeding trial was conducted for 16 weeks.

System and water

Feeding trials were conducted in a recirculating aquaculture system (Appendix 3). Fish were reared in one of 24 gray 110-L experimental culture tanks. Each tank was equipped with a screened bottom drain and an external standpipe (Appendix 4). The water exited the tank via the bottom drain, through

the standpipe and into a plastic gutter that channeled the water to a primary solids removal tank. Large solids were removed by passing incoming water through a screen. The screen was removed and cleaned weekly. Fine particulate matter was passively removed within a 590-L rectangular settling tank. Water passed from the settling tank to the biofiltration level tank. Biofiltration level was accomplished using a 440-ft^2 rotating biological contact filter and approximately 0.5-m^3 shredded plastic in a bio-bag were used as biofilter media. Nitrifying bacteria which colonized the filter media converted ammonia to nitrite and then to nitrate. The biofiltration level tank was heavily aerated to provide appropriate dissolved oxygen (DO) levels to maintain the nitrifying bacteria. Water was pumped from the biofilter to the experimental culture tanks.

Well water was used to fill the reuse system and to replace water lost by evaporation, cleaning/maintenance, or during weighing procedures. Water temperature was maintained at ambient room temperature (21±2 °C). Average water exchange rates were 5.5 L/min. The pH and total ammonia (TAN) of the culture water were monitored every two weeks. The pH was measured using a Fisher Scientific, Accumet model 25 pH/ion meter with the appropriate probe and calibration. Total ammonia levels were monitored using a HACH ammonia nitrogen test-kit (model NI-8). Levels were maintained within the ranges considered safe for yellow perch (pH ranged from 7.8 to 8.3 and the total ammonia levels remained below 0.4 ppm).

Fish

Separate stocks of male and female yellow perch were obtained from
Willow Creek Aquaculture (Wautoma, Wisconsin) and transported to Michigan
State University. Fish were separated by gender using the following criteria by
personnel of Willow Creek Aquaculture:

- Males: fish were selected from a stock of fish that had been previously
  identified as males. Gender was re-checked by applying pressure to the
  abdomen of each individual. Only fish that produced milt were identified
  as males.

- Females: fish were selected from a stock of offspring originating from a
  mating between masculinized females and normal females (Malison and
  Garcia-Abiado 1986).

Each gender stock was brought to MSU in a separate transport unit. Each
gender stock was held in a separate holding tank at the Upper River Laboratory
for a minimum of two weeks to acclimate them to their new environmental
conditions as described above.

Each experimental culture tank was stocked with eight 110 – 150-mm TL
fish using a totally random stocking scheme. Tanks were randomly stocked by
the following method:

1. Tanks were assigned to male or female stocks using a random
   numbers table.

2. A single fish was taken from the holding tank and placed into the appropriately assigned experimental tank. Another fish was not placed into this tank until all tanks contained an equal number of fish.

Each experimental culture tank was covered with a screen to minimize the impact of external visual stimuli from movement around the culture tanks. Fish were fed three times daily during a two-week acclimation period. Another group of 8 males and 8 females was euthanized using MS-222 to serve as reference fish for proximate analysis.

Feed and Feeding Trials

All fish were fed a commercial trout diet, PURINA AquaMax Grower 400 (lot A-5D04), containing at least 45% crude protein and at least 16% crude lipids. The feed was frozen at $-20^{\circ}C$ to maintain freshness throughout the experimental period. Feeding levels were 0.5, 1.0 2.0 and 4.0% wet-weight/day. The 4.0% ration level was decreases to 3.0% on day 3 of the experiment because excess feed accumulated in the tanks fed the 4.0% ration level.

The wet weight of fish was determined by the following method:

- The tank was drained ½ capacity for easier fish collection.

- All fish in a single tank were collected in a net and placed in a tared 2-gallon pale + water.

- The pale + water + fish was weighed to the nearest 0.1 gram.

- Fish were placed back in the tank and the water was added to fill the tank back to the original level.

Three replicate tanks of fish were used for each feeding level and for each gender. All feed levels were fed three times daily, at 0800, 1300, and 1800-h. Feed levels were adjusted every two weeks.

Proximate Analysis

At the completion of the growth trials, all fish were euthanized using MS-222 at a dosage rate of 1-g/L for a minimum of 10 minutes. Each replicated group of fish were ground and stored at temperatures of –20°C. Proximate analysis of the pre-treatment control, experimental fish and feed samples were determined using standard AOAC (1990) methods for moisture, total ash, crude fat, crude protein, and total gross energy on a dry matter basis. Crude protein levels were determined for 1-g samples using a Leco 2000 FP nitrogen analyzer in the Animal Science Department, Michigan State University. Moisture was determined on 1-g samples at a temperature of 105°C ±1 for 48 hours. Total ash samples were determined for 1-g samples using a muffle furnace for 18 hours at a temperature of 550°C. Crude fat was determined for 1-g samples using ether extraction in a Sockslet Apparatus. A Parr 1241 Bomb Calorimeter was used to determine gross energy for each tank on 1-g samples. All proximate analysis values were calculated on a percent dry matter basis.

Feed, protein, lipid, and energy efficiency were determined for all ration level levels and genders. The efficiency values were determined based on the weight of the component (feed, protein, lipid, energy) gained per gram of component fed.

Statistical Analysis

A non-linear regression model provide by Dr. Mercer, University of Kentucky at Lexington, was used to determine R_{max}, K_m, b, and n of the predominately male and female stocks. A one-way ANOVA and Tukey test (SYSTAT 9, 1999) were used to compare growth, ash, crude protein, crude fat and gross energy within each gender. A t-test was used to compare between genders at ration level levels. Differences were considered significant if $P \leq 0.05$.

Results

Secondary Sex Characteristics

Forty yellow perch, 130.0 ± 9.4-mm, were examined to determine the effectiveness of each potential secondary sex characteristic in determining gender. The sample of yellow perch was comprised of 23 males and 17 females as verified by dissection and examination of the gonads. A comparison, by gender, of the mean, standard error of the mean, and standard deviation for ED, ML, MW, MH, SL, TL measurements and the ratios of ED, ML, MW, MH to SL and TL are summarized in Table 2.1. The similarity of the means and the high variance and standard deviation indicated that statistical tests were not necessary to determine if characteristics differed between genders.

An otoscope was inserted into the urogenital opening of all 40 yellow perch to determine if it was possible to locate the seminal duct of males. No seminal ducts were observed in any of the male fish. The use of a food grade dye did not enhance identification of the seminal duct. No seminal ducts were observed in any fish.

Using the shape of the urogenital opening while pressure was applied to the abdomen (Malison and Kayes, in print) was not a reliable method to identify gender of 130 ± 9.4 mm fish. Only 24 of 40 fish were correctly identified to gender based on the shape of their urogenital opening. Placing a small amount of food grade dye over the urogenital opening failed to aid in identifying any morphological differences between males and females.

Table 2.1—— The mean, standard error of the mean, and standard deviation for eye diameter (ED), maxillary length (ML), mouth width (MW), mouth height (MH), total length (TL), and standard length (SL) by gender for yellow perch (*Perca flavescens*), as well as the ratios between ED, ML, MW, and MH to SL and TL.

	MALES			FEMALES		
	MEAN	STANDARD ERROR OF THE MEANS	STANDARD DEVIATION	MEAN	STANDARD ERROR OF THE MEANS	STANDARD DEVIATION
ED	5.79	0.16	0.58	6.19	0.12	0.63
ML	12.79	0.35	1.31	13.42	0.16	0.81
MW	12.43	0.33	1.22	13.39	0.16	0.80
MH	12.79	0.26	0.98	13.58	0.21	1.07
TL	126.14	2.84	10.61	132.54	1.68	8.56
SL	104.86	2.28	8.55	110.92	1.31	6.69
ED: SL	0.055	0.002	0.006	0.056	0.001	0.005
ML: SL	0.122	0.003	0.010	0.121	0.001	0.007
MW :SL	0.119	0.003	0.011	0.121	0.001	0.007
MH: SL	0.123	0.004	0.013	0.123	0.002	0.011
ED: TL	0.046	0.001	0.005	0.047	0.001	0.004
ML: TL	0.101	0.002	0.008	0.101	0.001	0.006
MW :TL	0.099	0.002	0.009	0.101	0.001	0.006
MH: TL	0.102	0.003	0.011	0.103	0.002	0.009

Saturation Level Kinetics

Originally, we had planned to use the saturation level kinetics model to compare and contrast differences between all male, all female, and mixed gender stocks of yellow perch. However, confirmation of the gender of male and female stocks by dissection indicated that the purported all male stock was comprised of only 71.2% males while the all female stocks were comprised of 99.0% female (95 of 96). Since our mixed gender stock was comprised of 68.6% males, a mixed gender experiment was not conducted. The purported all male stock will be referred to as the predominantly-male stock (PM) in all future references.

Growth curves were developed for the PM and all-female (AF) stocks of yellow perch fed varying levels of diet. R^2 for growth curves are not presented in these results since the kinetics model developed by Morgan et al. (1975) maximizes the R^2 value. The proximate analysis of the feed and whole body tissues for each gender group (Table 2.2) were used to calculate the intake and response relationships for crude protein, fat, gross energy, and ash. Each point on each figure represents the mean of three observations at each intake level. Points above the abscissa represent actual observations of a positive growth response. The model described by King was used to generate the points below

the abscissa (b). The shape of the curves (n), the K_m curve parameter, and

calculated maintenance value and optimal value were similar between both

Table 2.2— Proximate analysis, on dry matter basis, of a commercial feed, Purina Aquamax Grower 400 (lot A-5d04) and ±SEM that was fed at ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day for 112 days to a predominately-males stock and an all-female stock of yellow perch (*Perca flavescens*).

	Percent Protein	Percent Lipid	Percent Ash	Energy (Kcal)	Moisture
Feed	46.1±1.4	16.4±0.4	9.0±0.1	5.14±0.03	NA
PM 0.5	63.0±0.3	19.0±0.9	12.0±1.6	4.73±0.12	69.8±0.6
AF 0.5	62.2±0.9	22.5±1.2	13.9±0.6	4.73±0.06	68.6±0.7
PM 1.0	59.4±2.1	23.9±3.2	11.8±2.3	4.59±0.76	67.9±0.8
AF 1.0	58.8±0.9	25.3±2.5	8.1±2.9	5.05±0.06	67.1±0.5
PM 2.0	59.9±0.4	23.2±1.7	8.7±3.0	4.92±0.08	67.8±0.5
AF 2.0	61.7±1.5	25.8±3.1	11.1±1.7	4.89±0.17	67.6±0.4
PM 3.0	59.5±0.7	22.5±0.7	6.4±1.4	5.09±0.13	68.1±0.6
AF 3.0	61.1±2.1	27.7±1.2	5.4±2.8	5.25±0.08	67.8±0.4

stocks. The R_{max} curve parameter, and ordinate intercept (b) varied between genders.

Growth

Figure 2.1 represents the mean growth rates in grams per day for PM and AF perch fed a commercial diet. Table 2.3 summarizes the growth parameters calculated using the kinetics model.

The R_{max} which represented the maximum theoretical response, was significantly different between stocks. The R_{max} calculated for the PM over the 112-day experimental period was 1.112-g/day/tank while the R_{max} calculated for the AF was 1.853-g/day/tank. The K_m (theoretical amount of feed per gram of fish that produces a growth response equal to half the R_{max}. or the efficiency estimate) observed for male and female stocks (PM=0.575, and AF=0.592) were not significantly different. The calculated slope factor (n) indicated that the growth response was a sigmoidal shaped curve (n>1) for both genders (Table 2.3).

The b which represented the ordinate intercept or the theoretical response to a dietary intake of zero, was generated using a kinetics model provided by Dr. Darrel King (Michigan State University, personal communication); b = –0.348 for the AF stock and b = –0.115 for the PM stock. The calculated numbers were used to aid in generating the other model parameters. The $I_{r=0}$ which represented the maintenance level or theortical Y = 0, values were similar between stocks at 0.373 for PM and 0.424 for AF. The I_{mx} (optimum feeding level = a intercept of a $45°$ line tangent to the growth curve which represents the greatest amount of fish
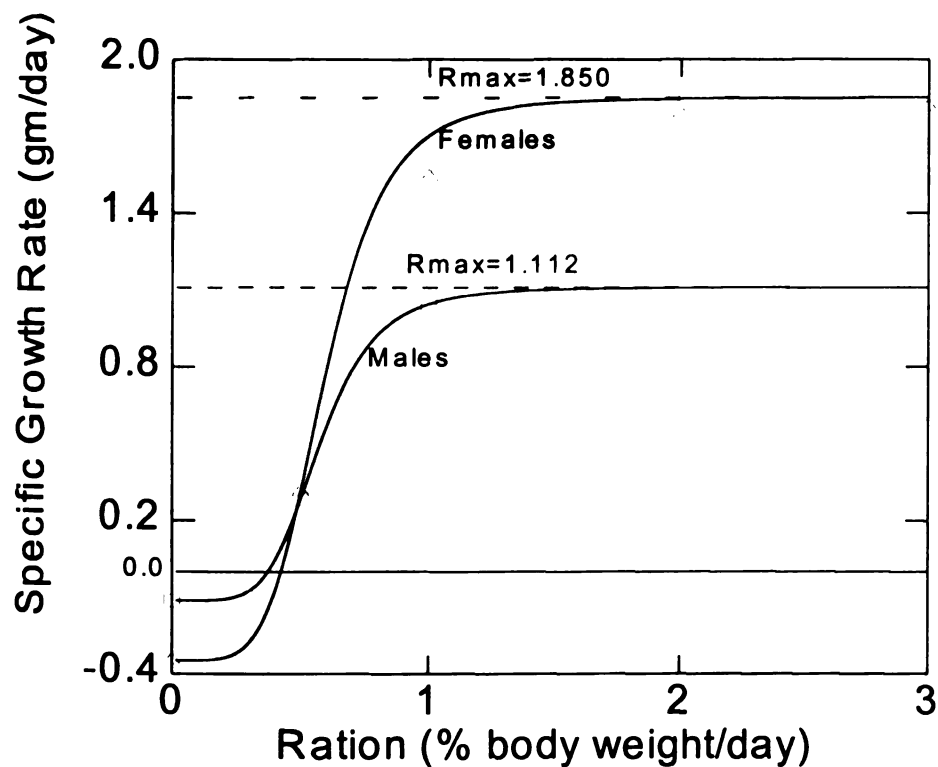
Figure 2.1---- Daily specific growth rate of male and female yellow perch (*Perca flavescens*) (141.5+-11.6 mm TL) reared in a recirculating aquaculture system fed a commercial diet (Purina AquaMax 400) at 0.5, 1.0, 2.0, and 3.0% body weight per day for 112 days. Δ=Females (r^2=0.96), =Males (r^2=0.88).

Table 2.3---- Parameter estimates for Mercer's saturation level kinetic model for yellow perch (Perca flavescens) (141.5±11.6 mm TL) fed at ration levels of 0.5, 1.0, 2.0 and 3.0% body weight per day for 112 days. Basal metabolic maintenance level is $I_{r=0}$, and optimal feeding level is I_{mx}.

Parameter	Predominately-males	All-females
b	-0.115	-0.348
R_{max}	1.112	1.853
K_m	0.575	0.592
n	5.242	4.995
$I_{r=0}$	0.373	0.424
I_{mx}	0.76	0.78

growth on the least amount of feed) values were similar between genders at 0.76

and 0.78-g/day for PM and AF, respectively.

Maximum feed efficiency levels, which represented the greatest rate of

gain per gram diet fed, were calculated for both stocks (Table 2.4). The

maximum calculated feed efficiency level was significantly greater for female

stocks compared to male stocks at 0.52 and 0.34 grams gained per gram fed for

AF and PM, respectively. The maximum feed efficiency levels were achieved at

a feeding rate of 1.0% for both stocks. Feed efficiency increased from the 0.5%

ration level to the 1.0% ration level and decreased at ration level levels greater

than 1.0%. However, the feed efficiency levels for the AF stock was greater than

the feed efficiency for the PM stock at all feeding levels.

Table 2.4— Mean feed efficiencies (weight gained/weight fed) and ±SEM of predominately-male and all-female stocks of yellow perch (Perca flavescens) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day for 112 days. Corresponding letters indicate no statistical difference between means (p>0.05).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	0.21 ± 0.04^{ace}	0.34 ± 0.05^{b}	0.17 ± 0.03^{cde}	0.11 ± 0.01^{e}
FEMALE	0.23 ± 0.01^{ad}	0.52 ± 0.03^{b}	0.26 ± 0.01^{a}	0.17 ± 0.01^{ac}

Crude Protein

The CP content in Table 2.5 was determined as a percent CP per gram of dry matter. The mean percent CP level ranged from 59.4 to 63.0 for the PM stock while the mean percent CP level ranged from 58.8 to 62.2 for the AF stock. There were no significant differences ($p > 0.05$) in percent CP concentration level within or between genders.

Protein Efficiency

The maximum protein efficiency (protein gained for each gram of protein fed) was determined for both stocks (Table 2.6). The maximum calculated protein efficiency level was significantly greater ($P = 0.02$) for AF stock compared to PM stock at 0.47 and 0.32 grams gained per gram fed, respectively. Maximum protein efficiencies were calculated for feed levels of 1.0% for both stocks. Protein efficiency increased from the 0.5% ration level to the 1.0% ration level and decreased at ration level levels greater than 1.0%. The protein efficiency level for the AF stock was greater than the protein efficiency for the PM stock at all feeding levels.

Table 2.5---- Mean crude protein levels (% dry matter) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. No statistical difference was identified between means ($p > 0.05$).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	63.0±0.3	59.4±2.1	59.9±0.4	59.5±0.7
FEMALE	62.2±0.9	58.8±0.9	61.7±1.5	61.1±2.1

Table 2.6---- Mean protein efficiencies (protein gained/dietary protein) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. Corresponding letters indicate no statistical difference between means ($p > 0.05$).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	0.21±0.03[ac]	0.32±0.05[bc]	0.15±0.02[a]	0.10±0.01[a]
FEMALE	0.23±0.01[a]	0.47±0.2[b]	0.25±0.01[a]	0.17±0.01[c]

Crude Lipids

The percent lipid levels (% lipid/gram of dry matter) are summarized in Table 2.7. The PM mean percent lipid concentration level ranged from 19.0 to 23.9. The AF mean percent lipid concentration level ranged from 22.5 to 27.7. No differences ($p > 0.05$) were identified between stocks. The saturation level kinetics model was not used to describe the response because of the lack of significance between the treatments and genders.

Lipid Efficiency

The maximum lipid efficiency (lipid gained for each gram of lipid fed) was determined for both AF and PM stocks (Table 2.8). The maximum calculated lipid efficiency level was significantly greater for AF stock compared to PM stock at 0.48 and 0.36 grams gained per gram fed, respectively. Maximum lipid efficiencies were calculated at feeding levels of 1.0% wet-weight per day for both stocks. Lipid efficiency increased from the 0.5% ration level to the 1.0% ration level and decreased at ration level levels greater than 1.0%. However, the lipid efficiency level for the AF stock was greater than the lipid efficiency for the PM stock at all feeding levels.

Table 2.7—— Mean crude lipid levels (% dry matter) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. No statistical difference was identified between means (p>0.05).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	19.0±0.9	23.9±1.2	23.2±1.7	22.5±0.7
FEMALE	22.5±1.2	25.3±2.5	25.8±3.1	27.7±1.2

Table 2.8—— Mean lipid efficiencies (lipid gained/dietary lipid) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. Corresponding letters indicate no statistical difference between means (p>0.05).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	0.18±0.04[ab]	0.36±0.09[a]	0.17±0.03[ab]	0.11±0.01[b]
FEMALE	0.26±0.11[ab]	0.48±0.21[ab]	0.29±0.12[ab]	0.31±0.07[ab]

Energy

The gross energy content (Kcal/gram) results are summarized in Table 2.9. The mean percent gross energy concentration level ranged from 4.73 to 5.25 Kcal/g for AF stocks. The mean gross energy ranged from 4.59 to 5.09 Kcal/g for PM stocks. There were no significant differences within gender groups ($p > 0.05$). The saturation kinetics model was not used to describe the response because of the lack of significance between the treatments and genders.

Energy Efficiency

The maximum energy efficiency (energy gained for each gram of energy fed) was determined for AF and PM stocks (Table 2.10). The maximum calculated energy efficiency level was greater for AF stocks compared to PM stocks at 0.53 Kcal and 0.31 Kcal gained per Kcal fed, respectively. Calculated maximum energy efficiencies were observed at feeding levels of 1.0% wet-weight per day for both stocks. Energy efficiency increased from the 0.5% ration level to the 1.0% ration level and decreased at ration level levels greater than 1.0%. However, the energy efficiency level for the AF stock was greater than the energy efficiency for the PM stock at all feeding levels.

Table 2.9---- Mean energy levels (Kcal/gram) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. Corresponding letters indicate no statistical difference between means (p>0.05).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	4.73 ± 0.12^a	4.59 ± 0.79^a	4.92 ± 0.08^{ab}	5.09 ± 0.13^{ab}
FEMALE	4.73 ± 0.06^a	5.05 ± 1.06^{ab}	4.89 ± 0.17^{ab}	5.25 ± 0.08^b

Table 2.10---- Mean energy efficiencies (Kcal gained/dietary energy) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. Corresponding letters indicate no statistical difference between means (p>0.05).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	0.20 ± 0.03^{ab}	0.31 ± 0.05^{ac}	0.17 ± 0.03^{ab}	0.11 ± 0.01^b
FEMALE	0.23 ± 0.01^a	0.53 ± 0.3^c	0.26 ± 0.01^a	0.20 ± 0.01^a

Ash

The mean ash level at each ration level for AF and PM stocks are summarized in Table 2.11. The mean ash level ranged from 5.4 to 13.9 percent for AF stocks. The mean ash level ranged from 6.4 to 12.0 percent to PM stocks. The mean ash level (% dry matter) of whole yellow perch tissue differed between the 0.5% ration level and the other three ration levels fed to AF stocks. There was no significant difference ($p > 0.05$) between ration level levels for PM stocks.

Table 2.11—— Mean ash levels (% dry matter) and ±SEM of predominately-male and all-female stocks of yellow perch (*Perca flavescens*) (141.5 ± 11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day. Corresponding letters indicate no statistical difference between means ($p > 0.05$).

Ration level (% body weight fed per day)	0.5	1.0	2.0	3.0
MALE	12.0 ± 1.63^a	11.8 ± 2.29^a	8.7 ± 2.97^{ab}	6.4 ± 1.35^{ab}
FEMALE	13.9 ± 0.63^a	8.1 ± 2.87^{ab}	11.1 ± 1.66^{ab}	5.4 ± 2.81^b

Discussion

The primary focus of this discussion is on the determination of the bioenergetic responses of male, female, and mixed gender populations of yellow perch which may help commercial farmers develop culture strategies to avoid gender based growth differences. Previous research has not focused on the bioenergetic implications of gender on differences in growth.

Secondary Sex Characteristic

Although many fish exhibit sexual dimorphic characteristics, yellow perch genders are not readily distinguishable by color or other gross anatomical features. A reliable secondary sex characteristic to identify the gender of $130 \pm$ 9.4 mm yellow perch was not identified. No differences were identified between the ED, ML, MW, and MH of male and female yellow perch. The ratio of ED to TL was not different between genders of yellow perch. Female eels (Buellens et al. 1997) had a smaller eye diameter to total length ratio than male eels.

Since female yellow perch do not have an ovoduct connected to the urogenital opening (Schott 1978), it was hoped that we could identify males by the presence of a duct entering the urethra. However, the point of attachment of the seminal duct to the urethra could not be located using an otoscope to identify males. Adding dye to the urogenital opening did not enhance our ability to visually locate the duct.

Malison and Kayes (unpublished data) indicated that pressure applied anterior to the abdomen of yellow perch greater than 110-mm TL caused the urogenital opening to appear V-shaped in the females and crescent shaped in

the males. They indicated that they successfully identified 80% of the gender of yellow perch using this method. However, we only observed a 60% success rate using this method. In conversations with Malison, he noted that the success rate decreased with smaller-sized fish. Although they observed a success rate of 80% overall, the gender of only 60% of fish less than 150 mm were accurately identified while the gender of nearly 100% of the fish over 150 mm were correctly identified. The fish used in our study had never spawned, while the larger fish used by Malison and Kayes (unpublished data) may have spawned once. When female fish spawn, eggs rupture through a thin membrane and exit the urogenital opening. If the female fish had spawned, the resulting scar tissue interior to the urogenital opening may have stretched differently compared to non-scar tissue of younger, non-spawned female fish and male fish.

Saturation Level Kinetics

The primary goal of this study was to use the saturation level kinetic model (Morgan et al. 1975) to describe growth of yellow perch as a function of dietary intake. The growth parameters for the saturation level kinetics models were generated using the variable wet weight gain between feeding levels as the response. Weight gain can be calculated at any intake level using the model equation after estimating the parameters (R_{max}, K_m, b) I_{mx} and $I_{r=0}$. Each of these parameters interacts with one another either directly or indirectly. R_{max}, K_m, n, and b define the response by the following equation;

$$r = \frac{b(K_m)^n + R_{max}I^n}{(K_m)^n + I^n}$$

The maximum ration level originally fed in this study (4%wet eight per day) was one percent higher the maximum feeding rates used by commercial yellow perch producers in the North Central Region (personal communications, S. Genson, Willow Creek Aquaculture, WI and C. Starr, Bay Port Aquaculture, MI, 1998). Commercial yellow perch producers typically feed at a rate of 3%-wet weight per day. Based on the highest feeding rate, replicate tanks of fish were fed decreasing graded levels of 2.0, 1.0, and 0.5% of body-wet weight/day. After the initiation of the growth trial, fish feed the 4.0% rate did not consume all feed fed. Consequently, the maximum rate was reduced to 3.0%.

Initially, we had anticipated that the fish fed at 0.5% rate would loose weight; however, fish fed at the 0.5% rate exhibited a positive growth response. A negative growth response was needed to generate R_{max}, K_m, and n parameters using the model developed by Morgan et al. (1975). A model described by Dr. Darrel King (personal communication) was used to determine a theoretical starvation point equal to the response at a ration level of 0.0% per day. The theoretical starvation point was used with observed growth responses to generate the growth curves for AF and PM stocks (Table 2.3).

Melard et al. (1996) calculated the growth response of a mixed gender stock of European perch, Perca fluvitilis, using a four-parameter saturation level kinetics model (Mercer 1982). The maximum growth response for P. fluvitilis was 1.8-g/day for fish up to 300 grams total wet-weight. The maximum growth response for yellow perch in our study was calculated as 1.85 and 1.11-g/day for AF and PM stocks, respectively. The ration level for optimum growth efficiency

for European perch was 1.0% wet weight per day compared to 0.78% and 0.76% wet weight per day calculated for the AF and PM yellow perch stocks, respectively. Other kinetics parameters were similar between the two studies. The greater responses observed by Melard et al. (1996) for mixed stocks may have resulted from a larger percentage of females in their stock compared to our PM stock. However, the gender ratio was not reported in their study.

The maximum growth rate of the PM stock was approximately 60% of the growth rate of the AF stock. Only a slight difference was found when comparing I_{mx} or $I_{r=0}$ between genders. This observation implies that females were more efficient at converting feed into body mass at ration level levels greater than 1%. This result corresponds with the earlier observations that female yellow perch grow faster than males once the fish reaches a TL of 100 to 110-mm (Malison et al. 1985 and 1986, Malison and Garcia-Abiado 1996, Schott et al. 1978).

Proximate Analysis

The whole body proximate analysis values for protein, lipid, ash, energy, and moisture content determined in our study were similar to the values observed by Rienitz and Austin (1980) and Tidwell et al. (1999). Rienitz and Austin (1980) compared growth and whole body proximate analysis between fish fed four potential yellow perch diets. The proximate analysis of the fish fed a diet containing 53.1% crude protein and 15.4% crude fat yielded crude protein, crude fat, and ash levels of 66.7, 16.5, and 15.3% dry weight, respectively. We observed crude protein, crude fat, and ash levels of 60.7, 23.7, and 9.7% dry weight, respectively when fed a diet containing 46.1% crude protein, 16.4%

crude fat, and 5.14% ash. This may account for the slight differences observed in whole body proximate analysis between the two studies. Tidwell et al. (1999) compared growth and whole body proximate analysis of yellow perch fed a commercial salmonid diet containing 45% crude protein and 16% crude lipid three temperatures, 20, 24, and 28°C. The body composition of their fish did not differ between any of the temperatures. At the 20°C treatment, the level of whole body crude protein, crude fat, and ash levels were 59.7, 27.5 and 11.1%, respectively. The elevated crude fat level observed by Tidwell et al. (1999) compared to our study may have been an affect of not separating the genders or females producing eggs.

Whole body crude protein levels as a percent of dry weight were not significantly different between genders or within gender stocks regardless of ration level in our study. Robards et al. (1999) compared whole body crude protein and lipid/energy composition of sand lances (*Ammodytes hexapterus*) by gender and season from a capture fishery. They observed that crude protein did not differ significantly between genders.

Whole body lipid content of the AF stock was not significantly higher than the whole body lipid content of the PM stock (Table 2.7) even though many of the females had developing eggs by the end of our study. Gonad development affects the growth rate of yellow perch (Hayes and Taylor 1990). They found that the growth rate slows as yellow perch reach sexual maturity. Robards et al. (1996) determined that the lipid and energy content in female sand lances was greater than the lipid or energy content in the males. The gender differences in

lipid or energy content only appeared once the sand lances reached a TL greater than 80-mm and female fish increased lipid stores during egg production. Fish in our study may not have reached that level of egg development. Hokanson (1977) determined that yellow perch require a 160-day chilling period (water temperature less than 6°C) for proper egg development. Fish in our experiment were maintained at a constant temperature of 21°C.

Typically, digestion efficiencies in fish have been observed to decrease as feeding levels increase (Smith 1978). Figures 2.2, 2.3, and 2.4 compare protein, lipid, and energy digestion efficiencies, respectively, between gender stocks at the four ration levels fed. At the 0.5% body-wet weight/day ration level, the calculated digestion efficiencies for AF and PM stocks were lower than the digestion efficiencies calculated for the 1.0% body-wet weight/day ration level. The lower digestion efficiencies were probably due to the use of the dietary components for maintenance energy. The lower digestion efficiencies calculated at feed rates greater than 1.0% body-wet weight/day were probably caused by reduced conversion of the feed to tissue. The decline in digestion efficiency at ration levels greater than 1.0% body-wet weight/day were caused by over feeding. The calculated digestion efficiency values were similar to those reported by Fiogbe' et al. (1996) for juvenile P. fluviatilis. Fiogbe' et al. (1996) determined that the protein efficiency was 0.38 grams of protein grained for a gram of protein fed. Juvenile fish used much of their dietary energy for somatic growth during a growth phase characterized by a rapid increase in body size. Because female

Figure 2.2— Mean protein efficiencies (protein gained/dietary protein) of predominately-male (PM) and all-female (AF) stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day for 112 days. Y-bars represent the standard error of the mean.
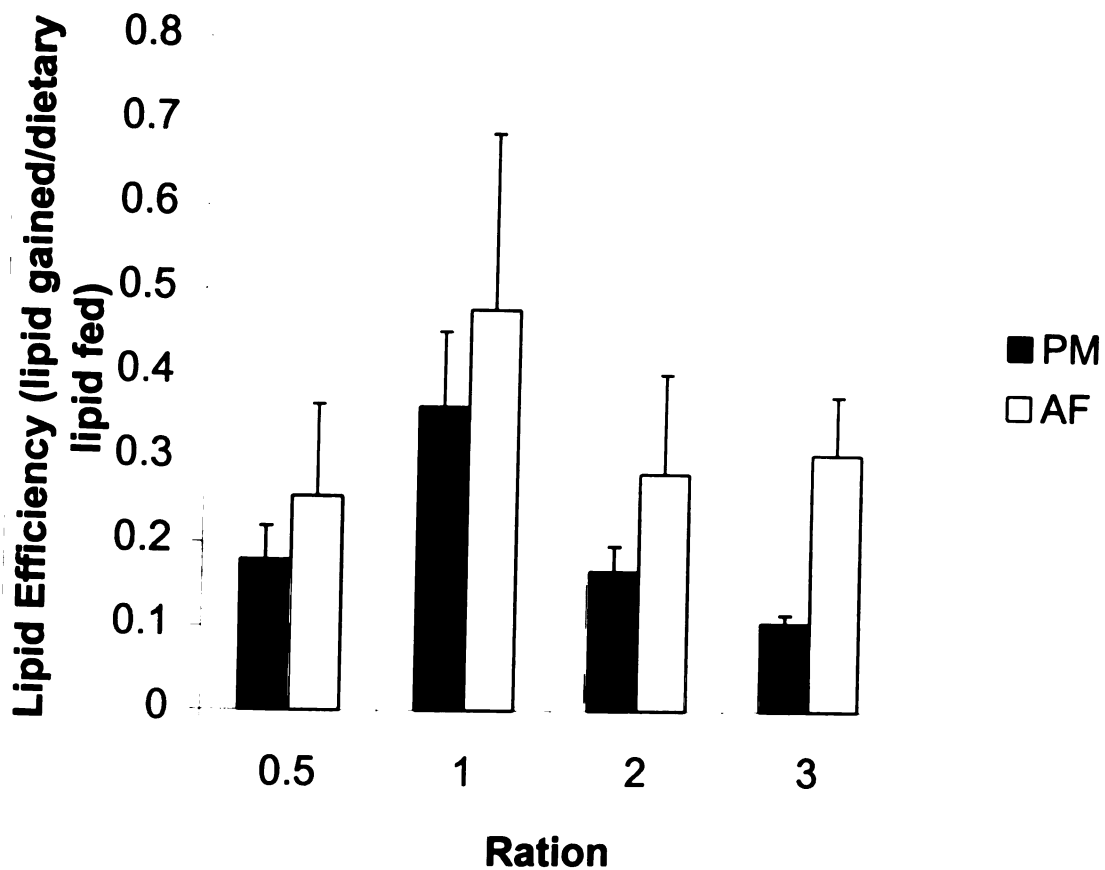
Figure 2.3---- Mean lipid efficiencies (lipid gained/dietary lipid) of predominately-male (PM) and all-female (AF) stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day for 112 days. Y-bars represent the standard error of the mean.
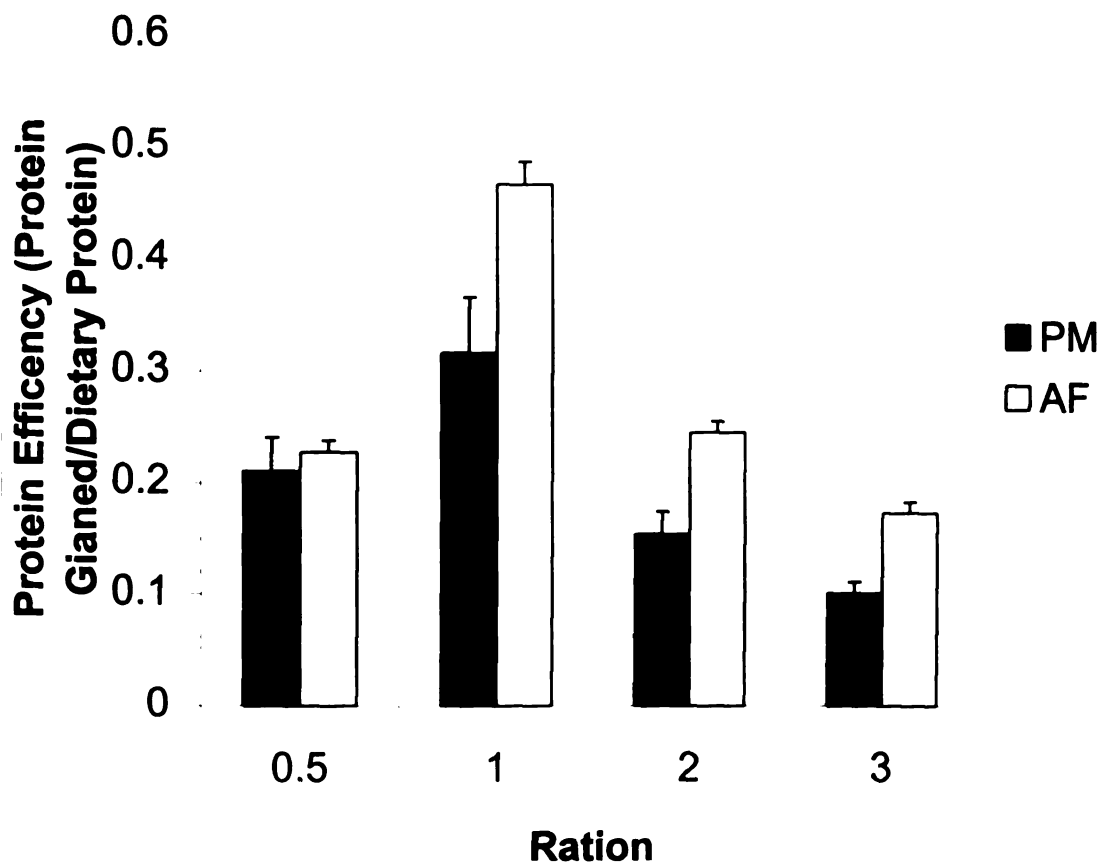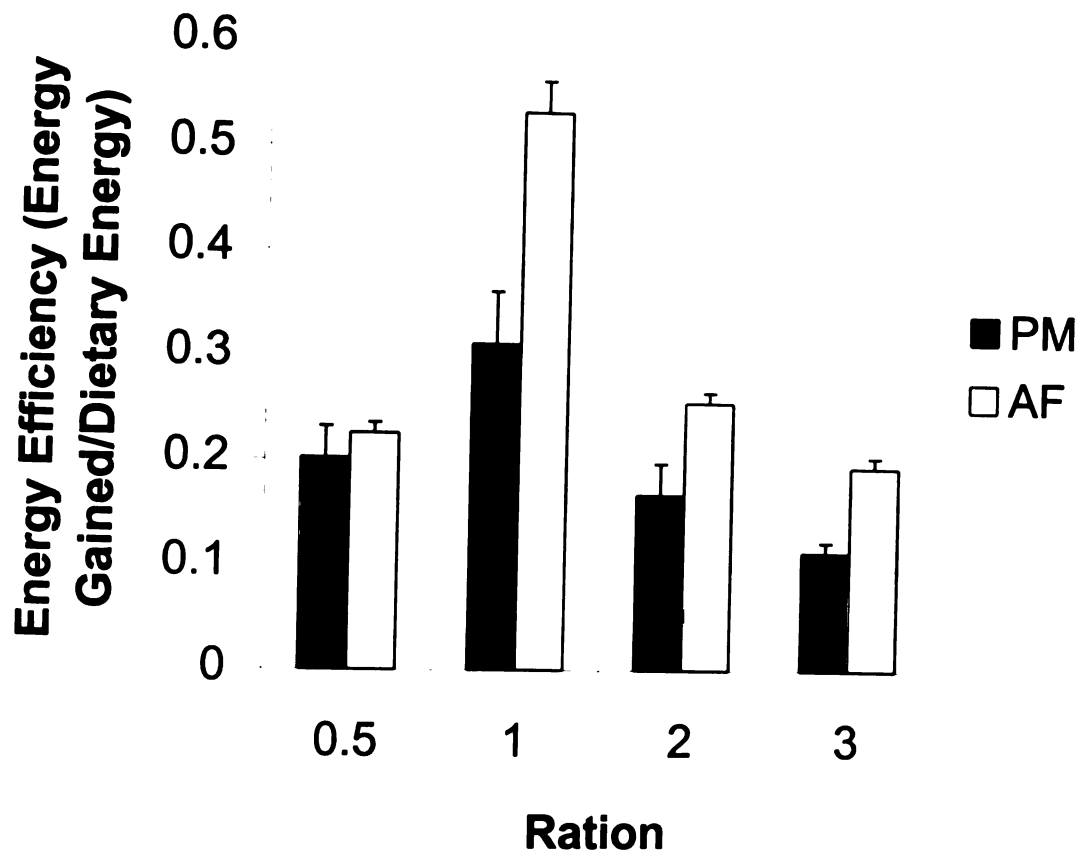
Figure 2.4---- Mean energy efficiencies (energy gained/dietary energy) of predominately-male (PM) and all-female (AF) stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day for 112 days. Y-bars represent the standard error of the mean.

fish used in this study had initiated egg development, some energy was directed toward gonadal development. This could account for the slight differences in protein efficiency between this study and Fiogbe' et al. (1996).

The whole body lipid digestion efficiencies for the AF at feed rates greater than 1% wet-weight per day may indicate that females hand an increased ability to assimilate lipids from the diet. The developing eggs within the AF stock may have caused the increase lipid efficiencies in fish fed at 3.0% of their body-wet weight, because the major component of a fish eggs is lipids. The different energy content of male and female energy content may be explained by the increased amount of lipid in the females. According to Fish Feed Technology (1978), lipid has a gross energy level of 9.0-Kcal/g where as the protein has a gross energy content of 5.65-Kcal/g. Since the females contained eggs, a slightly higher gross energy content would be observed when comparing the AF and PM stocks.

Feed efficiency (Figure 2.5) followed the same response pattern as protein, lipid, and energy digestion efficiencies (Figures 2.2, 2.3, 2.4). This should be expected since the feed efficiency response is a combination of nutritional factors relative to the weightgained by the fish. No differences in feed efficiency were observed between genders at the 0.5, 1.0%, and 2.0% wet-weight per day ration level levels. However, significant differences were observed between genders fed at the 3.0% wet-weight per day ration level. This indicated that the AF stock was more efficient at converting the feed into body

Figure 2.5---- Mean feed efficiencies (weight gained/weight of feed fed) of predominately-male (PM) and all-female (AF) stocks of yellow perch (*Perca flavescens*) (141.5±11.6-mm TL) fed ration levels of 0.5, 1.0, 2.0, and 3.0% body-wet weight per day for 112 days. Y-bars represent the standard error of the mean.
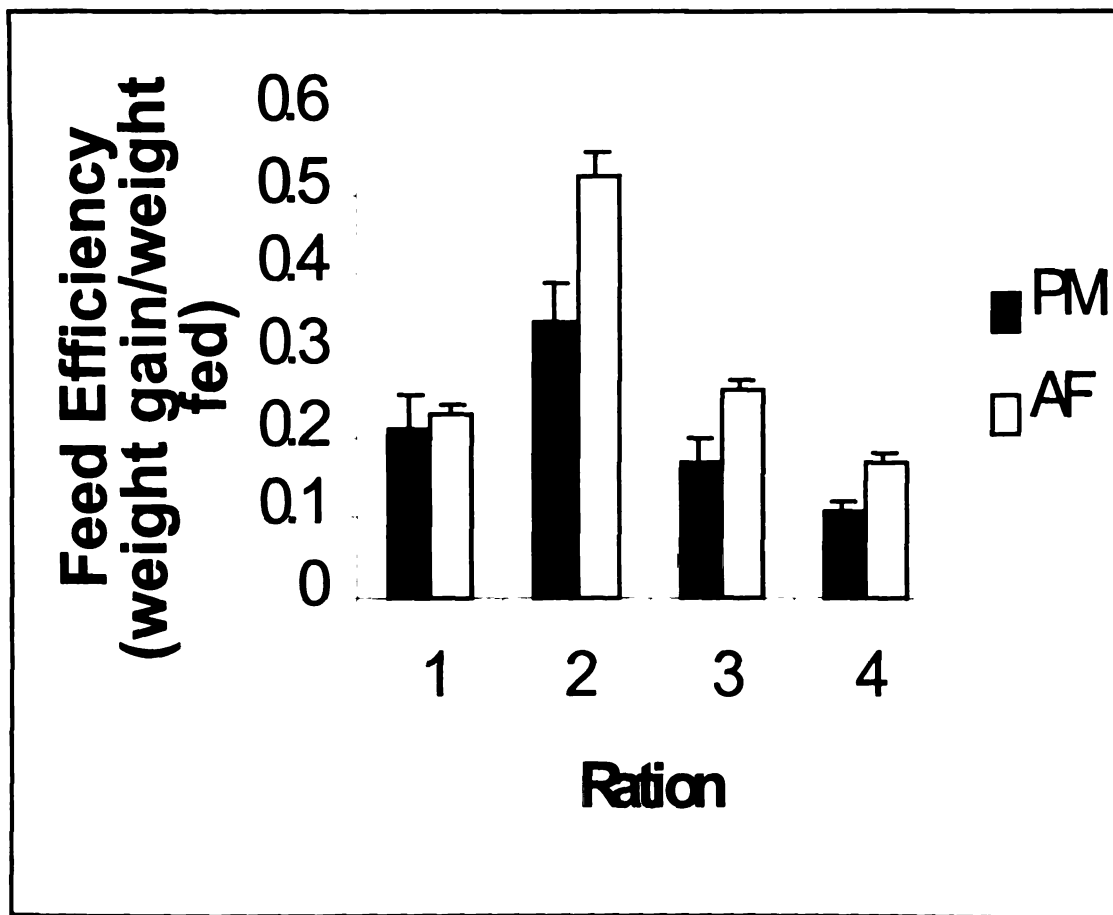
mass compared to the PM stock which corresponds to the ration level levels fed by commercial yellow perch producers.

The calculated efficiency values indicated that the AF stock had a greater feed assimilation at the 3.0% ration level compared to the PM stock. As the feed rate was lowered (became restricted), the response between genders was similar. Similarly, both stocks had comparable basal metabolic requirements ($I_{r=0}$). A significant difference in growth was observed between the AF and PM stocks at a ration level greater than or equal to 1.0% wet-weight per day. The 1.0% wet-weight per day ration level appeared to be near the optimal ration level for yellow perch fed the diet used in our study. The growth rate for the PM stock fed at the calculated optimal ration level (I_{mx}) was 0.88 g/tank/day, whereas the growth rate for the AF stock at the calculated optimal ration level (I_{mx}) was 1.41 g/tank/day. The AF stock had a 38% greater growth response compared to the PM stock.

A large variance was observed in the ash levels within both gender stocks. Mean ash levels greater than 12.0% and lower than 6.0% were observed for both stocks. A few ash samples were observed that contained relatively large bone fragments. This indicated that the samples may not have been completely homogenized. This could have accounted for the high variation within stocks fed at the same feed ration level.

Gender related differences in feeding behavior could account for the differences in growth rates between the AF and PM stocks. In this study, the majority of yellow perch in the PM stock did not aggressively feed when the feed

was fed. The AF stock demonstrated a more uniform and aggressive feeding activity when the feed was fed.

Frequency of feeding and time between feeding may also affect the utilization of feeds. Nile tilapia grew more efficiently when fed 3 times daily instead of 5 (Riche 2000). Riche (2000) also observed that the optimal interval between feedings was 4 – 5 hours, depending on the energy and composition of the diet. Shorter time intervals between feedings increased gastric motility and reduced digestion efficiency. *Perca fluvitilis* grew best on restricted food supplies (< 2.0% wet body weight/day) if the feed was evenly distributed over 6 feedings per day, however, the fish grew best when fed only 3 times daily if the ration level was 2.0% wet-weight per day (Kestemont and Melard 1996). Yellow perch were fed three times each day at time intervals of approximately 4 hr in our study. The optimal number of feedings and time interval between feedings should be determined for yellow perch to ensure that the gastric mobility is paired with the efficiency of processing and absorbing the feed.

The mixed gender PM stocks may have limited our ability to statistically differentiate between yellow perch genders when comparing calculated growth parameters and proximate analysis values. The PM stock was 18% female and the AF stock was 99% female. The fish within the AF stock were more uniform in size then those of the PM stock. This may have been the result of the number of females within the PM stock. The females within the AF stock tanks may have consumed similar amounts of food resulting in a more uniform sized distribution

while the females in the PM stock tanks may have consumed larger portions of the ration level resulting in a larger sized variance.

Summary and Conclusion

Secondary sex characteristics could not be identified to reliably identify the gender of yellow perch. The PM stock contained 18% females while the AF stock was 99% female. The mixed gender PM stocks may have limited our ability to statistically differentiate between the gender stocks when comparing calculated growth parameters and proximate analysis values. Therefore, without a reliable means to separate genders, commercial yellow perch producers cannot develop strategies to separate stocks.

The growth of the AF stock was significantly greater than PM stock when fish were fed at 3% body weight per day. At ration levels of 2% or less, the observed growth rates of the gender stocks were not significantly different. However, at the calculated optimal ration level (I_{mx}) of 0.8% body weight per day (Morgan et al. 1975), the female growth rate was projected to exceed the growth rate of males by nearly 1.5 times. The digestion efficiency of both stocks followed a similar pattern to the growth response. The greatest digestion efficiency was observed for the feed rate of 1.0% per day.

Other researchers have focused on developing technologies to culture female stocks to avoid differences in gender based growth differences. This strategy would decrease the time required to culture yellow perch to marketable size. Future research should focus on the development of a balanced yellow perch feed and feeding strategies that optimize the growth of both genders. The optimal number of feedings and time interval between feedings should be

determined for yellow perch to ensure that the gastric mobility is paired with the efficiency of processing and absorbing the feed.

Genetic selection and domestication of yellow perch may also increase growth rates and decrease gender-based differences in growth. Genetic selection of the fastest growing male and female perch reared under intensive culture conditions should decrease the high variability in growth rates among the offspring of wild captured yellow perch. This may decrease the length of grow-out time for yellow perch.

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APPENDICES

APPENDIX 1

Artemia Culture

Two-liter soda bottles were modified and used for culture tanks. The bottom of the bottle was removed. An air stone was glued through the cap of the bottle. The bottle was inverted and supported on a ring stand. Approximately 1.75 liters of water was added to each of the five culture tanks. Aeration was supplied gently to each tank. One gram of NaCl was added to the water. *Artemia* spp. (Great Salt Lake strain) cysts, 0.5-g, were stocked into the culture tank. Constant light was supplied. The initiation of the cultures was staged so that the first hatch nauplii would be ready at the appropriate feeding time, 24-h after stocking cysts.

Artemia spp. were harvested by turning off the aeration and allowing the cyst shells to settle for 15 minutes. All room lights were turned off and a single light was placed beside the bottle to concentrate the *Artemia*. A siphon was used to extract the congregated *Artemia* spp. All *Artemia* spp. were placed into a graduated cylinder. Water was added to bring the level to 200ml. The *Artemia* spp. was mixed thoroughly and 50ml were placed into four separate beakers. Each beaker was used to feed a single tank. This process was repeated 5 times daily.
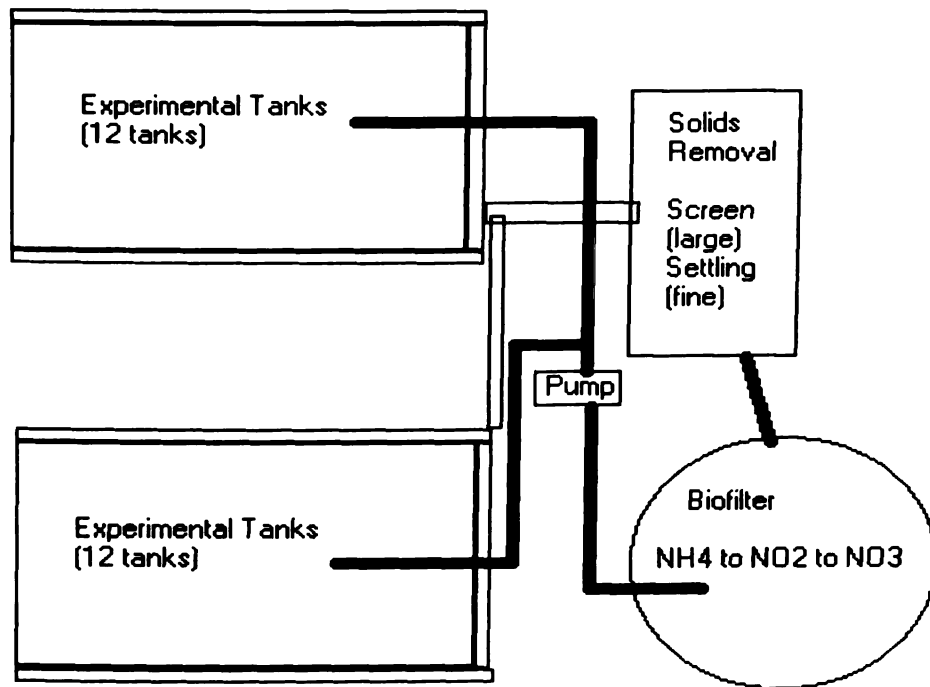
APPENDIX 2

Vinegar Eel (*Tirbatrix aceti*) Culture

Three gallons of apple cider vinegar was mixed with two gallons of water in a 6 gallon pale. One large apple was cut into slices and added to the diluted vinegar. The solution was loosely covered and stored at room temperature. After 6 weeks, a sample was analyzed to determine the presence of vinegar eels. Vinegar eels were present at a high concentration.

Prior to feeding, the vinegar eel culture tank was stirred to evenly distribute the nematodes. A 4 X 4-in scrub pad was submerged in the solution. The scrub pad was rinsed with well water into a 1L beaker. The content of the beaker was diluted to 1-L. A subsample of 250-ml of the mixture was poured Into 4 separate smaller containers. Each container was used to feed one tank. This process was repeated for each of the five feedings.

Appendix A, 3.1-- The recirculation aquaculture system used to rear yellow perch (*Perca flavescens*) for 112-day experimental period. Water was pumped from the biofilter, rotating biological contact filter and shredded, to the experimental culture tanks. The water flowed from the experimental culture tanks into a gutter system that channels the water into a primary solids removal system, screens. Small-suspended solids were passively removed in a large settling tank. Water exited the passive solids removal tank and entered the biofilter.

APPENDIX 4

Appendix A. 4.1-- Water depth was determined by the height of the external standpipe. A ball valve at the tanks lowest point aided in large solids removal by allowing a small settling area above the valve. Opening the valve removed all the accumulated solids.

Water inflow

Experimental Tank

Exit

External Standpipe

Ball valve
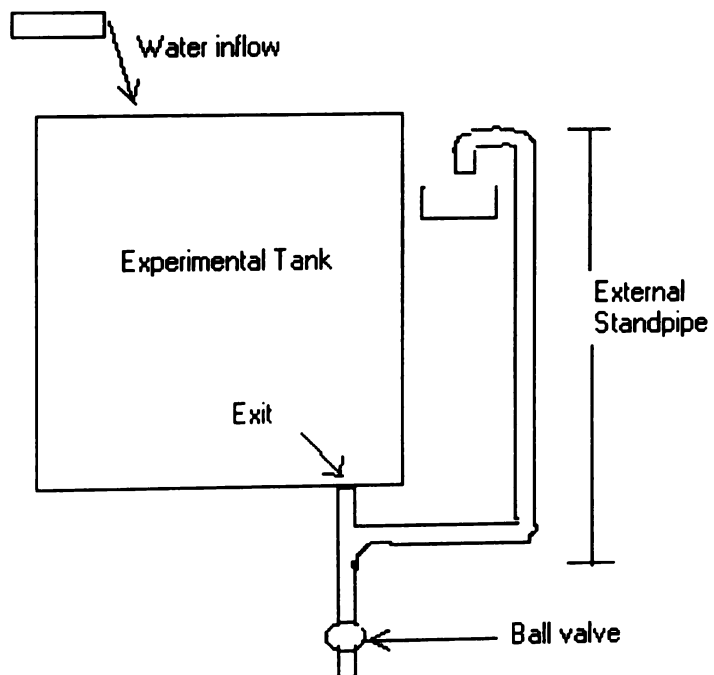
(External standpipe used in the recirculating aquaculture system to rear yellow perch (*Perca flavescens*)).