ABSTRACT

Title of Thesis: UNDERSTANDING MATERNAL EFFECTS AS A RECRUITMENT MECHANISM IN LAKE MICHIGAN YELLOW PERCH (*PERCA FLAVESCENS*)

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Changes that have occurred in the abundance and trait distribution of adult Lake Michigan yellow perch (*Perca flavescens*) suggest that maternal effects on larval traits may be substantially influencing the recruitment of this heavily exploited species. Maternal effects on yellow perch larvae at hatching and through 32 days post hatch (dph) were investigated in ten maternal lines to test the null hypotheses of no effect of maternal condition on offspring condition at hatching, no persistence of maternal effects under conditions of starvation and high food availability, and no difference in offspring survival under conditions of starvation and high food availability. Maternal effects were detectable at hatching and likely result in differences among females in size, age, gonadal somatic index, and egg production. Maternal effects at hatching were expressed by differences in larval total length, yolk volume, dry weight, and DNA quantity. Maternal effects persisted under conditions of starvation to 6 dph, after which point virtually all

larvae had perished. Maternal effects resulted in a twofold difference in resistance to starvation among the maternal lines. Larvae that exhibited the lowest resistance to starvation were long with small yolk volumes, while those exhibiting the highest resistance to starvation were short with large yolk volumes. Under high food availability maternal effects persisted to 32 dph, and resulted in threefold differences in survival among the maternal lines. No clear mechanism was identified to account for these survival differences. The observed maternal effects in Lake Michigan yellow perch may have substantial implications on recruitment.

UNDERSTANDING MATERNAL EFFECTS AS A RECRUITMENT MECHANISM IN LAKE MICHIGAN YELLOW PERCH (*PERCA FLAVESCENS*)

by

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PREFACE

This research reflects a portion of a combined multi-institutional, multi-agency, multi-state, collaborative effort to understand the mechanisms responsible for the recruitment failure and subsequent population decline observed throughout the last decade in Lake Michigan yellow perch *Perca flavescens*. While the shear size of this research required the assistance of many individuals, the results, thoughts, ideas and conclusions presented in this thesis are my own. Upon the successful defense of this research, my intention is to submit three of the five chapters of this thesis as co-authored manuscripts for review and hopefully eventual publication

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Chapter 1

Introduction

"The opinion generally prevalent hitherto was that the renewal of the stock of fish took place, as in the case of the increase of any human population, by means of a more or less constant annual increment in the form of new individuals; the results here arrived at, however, indicate that this renewal, in the case of the species investigated, is of a highly irregular nature."

Dr. Johan Hjort, 1914

Throughout much of the past century fisheries scientists have sought to predict and understand the mechanisms that regulate recruitment of exploited species. Since Hjort's landmark publication in 1914, fisheries scientists have recognized that recruitment is highly variable from one year to the next, and a substantial amount of research over the past 85 years has focused on unraveling the mechanisms that control and regulate recruitment. It is generally believed that year-class strength in finfish is determined during the first few months of life (Houde 1987; Pepin 1989; Leggett and Deblois 1994). Historically, much of the previous research conducted on recruitment has focused on population-level assessments. However, in recent years many recruitment mechanisms have been found to be size-dependent and hence affected by variation among individuals (Miller et al. 1988). Recognition of the potential importance of inter-individual variability suggests that recruitment research may be more successful if it were to focus on individuals rather than populations (Crowder et al. 1989).

Starvation induced mortality was the focal point of much of the past century's research since Hjort's 'critical period' hypothesis in 1914. Hjort (1914) hypothesized that mass larval mortality, and thus poor recruitment, would ensue if food abundance were low during the larval transition phase from endogenous to exogenous food sources. Alternatively, if food availability were high during this critical period, larval mortality would be low and subsequent good recruitment would occur (Hjort 1914). Leggett and

Deblois (1994) reviewed the pertinent literature and concluded that there was insufficient evidence to support Hjort's 'critical period' hypothesis as a strong driving factor in recruitment variation. Revision and broader generalization of Hjort's hypothesis by Cushing in 1972 (reviewed in Leggett and Deblois 1994) yielded the 'match-mismatch' hypothesis. Cushing hypothesized that larval mortality, and thus recruitment, was regulated by the temporal overlap between larval hatching and the seasonal production of plankton (reviewed in Leggett and Deblois 1994). Again, Leggett and Deblois (1994) found a lack of evidence to support this hypothesis as a strong driving factor in recruitment variation. Furthermore, mortality due to starvation depends not only on the food availability, but also on the individual larva's resistance to starvation (Rice et al. 1987). Resistance to starvation increases with hatching size (Hunter 1981; Miller et al. 1988) and begins to increase dramatically as feeding begins and energy reserves begin to increase, and as increases in mouth gape and swimming ability provide a wider range of available food sizes to the larvae (Blaxter 1969; Hunter 1981; Miller et al. 1988).

Larval mortality directly attributable to predation has yielded one hypothesis that has been under constant review, the 'bigger is better' hypothesis. The 'bigger is better' hypothesis contends that larger larvae are less susceptible to predation than smaller larvae (reviewed in Leggett and Deblois 1994). However, in mesocosm and microcosm studies Litvak and Leggett (1992) found selectivity for older larvae when size was held constant, no selectivity when age was held constant and size was variable, and selectivity for larger larvae when both size and age were variable as they would be within a cohort. These findings counter the 'bigger is better' hypothesis. Litvak and Leggett (1992) pointed out that larger larvae swim more than smaller larvae and therefore may encounter more

predators, thus the selection for larger larvae may be confounded with increased encounter probability.

The lack of evidence supporting the 'critical period' and the 'match-mismatch' hypotheses suggests that while starvation experienced at hatching may alone result in some mortality, it is not likely a controlling factor of recruitment by itself. The evidence for the 'bigger is better' hypothesis suggests that while predation is obviously of great importance in controlling recruitment, a clear understanding of how this agent operates independently cannot be established. If neither starvation nor predation can be determined and defined conclusively as independent agents that control recruitment then what can? Much recent research has begun to examine starvation not as a stand-alone recruitment controller, but as a modifier to predation. Starvation can increase vulnerability to predation through reductions in avoidance capabilities. Starvation can also result in reduced growth rates, which can lengthen stage duration and thus increase vulnerability to predation. Such interactions are more likely to control recruitment than starvation or predation alone.

Interest in the role of starvation-mediated larval mortality has prompted researchers to evaluate the condition or "health" of larvae in the field. Condition has been used as a measure that indexes past growth, as indicated by recent feeding history, and likely future survival based on selectivity. Poorly conditioned larvae, resulting from reduced feeding (i.e. starvation), have been shown to exhibit reduced growth rates (Buckley 1984). Starvation-induced reductions in growth rate can potentially lead to selective mortality (i.e. predation). Morphological, histological and biochemical indices have been historically used to assess the condition of larval fish (see review by Ferron

and Leggett 1994). Of these measures, biochemical indices of condition, particularly nucleic acid based indices, are the most sensitive indicators of recent feeding history, thus extensive research has been conducted on various methodologies (Buckley 1980; 1984; Ferron and Leggett 1994).

Since 1970 when Sutcliffe (reviewed in Bergeron 1997) demonstrated that the growth of small organisms could be estimated from their ribonucleic acid (RNA) content, much attention and interest has been focused on such methodologies. A commonly employed biochemical method of measuring larval fish growth is the use of the ratio of RNA to deoxyribonucleic acid (DNA) (see reviews by Ferron and Leggett 1994; Bergeron 1997). The ratio of RNA:DNA has also been used as a measure of larval nutritional condition (see reviews by Bergeron 1997; Ferron and Leggett 1994). The assumptions behind the RNA:DNA ratio are that the amount of DNA in the somatic cells of individual species is relatively constant (Buckley 1980, 1984), and that the amount of RNA in the cell changes as a function of protein synthesis (Bulow 1970). In more general terms, larvae of good condition will tend to have a higher RNA:DNA ratio than larvae of poorer condition. Improvements in our understanding of larval growth and mortality may be accomplished through the use of biochemical techniques such as the ratio of RNA:DNA.

During early life, selective mortality may operate differentially on individual larval traits (Houde 1997; Miller 1997). Parental contributions to the phenotypic expression of larval traits can be either genetic or environmental (Chambers 1993). Environmental factors expressed via parental contributions are generally of a maternal origin and are expressed in the early life stages of the offspring (Chambers 1993). These

maternal contributions are commonly referred to as maternal effects and can be defined as comprising "a class of phenotypic effects that parents have on phenotypes of their offspring that are unrelated to the offspring's own genotype" (Bernardo 1996). Maternal effects have potential implications on the susceptibility of a cohort to starvation and predation. Heavy exploitation may pass a spawning population through a phenotypic bottleneck through the removal of adults from the population that exhibit desired traits (i.e. large fish). This selective removal of adults could select for larval traits that exhibit lowered resistance to starvation and increased vulnerability to predation, and could potentially result in a substantial impact on recruitment.

Lake Michigan yellow perch (*Perca flavescens*) have experienced shifts in the distribution of the phenotypic traits of the spawning population through high levels of selective exploitation. An overall reduction in the spawning stock biomass has shifted the age structure of the population from a broad distribution to one dominated by adults greater than 4 years of age (Wisconsin Department of Natural Resources Fish Unit (WDNRFU), 600 E. Greenfield Ave., Milwaukee, WI 53204, USA personal communication). The commercial and recreational fisheries target the larger and faster growing female portion of the population. Subsequently, shifts in the population sex ratio have occurred over the past decade such that the population in 1996 was dominated by a male composition of 90% (WDNRFU personal communication). Furthermore, female growth rates have shifted dramatically since the 1980s. Females in the mid 1980s reached a size of about 250 mm at an age of 12 years, by the mid 1990s females were reaching the same size at an age of only 4 years (S.M. Shroyer, Department of Biology, Ball State University, Muncie, IN 47306-0440, USA personal communication).

Historically, yellow perch have provided one of the most important commercial and recreational fisheries in Lake Michigan (Rakoczy and Rogers 1987; GLFC 1995; Francis et al. 1996). However, the recent decline of yellow perch in Lake Michigan and the truncation of the trait distribution of the spawning population suggest that maternal effects might be affecting the recruitment potential of the population. Yellow perch are the only native Great Lakes fish that has sustained substantial, uninterrupted commercial and recreational harvests throughout the past century. Between 1889, when Lake Michigan yellow perch catch records were first recorded, and 1960, annual landings averaged 1090 metric tons (Baldwin et al. 1979). After 1960, commercial and recreational harvests of yellow perch fluctuated in response to a fluctuating population level (Francis et al., 1996). Population booms of the non-native Alewife (*Alosa pseudoharengus*) in the 1960s caused large yellow perch declines (Crowder 1980). Rebounds in the yellow perch population began to occur by 1972 and by the early 1980s the yellow perch population was at a substantially high level (GLFC 1995; Francis et al. 1996). However, the population declined dramatically in the early 1990s and failed to recover throughout the last decade. The Yellow Perch Task Group (YPTG), a subcommittee of the Lake Michigan Committee, was established by the Great Lakes Fishery Commission in 1994 to provide analysis, technical information and advice on management alternatives toward resolving the continuing and rapid decline of the yellow perch population in Lake Michigan. The YPTG identified poor reproduction and recruitment failure as primary reasons for the continued decline of the Lake Michigan yellow perch population. The population has experienced practically constant recruitment failure since 1990. The production of weak year-classes in 1996 (WNRB

1996) and 1997 (personal observation) was an ominous reminder of the continuing recruitment failure. The recruitment failure of the Lake Michigan yellow perch population has prompted an intense regional effort to understand what went wrong. Researchers from around Lake Michigan are investigating many aspects of recruitment processes.

Yellow perch spawning takes place during the months of May and June in Lake Michigan when the shallow inshore waters warm to temperatures of about 10 °C. Adult yellow perch will move inshore from the deeper offshore waters and begin to congregate on spawning grounds around the lake. Spawning normally occurs at night and is marked by a unique courtship. A single female will lead two side by side lines of males around a circuitous course before she releases her accordion-like gelatinous egg skein (Werner 1980). The males then fertilize the egg skein. The accordion-like architecture of yellow perch egg skeins coupled with their semi-buoyant nature allows the egg strands to slightly compress and expand with the water currents, creating a pumping action which moves a fresh supply of water across the eggs. Fecundity has been documented to range between 950 to 210,000 eggs per female, with an average fecundity between 20,000 and 30,000 eggs per female (Collette et al. 1977; Werner 1980). Incubation of yellow perch eggs in Lake Michigan can range between 14 and 21 days depending on water temperature and the rate of warming (personal observation). After hatching, the larvae move up into the upper three meters of the water column where they begin feeding on zooplankton within the first couple days (Forney 1971; Mills and Forney 1981). At a size of approximately 25 to 30 mm, yellow perch begin to make a transition to demersal life (Forney 1971; Mills and Forney 1981). Yellow perch remain demersal in the inshore

waters until they reach a size of about 65 mm, at which point they begin to migrate offshore (Forney 1971). Yellow perch are recruited to the fishery between ages 2 and 3.

Year class strength in both yellow perch (*P. flavescens*) and European perch (*P. fluviatilis*) are known to exhibit high inter-annual variation in many systems (Forney 1971). The variation in year class strength has been attributed to predatory control in many systems. Yellow perch year class strength has been shown to be controlled by walleye (*Stizostedion vitreum*) predation on age-0 yellow perch in Lake Oneida (Mills et al. 1987), by northern pike (*Esox lucius*) in Hemming Lake (Lawler 1965), and by muskellunge (*Esox masquinongy*) in lakes where it was stocked (Forney 1971). There has been some limited evidence for the role of predation in the Great Lakes systems. In an embayment off of Lake Ontario Mason and Brandt (1996) documented extensive predation by alewives on larval yellow perch. The evidence is less clear on a lake wide basis. For example, in Lake Michigan there is some evidence that yellow perch year class strength is affected by alewife (*Alosa pseudoharengus*) predation on larval yellow perch and competition between alewife and yellow perch in some areas of the lake (Shroyer and McComish 1998). However, by and large it is not believed that year class strength in Lake Michigan is controlled through predation as it is in other systems.

The truncation of the spawning stock traits in recent years coupled with the selective mortality that may operate differentially on individual larval traits have lead to the focus of this research on the role of maternal effects as a controlling factor of recruitment in Lake Michigan yellow perch. The goals of this research were as follows:

1. To determine the presence of maternal effects in Lake Michigan yellow perch
2. To determine the influence of maternal effects on the susceptibility of larvae to starvation in Lake Michigan yellow perch, and;
3. To determine the implications maternal effects have on the recruitment of yellow perch in Lake Michigan.

Thesis Structure

Chapter 2 is a determination of whether maternal effects are present in Lake Michigan yellow perch. Background information on changes that have occurred to the population structure over the past 15 years is presented to lay the foundation for examining maternal effects. The presence of maternal effects in newly hatched yellow perch larvae was detected. The correlation between the maternal traits and the traits of the offspring were investigated. Conclusions on which offspring traits can be expected given a set of maternal traits are drawn. Furthermore, the roles of trade-offs between traits that occur at both the maternal and offspring levels are examined. While the importance of maternal effects expressed as differences in hatching success among the maternal lines are examined, their further importance is merely suggested here and is not empirically evaluated.

Chapter 3 is the first of two chapters that empirically evaluate the importance of the maternal effects detected in Chapter 2. In this chapter, the susceptibility of larvae from different maternal lines to starvation is investigated. Differences in survival and differences in the changes that occur in individual larval traits are tracked. The importance of maternal effects and size-dependent recruitment mechanisms are evaluated in terms of resistance to starvation.

Chapter 4 continues the evaluation of the importance of maternal effects detected in Chapter 2. In this chapter, the implications of persistent maternal effects through metamorphosis on the recruitment of yellow perch in Lake Michigan are investigated. Differences in not only survival, but also differences in the change of individual larval traits are tracked. The importance of maternal effects and size-dependent recruitment mechanisms are evaluated and discussed.

Finally, in Chapter 5 the implications of the results on the rebuilding of yellow perch in Lake Michigan and on the long-term future management of the stock are discussed. Furthermore, the utility of this research and implications for understanding recruitment mechanisms in general are discussed. Advice to managers about the dangers of phenotypic bottlenecking caused by high exploitation and uncertain stock assessments are given.

References

Baldwin, N.S., R.W. Saalfeld, M.A. Ross, and H.J. Buettner. 1979. Commercial fish production in the Great Lakes 1867-1977. Great Lakes Fishery Commission Tech. Rep. No. 3.

Bergeron, J.P. 1997. Nucleic acids in icthyoplankton ecology: a review, with emphasis on recent advances for new perspectives. J. Fish Biol. **57(Supplement A)**: 284-302.

Bernardo, J. 1996. Maternal effects in animal ecology. Amer. Zool. **36**: 83-105.

Blaxter, J.H.S. 1969. Development: eggs and larvae, p. 177-252. In: W.S. Hoar and D.J. Randall (eds.) Fish Physiology. Vol. III. Reproduction and growth. Academic Press, New York, New York.

Buckley, L.J. 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. **80**: 291-298.

Buckley, L.J. 1980. Changes in the ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. Fish. Bull. **77**: 703-708.

Bulow, F.J. 1970. RNA-DNA ratios as indicators of recent growth rates of a fish. J. Fish. Res. Board Canada. **27**: 2343-2349.

Chambers, R.C. 1993. Phenotypic variability in fish populations and its representation in individual-based models. Trans. Am. Fish. Soc. **122** 404-414.

Collette, B.B., M.A. Ali, K.E.F. Hokanson, M. Nagiec, S.A. Smirnov, J.E. Thorpe, A.H. Weatherly, and J. Willemsen. 1977. Biology of percids. J. Fish. Res. Board Can. **34**: 1890-1899.

Crowder, L.B. 1980. Alewife, smelt and native fishes in Lake Michigan: competition or predation? Env. Biol. Fish. **5**: 225-233.

Crowder, L.B., J.A. Rice, T.J. Miller, E.A. Marschall. 1989. Empirical and theoretical approaches to size-based interactions and recruitment variability in fishes, p. 237-255. In: DeAngelis, D.L. and L.J. Gross (eds.) Individual-Based Models and Approaches in Ecology. Chapman and Hall, New York, New York.

Ferron, A. and W.C. Leggett. 1994. An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. **30**: 217-304.

Forney, J.L. 1971. Development of dominant year classes in a yellow perch population. Trans. Am. Fish. Soc. **100(4)**: 739-749.

Francis, J.T., S.R. Robillard, and J.E. Marsden. 1996. Yellow perch management in Lake Michigan: a multi-jurisdictional challenge. Fisheries. **21(2)**: 18-20.

Great Lakes Fishery Commission (GLFC). 1995. Fish community objectives for Lake Michigan. Great Lakes Fishery Commission Special Publication 95-3.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P-V Réun. Cons. Int. Explor. Mer. **20**: 1-228.

Houde, E.D. 1997. Patterns and consequences of selective processes in teleost early life histories, pp 173-196. In: R.C. Chambers and E.A. Trippel (eds.) Early Life History and Recruitment in Fish Populations. Chapman and Hall, New York, New York.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Amer. Fish. Soc. Symp. **2**: 17-29.

Hunter, J.R. 1981. Feeding ecology and predation of marine fish larvae, p. 33-79. In: R. Lasker (ed.) Marine Fish Larvae: Morphology, Ecology, and Relation to Fisheries. University of Washington Press, Seattle, Washington.

Lawler, G.W. 1965. Food of the pike, *Esox lucius*, in Heming Lake, Manitoba. J. Fish. Res. Board Canada. **22**: 1357-1377.

Leggett, W.C. and E. Deblois. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages. Neth. J. Sea. Res. **32**(2): 119-134.

Litvak, M.K. and W.C. Leggett. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Prog. Ser. **81**: 13-24.

Mason, D.M. and S.B. Brandt. 1996. Effect of alewife predation on survival of larval yellow perch in an embayment of Lake Ontario. Can. J. Fish. Aquat. Sci. **53**: 1609-1617.

Miller, T.J. 1997. The use of field studies to investigate selective processes in fish early life history. In R.C. Chambers and E.A. Trippel (eds.) Early Life History and Recruitment in Fish Populations. Chapman and Hall, New York, New York.

Miller, T.J., L.B. Crowder, J.A. Rice, and E.A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657-1670.

Mills, E.L. and J.L. Forney. 1981. Energetics, food consumption, and growth of young yellow perch in Oneida Lake, New York. Trans. Am. Fish. Soc. **110**: 479-488.

Mills, E.L., J.L. Forney, and K.J. Wagner. 1987. Fish predation and its cascading effect on the Oneida Lake food chain. pp. 118-131. In: W.C. Kerfoot and A. Sih (eds.).

Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover, New Hampshire, USA.

Pepin, P. 1989. Predation and starvation of larval fish: a numerical experiment of size- and growth-dependent survival. Biol. Ocean. **6**: 23-44.

Rakoczy, G. and R. Rogers. 1987. Sport fishing catch and effort from Michigan waters of Lakes Michigan, Huron and Erie, and their important tributary streams. April 1, 1986 – March 31, 1987. Michigan Dept. Nat. Res., Fish. Div., Fish. Tech. Rep. No. 87-6A. 58 pp.

Rice, J.A., L.B. Crowder, and F.P. Binkowski. 1987. Evaluating potential sources of mortality for larval bloater (*Coregonus hoyi*): starvation and vulnerability to predation. Can. J. Fish. Aquat. Sci. **44**: 467-472.

Shroyer, S.M. and T.S. McComish. 1998. Forcasting abundance of quality-size yellow perch in Indiana waters of Lake Michigan. N. Amer. J. Fish. Manag. **18**: 19-24.

Sutcliffe, W.H., Jr. 1970. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Board Can. **27**: 606-609.

Werner, R.G. 1980. Freshwater Fishes of New York State. Syracuse University Press. Syracuse, NY. 186 pp.

Wisconsin Natural Resources Board (WNRB) Memorandum. 1996. File Ref. #3600.

Wootton, R.J. 1990. Ecology of Teleost Fishes. Chapman and Hall, New York, New York. 404 pp.

Chapter 2

Understanding Maternal Effects as a Recruitment Mechanism in Lake Michigan Yellow Perch (*Perca flavescens*)

I. Presence of Maternal Effects

Abstract

Changes that have occurred in the phenotypic distribution of adult yellow perch (*Perca flavescens*) in Lake Michigan suggest that maternal effects on larval traits may be substantially influencing the recruitment of this heavily exploited species. We investigated maternal effects on yellow perch larvae at hatching in ten maternal lines to test the null hypothesis of no effect of maternal condition on offspring condition. Analyses lead to a rejection of the null hypothesis and indicate that the observed maternal effects likely result from differences among females in size, age, gonadal somatic index, and egg production. The observed maternal effects are expressed in the offspring by differences in larval total length, yolk volume, dry weight, and DNA quantity. Correlation analyses indicated trade-offs within female traits between GSI and all other female traits measured, and within larval traits between total length and yolk-volume. The observed trade-offs have potentially substantial implications on the offspring's vulnerability to starvation and predation. We conclude that yellow perch exhibit maternal effects that are expressed in the distribution of larval traits at hatching.

Introduction

Despite the large focus of energy that has been directed towards understanding and predicting recruitment, recruitment variability is the single least understood process in fisheries science (Houde 1987). The difficulty stems from the "synergism of variable processes" that regulate recruitment (Houde 1987). These processes include, but are not limited to, interactions between spawners and their characteristics, recruit characteristics, larval growth and mortality, and trophic interactions together with the effects of the physical environment in which both the spawners and recruits exist (Laurence 1988; Crowder et al. 1989). This complexity underlies the high inter-annual variability characteristic of recruitment.

Recruitment variability is likely attributable to processes in early life history (Houde 1987; Pepin 1989; Leggett and Deblois 1994). The extreme variability in vital rates that characterizes early life stages produces larvae with a wide range of characteristics on which phenotypic selection may act (Rice et al. 1993). Recognition of the potential importance of inter-individual variability has led to suggestions that recruitment research may be more successful if it were to focus on individuals rather than populations (Crowder et al. 1989). Many recruitment mechanisms have been found to be size-dependent (Miller et al. 1988). Because of this size-dependence, small initial size differences within a population can result in very large survival and recruitment differences (Adams and DeAngelis 1987; Crowder et al. 1989; Rice et al. 1993).

Previous research has shown that observed variation in the early life history traits among individuals of many finfish species has been linked to maternal influences (Chambers and Leggett 1992, 1996). Maternal effects can be defined as comprising "a

class of phenotypic effects that parents have on phenotypes of their offspring that are unrelated to the offspring's own genotype" (Bernardo 1996). Maternal influences result from differential reproductive investment by males and females. While both male and female gametes are required for successful fertilization, it is the female's contribution to the egg that provides the nutritional requirements essential for proper development. Subsequent to hatching, it is again the investment of the female that provides the nutritional sustenance that is required for larval survival until first feeding. These investments by the female can be measured by the morphological and biochemical condition of the subsequent offspring. In contrast, paternal investment and its impact on subsequent survival is believed to be small. Recent research on parental influences in Atlantic cod (*Gadus morhua*) did not show any consistent effects of male size on fertilization success (Rakitin et al. 1999). Furthermore, differences in hatching success and survival in Atlantic cod could not be explained by differences in male size nor sperm density (Rakitin et al. 1999).

Much of the previous research on maternal effects has focused on detecting maternal effects in the phenotypic traits of eggs. Significant female effects on egg sizes have been observed in Atlantic salmon *Salmo salar* (Kazakov 1981), chum salmon *Oncorhynchus keta* (Beacham and Murray 1985), rainbow trout *O. mykiss* (Gall 1974), capelin *Mallotus villosus* (Chambers et al. 1989), Atlantic cod (Knutsen and Tilseth 1985; Chambers and Waiwood 1996), haddock *Melanogrammus aeglefinus* (Hislop 1988), Atlantic silverside *Menidia menidia* (Bengston et al. 1987), black porgy *Acanthopargrus schlegeli* (Huang et al. 1999), winter flounder *Pseudoplueronectes americanus* (Buckley et al. 1991), and yellowtail flounder *Pleuronectes ferrugineus* (Benoit and Pepin 1999),

among others. Chambers and Leggett (1996) also identified Atlantic herring *Clupea harengus*, walleye pollock *Theragra chalcogramma*, striped bass *Morone saxatilis*, turbot *Scophthalmus maximus*, and flathead sole *Hippoglossoides elassodon*, as species that exhibited significant maternal effects on egg size in their review of maternal effects. In contrast, research on maternal effects on the size, condition and growth rates of larvae has been limited (Benoit and Pepin 1999). Significant maternal effects in larval traits have been observed in a few species. The traits for which these effects have been documented are size at hatch (chum salmon, Beacham and Murray 1985; Atlantic silversides, Bengston et al. 1987), yolk volume at hatching (chum salmon, Beacham and Murray 1995; capelin, Chambers et al. 1989), and size at metamorphosis (winter flounder, Chambers and Leggett 1992). Chambers and Leggett (1996) concluded, "body size during larval life was likely to be significantly influenced by maternal effects." For most other species where significant maternal effects are reported in larval traits, such correlations are inappropriately extrapolated from significant maternal effects in phenotypic egg traits (Pepin and Miller 1993; Benoit and Pepin 1999). Of those studies in which maternal effects in larval traits have been documented, few have used biochemical traits such as the ratio of RNA to DNA to evaluate larval condition.

Potentially, maternal effects can substantially impact the recruitment dynamics of heavily exploited populations. Since maternal effects are an expression of the phenotypic characteristics of the spawning population, overfishing can lead to phenotypic bottlenecking which can cause the population to shift from its fitness peak (Solemdal 1997). Changes in the distributions of size, age and condition of the spawning population that result from exploitation can potentially cause changes in egg production, fertilization

success, and hatching success (Rjinsdorp 1989). Furthermore, changes in the size and condition distribution of newly hatched offspring of a population can potentially lead to changed susceptibilities to starvation and predation. In theory, such shifts can result in increased mortality, decreased recruitment, and ultimately recruitment failure.

Historically, yellow perch (*Perca flavescens*) has provided one of the most important commercial and recreational fisheries in Lake Michigan (Francis et al. 1996). Substantial harvests have been taken over the past century, even in the face of perturbations such as the alewife invasion and its subsequent population boom in the late 1960s. However, the population declined dramatically in the early 1990s and has failed to recover throughout the last decade. The Lake Michigan Yellow Perch Task Group, established in 1994 by the Great Lakes Fishery Commission to provide analysis, technical information and advice on yellow perch management, identified poor reproduction and recruitment failure as primary reasons for the continued decline of the Lake Michigan yellow perch population. The population has experienced practically constant recruitment failure since 1990. The production of weak year classes in 1996 (WNRB 1996) and 1997 (personal observation) was an ominous reminder of the continuing recruitment failure.

In 1998, the Lake Michigan yellow perch population level was reported to be 20% of 1990 levels (Wisconsin Department of Natural Resources Fish Unit (WDNRFU), 600 E. Greenfield Ave., Milwaukee, WI 53204, USA unpublished data). Winter gillnet surveys catch per unit efforts (CPUE) declined from around 4000 to 100 yellow perch per 1000 ft of gillnet per night between 1986 and 1998 (Fig. 1). The age structure of the population changed over this period as well (WDNRFU personal communication). In the

mid and late 1980s, the population was dominated by yellow perch age 2-4 years with a relatively small portion of the total population exceeding 4 years of age. By 1994, the population had shifted to one composed of almost exclusively greater than 4-year-old yellow perch (Fig. 1). The same WDNRFU winter gillnet surveys have also shown a shift in the sex ratio of the population from a ratio of almost 50% males to 50% females in the mid 1980s to a ratio of 90% males to 10% females in the mid 1990s. In response to the population decline, the average growth rates of female yellow perch have increased substantially since 1983 (S.M. Shroyer, Department of Biology, Ball State University, Muncie, IN 47306-0440, USA personal communication). In the early and mid-1980s female yellow perch reached a size of about 250 mm at an age of 12 years, while in the mid-1990s, females were reaching that same size at an age of only 4 years (Fig. 2).

The changes in the population structure that have occurred over the past decade reflect the mark of an extremely size selective fishery, which targets the faster growing and larger female yellow perch. The high fishing mortality rate, the highly size-selective nature of the fishery and a lack of recruitment have caused the overall population decline and shifts in the sex ratios which further weakened reproduction and recruitment. The changes in the female portion of the Lake Michigan yellow perch population structure suggest that the distribution of female phenotypes has been significantly altered. Large females that are predominantly greater than 4 years of age, but that are historically young for their size, dominate the female portion of the population (Figs. 1 and 2). The lack of recruitment further fuels this phenotypic bottleneck. These changes suggest that maternal effects may play a dominant role in the recruitment dynamics of yellow perch in Lake Michigan.

Given the changes that have occurred in the Lake Michigan yellow perch population structure, this study aimed to investigate the role of maternal effects within the population. The objectives of this study were threefold; to determine whether maternal effects on larvae of Lake Michigan yellow perch were present; to determine which larval traits accounted for the maternal effects if found, and; to determine which female traits correlate with the larval traits that account for the observed maternal effects if found.

Materials and Methods

We investigated our objectives within a single experiment, which involved quantifying the relationship between ten different females and their offspring. All females were collected in Lake Michigan. Subsequent experimental work was conducted at the University of Wisconsin's WATER Institute, Milwaukee, WI. The experiment was conducted as a randomized complete block design, consisting of three blocks of 20 tanks with two replicate tanks per female per block.

Collection of Parental Brood Stock and Gametes

Yellow perch were collected off Green Can Reef (42° 50' 00" N, 87° 50' 00"W), a historic yellow perch spawning ground offshore of Milwaukee, WI, using graded mesh gillnet (mesh from 2 ¼ to 2 ¾ bar) in June of 1998. Independent bottom sets were made between 10 - 15 m. of water in the afternoon prior to retrieval. Nets soaked overnight. Upon retrieval, all fish were carefully removed from the gillnet and their sex was determined. Female yellow perch were determined to be green (not ready to spawn, eggs not fully hydrated), ripe (eggs fully hydrated and easily expressed) or spent. Male yellow

perch were characterized as green (not ready to spawn, no sperm), ripe (sperm readily expressed), or spent. A minimum of 25 ripe males was held in an onboard holding tank until ripe females were captured. As ripe female yellow perch were captured, their eggs were immediately expressed into separate large stainless steel mixing bowls. The separate egg strands were then carefully transferred to Ziploc bags and weighed. The eggs were transferred back to the separate stainless steel mixing bowls and fertilized with the milt from no less than three and no greater than eight males. The number of males used to fertilize the eggs was determined based upon each male's sperm production. After approximately one minute, fresh lake water was added to the mixing bowls to keep the eggs wet and at ambient temperature. After a minimum of three minutes, the water was drained from the bowls and the fertilized eggs were rinsed with fresh lake water. The fertilized egg skeins were then transferred to separate 2-gallon Ziploc bags and filled with fresh lake water. The bags were stored in large coolers containing ice in a separate compartment until transported back to the laboratory. The females and males used to produce each fertilized egg skein were sacrificed, and kept in separate Ziploc bags on ice for transportation back to the laboratory.

Due to the population decline of yellow perch in Lake Michigan it was impossible to obtain more than five ripe female yellow perch on any one given day, despite the use of more than two miles of commercial gillnet. As a result, egg skeins from five female yellow perch were obtained on 2 June 1998; five additional egg skeins were obtained on 4 June 1998.

Determination of Maternal Characteristics

We quantified eight maternal traits (Table 1). The total length and weight of each female were measured in the laboratory. Males were also measured, although these data are not reported. Fulton's condition factor (k) was calculated for each female as $k = (\text{weight}/\text{length}^3) \times 100{,}000$. Additionally, the body depths at the anterior base of the first dorsal fin and the posterior base of the second dorsal fin were measured. The sagital otoliths of each female were removed for subsequent age analysis. Each female's gonadal somatic index (GSI) was determined as:

$$GSI = \frac{(\text{Wet Weight of Eggs} + \text{Wet Weight of Stripped Ovary})}{(\text{Wet Weight of Female} + \text{Wet Weight of Eggs} + \text{Wet Weight of Stripped Ovary})}$$

When the ovary from each female was removed there were some green eggs remaining in each ovary. These eggs were determined to be eggs that would not have been released during spawning and would most likely have been reabsorbed by the female; therefore our estimates of total egg production for each female are based solely on the ripe eggs expressed. Since the green eggs remaining in the ovary were assumed to be eggs that would have been reabsorbed, their weight was included in the weight of the stripped ovary.

Egg Sampling

The total volume of each female's egg skein was determined in the laboratory. Independent 1-ml sub-samples (n=3) of each egg skein were taken and the eggs in each sub-sample were enumerated. The total egg production per female was defined as the product of the skein volume and the average egg concentration per unit volume. An additional sub-sample of each female's eggs was videotaped for subsequent analysis of

morphometry (n=15). For unknown reasons, the gelatinous matrix of $Female_2$ did not hold together. While we were able to get egg production estimates for $Female_2$, we did not videotape her eggs to avoid risking loss of too many eggs. The egg skeins obtained on 2 June 1998 were kept separated, but held in a single mass rearing tank until 4 June 1998 when the second set of egg skeins was obtained. Because of the fragile gelatinous matrix of the egg skein from $Female_2$, her eggs were held separately in a 10-gallon flow-through aquarium.

Husbandry of Eggs

Once egg skeins from ten females were available, the eggs were randomly assigned to and incubated in flow-through, temperature-controlled 10-gallon aquaria. Based upon the egg production of each female, and the previously determined number of eggs per milliliter (checked again on 4 June 1998 to adjust for swelling associated with water hardening), a volume of skein equivalent to ~2000 eggs was placed into each tank assigned to that female. The egg skein for $Female_5$ was crushed in transport and some eggs were damaged; the total number of usable eggs for $Female_5$ was only 1400 per tank.

Hatching began on 14 June 1998 in all tanks. Hatching was encouraged by gently mixing the water and the broken egg skeins to allow those larvae trapped within the gelatinous matrices to be freed. The broken egg skeins, and any dead eggs and larvae were removed.

Larval Sampling

We sampled 15 larvae at hatch from each tank systematically from the first tank in the first block to the last tank in the last block. Larvae from each tank were sampled five at a time to prevent mortality during sample processing. The larvae were first anesthetized with Tricaine Methansulfonate (MS-222), and then videotaped for analysis of morphometry. We sampled 12 larval traits, of which 9 were morphometric measures and 3 were biochemical measures of condition (Table 1). The individual larvae were then placed into cryovials and flash-frozen in liquid nitrogen, then stored at –80 °C for subsequent extraction of nucleic acids.

Post-Sampling Processing of Eggs and Larvae

Analysis of morphometric landmarks of the eggs and larvae was conducted with Optimas imaging analysis system (v 6.1, Media Cybernetics, Takoma Park, MD) at the Chesapeake Biological Laboratory in Solomons, MD on videotaped images. Calibrations for imaging analysis were derived from videotaped images of micrometers taken before sampling of both the eggs and the larvae. The two-dimensional surface area (calculated internally by Optimas from the outline of the egg or egg yolk) and three sides of a triangle inscribed within the outline of each female's individual eggs and egg yolks were measured. Total egg volume and egg yolk volumes were then determined based upon the following equations adapted from Miller et al. (1995).

$$\text{Volume}(\text{mm}^3) = \frac{4}{3}\pi R^2$$

$$R = \frac{abc}{4K}$$

$$K = \sqrt{s(s-a)(s-b)(s-c)}$$

$$s = \frac{a+b+c}{2}$$

Where R is the estimated radius of the sphere and a, b and c represent the three triangle sides inscribed within each egg and egg yolk.

Total length, eye diameter, body depth at the insertion point of the pectoral fin, body depth at the insertion point of the first dorsal fin (inclusive of the yolk-sac), and body depth at the insertion point of the anus were measured for each individual larva sampled from each female. The two-dimensional surface area of each larva's yolk-sac (calculated internally by Optimas from the outline of each larva's yolk-sac) was measured along with the major and minor yolk axes. Total yolk-sac volume was then estimated as:

$$\text{Volume}\,(\text{mm}^3) = \frac{4}{3}(\text{Length of the Minor Yolk Axis}) \cdot (\text{Surface Area})$$

The two-dimensional body area of each larva (calculated internally by Optimas from the outline of each larva) was also measured, and a calibration from other newly hatched yellow perch larvae between two-dimensional body area and dry weight allowed estimation of individual larval dry weight (Dry weight (μg) = $[(2 \times 10^{-6}) \times (\text{Body Area})^2 + (6 \times 10^{-5}) \times (\text{Body Area}) + 3 \times 10^{-5}] \times 1000$, $n = 171$, $r^2 = 0.9836$, $p = 0.0001$; Appendix I).

Nucleic acids were extracted from each individual larva. Larvae were removed from –80 °C storage and placed on ice. Whole larvae were placed in individual microcentrifuge tubes containing 150 µl of 1% sarcosyl solution (N-lauroylsarcosine: Sigma L-5125). The microcentrifuge tubes were vortexed at high speed for 1 hour to fully break down the tissue and dissociate the nucleic acids. After 1 hour of vortexing, 1.35 ml of Tris buffer (5mM Tris-HCL (Trizma Base: Sigma T-8524, HCL: Sigma H-1758), 0.5 mM EDTA (Sigma E-5134), pH 7.5) was added to each microcentrifuge tube to dilute the total nucleic acid concentration. Tubes were mixed by inversion, and centrifuged at 14,000 g for 15 minutes to separate any remaining tissue structures. 75 µl Aliquots of the supernatant from each microtube were combined with 75 µl of Ethidium Bromide (EB) (ISC Bioexpress C-5515-10), a nucleic acid specific fluorophore, in 96-well microplates. Dilution series of calf liver rRNA (Sigma R-0889) and calf thymus DNA (Sigma D-4764) standards, blanks of 0.1% sarcosyl, and a 9 dph yellow perch homogenate standard were also combined with EB (75 µl : 75 µl). The microplate was shaken at low speed for 5 minutes to fully mix the sample and EB. The microplate was read on a fluorescence plate reader (Ex = 545 nm, Em = 575 nm) (IDEXX FCA-VIP, IDEXX Corp., Portland, ME) to determine total nucleic acid (TNA) fluorescence in each sample. 7.5 µl of RNase A (Sigma R-6513) was then added to each well of the microplate. The plate was shaken at low speed for 20 minutes to digest the RNA in each sample, followed by fluorescence determination of the DNA in each sample. The fluorescence due to RNA was then determined for each sample by subtraction of the DNA fluorescence from the TNA fluorescence. Calibration curves for RNA and DNA were generated from the fluorescence values obtained for the appropriate dilution series.

The concentrations of RNA and DNA in each sample were then determined from the standard calibration curves, and the ratio of total RNA (concentration*1.5 ml) to total DNA (concentration*1.5 ml) in each sample was calculated. To reduce error associated with any one day's reading, only a few of the larvae from an individual tank were read on a given day.

Statistics

Separate statistical analyses were conducted to address our three objectives. All statistical analyses were conducted using SAS (v 6.12, SAS Institute, Inc., Cary, NC). We used 12 larval and eight maternal traits in our analyses (Table 1). The presence of significant maternal effects at hatching in the offspring of the ten females was determined by multivariate analysis of variance (MANOVA; SAS Proc GLM) on all larval traits at hatching. The MANOVA was based on the mean value of each larval trait within each tank, for each female nested within her date of fertilization (2 June or 4 June 1998). Significance of MANOVA was determined based upon a Wilks Lambda statistic with 96 and 259.507 degrees of freedom.

The larval traits that accounted for the observed maternal effects at hatch were determined by univariate analysis of variance (ANOVA; SAS, Proc Mixed) on biologically important larval traits within each tank, for each female nested within her date of fertilization. Significance of each ANOVA was determined based on an F statistic with 8 and 48 degrees of freedom.

To determine which female traits correlated with the larval traits that accounted for the observed maternal effects at hatch we performed a canonical correlation analysis

on the female and larval data (SAS, Proc Cancorr). Significance of the overall canonical correlation analysis was based on a Wilks Lambda statistic with 80 and 274.95 degrees of freedom. We report the trait loadings on significant canonical correlation vectors only.

Results

The females that we did catch had a fairly wide distribution of phenotypic traits. Female total length ranged from 216 mm to 287 mm and female weight ranged from 113 g to 261 g (Table 2). Females ranged in age from 2 years to 6 years (Table 2). The GSIs ranged from 0.22 to 0.63, while egg production ranged from 11,400 to 36,720 eggs (Table 2). Correlation analysis indicated that female total length, female weight, Fulton's condition factor k, female body depth, age, and egg production were all positively correlated with one another, and negatively correlated with GSI. This suggests a tradeoff between the GSI of the female and the female's size, age and egg production.

Mean hatching success of the eggs was highly variable among the ten maternal lines. The lowest mean number of offspring observed was for $Female_2$, 89 ± 20, representing a 4.5 % ($n_{initial} = 1968 \pm 124$) hatching success (Table 3). Excluding $Female_2$, given the poor quality of her egg skein, hatching success narrowed to a range of 896 ± 181 offspring for $Female_3$, representing 44.8 % ($n_{initial} = 2000 \pm 324$) hatching success, to 82.5 % (1148 ± 198 ($n_{initial} = 1392 \pm 156$)) for $Female_5$ (Table 3).

At hatching, 90 larvae from each female were sampled. There was a loss of a portion of videotape record for each female due to a problem with the video recording device that was not evident until analysis of the videotape began. Means of individual larval traits are based on variable sample sizes ranging from 67 to 81 larvae per female

(Table 4). The means of individual larval morphometric traits varied among females by between 10.2 % (mean larval eye diameter, followed by 10.3 % variation in mean larval body depth at the insertion of the pectoral fin) and 29.3 % (mean RNA) (Table 4). Mean RNA ranged from 2.7 ± 0.63 μg to 3.8 ± 0.55 μg while DNA ranged from 1.7 ± 0.21 μg to 2.3 ± 0.30 μg between the hatchlings (Table 4). Despite relatively large differences in the mean quantity of RNA and DNA in the larvae, the mean ratio of RNA:DNA only varied between 1.5 ± 0.32 and 1.8 ± 0.78 among the offspring of the ten maternal lines (Table 4).

Correlation analysis of tank mean values for 10 larval traits showed a highly variable covariance structure (Fig. 3). Some trait pairs were highly correlated (e.g. larval body depth at the insertion of the pectoral fin and larval body depth at the insertion of the anus ($r = 0.8297$, $n = 60$, $p = 0.0001$); larval total length and larval dry weight ($r = 0.7093$, $n = 60$, $p = 0.0001$); RNA and RNA:DNA ratio ($r = 0.7406$, $n = 60$, $p = 0.0001$). However, other trait pairs exhibited no significant correlation (e.g. larval total length and larval yolk volume ($r = -0.1309$, $n = 60$, $p = 0.3188$); larval yolk volume and larval dry weight ($r = 0.0527$, $n = 60$, $p = 0.6893$). Both negative and positive correlations were present in the morphometric data (Fig. 3). Nucleic acid-based traits showed strong correlations internally. However, with the exception of a correlation between DNA and larval dry weight, there were only weak correlations between nucleic acid-based traits and morphometric ones (Fig. 3).

We were able to reject the null hypothesis of no overall maternal effect among the offspring of the ten maternal lines for larval traits based upon MANOVA results ($F_{96, 259.507} = 2.21$, $p = 0.0001$). Univariate ANOVA indicated an overall significant difference

between the offspring of the ten maternal lines for larval total length, larval yolk volume, larval dry weight, and DNA (Table 5; Figs. 4, 5, 6, 7).

There was a significant canonical correlation between maternal and larval traits ($F_{80, 274.95} = 4.51$, $p = 0.0001$). The canonical correlation analysis indicated that canonical correlation vectors 1, 2 and 3 were the only significant vectors, with vector 1 explaining 54% of the variance, vector 2 explaining 33.6%, and vector 3 explaining 5.5% of the variance. Because canonical correlation vector 3 explains only 5.5% of the variance, we did not consider this vector further. The female traits, age, GSI and egg production were strongly associated with the Female 1 canonical variable. Female age and egg production drive canonical variable Female 1 in a negative direction while GSI drives it in a positive direction (Table 6). This provides further evidence for a potential trade-off between female GSI and other traits such as egg production and age. The canonical variable Female 2 is a strong positive descriptor of female size and age (Table 6). The larval traits, larval total length, larval yolk volume and larval body depth at the insertion of the first dorsal fin are strongly associated with the Larval 1 canonical variable. Larval total length drives the canonical variable Larval 1 in a positive direction while larval yolk volume and larval body depth at the insertion of the first dorsal fin drive it in a negative direction (Table 7). This suggests a potential trade-off between larval total length and larval yolk volume. The canonical variable Larval 2 is a strong positive descriptor of larval size and nucleic acid content (Table 7). Furthermore, 54% of the variation we observed among the larvae of the ten maternal lines was explained most by differences in larval total length, larval yolk volume and larval body depth at the insertion of the first dorsal fin, and was associated most with differences in female GSI, egg production and

age (Table 8). An additional 33.6% of the variation among the larvae of the ten maternal lines was expressed most in larval size (particularly larval dry weight) and nucleic acid content, and was most associated with differences in female size and age (Table 8).

Discussion

We detected significant maternal effects at hatch among the offspring of the ten yellow perch females. Analyses indicate that these effects likely result from differences among females in size, age, GSI, and egg production. Furthermore, larvae express the maternal effects by differences in larval total length, larval yolk volume, larval dry weight and DNA quantity. However, we could not find evidence of significant differences among the eggs of the different females.

Previous research on maternal effects has focused largely on correlations between the phenotypic traits of the female and the phenotypic traits of her eggs. There has been substantially less focus on the relationship between female phenotypic traits and the phenotypic traits of her offspring, both immediately following hatching and throughout the larval stage. This focus is perhaps driven by the need of fisheries scientists to predict recruitment as early as possible in the fish's ontogeny and by the underlying hypotheses that egg size is predictive of larval size and subsequent performance (see reviews by Chambers and Leggett 1996; Chambers and Waiwood 1996). Also, propagule size is an obvious expression of maternal effects that should correlate with the female's phenotype (Bernardo 1996; Solemdal 1997). In light of this, our failure to detect maternal effects in the eggs of yellow perch is surprising. We believe that our lack of data on egg traits most likely accounts for the results observed. The information that we were able to collect,

egg volume and yolk volume, bore no relationship to either maternal phenotype or larval phenotype. Had we been able to collect more data on the female's eggs (e.g. dry weight and lipid content), maternal effects in the egg stage of yellow perch may have been expressed.

Our inability to detect maternal effects in the egg stage of yellow perch was not a precursor to a lack of detectable maternal effects in the newly hatched offspring. Differences in the phenotypic traits of the hatchlings from the ten maternal lines were significantly explained by difference in the phenotypic traits of the females. Previous research in other species has shown that maternal effects were expressed in hatching length, size, yolk volume, and oil globule volume (Chambers et al. 1989; Buckley et al. 1991; Chambers and Leggett 1996). Our findings suggest that in yellow perch maternal effects are expressed most in larval length, yolk volume, dry weight and DNA content (Figs. 4, 5, 6, 7) (Table 5). We can ignore the expression of maternal effects in larval body depth at the insertion of the first dorsal fin because this measure is inclusive of the yolk-sac and is likely to be driven by yolk volume. Since the quantity of DNA in a cell is relatively constant within a given species, we expect a higher number of cells in larger larvae than in smaller larvae, and thus a higher quantity of DNA. Therefore, there should be a correlation between larval size and DNA content. Thus, if maternal effects were expressed in one trait they would be expressed in the other. Near allometric relationships have been shown between larval length and total DNA for capelin, cunner *Tautogolarbus adspersus*, and radiated shanny *Ulvaria subbifurcata* (Pepin et al. 1999). Our results provide further evidence for this pattern.

A lack of significant maternal effects in the RNA content and RNA:DNA ratio of the hatchlings from the ten maternal lines indicates that condition of larvae was not significantly different at hatching (Table 5) suggesting that differences in protein synthesis may not be exhibited until larvae begin feeding on exogenous food sources, or perhaps until they have utilized much of their endogenous food source. Changes in condition are likely to occur at different rates once the larvae begin depleting their yolk–sacs and body reserves, and once they begin first feeding. Such variability in RNA:DNA ratios of first feeding larvae has been shown for Atlantic herring (Clemmensen 1994), Iberian sardine *Sardina pilchardus* (Chicharo 1998) and red drum *Sciaenops ocellatus* (Rooker et al. 1997).

The age distribution of females in this study was surprisingly wide, given the recent population structure changes that have occurred. Our initial expectations had been that all mature female yellow perch caught in 1998 would be greater than 4 years old. Female GSI, age, size and egg production are the key female phenotypic traits that explained differences in the size, yolk volume and DNA content of offspring (Table 8). There appears to be a potential trade-off between the GSI of the female and her age, size and egg production. It appears that old, large females with high egg production exhibit low GSI values, while younger, smaller females have high GSI values while producing fewer eggs. The strong association between larval total length and larval yolk volume and female GSI, egg production and age suggests that it is these female traits that potentially determine the length and amount of yolk a larva may have at hatching. Additionally, the association between larval size and nucleic acid content and female size and age suggests that it is these female traits that potentially determine the size and

amount of nucleic acids of a larva at hatching. These results suggest that the traits of larvae produced by inexperienced spawners (i.e. spawners of a young age) may be very different from the traits of larvae produced by experienced spawners. While previous studies of maternal effects have not shown trade-offs between larval characteristics that are driven by female characteristics, some correlations have been found. Larval size has been positively correlated with female size (Bernardo 1996), and female size and age have been positively correlated with fecundity (Hislop 1988; Buckley et al. 1991; Chambers and Waiwood 1996).

The canonical correlation analysis clearly identifies a trade-off between hatchling total length and larval yolk volume within canonical vector 1. Furthermore, evidence for this trade-off is observed in the box plots for hatchling total length and yolk volume. Across all maternal lines, those hatchlings with the highest total lengths exhibit the lowest yolk volumes, and vise-versa. The non-significant correlation between larval total length and yolk volume is likely due to the variation of those larval traits within maternal lines caused by a few unique individuals (outliers). Despite these correlation results, there is a trend in the data suggesting that a negative relationship between larval total length and yolk volume does exist, and may have become more evident with a larger sample size. The trade-off between larval size and yolk volume has potentially substantial implications on the larvae's vulnerability to starvation and predation.

Larger larvae are thought to have an advantage over smaller larvae in that they have been shown to swim faster and thus avoid predators more easily, search greater distances for food, capture larger sizes and quantities of prey, and survive periods of food shortages longer (Knutsen and Tilseth 1985; Miller et al. 1988; Chambers et al. 1989;

Chambers and Leggett 1996;). However, this does not take into consideration the dangers of initial starvation, or the consequences of potential spatial and temporal overlap with prey. Should a larva be faced with a lack of food upon hatching, it will be solely dependent upon its yolk-sac and body reserves for survival. Our results indicate a trade-off potentially exists between larval size and yolk volume, and that trade-off is driven by key characteristics of the female spawning stock. Under circumstances of food limitation immediately after hatching, we would expect smaller larvae with larger yolk-sacs to have a survival advantage over larger larvae with smaller yolks, in terms of survival. However, assuming that the food limitation is not indefinite, then we would begin to expect the larger larvae to again have a survival advantage over the smaller larvae as feeding and predator avoidance begin to become important. This suggests that potentially, bigger is not necessarily better in all cases.

Heavily exploited fisheries, especially those which target faster growing, larger females will be much more susceptible to the phenotypic bottlenecking of the female population and may subsequently be at higher risk to recruitment failure when environmental conditions shift. Solemdal (1997) argues that overfishing of the female portion of the spawning stock will cause a population to shift from its fitness peak since maternal effects are phenotypic. We believe that phenotypic bottlenecking, resulting from overfishing of the female portion of the population, was a strong component of the causes of the recruitment failure of yellow perch in Lake Michigan. The Lake Michigan ecosystem has dramatically changed over the past decade and a half with the introduction of many new exotic species, particularly the zebra mussel (*Dreissena polymorpha*). It is likely that changes in the environment and high fishing pressure, have resulted in stresses

on the population. No single recruitment mechanism is likely to be responsible for recruitment variation and failure, rather a combination of many mechanisms is likely responsible (Leggett and Deblois 1994). However, Solemdal (1997) has argued that because of the complex nature of recruitment mechanisms, maternal effects may be the only recruitment mechanism that can be effectively managed in a fishery through regulation.

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References

Adams, S.M. and D.L. DeAngelis. 1987. Indirect effects of early bass-shad interactions on predator population structure and food web dynamics, p. 103-117. In: W.C. Kerfoot and A. Sih (eds.) Predation: Direct and Indirect Impacts on Aquatic Communities. University of New England Press, Hanover, N.H.

Beacham, T.D. and C.B. Murray. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhyncus keta*) from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. **42**: 1755-1765.

Bengston, D.A., R.C. Barkman, and W.J. Berry. 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. J. Fish. Biol. **31**: 697-704.

Benoit, H.P. and P. Pepin. 1999. Interaction of rearing temperature and maternal influence on egg development rates and larval size at hatch in yellowtail flounder (*Pleuoronectes ferrugineus*). Can. J. Fish. Aquat. Sci. **56**: 785-794.

Bernardo, J. 1996. Maternal effects in animal ecology. Amer. Zool. **36**: 83-105.

Buckley, L.J., A.S. Smigielski, T.A. Halavik, E.M. Caldarone, B.R. Burns, and G.C. Laurence. 1991. Winter flounder *Pseudopleuornectes americanus* reproductive success. II. Effects of spawning time and female size on size, composition and viability of eggs and larvae. Mar. Ecol. Prog. Ser. **74**: 125-135.

Chambers, R.C. and W.C. Leggett. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. Amer. Zool. **36**: 180-196.

Chambers, R.C. and W.C. Leggett. 1992. Possible causes and consequences of variation in age at size and metamorphosis in flatfishes (*Plueronectiformes*): An analysis at the individual, population, and species levels. Neth. J. Sea. Res. **29**: 7-24.

Chambers, R.C. and K.G. Waiwood. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. **53**: 1986-2003.

Chambers, R.C., W.C. Leggett, J.A. Brown. 1989. Egg size, female effects, and the correlations between early life history traits of Capelin, *Mallotus villosus*: An appraisal at the individual level. Fish. Bull. **87**: 515-523.

Chicharo, M.A. 1998. Nutritional condition and starvation in *Sardina pilchardus* (L.) larvae off southern Portugal compared with some environmental factors. J. Exp. Mar. Biol. Ecol. **225**: 123-137.

Clemmensen, C. 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Mar. Biol. **118**: 377-382.

Crowder, L.B., J.A. Rice, T.J. Miller, E.A. Marschall. 1989. Empirical and theoretical approaches to size-based interactions and recruitment variability in fishes, p. 237-255. In: DeAngelis, D.L. and L.J. Gross (eds.) Individual-Based Models and Approaches in Ecology. Chapman and Hall, New York, New York.

Francis, J.T., S.R. Robillard, and J.E. Marsden. 1996. Yellow perch management in Lake Michigan: a multi-jurisdictional challenge. Fisheries. **21**(2): 18-20.

Gall, G.A.E. 1974. Influence of size of eggs and age of female on hatchability and growth in rainbow trout. Calif. Fish and Game. **00(1)**: 26-35.

Hislop, J.R.G. 1988. The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. J. Fish. Biol. **32**: 923-930.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Amer. Fish. Soc. Symp. **2**: 17-29.

Huang, W, T. Chui, and C. Shih. 1999. Effects of maternal conditions on early life history traits of black porgy *Acanthopagrus schlegeli*. J. Appl. Ichthyol. **15**: 87-92.

Kazakov, R.V. 1981. The effect of the size of Atlantic salmon, *Salmo salar* L., eggs on embryos and alevins. J. Fish. Biol. **19**: 353-360.

Knutsen, G.M. and S. Tilseth. 1985. Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. Trans. Am. Fish. Soc. **114**: 507-511.

Laurence G.C. 1988. Toward a consensus paradigm of early life stage survival of fishes and recruitment—research in the face of variability and uncertainty. ICES ELH No. 11.

Leggett, W.C. and E. Deblois. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages. Neth. J. Sea. Res. **32**(2): 119-134.

Miller, T.J., T. Herra, and W.C. Leggett. 1995. An individual-based analysis of the variability of eggs and their newly hatched larvae of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. Can. J. Fish. Aquat. Sci. **52**: 1083-1093.

Miller, T.J., L.B. Crowder, J.A. Rice, and E.A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657-1670.

Pepin, P. 1989. Predation and starvation of larval fish: a numerical experiment of size- and growth-dependent survival. Biol. Ocean. **6**: 23-44.

Pepin, P. and T.J. Miller. 1993. Potential use and abuse of general empirical models of early life history processes in fish. Can. J. Fish. Aquat. Sci. **50**: 1343-1345.

Pepin, P., G.T. Evans, and T.H. Shears. 1999. Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field. ICES J. Mar. Sci. **56**: 697-706.

Rakitin, A., M.M. Ferguson, and E.A. Trippel. 1999. Sperm competition and fertilization success in Atlantic cod (*Gadus morhua*): effect of sire size and condition factor on gamete quality. Can. J. Fish. Aquat. Sci. **56**: 2315-2323.

Rice, J.A., T.J. Miller, K.A. Rose, L.B. Crowder, E.A. Marschall, A.S. Trebitz, D.L. DeAngelis. 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. **50**: 133-142.

Rijnsdorp, A.D. 1989. Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.). J. Cons. Int. Explor. Mer. **46**: 35-51.

Rooker, J.R., G.J. Holt, and S.A. Holt. 1997. Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. Mar. Biol. **127**: 387-394.

Solemdal, P. 1997. Maternal effects – a link between the past and the future. J. Sea. Res. **37**: 213-227.

Wisconsin Natural Resources Board (WNRB) Memorandum. 1996. File Ref. #3600.

Table 1. Maternal and larval traits measured in each of the yellow perch (*Perca flavescens*) females and sampled offspring of the ten maternal lines.

Maternal Traits		Larval Traits
	Linear measures	
Total Length (mm)		Total Length (mm)
Weight (g)		Eye Diameter (mm)
Body Depth at the Anterior Base of the First Dorsal Fin (mm) (FBD_1)		Minor Yolk Axis (mm)
Body Depth at the Posterior Base of the Second Dorsal Fin (mm) (FBD_2)		Major Yolk Axis (mm)
Age (years)		Body Depth at the Insertion Point of the Pectoral Fin (mm) (BDIP)
Egg Production (# Eggs)		Body Depth at the Insertion Point of the First Dorsal Fin (mm) (BDID)
		Body Depth at the Insertion Point of the Anus (mm) (BDIA)
	Biochemical measures	
		RNA Content (µg) (RNA)
		DNA Content (µg) (DNA)
	Volume measures	
		Yolk Volume (mm^3)
	Derived measures	
Fulton's Condition Factor k (g/mm^3)		Dry Weight (µg or mg)
Gondal Somatic Index (GSI)		RNA:DNA Ratio

Table 2. Phenotypic characteristics of the ten female yellow perch (*Perca flavescens*) caught off Green Can Reef 42° 50' 00" N, 87° 50' 00" W), Lake Michigan. Females 1-5 were collected on 2 June 1998, and Females 6-10 were collected on 4 June 1998. All trait abbreviations are as defined in Table 1.

Female	Total Length (mm)	Weight (g)	Fulton's Condition Factor (k)	Age (Years)	FBD_1	FBD_2	GSI	Egg Production
1	250	145.3	0.929856	4	53	47	0.3589	22560
2	227	124.7	1.066078	4	46	44	0.6284	11400
3	280	255.6	1.164222	4	72	57	0.5676	36075
4	249	151.3	0.980228	2	54	43	0.6170	23560
5	256	169.9	1.012683	3	55	46	0.3824	11600
6	285	261.4	1.29244	5	65	61	0.2869	36720
7	287	255.7	1.081730	6	65	62	0.2168	29440
8	216	113.1	1.122479	3	47	40	0.4064	30600
9	222	115.7	1.057760	3	45	41	0.2875	21970
10	242	115.4	0.814465	4	49	46	0.3989	32760

Table 3. Mean initial number of eggs (± SD) in each female's replicate tank (n = 6), mean number of offspring at hatching (± SD), and percent hatching success in the ten yellow perch maternal lines.

Female	Mean Initial Number of Eggs (n=3)	Mean Initial Number of Offspring (Hatching Success) (n=6)	Hatching Success (%)
1	2002 ± 14	1615 ± 289	80.7
2	1968 ± 11	89 ± 20	4.5
3	2000 ± 18	896 ± 181	44.8
4	2024 ± 15	1538 ± 133	76.0
5	1392 ± 12	1148 ± 198	82.5
6	2040 ± 12	1269 ± 228	62.2
7	2020 ± 11	1610 ± 290	79.7
8	2002 ± 6	1061 ± 381	53.0
9	2032 ± 14	1379 ± 164	67.9
10	1965 ± 17	1287 ± 188	65.5

Table 4. Mean phenotypic characteristics (± SD) of the yellow perch (*Perca flavescens*) offspring of the ten maternal lines sampled at hatching on 14 June, 1998. All trait abbreviations are as defined in Table 1.

Female	N	Mean Total Length (mm)	Mean Eye Diameter (mm)	Mean Yolk-sac Volume (mm3)	Mean BDIP (mm)	Mean BDID (mm)	Mean BDIA (mm)	Mean Dry Weight (mg)	Mean RNA (μg)	Mean DNA (μg)	Mean RNA:DNA Ratio
1	80	6.1 ± 0.47	0.38 ± 0.044	0.05 ± 0.015	0.7 ± 0.091	0.63 ± 0.087	0.56 ± 0.083	0.22 ± 0.023	3.4 ± 0.98	2.1 ± 0.34	1.7 ± 0.69
2	70	6.2 ± 0.39	0.39 ± 0.042	0.04 ± 0.014	0.68 ± 0.093	0.64 ± 0.094	0.54 ± 0.021	0.22 ±0.019	3.2 ± 0.93	2.2 ± 0.30	1.5 ± 0.45
3	78	6.1 ± 0.47	0.39 ± 0.05	0.04 ± 0.012	0.68 ± 0.086	0.66 ± 0.091	0.55 ± 0.087	0.22 ± 0.022	3.4 ± 0.5	2.1 ± 0.37	1.7 ± 0.47
4	81	6.2 ± 0.46	0.38 ± 0.044	0.05 ± 0.015	0.73 ± 0.088	0.67 ± 0.083	0.59 ± 0.079	0.23 ± 0.022	3.4 ± 0.66	2.2 ± 0.29	1.6 ± 0.43
5	76	6.1 ± 0.44	0.39 ± 0.043	0.05 ± 0.015	0.69 ± 0.086	0.64 ± 0.087	0.56 ± 0.081	0.22 ± 0.017	3.2 ± 0.6	2.1 ± 0.25	1.5 ± 0.32
6	77	5.5 ± 0.48	0.42 ± 0.047	0.06 ± 0.012	0.67 ± 0.087	0.75 ± 0.085	0.53 ± 0.069	0.20 ± 0.018	3 ± 0.61	1.9 ± 0.31	1.6 ± 0.71
7	67	6.1 ± 0.41	0.39 ± 0.045	0.07 ± 0.017	0.72 ± 0.077	0.8 ± 0.11	0.57 ± 0.088	0.24 ± 0.024	3.8 ± 0.55	2.3 ± 0.30	1.6 ± 0.29
8	75	5.6 ± 0.4	0.38 ± 0.048	0.06 ± 0.015	0.69 ± 0.091	0.8 ± 0.14	0.51 ± 0.078	0.2 ± 0.019	2.9 ± 0.68	1.8 ± 0.38	1.7 ± 0.67
9	70	5.5 ± 0.43	0.35 ± 0.04	0.05 ± 0.011	0.65 ± 0.081	0.73 ± 0.096	0.51 ± 0.075	0.19 ± 0.016	2.7 ± 0.63	1.7 ± 0.21	1.6 ± 0.41
10	78	5.7 ± 0.42	0.42 ± 0.036	0.07 ± 0.014	0.69 ± 0.096	0.76 ± 0.112	0.51 ± 0.081	0.21 ± 0.018	3.3 ± 0.53	1.9 ± 0.42	1.8 ± 0.78

Table 5. Univariate ANOVA results for tests of significant maternal effects, for individual larval traits, among the offspring of the ten maternal lines (n = 752). All trait abbreviations are as defined in Table 1. Univariate tests were performed with 8 and 48 degrees of freedom.

Larval Trait	F	Pr>F
Mean Total Length	5.86	0.0001
Mean Eye Diameter	0.55	0.8096
Mean Yolk Volume	3.45	0.0032
Mean BDIP	1.69	0.1242
Mean BDID	2.04	0.0617
Mean BDIA	1.62	0.1437
Mean Dry Weight	10.78	0.0001
Mean RNA	1.68	0.1284
Mean DNA	7.31	0.0001
Mean RNA:DNA Ratio	0.00	1.0000

Table 6. Canonical correlation analysis: female loading results for the eight female phenotypic traits of the ten female yellow perch (*Perca flavescens*). All trait abbreviations as defined in Table 1.

	Canonical Variables	
Female Traits	**Female 1**	**Female 2**
Total Length	0.0140	0.5477
Weight	-0.0476	0.4185
Fulton's Condition Factor (k)	-0.0474	-0.1657
Age	-0.4114	0.4898
GSI	0.6980	-0.0551
Egg Production	-0.5160	0.0808
FBD_1	0.0800	0.4096
FBD_2	-0.1976	0.4985

Table 7. Canonical correlation analysis: larval loading results for the ten larval phenotypic traits from the ten yellow perch (*Perca flavescens*) maternal lines. All trait abbreviations as defined in Table 1.

	Canonical Variables	
Larval Traits	**Larval 1**	**Larval 2**
Total Length	0.6226	0.4855
Eye Diameter	0.4139	0.2733
Yolk Volume	-0.6858	0.4244
BDIP	0.2217	0.4417
BDID	-0.7681	0.2451
BDIA	0.3847	0.3566
Dry Weight	0.3887	0.7954
RNA	0.1192	0.5172
DNA	0.4416	0.7385
RNA:DNA Ratio	-0.1574	0.0429

Table 8. Canonical correlation analysis: results for the eight female phenotypic traits and the 12 larval phenotypic traits of the ten maternal lines. Canonical correlation vector 1 explains 54% of the variation while canonical correlation vector 2 explains 33.6% of the variation. Canonical correlation vector 1 is positively driven by female GSI and negatively driven by egg production. Canonical correlation vector 2 is positively driven by female size and age. All traits abbreviations as defined in Table 1.

Larval Trait	Vector 1 (Driven by Female GSI (+) and Female Egg Production (-)	Vector 2 (Driven by Female Size and Age) (+)
Proportion of Variance	54 %	33.6 %
Total Length	0.5852	0.4410
Eye Diameter	0.3891	0.2483
Yolk Volume	-0.6446	0.3856
BDIP	0.2804	0.4013
BDID	-0.7220	0.2226
BDIA	0.3616	0.3240
Dry Weight	0.3653	0.7226
RNA	0.1120	0.4698
DNA	0.4151	0.6710
RNA:DNA Ratio	-0.1480	0.0390

Figure Legends

Figure 1. Annual yellow perch (*Perca flavescens*) catch per unit of effort (number of fish per 1000 ft. of gillnet per night) from the WDNRFU winter gillnet survey over Green Can Reef (42° 50' 00" N, 87° 50' 00" W) (a historic yellow perch spawning ground) in Lake Michigan from 1986 to 1998.

Figure 2. Average size at age for Lake Michigan female yellow perch (*Perca flavescens*) collected from fishery independent surveys conducted by Ball State University in Illinois waters from 1983 to 1995 (S.M. Shroyer, Department of Biology, Ball State University, Muncie, IN 47306-0440, USA "personal communication").

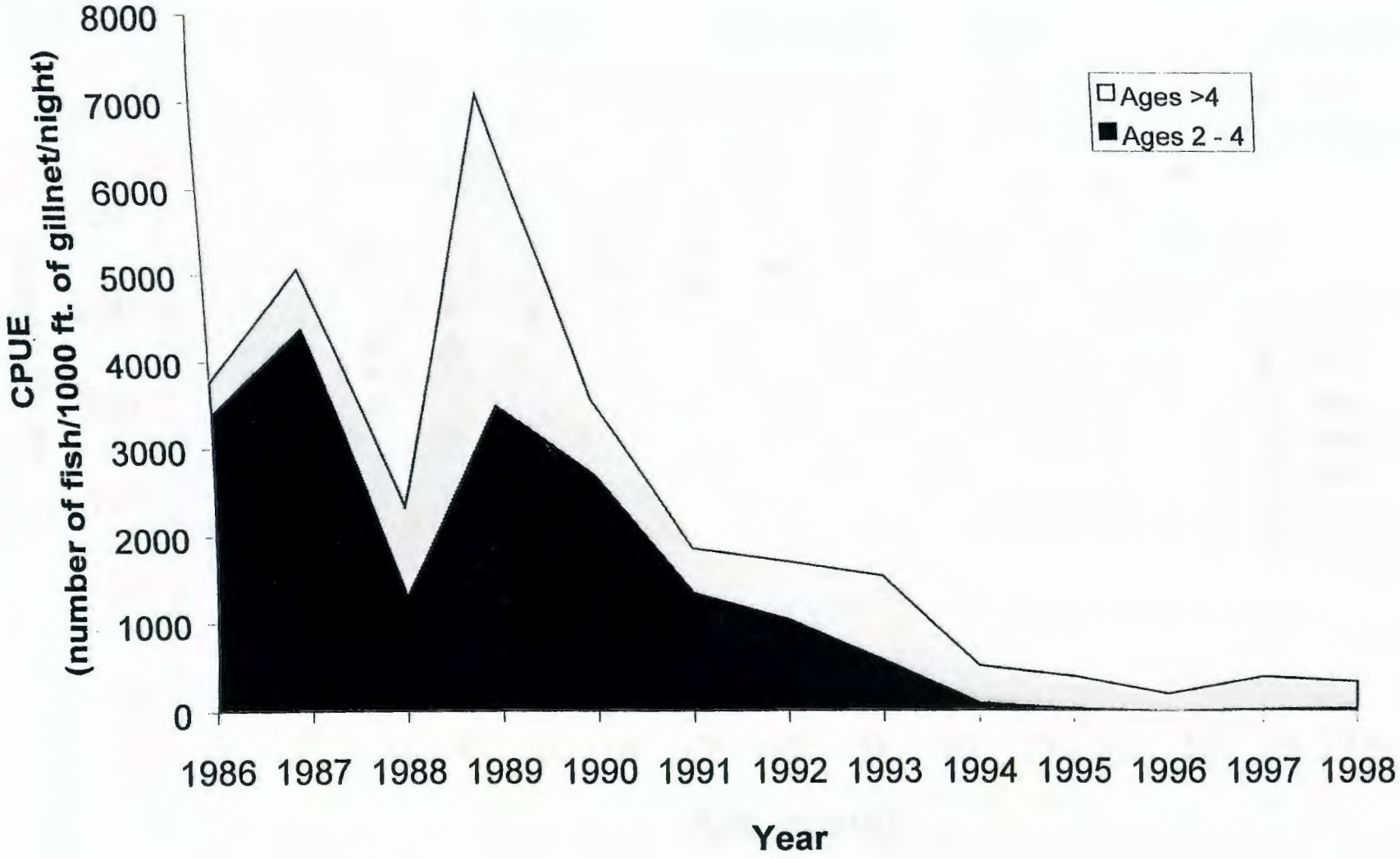
Figure 3. Bivariate correlations among 10 larval traits of yellow perch (*Perca flavescens*). Data plotted are mean values of each trait based on analysis of between 67-81 larvae in each of 60 tanks that comprised the experiment.

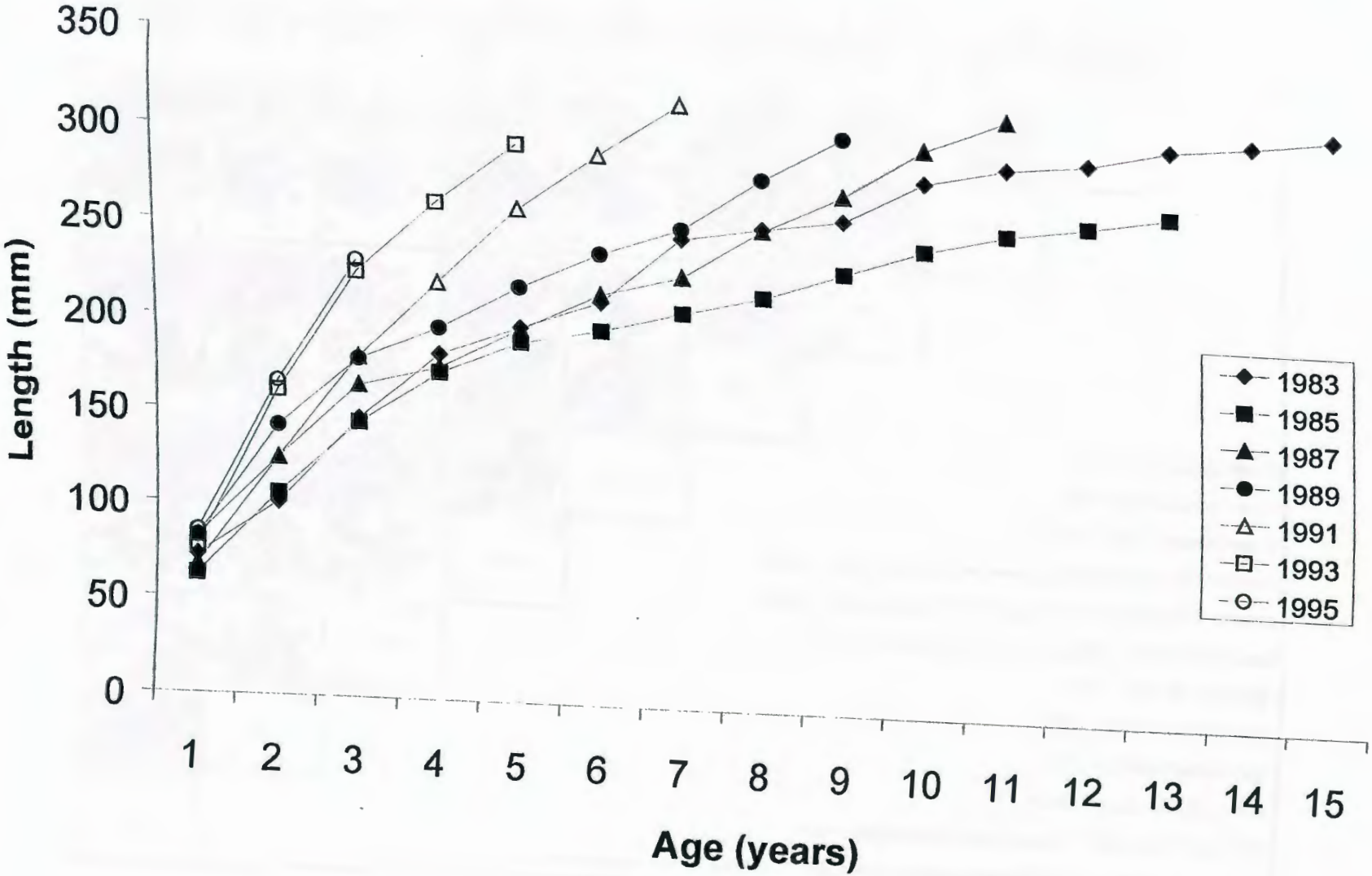
Figure 4. Box and whiskers plot of total length of yellow perch (*Perca flavescens*) offspring from ten maternal lines at hatching. The upper and lower limits of the box represent the 3rd (Q_3) and 1st (Q_1) quartiles, respectively. The solid horizontal line within the box represents the median value of the data. The dashed horizontal line within the box represents the mean value of the data. The upper and lower extremes of the whiskers represent (Q_3+1.5*(Q_3-Q_1)) and (Q_1-1.5*(Q_3-Q_1)), respectively. Data outside of the whiskers are considered outliers, and are represented by open circles. Closed circles represent the minimum and maximum values of the data.

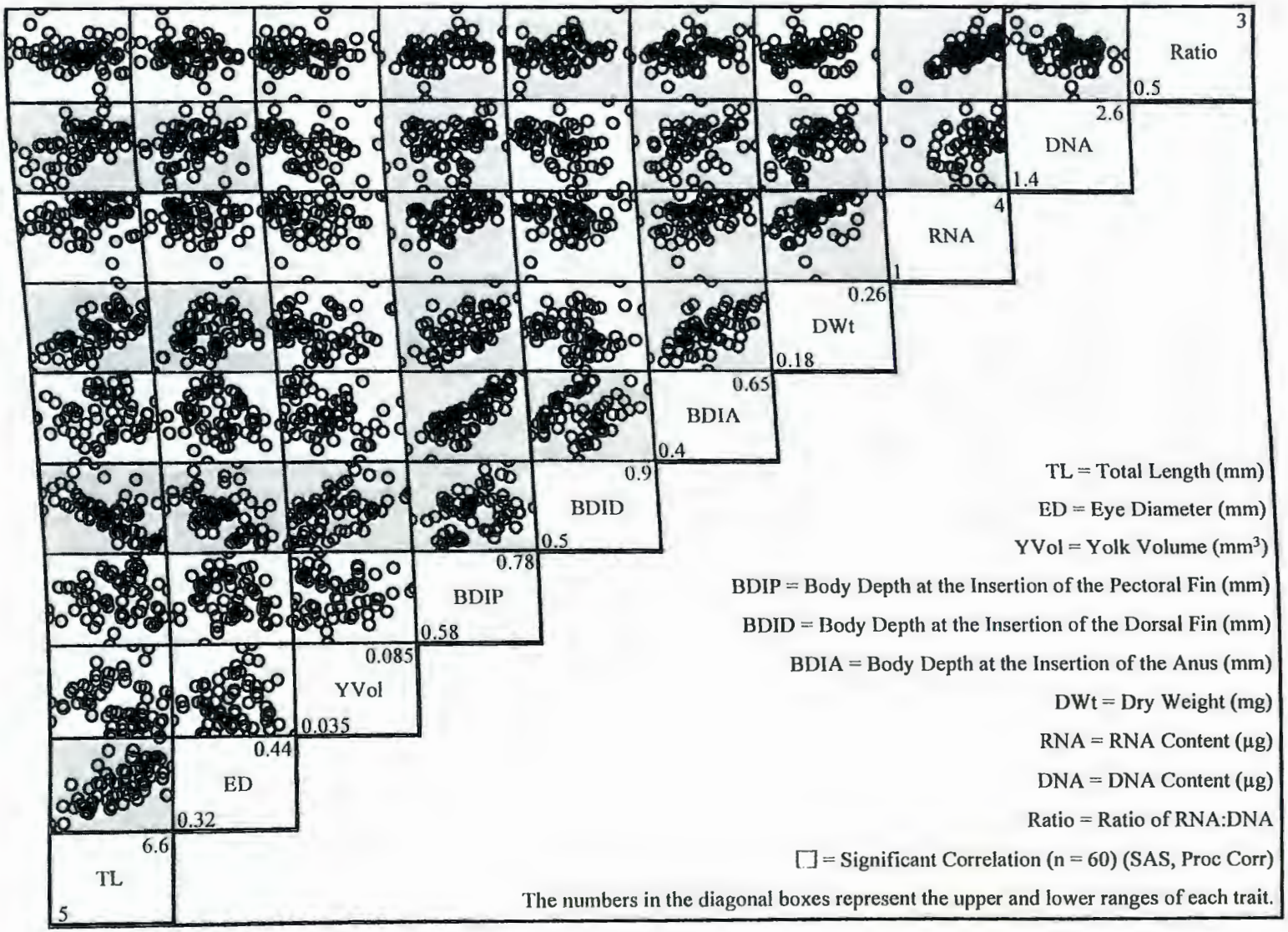
Figure 5. Box and whiskers plot of dry weight of yellow perch (*Perca flavescens*) offspring from ten maternal lines at hatching. Box-whisker antennae as defined in Fig. 4.

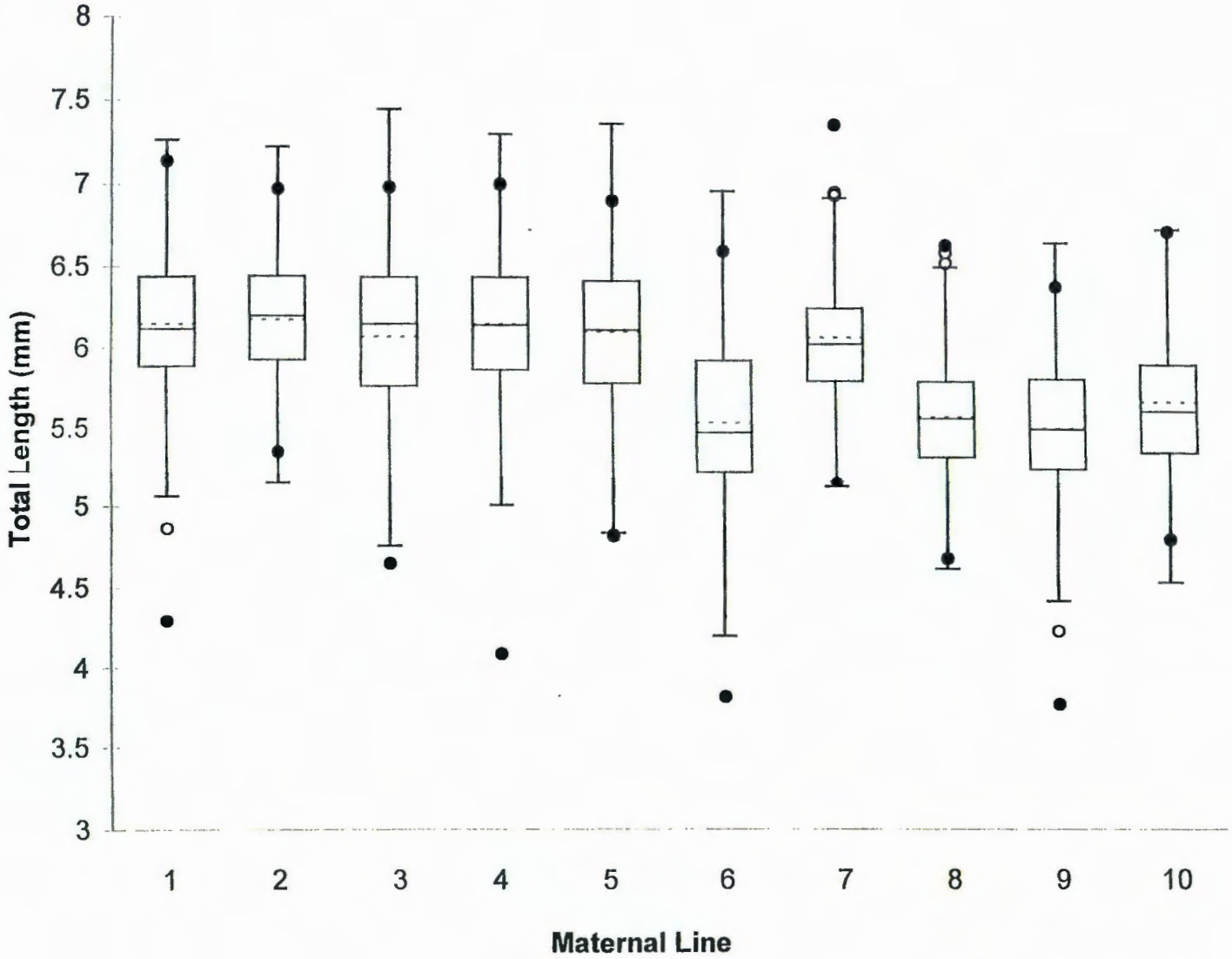
Figure 6. Box and whiskers plot of yolk volume of yellow perch (*Perca flavescens*) offspring from ten maternal lines at hatching. Box-whisker antennae as defined in Fig. 4.

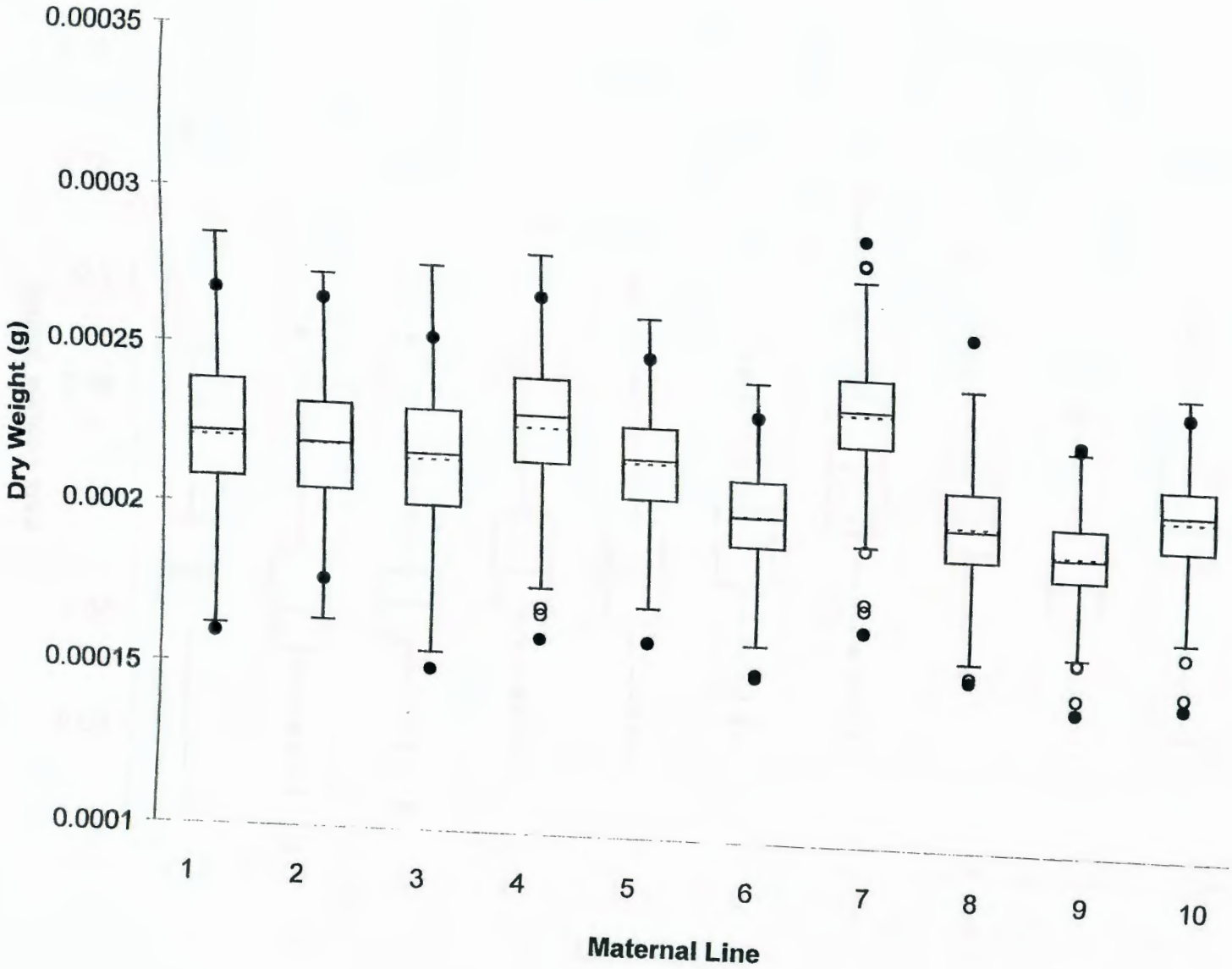
Figure 7. Box and whiskers plot of DNA content of yellow perch (*Perca flavescens*) offspring from ten maternal lines at hatching. Box-whisker antennae as defined in Fig. 4.


8000
7000
6000
5000
4000
3000
2000
1000
0
CPUE
(number of fish/1000 ft. of gillnet/night)
Ages >4
Ages 2 - 4
1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998
Year


Length (mm)
350
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Age (years)
1983
1985
1987
1989
1991
1993
1995


3
Ratio
0.5
2.6
DNA
1.4
4
RNA
1
0.26
DWt
0.18
0.65
BDIA
0.4
0.9
BDID
0.5
0.78
BDIP
0.58
0.085
YVol
0.035
0.44
ED
0.32
6.6
TL
5
TL = Total Length (mm)
ED = Eye Diameter (mm)
YVol = Yolk Volume (mm3)
BDIP = Body Depth at the Insertion of the Pectoral Fin (mm)
BDID = Body Depth at the Insertion of the Dorsal Fin (mm)
BDIA = Body Depth at the Insertion of the Anus (mm)
DWt = Dry Weight (mg)
RNA = RNA Content (µg)
DNA = DNA Content (µg)
Ratio = Ratio of RNA:DNA
☐ = Significant Correlation (n = 60) (SAS, Proc Corr)
The numbers in the diagonal boxes represent the upper and lower ranges of each trait.


8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
Total Length (mm)
1
2
3
4
5
6
7
8
9
10
Maternal Line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0.00025
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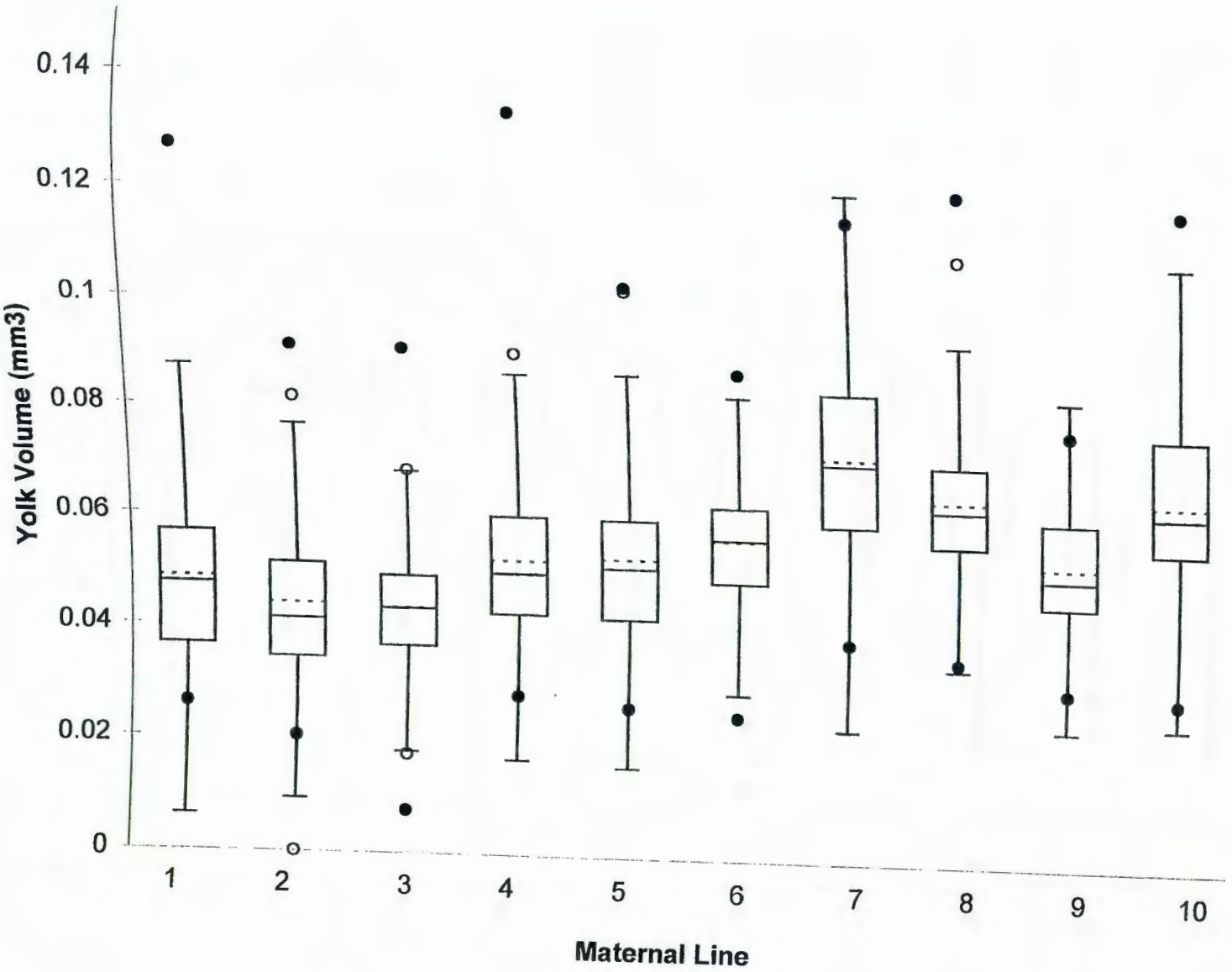
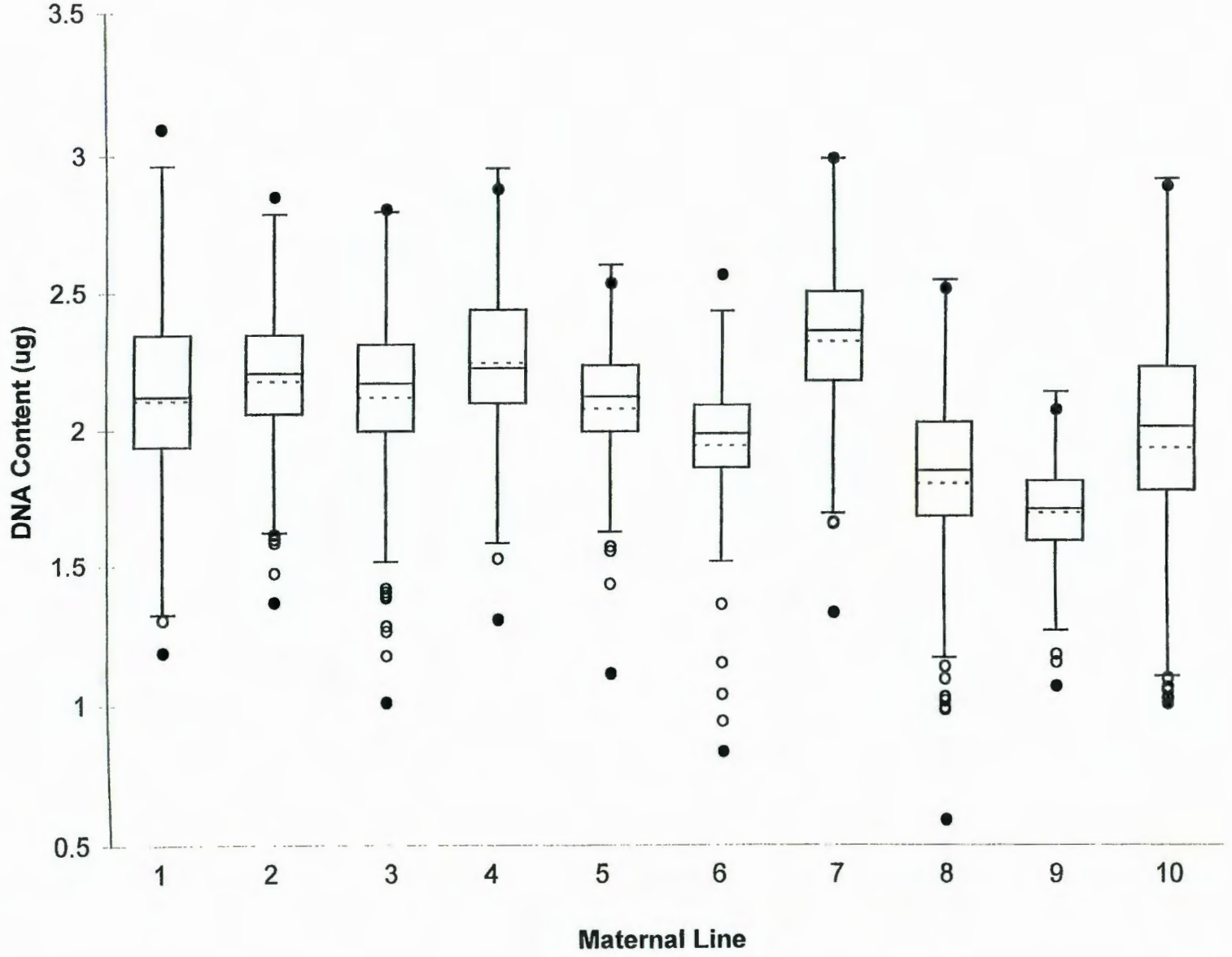
0.00015
0.0001
Dry Weight (g)
1
2
3
4
5
6
7
8
9
10
Maternal Line


0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Yolk Volume (mm3)
1
2
3
4
5
6
7
8
9
10
Maternal Line


3.5
3
2.5
2
1.5
1
0.5
DNA Content (ug)
1
2
3
4
5
6
7
8
9
10
Maternal Line

Chapter 3

Understanding Maternal Effects as a Recruitment Mechanism in Lake Michigan Yellow Perch (*Perca flavescens*)

II. Resistance to Starvation

Abstract

Maternal effects have been detected previously in Lake Michigan yellow perch (*Perca flavescens*). The persistence and potential importance of maternal effects under conditions of food limitation were examined between hatching and the time of 100% mortality in ten maternal lines. Furthermore, the importance of maternal effects were evaluated under such conditions to test the null hypothesis of no difference among the maternal lines in their resistance to starvation. By 4 days post hatch (dph) nearly all larvae had fully absorbed their yolk-sacs. Maternal effects were detectable at hatching and at four and six dph, but not at two dph. Analyses lead to a rejection of the null hypothesis and indicated that the detected maternal effects resulted in differences of a factor of two among the maternal lines in resistance to starvation. Furthermore, larvae that exhibited the lowest resistance to starvation were long and had small yolk volumes, while larvae that exhibited the highest resistance to starvation were characterized as short with and large yolk volumes. Rates of mortality differed among the yellow perch maternal lines. There was evidence for a mortality threshold based on an RNA:DNA ratio of about 1.15. The observed differences in resistance to starvation can have substantial implications for recruitment success. Maternal effects in yellow perch result in differential mortality of larval progeny under conditions of starvation.

Introduction

Recruitment in fishes can vary substantially from year to year, a fact that has been recognized since 1914 when Johan Hjort first addressed it. For over 80 years fisheries scientists have sought to understand what mechanisms may control recruitment and cause the high inter-annual variability observed in many exploited fish species. One potential controlling mechanism of recruitment that received substantial attention during the early 1900s and throughout most of the 20th century was starvation.

The magnitude of mortality directly attributable to starvation, and thus the magnitude of starvation effects on recruitment remain unclear at the present time (Leggett and Deblois 1994). Starvation-induced mortality depends not only on food availability but also on the individual larva's resistance to starvation (Rice et al. 1987). Research has recognized that starvation does not affect all individuals equally. Resistance to starvation increases with larval size (Hunter 1981; Miller et al. 1988), and increased resistance to starvation affords greater flexibility in encountering food and thus increases the chance for survival (Chambers et al. 1989).

Recently, researchers have recognized that even if the importance of food limitation as a direct recruitment controller may be small, its importance as a modifier to predation may be quite large. Food limitation can reduce escape swimming speeds (Elliot and Leggett 1997; Litvak and Leggett 1992) thus increasing a larva's vulnerability to predation. Furthermore, food limitation can lead to reduced growth rates (Buckley 1984), which can result in lengthened stage durations and thus increased exposure to predation (Houde 1987; Bertram et al. 1997). Furthermore, food limitation reduces larval body tissues and reserves that are essential for survival. Such starvation-induced changes

in larval traits may result in increased vulnerability to predation. Larvae that are more resistant to starvation will exhibit reduced rates of change in larval size and thus, are likely to be less vulnerable to predation than larvae that are less resistant to starvation. Larvae that exhibit high resistance to starvation are more likely to exhibit greater swimming ability that will allow them to better avoid and elude predators (Rice et al. 1987) and search longer and further for suitable prey (Miller et al. 1988) than larvae with low resistance to starvation.

Maternal effects can control larval size at hatching (Beacham and Murray 1985; Bengston et al. 1987; Chambers et al. 1989; Chambers and Leggett 1992, and this thesis - Chapter 2). Additionally, research on maternal effects has shown that larger females typically produce larger larvae that are more resistant to starvation (Huang et al 1999). Previously, we have shown that maternal effects were present at hatching in ten maternal lines of Lake Michigan yellow perch (*Perca flavescens*) (Chapter 2). These maternal effects were primarily driven by female size and age and were expressed in the hatchlings by differences in larval total length, yolk volume, dry weight and DNA content (Chapter 2).

Research has shown that yellow perch larvae can exhibit high sensitivity to food limitation. Yellow perch larvae, fed on exogenous food sources until they reached a length of 10 mm, experienced 50% mortality in an average of 6 days after exogenous feeding was experimentally limited (Letcher et al. 1996). Letcher et al. (1996) also measured time to 50% inactivity, the time at which 50% of the larvae within a tank were laying inactive on the bottom. Time to 50% inactivity in yellow perch occurred, on average, 2.6 days before the onset of 50% mortality (Letcher et al. 1996).

These findings, when coupled with previous findings of maternal effects in yellow perch, lead us to evaluate the importance of maternal effects on differences in resistance to starvation among hatchlings of different maternal lines. As recruitment mechanisms such as starvation have been found to be size-dependent (Miller et al. 1988), we hypothesized that the offspring of the ten maternal lines would have varying vulnerabilities to starvation due to the initial differences in larval size and yolk volume that were observed at hatching (Chapter 2). This study aimed to investigate the importance of maternal effects on the resistance to starvation in Lake Michigan yellow perch larvae. We sought to determine if differences in larval traits observed at hatching resulted in differences in survival, expressed as resistance to continuous starvation from hatching. Additionally, we wished to quantify which larval traits most accounted for differences in resistance to starvation if found, and whether the maternal effects expressed at hatching persisted throughout the duration of starvation.

Materials and Methods

We investigated our objectives within a single experiment, which involved monitoring and quantifying several larval traits and survival of larval yellow perch when starved from hatch. All larvae used were the offspring of the ten maternal lines described in Chapter 2. Experimental work was conducted at the University of Wisconsin's WATER Institute, Milwaukee, WI. The experiment was conducted as a randomized complete block design, consisting of three blocks of 20 10-gallon flow-through aquaria with two replicates per maternal line per block.

Full details of the collection and husbandry of eggs and larvae are given in Chapter 2, and are only summarized here. Male and female yellow perch were collected on 2 June and 4 June 1998 in the vicinity of Green Can Reef (42° 50' 00" N, 87° 50' 00" W) off Milwaukee, WI. Eggs were fertilized in the field and returned to the lab. Egg volumes, sufficient to produce approximately 2000 larvae, were incubated in each of 60 10-gallon flow-through, temperature-controlled aquaria.

Husbandry of Larvae

Hatching began on 14 June 1998 in all tanks. After the removal of the broken egg skeins and any dead eggs and larvae, the larvae in all 60 tanks were sampled for the presence of maternal effects at hatch. After sampling at hatch, one of two treatments, fed or starved, was randomly applied to one replicate tank of each maternal line within each block. The 30 starved treatment tanks were not given any exogenous food throughout the duration of the experiment. The flow rate and temperature in the tanks were consistent with successful yellow perch rearing conditions established at the WATER Institute from previous research. Mortalities in each tank were removed daily and enumerated.

Larval Sampling

At hatching, 15 larvae were sampled from each tank. Subsequently, five larvae were sampled every two days post hatch (dph) until 100% mortality occurred in all tanks. We quantified 12 larval traits (9 morphometric and 3 biochemical measures of condition - Table 1) for each larva sampled from each tank on each sampling day. Larvae were anesthetized with Tricaine Methansulfonate (MS-222), and then videotaped for analysis

of morphometry. Individual larvae were placed into cryovials, flash-frozen in liquid nitrogen, and then stored at –80 °C for subsequent extraction of nucleic acids.

Post-Sampling Processing of Larvae

Morphometric analysis of the larvae was conducted on videotape images of larvae using Optimas (v 6.1, Media Cybernetics, Takoma Park, MD) as defined in Chapter 2. Larval total length, eye diameter, two-dimensional yolk surface area (calculated internally by Optimas from the outline of the yolk), the major and minor axes of the yolk, body depth at the insertion point of the pectoral fin, body depth at the insertion point of the first dorsal fin (inclusive of yolk-sac), body depth at the insertion point of the anus, and body area (calculated internally by Optimas from the outline of the larvae) were measured for each individual larva sampled (Table 1). From these primary measurements yolk-sac volume and dry weight were estimated using methods defined in Chapter 2. Extraction and determination of nucleic acids was also performed as described in Chapter 2, with two exceptions. Microplates were read on a fluorescence plate reader at Ex = 590 nm, Em = 625 nm (Spectra Max Gemini, Molecular Devices Corp., Sunnyvale, CA). Furthermore, growth and development of the larvae following absorption of their yolk-sacs required an additional dilution of the extracted nucleic acid supernatant (Table 2). This additional dilution factor was used in the conversion of RNA and DNA concentration to RNA and DNA content (concentration*1.5 ml*additional dilution factor).

Statistics

Separate statistical analyses were conducted to address each of our objectives. All statistical analyses were conducted using SAS (v 6.12, SAS Institute, Inc., Cary, NC). For all traits except larval yolk volume, the analyses were conducted from hatching through six dph. Virtually 100% mortality had occurred by seven dph in all tanks. The persistence of significant maternal effects throughout the duration of the experiment (time at 100% mortality in all tanks) was determined by multivariate analysis of variance (MANOVA; SAS Proc GLM) on all larval traits, except survival, for each sampling day. The MANOVA was based on the mean value of each larval trait within each starved tank, for each maternal line nested within the date of fertilization (2 June or 4 June 1998). Significance of MANOVA was determined based upon a Wilks' Lambda statistic with varying degrees of freedom.

Variation in survival over time and among the maternal lines was evaluated by repeated measures analysis of variance (MANOVA; SAS Proc GLM). The proportion of larvae surviving on each day was arcsine transformed before analysis. The repeated measures analysis was based on the mean value of survival within each tank, for each maternal line on each sampling day. Repeated measures analysis was conducted 0, 2, 4 and 6 dph. Significance of the test of the null hypothesis of no significant difference in survival over time (i.e. time effect) was determined based upon a Wilks' Lambda statistic with 3 and 16 degrees of freedom. Significance of repeated measures analysis for the test of the null hypothesis of no difference in survival among the offspring of the ten maternal lines over time (i.e. interaction between mother and time) was determined based upon a

Wilks' Lambda statistic with 27 and 47.37 degrees of freedom. This test of significance examines whether the offspring of the maternal lines were dying at different rates.

The repeated measures analysis was conducted on the sampling dates indicated above for all larval traits. The analysis was based on the mean value of each larval trait within each tank, for each maternal line on each sampling day. However, by four dph, full yolk-sac absorption had been observed in all but two of 150 sampled larvae. Therefore, repeated measures analysis for larval yolk volume was only conducted from hatching through two dph. Significance of repeated measures analysis for the test of the null hypothesis of no variation in the larval traits (Table 1) over time was determined based upon a Wilks' Lambda statistic with 3 and 16 degrees of freedom for the morphometric traits, with 1 and 20 degrees of freedom for larval yolk volume and with 3 and 15 degrees of freedom for the biochemical traits. Differences among the starved offspring of the maternal lines over time were determined by repeated measures analysis on each larval trait at each sampling day. Significance of repeated measures analysis for the test of the null hypothesis of no difference among the offspring of each maternal line for the larval traits over time was determined based upon a Wilks' Lambda statistic with 27 and 47.37 degrees of freedom for the morphometric traits, with 9 and 20 degrees of freedom for larval yolk volume, and with 27 and 44.45 degrees of freedom for the biochemical traits. These tests of significance examine whether the ten maternal lines have different rates of change for a given larval trait over time. Significance of repeated measures analysis was determined based upon an F statistic with 9 denominator degrees of freedom for the test of the null hypothesis of no difference among the offspring of each maternal line for a given larval trait (i.e. mother effect). This test of significance is

analogous to evaluating differences in the absolute magnitude of a given larval trait among the offspring of the ten maternal lines.

Results

Significant maternal effects were detected at hatching and on two of the three post hatch sampling days. At two dph we were unable to reject the null hypothesis of no overall maternal effect among the offspring of the ten maternal lines for the larval traits based upon MANOVA results ($F_{80, 65.65} = 1.33$, $p = 0.1143$). However, at four dph and again on six dph we were able to detect a significant overall maternal effect among the offspring of the ten maternal lines based upon MANOVA results (Table 3).

Mortality rates differed among the offspring of the ten maternal lines by a factor of two. Time to 50% mortality ranged from two to six dph among the offspring of the ten maternal lines. The offspring of Maternal $Line_3$ were the least resistant to starvation, reaching 50% mortality by 2.7 ± 0.58 days. The offspring of Maternal $Line_6$ were the most resistant to starvation, reaching 50% mortality by 6.3 ± 0.58 days (Table 4). At 6 dph the proportion of larvae surviving varied from 10 ± 9.1 % to 54 ± 13.0 (Table 4). Repeated measures analysis showed that survivorship varied significantly over time (Table 5). We were able to reject the null hypothesis of no difference in the survivorship of the offspring of the ten maternal lines over time (Table 5). The rate of mortality was significantly different among the offspring of the ten maternal lines ($F_{27, 47.37} = 3.097$, $p = 0.0003$).

A positive relationship between mean larval yolk volume at hatching and mean time to 50% mortality was observed among the maternal lines (Fig. 1). However, no

strong relationships were observed between any of the other larval traits measured at hatching and time to 50% mortality. While there is a positive relationship between larval yolk volume at hatching and time to 50% mortality, there is a negative relationship between larval total length at hatching and time to 50% mortality (Fig. 1).

We quantified the trait variability at 50% mortality for the offspring of the ten maternal lines. Mean larval total length at the time of 50% mortality ranged from 5.7 ± 0.17 mm (Maternal $Line_9$) to 6.28 ± 0.096 mm (Maternal $Line_7$) (Fig. 2, Table 6). Larval yolk volume at the time of 50% mortality varied by a factor of two among the offspring of the ten maternal lines, ranging from 0.0373 ± 0.0014 mm^3 (Maternal $Line_2$) to 0.0689 ± 0.0066 mm^3 (Maternal $Line_7$) (Fig. 3, Table 6). Body depth at the insertion of the first dorsal fin ranged between 0.58 ± 0.042 mm (Maternal $Line_2$) and 0.71 ± 0.032 mm (Maternal $Line_7$). Larval dry weight ranged between 0.199 ± 0.0034 mg (Maternal $Line_8$) and 0.237 ± 0.0068 μg (Maternal $Line_7$) (Fig. 4, Table 6). RNA content at the time of 50% mortality ranged from 2.4 ± 0.18 μg (Maternal $Line_8$) to 3.5 ± 0.53 μg (Maternal $Line_7$) (Fig. 5), while DNA content did not exhibit large variation, ranging between 2.2 ± 0.19 μg (Maternal $Line_8$) and 2.9 ± 0.31 μg (Maternal $Line_7$) (Table 6). Condition, the ratio of RNA:DNA, at the time of 50% mortality also exhibited low variation, ranging between 1.1 ± 0.37 (Maternal $Line_1$) and 1.26 ± 0.074 (Maternal $Line_7$) (Fig. 6, Table 6).

A strong positive relationship was observed among the maternal lines between mean larval yolk volume at the time of 50% mortality and mean time to 50% mortality (Fig. 7). However, no strong relationship was observed between other larval traits measured at time of 50% mortality and time to 50% mortality. While there is a positive relationship between larval yolk volume at time of 50% mortality and time to 50%

mortality, there is a negative relationship between larval total length at time of 50% mortality and time to 50% mortality (Fig. 7).

Starvation induced changes in the traits of the offspring from the ten maternal lines were observed between hatching and the time of 100% mortality for all maternal lines. Between hatching and two dph, yolk absorption was highly variable among the offspring of the ten maternal lines. However, by four dph virtually all larvae had fully absorbed their yolk-sacs. Between hatching and two dph, larval yolk volume decreased by as much as 40 ± 17.7 % (Maternal $Line_4$) and by as little as 1 ± 29.7 % (Maternal $Line_{10}$). Some offspring of Maternal $Line_9$ had increased yolk volumes by two dph, thus the average change in yolk volume for Maternal $Line_9$ was negative. By six dph, large variation in body depth at the insertion of the first dorsal fin was observed in all maternal lines. Between hatching and six dph, body depth at the insertion of the first dorsal fin decreased by as much as 40 ± 3.1 % (Maternal $Line_{10}$) and by as little as 9 ± 38.7 % (Maternal $Line_7$) (Table 7). Body depth at the insertion of the first dorsal fin increased in some offspring of Maternal $Line_1$, as indicated by a mean change in body depth at the insertion of the first dorsal fin of −1 ± 52.2 %. Variation in the dry weight of the offspring from the ten maternal lines was much lower by six dph than for other traits. Larval dry weight decreased by six dph by as much as 12 ± 6.6 % (Maternal $Line_1$) and by as little as 1 ± 3.9 % (Maternal $Line_9$). Offspring from Maternal $Line_6$, Maternal $Line_7$ and Maternal $Line_{10}$ exhibited increases in larval dry weight over the six day period (Table 7). RNA content and the ratio of RNA:DNA decreased in all maternal lines by six dph while DNA increased. Decreases in the RNA content of the offspring from the ten maternal lines ranged from 47.3 ± 38.15 % (Maternal $Line_1$) to 69 ± 14.8 % (Maternal

$Line_4$) (Table 7), while decreases in the ratio of RNA:DNA ranged from 53 ± 42.1 % (Maternal $Line_1$) to 72.4 ± 10.46 % (Maternal $Line_4$) (Table 7). DNA content increased by as much as 42 ± 6.1 % (Maternal $Line_2$) by six dph, and by as little as 8 ± 12.5 % (Maternal $Line_4$) (Table 7).

All larval traits except body depth at the insertion of the pectoral fin varied significantly over time (Table 8). We were unable to reject the null hypothesis of no significant difference in the larval traits of the offspring of the ten maternal lines over time (Time*Mother Interaction in Table 8). This indicates that the change in larval traits observed over time was not significantly different among the offspring of the ten maternal lines. Furthermore, repeated measures ANOVA allowed us to reject the null hypothesis of no overall difference in larval total length, larval yolk volume, body depth at the insertion of the first dorsal fin and larval dry weight of the offspring of the ten maternal lines (Table 8). No significant differences in body depth at the insertion of the pectoral fin, body depth at the insertion of the anus, RNA content, and the ratio of RNA:DNA among the ten maternal lines were detected through repeated measures ANOVA (Table 8).

Discussion

We detected significant maternal effects at hatching and at 4 and 6 dph. There were no detectable maternal effects at two dph. There were significant differences in the survival of offspring from the ten maternal lines. Time to 50% mortality and the larval traits at time of 50% mortality varied greatly among the ten maternal lines. Furthermore, while the rate of mortality was significantly different among the offspring of the ten

maternal lines, the rates of change of all the larval traits during the 6 day period were not significantly different from one another.

It is not clear why we were unable to detect maternal effects at two dph. One possible explanation is that larval yolk volume was a strong driving factor in the analysis. Differential yolk absorption rates may have resulted in increased within maternal line variation in yolk-sac volume, resulting in an apparent reduction in between maternal line variation and thus a lack of detectable maternal effects. By four dph, virtually all larvae had fully absorbed their yolk-sacs and larval yolk volume was eliminated as a trait in the analysis for the detection of maternal effects at four dph. The absence of larval yolk volume at four and six dph in the MANOVA may have resulted in the significant detection of maternal effects among the offspring of the ten maternal lines for the remaining traits.

The rate of mortality differed significantly among the offspring of the ten maternal lines. Raising the question of which traits could have accounted for the differences. We found significant differences in larval size and yolk volume among the offspring of the ten maternal lines. While the absolute measures of each of these traits were significantly different among the offspring of the ten maternal lines, the rate of change of these traits were not significantly different among the ten maternal lines. Furthermore, the non-significant differences in the rates of change of the larval traits among the offspring of the ten maternal lines indicates that differences in survival can only be attributed to differences in larval size and yolk volume at hatch. It is probable that the absolute magnitude of larval size and yolk volume would result in survival

differences of the offspring due to size-dependent susceptibilities to starvation (Hunter 1981; Miller et al. 1988).

A two-fold difference in the time to 50% mortality was observed among the offspring of the ten maternal lines. Maternal $Line_2$ and Maternal $Line_3$ exhibited the lowest resistance to starvation. The offspring of Maternal $Line_2$ and Maternal $Line_3$ were characterized by the lowest hatching successes, long total length and small yolk volume (Chapter 2). The offspring of Maternal $Line_6$ exhibited the highest resistance to starvation and were characterized by moderate hatching success, short total length and large yolk volume (Chapter 2). This suggests that larval total length and larval yolk volume are important factors in determining a larva's resistance to starvation. Furthermore, it appears as if the trade-off between larval size and larval yolk volume observed at hatching is more than an anomaly (Chapter 2). While previous research has demonstrated that larger larvae typically have larger yolk-sacs and are more resistant to starvation than smaller larvae (Hunter 1981, Miller et al. 1988), these results suggest that in yellow perch a trade-off between larval total length and larval yolk volume exists and that it is not the size of the larva that determines resistance to starvation, but rather the relative size of the yolk-volume.

When we examine the individual larval traits at the time of 50% mortality we find that differences in larval yolk volume and body depth at the insertion of the first dorsal fin, which is inclusive of larval yolk volume, characterize differences observed in the time to 50% mortality. Furthermore, we find that larvae with large yolk volumes at hatching exhibit longer times to 50% mortality. This provides further support to the conclusion regarding the importance of larval yolk volume at hatch. Offspring of

Maternal $Line_2$ and Maternal $Line_3$ had small yolk volumes and shallow measures of body depth at the insertion of the first dorsal fin while the offspring of Maternal $Line_6$ had large yolk volumes and relatively deep measures of body depth at the insertion of the first dorsal fin. This suggests that the yolk volume and the body depth at the first dorsal fin in newly hatched larvae are important predictors of resistance to starvation, as measured by time to 50% mortality.

The lack of variability in the ratio of RNA:DNA among the ten maternal lines at the time of 50% mortality is striking. The consistency among maternal lines suggests that there may be a minimum threshold in the ratio of RNA:DNA that is necessary for survival. Our results indicate that an estimate for this mortality threshold in yellow perch based on an RNA:DNA ratio is about 1.15. This estimate is similar to values reported for other species under conditions of starvation. Reported values for RNA:DNA ratios at or near the 'point of no return' have been reported for Atlantic herring *Clupea harengus* larvae as 1.1 and 1.2 (Clemmesen 1987; Ueberschär and Clemmesen 1992, respectively), for striped bass *Morone saxatilis* larvae as 1.2 (Wright and Martin 1985), for North Sea plaice *Pleuronectes platessa* larvae as 1.0 (Hovenkamp 1990), and for turbot *Scophthalmus maximus* larvae as 1.3 (Clemmesen 1987).

The changes observed in the larval traits over the duration of starvation were by and large expected, and indicate which body morphometrics are first reduced when food availability is low. The yolk is the only food source that larvae have during periods of starvation, so naturally larval yolk volume would decrease. Decreases in dry weight of larvae also were expected due to the depletion of reserves and metabolic expenditures. Starvation-induced reductions in dry weight of 10-25 mm larval yellow perch have been

reported as high as 53% reduction from original mass (Letcher et al. 1996). We observed only 1-12% reductions in dry weight as a consequences of starvation. The differences in dry weight reductions of starving yellow perch may be attributable to age differences. Decreases in the RNA content and RNA:DNA ratio of the larvae throughout starvation also were expected. The condition of larvae decreases in the absence of food. The decline of RNA and the ratio of RNA:DNA during starvation has been observed for many species in the field and in the laboratory (Buckley 1980; 1984; Clemmesen 1987; Westerman and Holt 1994; Rooker and Holt 1996; Elliot and Leggett 1998).

With the exception of the observed increase in DNA content, the observed increases in some larval traits were striking. Previous research has shown that increases in DNA content during periods of low food availability have been shown to be indicative of starvation when coupled with decreases in RNA content (Buckley 1979; 1981; Clemmesen 1994). Given that the quantity of DNA per cell is relatively constant within somatic tissues of a species (Clemmesen 1994), we would conclude that cell proliferation is continuing even though food is limited. The observed increases in some maternal lines in mean larval total length, body depth at the insertion point of the pectoral fin, body depth at the insertion point of the first dorsal fin, body depth at the insertion point of the anus, and dry weight may be indicative of within maternal line differences in resistance to starvation.

Much of the previous research on maternal effects has neglected to focus on the expression of maternal effects in larval traits, and even less research has focused on the empirical evaluation of maternal effects on the survival of offspring. Many previous studies have only assumed that maternally influenced differences in larval traits at

hatching, primarily larval size, result in differences in survival (Chambers and Leggett 1996, Solemdal 1997). These assumptions have been based upon findings that larval survival is size-dependent (Houde 1987; Hunter 1981; Miller et al. 1988). One example of direct empirical evidence of significant maternal effects on the survival of offspring under conditions of starvation has been shown for black porgy *Acanthopagrus schlegeli* (Huang et al. 1999). Huang et al. (1999) observed differences in resistance to starvation among the offspring of 30 maternal lines. They found that larger females produced larger offspring that were more resistant to starvation than smaller offspring from smaller females (Huang et al. 1999). Our findings contradict those found in black porgy. We have found that small females produce short larvae with low dry weight that have large yolk-sacs and that are more resistant to starvation than the longer larvae with high dry weight and small yolk-sacs produced by large females. This contradiction in findings suggests that maternal effects may operate differently across species.

Understanding differences in the survival of offspring among different maternal lines under conditions of starvation is important to the understanding of what drives recruitment. If females with favorable phenotypic traits produce larvae that have a high resistance to starvation, than a portion of those females should be managed to conserve their traits to ensure against environmental uncertainty. Many changes have occurred in Lake Michigan over the past decade that have lead to changes in plankton abundance, consequently, it is important to protect yellow perch females with a wide distribution of traits that will produce larvae with a wide distribution of traits, in order to protect the population from environmental uncertainty and continued recruitment failures.

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References

Beacham, T.D. and C.B. Murray. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhyncus keta*) from Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. **42**: 1755-1765.

Bengston, D.A., R.C. Barkman, and W.J. Berry. 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. J. Fish. Biol. **31**: 697-704.

Bertram, D.F, T.J. Miller, and W.C. Leggett. 1997. Individual variation in growth and development during early life stages of winter flounder *Pleuronectes americanus*. Fish. Bull. **95**: 1-10.

Buckley, L.J. 1979. Relationship between RNA-DNA ratio, prey density and growth rate in Atlantic cod (*Gadus morhua*) larvae. J. Fish. Res. Board Can. **36**: 1497-1502.

Buckley, L.J. 1980. Changes in the ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. Fish. Bull. 77: 703-708.

Buckley, L.J. 1981. Biochemical changes during ontogenesis of cod (*Gadus morhua* L.) and winter flounder (*Pseudopleuronectes americanus*) larvae. Rapp P-v Réun Cons. perm. int. Explor. Mer. **178**: 547-552.

Buckley, L.J. 1984. RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. **80**: 291-298.

Chambers, R.C. and W.C. Leggett. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. Amer. Zool. **36**: 180-196.

Chambers, R.C. and W.C. Leggett. 1992. Possible causes and consequences of variation in age at size and metamorphosis in flatfishes (*Plueronectiformes*): An analysis at the individual, population, and species levels. Neth. J. Sea. Res. **29**: 7-24.

Chambers, R.C., W.C. Leggett, and J.A. Brown. 1989. Egg size, female effects, and the correlations between early life history traits of capelin, *Mallotus villosus*: an appraisal at the individual level. Fish. Bull. **87**: 515-523.

Clemmesen, C.M. 1987. Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. J. Cons. Int. Explor. Mer. **43**: 122-128.

Clemmesen, C.M. 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Mar. Biol. **188**: 377-382.

Elliott, J.K. and W.C. Leggett. 1998. Larval condition and vulnerability to predation: and analysis based on mixed-prey experiments. Can. J. Fish. Aquat. Sci. **55**: 626-630.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Amer. Fish. Soc. Symp. **2**: 17-29.

Hovenkamp, F. 1990. Growth differences in larval plaice *Pleuronectes platessa* in the Southern Bight of the North Sea as indicated by otolith increments and RNA/DNA ratios. Mar. Ecol. Prog. Ser. **58**: 205-215.

Huang, W., T. Chui, and C. Shih. 1999. Effects of maternal conditions on early life history traits of black porgy *Acanthopagrus schlegeli*. J. Appl. Ichthyol. **15**: 87-92.

Hunter, J.R. 1981. Feeding ecology and predation of marine fish larvae, p. 33-79. In: Lasker, R. (ed.) Marine Fish Larvae: Morphology, Ecology, and Relation to Fisheries. University of Washington Press, Seattle, Washington.

Leggett, W.C. and E. Deblois. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages. Neth. J. Sea. Res. **32(2)**: 119-134.

Letcher, B.H., J.A. Rice, and L.B. Crowder. 1996. Size-dependent effects of continuous and intermittent feeding on starvation time and mass loss in starving yellow perch larvae and juveniles. Trans. Am. Fish. Soc. **125**: 14-26.

Litvak, M.K. and W.C. Leggett. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Prog. Ser. **81**: 13-24.

May, R.C. 1974. Larval mortality in marine fishes and the critical period concept. In: J.H.S. Blaxter (ed.). The Early Life History of Fish. Springer-Verlag, Berlin. pp. 3-19.

Miller, T.J., L.B. Crowder, J.A. Rice, and E.A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can J. Fish. Aquat. Sci. **45**: 1657-1670.

Rice, J.A., L.B. Crowder, and F.P. Binkowski. 1987. Evaluating potential sources of mortality for larval bloater (*Coregonus hoyi*): starvation and vulnerability to predation. Can. J. Fish. Aquat. Sci. **44**: 467-472.

Rooker, J.R. and G.J. Holt. 1996. Application of RNA:DNA ratios to evaluate the condition and growth of larval and juvenile red drum (*Sciaenops ocellatus*). Mar. Freshwater Res. **47**: 283-290.

Solemdal, P. 1997. Maternal effects – a link between the past and the future. J. Sea. Res. **37**: 213-227.

Ueberschär, B. and C. Clemmesen. 1992. A comparison of the nutritional condition of herring larvae as determined by two biochemical methods – tryptic enzyme activity and RNA/DNA ratio measurements. ICES J. mar. Sci. **49**: 245-249.

Westerman, M. and G.J. Holt. 1994. RNA:DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. Mar. Biol. **121**: 1-9.

Wright, D.A. and F.D. Martin. 1985. The effect of starvation on RNA/DNA ratios and growth in larval striped bass, *Morone saxatilis*. J. Fish. Biol. **27**: 479-485.

Table 1. Larval traits measured in each of the yellow perch (*Perca flavescens*) offspring of the ten maternal lines.

Linear measures
Total Length (mm)
Eye Diameter (mm)
Body Depth at the Insertion Point of the Pectoral Fin (mm) (BDIP)
Body Depth at the Insertion Point of the First Dorsal Fin (mm) (BDID)
Body Depth at the Insertion Point of the Anus (mm) (BDIA)
Biochemical measures
RNA Content (μg) (RNA)
DNA Content (μg) (DNA)
Volume measures
Yolk Volume (mm^3)
Derived measures
Dry Weight (μg or mg)
RNA:DNA Ratio

Table 2. Dilution factors used in the fluorometric determination of RNA content and DNA content in the offspring of the ten yellow perch (*Perca flavescens*) maternal lines.

Age (dph)	Mean Total Length (mm)	Dilution Factor	Supernatant (µl)	0.1% Sarcosyl (µl)
0	5.9	0	1000	0
2	6.2	0	1000	0
4	7.1	2	500	500
6	6.1	2	500	500

Table 3. MANOVA results for tests of significance for all morphometric and biochemical larval traits, among the offspring of the ten yellow perch (*Perca flavescens*) maternal lines.

Day	df	F	Pr>F
0	80, 255.92	2.67	0.0001
2	80, 65.65	1.33	0.1143
4	72, 68.41	1.86	0.0053
6	72, 50.16	1.66	0.0298

Table 4. Mean time to 50% mortality and percent survival at 6 dph (± SD) (n = 3) of the offspring of the ten yellow perch (*Perca flavescens*) maternal lines over time, based upon the mean value for each tank per female on each sampling day.

Maternal Line	Minimum (days post hatch)	Maximum (days post hatch)	Mean (days post hatch)	% Survival at 6 dph
1	4	5	4.3 ± 0.58	17 ± 9.2
2	2	4	3 ± 1.2	28 ± 6.8
3	2	3	2.7 ± 0.58	10 ± 9.1
4	4	6	5 ± 1.2	38 ± 8.5
5	5	5	5 ± 0	28 ± 10.8
6	6	7	6.3 ± 0.58	44 ± 7.6
7	5	7	6 ± 1	49 ± 3.1
8	3	6	5 ± 1.2	36 ± 12.8
9	5	7	6 ± 1.2	54 ± 13.0
10	3	6	4 ± 1.5	42 ± 6.9

Table 5. Repeated measures ANOVA results for tests of significance among the yellow perch (*Perca flavescens*) offspring of the ten maternal lines (n = 118). Tests of significant differences among the offspring of the ten maternal lines were performed with 9 degrees of freedom (Mother). Tests of significant time effects were performed with 3 and 16 degrees of freedom (Time). Tests of significant differences among the offspring of the ten maternal lines over time (Time*Mother) were performed with 27 and 47.37 degrees of freedom.

	Mother at Age (dph)						
	0	**2**	**4**	**6**	**Mother**	**Time**	**Time*Mother**
Survival		**	**	**	**	***	***

Table 6. Mean larval traits at 50% mortality (± SD) (n = 3) of the offspring of the ten yellow perch (*Perca flavescens*) maternal lines over time, based upon the mean value for each tank per female on each sampling day. All trait abbreviations are as defined in Table 1.

	Maternal Line									
Larval Trait	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Total Length (mm)	6.2 ± 0.15	6.25 ± 0.057	6.1 ± 0.19	6.27 ± 0.097	6.12 ± 0.076	5.9 ± 0.23	6.28 ± 0.096	5.75 ± 0.082	5.7 ± 0.17	5.92 ± 0.017
Yolk Volume (mm^3)	0.04756 ± 0.00077	0.0373 ± 0.0014	0.0379 ± 0.0023	0.0466 ± 0.0049	0.0477 ± 0.0093	0.0576 ± 0.0084	0.0689 ± 0.0066	0.0593 ± 0.0021	0.0550 ± 0.0023	0.0630 ± 0.0061
BDIP (mm)	0.73 ± 0.092	0.68 ± 0.030	0.67 ± 0.010	0.72 ± 0.023	0.680 ± 0.0095	0.73 ± 0.088	0.78 ± 0.054	0.70 ± 0.012	0.66 ± 0.024	0.70 ± 0.026
BDID (mm)	0.60 ± 0.058	0.58 ± 0.042	0.59 ± 0.020	0.061 ± 0.043	0.058 ± 0.027	0.69 ± 0.032	0.71 ± 0.032	0.63 ± 0.035	0.63 ± 0.063	0.66 ± 0.049
BDIA (mm)	0.55 ± 0.062	0.52 ± 0.024	0.52 ± 0.011	0.054 ± 0.035	0.51 ± 0.015	0.55 ± 0.053	0.59 ± 0.027	0.49 ± 0.019	0.50 ± 0.034	0.51 ± 0.028
Dry Weight (mg)	0.213 ± 0.0065	0.213 ± 0.0048	0.208 ± 0.0073	0.224 ± 0.0078	0.2105 ± 0.00065	0.213 ± 0.0019	0.237 ± 0.0068	0.199 ± 0.0034	0.201 ± 0.0031	0.213 ± 0.0061
RNA (µg)	3 ± 1.2	3 ± 1.0	3.1 ± 0.60	2.9 ± 0.80	2.7 ± 0.41	2.5 ± 0.21	3.5 ± 0.53	2.4 ± 0.18	3 ± 1.2	2.7 ± 0.11
DNA (µg)	2.6 ± 0.37	2.7 ± 0.31	2.6 ± 0.29	2.7 ± 0.37	2.5 ± 0.16	2.3 ± 0.21	2.9 ± 0.31	2.2 ± 0.19	2.5 ± 0.73	2.4 ± 0.15
RNA:DNA Ratio	1.1 ± 0.37	1.1 ± 0.23	1.2 ± 0.16	1.1 ± 0.11	1.12 ± 0.093	1.116 ± 0.0082	1.26 ± 0.074	1.2 ± 0.29	1.1 ± 0.15	1.21 ± 0.079

Table 7. Mean percent decrease (± SD) (n = 3) in the larval traits of the yellow perch (*Perca flavescens*) offspring of the ten maternal lines sampled over six days, based upon the mean value for each tank per female on each sampling day. Negative percentages indicate an increase in the value of the larval trait. All trait abbreviations are as defined in Table 1.

	Maternal Line									
Larval Trait	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Total Length	3 ± 16.7	0.9 ± 4.3	0.2 ± 5.4	-2 ± 3.8	0.4 ± 5.1	-9 ± 5.6	-4 ± 2.5	-6 ± 1.3	-5 ± 3.2	-9 ± 4.1
BDIP	-32 ± 67.4	8 ± 14.1	-5 ± 6.8	16 ± 7.8	10 ± 10.3	-32 ± 65.3	-41 ± 61.8	-2 ± 5.1	6 ± 5.1	3 ± 11.9
BDID	-1 ± 52.2	28 ± 20.9	24 ± 15.5	32 ± 4.2	30 ± 7.2	14 ± 43.0	9 ± 38.7	34 ± 2.5	38 ± 2.3	40 ± 3.1
BDIA	-6 ± 51.6	11 ± 12.2	9 ± 17.0	31 ± 6.4	22 ± 12.6	-8 ± 48.9	-19 ± 59.5	12 ± 7.8	15 ± 8.5	14 ± 12.6
Dry Weight	12 ± 6.6	5 ± 9.8	9 ± 9.3	11 ± 9.9	8.3 ± 3.1	-2 ± 3.4	-1 ± 10.3	4 ± 3.4	1 ± 3. 9	-3 ± 4.0
RNA	47 ± 38.2	58 ± 6.6	56 ± 19.6	69 ± 14.8	61 ± 17.8	62 ± 19.2	52 ± 3.5	53 ± 23.9	52 ± 9. 5	59 ± 16.0
DNA	-22 ± 14.4	-42 ± 6.1	-28 ± 18.3	-8 ± 12.5	-15 ± 18.8	-17 ± 9.9	-37 ± 22.3	-37 ± 15.2	-28 ± 13.3	-28 ± 2.3
RNA:DNA Ratio	53 ± 42.1	54 ± 30.4	65 ± 17.2	72 ± 10.5	67 ± 11.6	68 ± 15.6	64 ± 1.6	67 ± 19.2	62 ± 8.9	69 ± 12.3

Table 8. Repeated measures ANOVA results for tests of significance among the yellow perch (*Perca flavescens*) offspring of the ten maternal lines. Tests of significant differences among the offspring of the ten maternal lines were performed with 9 degrees of freedom (Mother). Tests of significant time effects (Time) were performed with 3 and 16 degrees of freedom for all morphometric larval traits (n = 118), except yolk volume, which was performed with 1 and 20 degrees of freedom (n = 61). Tests of significant time effects (Time) were performed with 3 and 15 degrees of freedom for all biochemical larval traits (n = 117). Tests of significant differences among the offspring of the ten maternal lines over time (Time*Mother) were performed with 27 and 47.37 degrees of freedom for all morphometric larval traits, except yolk volume, which was performed with 9 and 20 degrees of freedom. Tests of significant differences among the offspring of the ten maternal lines over time (Time*Mother) were performed with 27 and 44.45 degrees of freedom for all biochemical larval traits. All trait abbreviations are as defined in Table 1.

	Mother at Age (dph)						
	0	**2**	**4**	**6**	**Mother**	**Time**	**Time*Mother**
Larval Total Length	**	*	***		***	***	
Larval Yolk Volume	***	***			***	***	
BDIP							
BDID	*				**	***	
BDIA						**	
Larval Dry Weight	***		***	**	***	**	
RNA						***	
DNA	**					***	
Ratio						***	

Figure Legend

Figure 1. Relationship between mean time to 50% mortality (n = 3) and mean larval total length ($r^2 = 0.2462$), yolk volume ($r^2 = 0.3434$) and dry weight ($r^2 = 0.0348$) at hatching (n = 751) for each maternal line (n=10) of yellow perch (*Perca flavescens*).

Figure 2. Box and whiskers plot of total length of yellow perch (*Perca flavescens*) offspring from the ten maternal lines at the time of 50% mortality (n = 3). The upper and lower limits of the box represent the 3rd (Q_3) and 1st (Q_1) quartiles, respectively. The solid horizontal line within the box represents the median value of the data. The dashed horizontal line within the box represents the mean value of the data. The upper and lower extremes of the whiskers represent (Q_3+1.5*(Q_3-Q_1)) and (Q_1-1.5*(Q_3-Q_1)), respectively. Data outside of the whiskers are considered outliers, and are represented by open circles. Closed circles represent the minimum and the maximum values of the data.

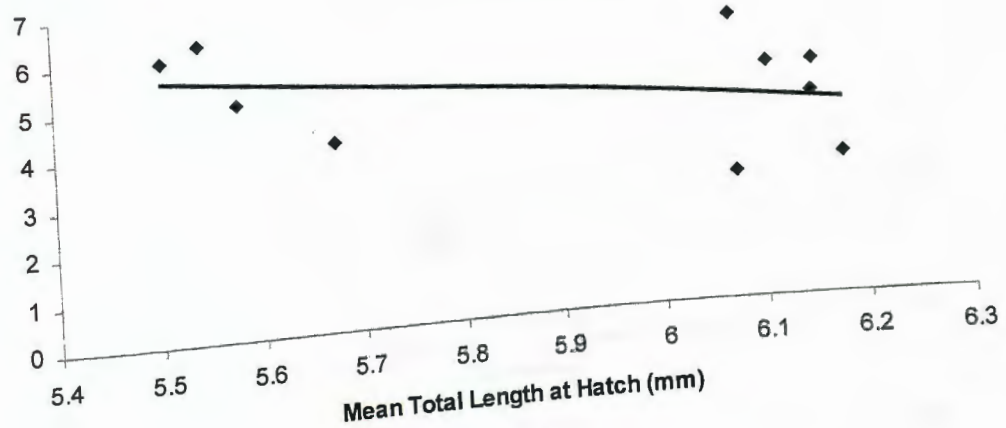
Figure 3. Box and whiskers plot of yolk volume of yellow perch (*Perca flavescens*) offspring from the ten maternal lines at the time of 50% mortality (n = 3). Box-whisker antennae as defined in Fig. 1.

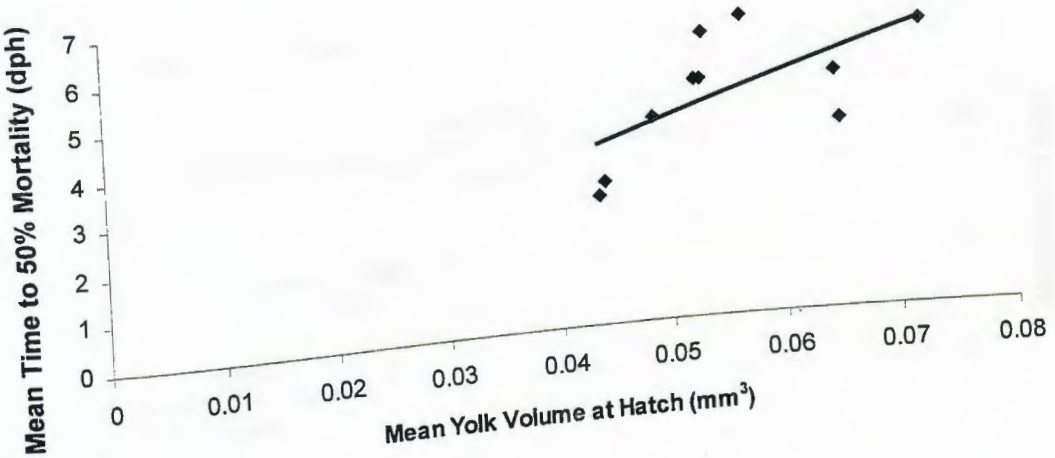
Figure 4. Box and whiskers plot of dry weight of yellow perch (*Perca flavescens*) offspring from the ten maternal lines at the time of 50% mortality (n = 3). Box-whisker antennae as defined in Fig. 1.

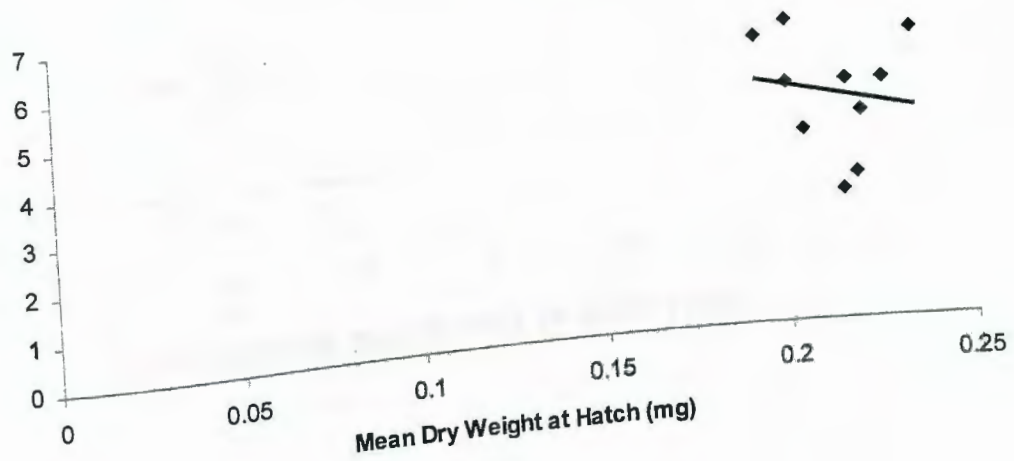
Figure 5. Box and whiskers plot of RNA content of yellow perch (*Perca flavescens*) offspring from the ten maternal lines at the time of 50% mortality (n = 3). Box-whisker antennae as defined in Fig. 1.

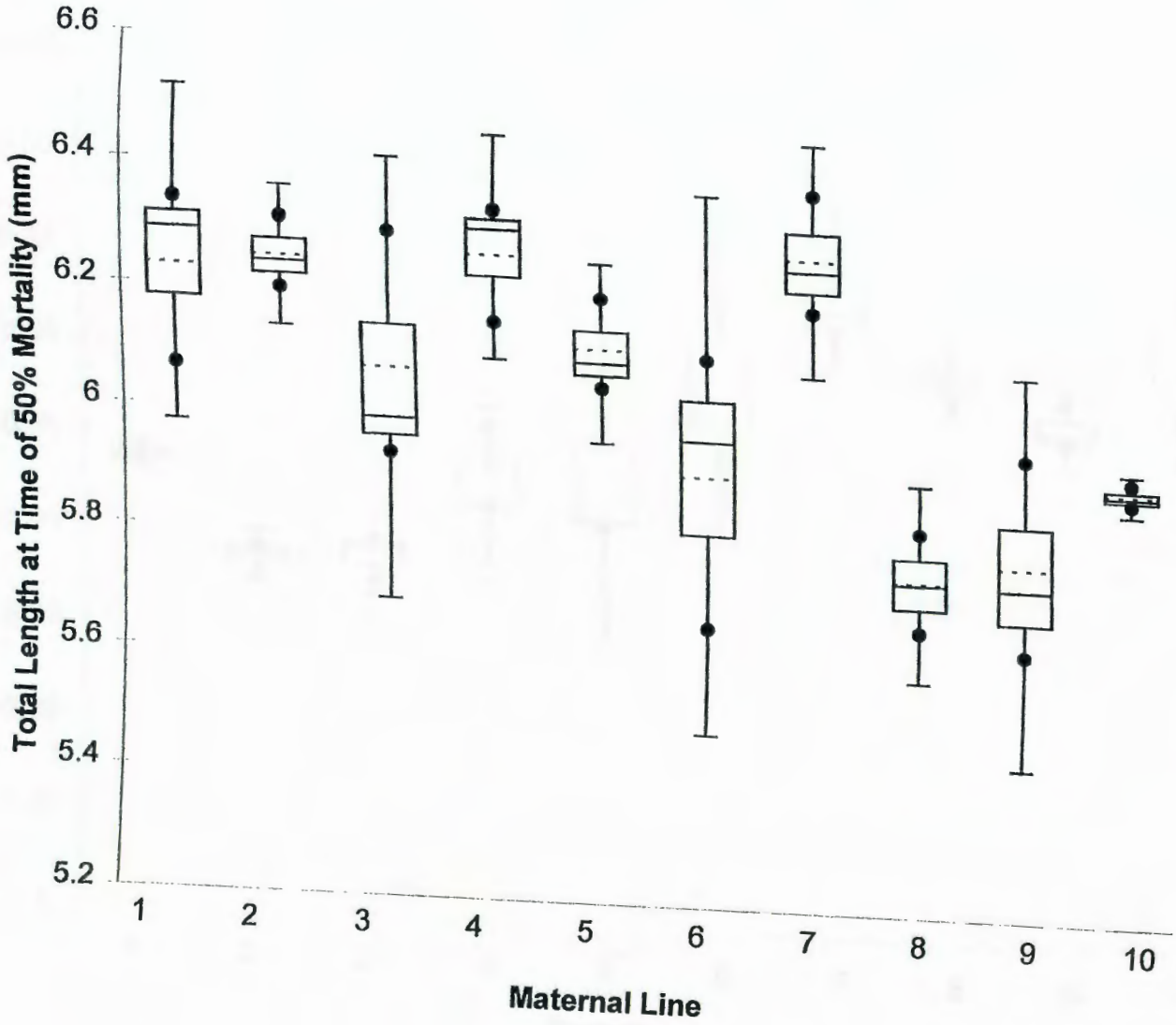
Figure 6. Box and whiskers plot of RNA:DNA ratio of yellow perch (*Perca flavescens*) offspring from the ten maternal lines at the time of 50% mortality (n = 3). Box-whisker antennae as defined in Fig. 1.

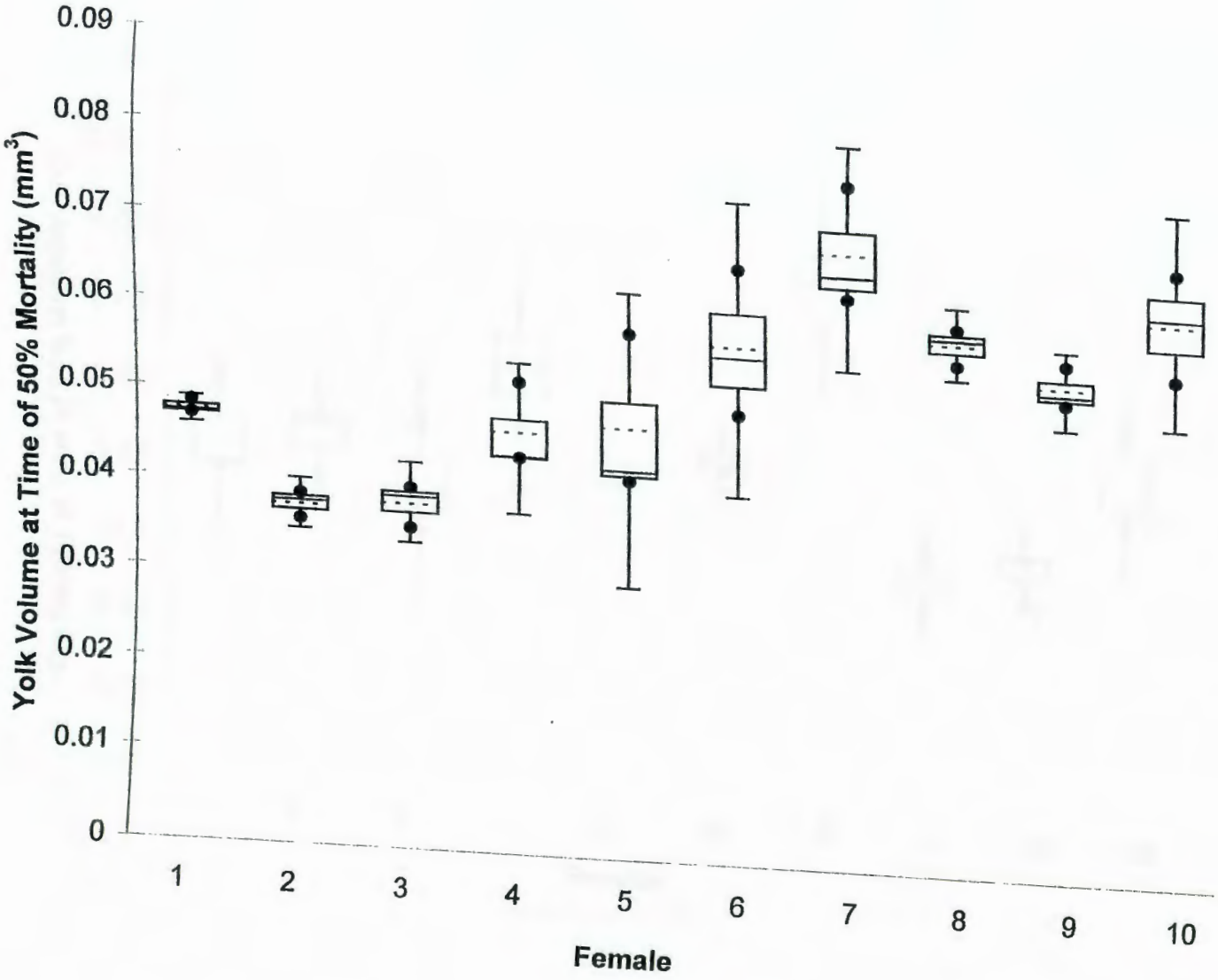
Figure 7. Relationship between mean time to 50% mortality (n = 3) and mean larval total length ($r^2 = 0.0797$), yolk volume ($r^2 = 0.491$) and dry weight ($r^2 = 0.0354$) at time of 50% mortality (n = 15) for each maternal line (n = 10) of yellow perch (*Perca flavescens*).

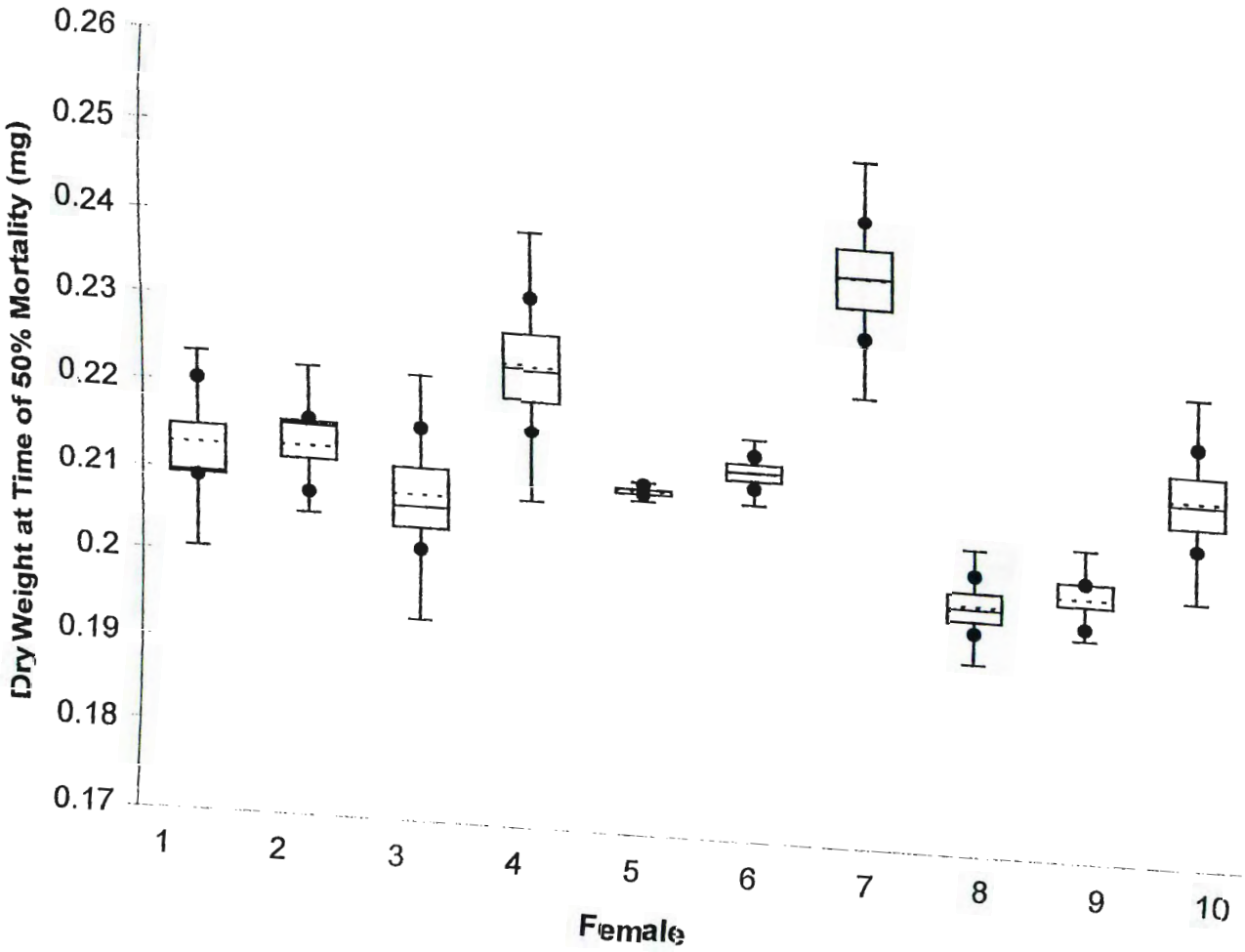

7
6
5
4
3
2
1
0
5.4
5.5
5.6
5.7
5.8
5.9
6
6.1
6.2
6.3
Mean Total Length at Hatch (mm)

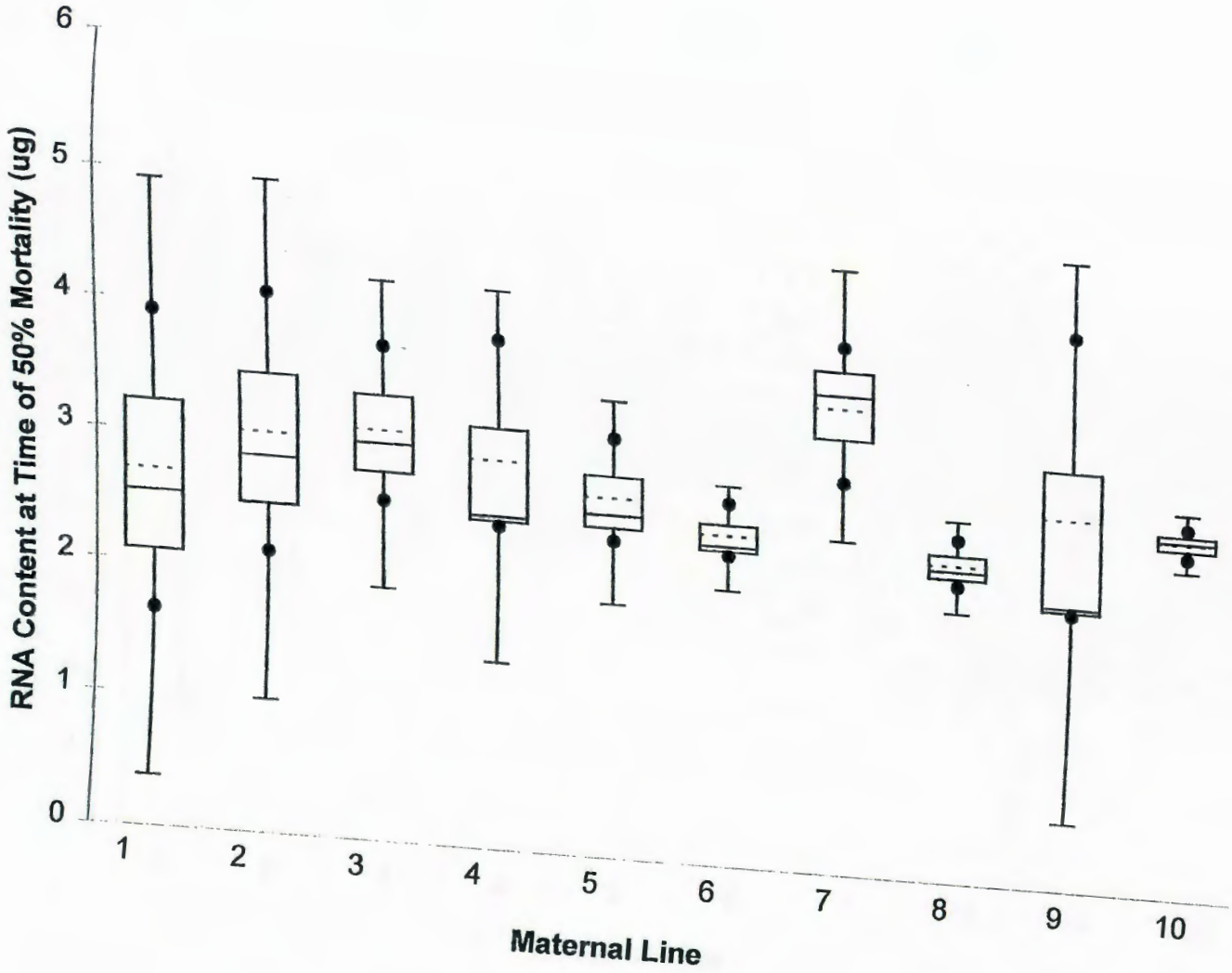

Mean Time to 50% Mortality (dph)
7
6
5
4
3
2
1
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Mean Yolk Volume at Hatch (mm3)

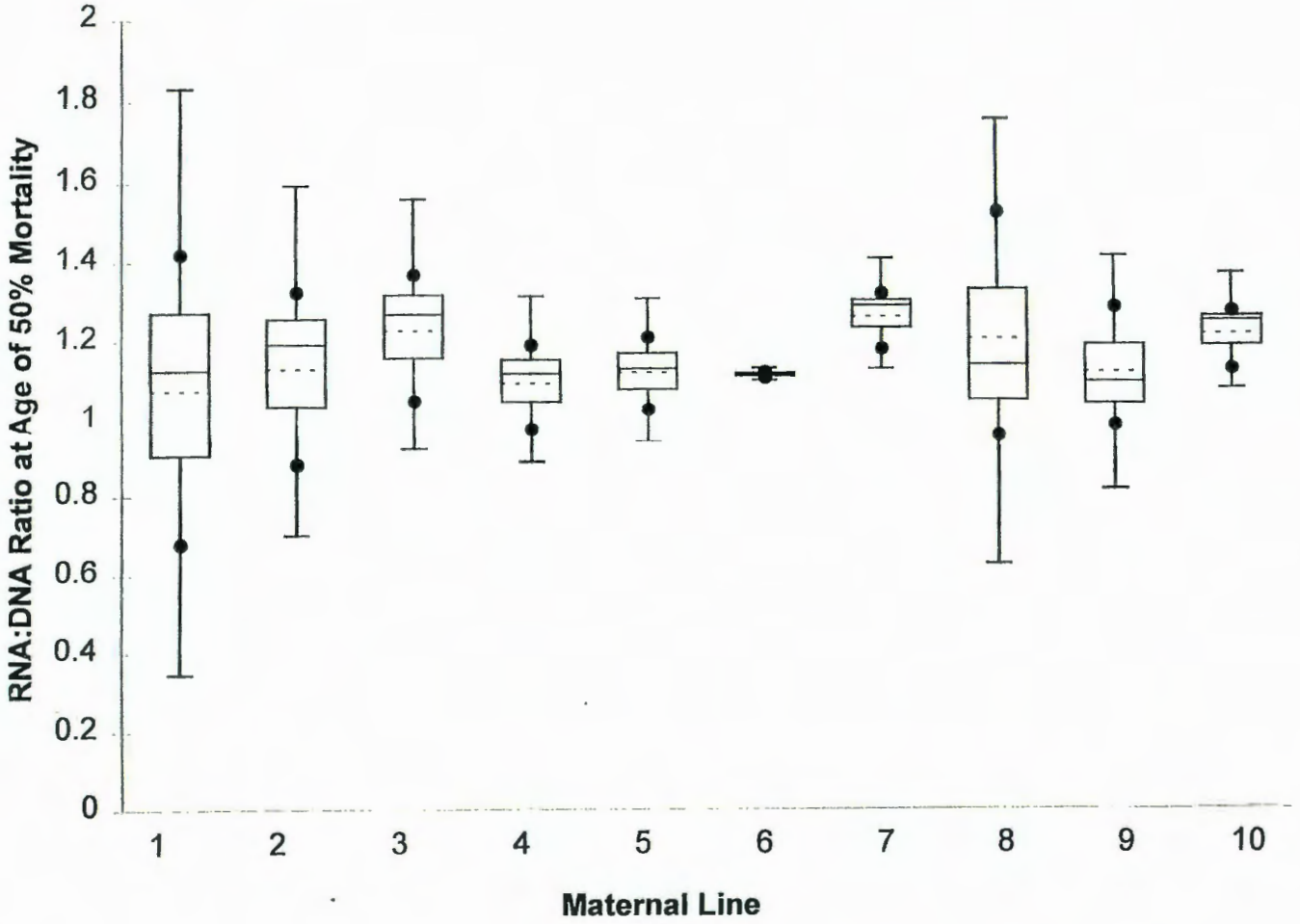

7
6
5
4
3
2
1
0
0
0.05
0.1
0.15
0.2
0.25
Mean Dry Weight at Hatch (mg)

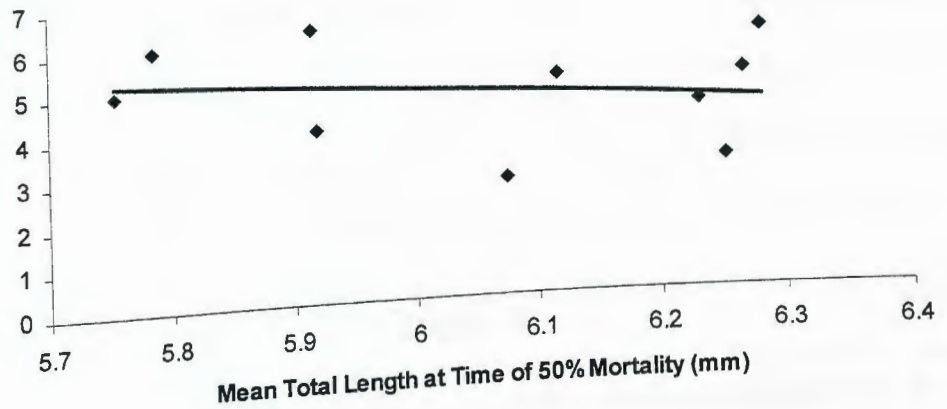

Total Length at Time of 50% Mortality (mm)
6.6
6.4
6.2
6
5.8
5.6
5.4
5.2
1
2
3
4
5
6
7
8
9
10
Maternal Line

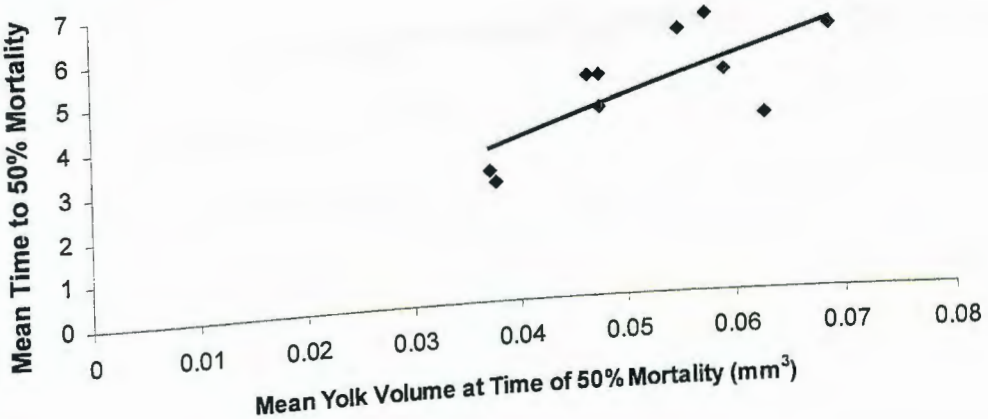

Yolk Volume at Time of 50% Mortality (mm3)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1
2
3
4
5
6
7
8
9
10
Female

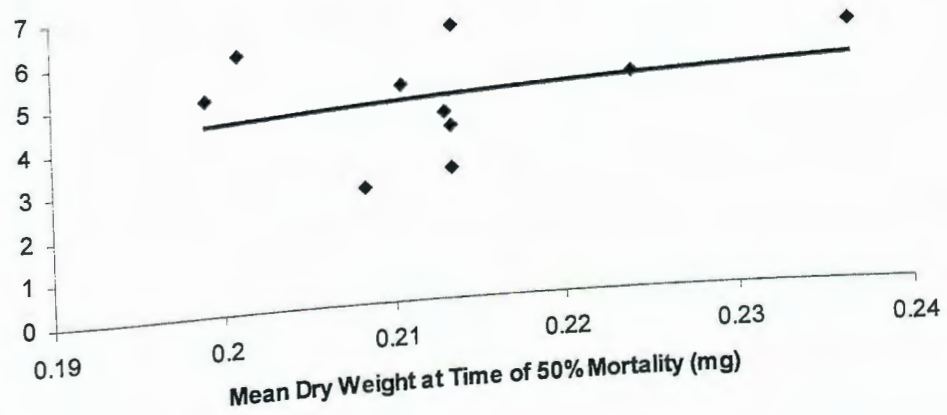

Dry Weight at Time of 50% Mortality (mg)
0.26
0.25
0.24
0.23
0.22
0.21
0.2
0.19
0.18
0.17
1
2
3
4
5
6
7
8
9
10
Female


RNA Content at Time of 50% Mortality (ug)
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
Maternal Line


RNA:DNA Ratio at Age of 50% Mortality
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
9
10
Maternal Line


7
6
5
4
3
2
1
0
5.7
5.8
5.9
6
6.1
6.2
6.3
6.4
Mean Total Length at Time of 50% Mortality (mm)


Mean Time to 50% Mortality
7
6
5
4
3
2
1
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Mean Yolk Volume at Time of 50% Mortality (mm3)


7
6
5
4
3
2
1
0
0.19
0.2
0.21
0.22
0.23
0.24
Mean Dry Weight at Time of 50% Mortality (mg)

Chapter 4

Understanding Maternal Effects as a Recruitment Mechanism in Lake Michigan Yellow Perch (*Perca flavescens*)

III. Implications for Recruitment

Abstract

Maternal effects in Lake Michigan yellow perch (*Perca flavescens*) have been detected, and the importance of maternal effects in resistance to starvation has been demonstrated. The goal of the present study was to determine the importance of maternal effects when food was not limited. We examined the persistence of maternal effects between hatching and 32 days post hatch (dph) in ten maternal lines and tested the null hypothesis of no difference in survival among maternal lines. Maternal effects were consistently detectable between hatching and four dph and between 16 and 32 dph. Detected maternal effects resulted in threefold differences in overall survival at 32 dph, and rejection of the null hypothesis. Furthermore, a three to four fold difference in the rate of mortality, measured as time to 50% mortality, was observed among the maternal lines. However, no clear mechanism was identified to account for these differences in survival. We conclude that maternal effects were detectable and present at least to 32 dph and resulted in differences in survival among maternal lines.

Introduction

A major goal of fisheries science and management is to predict the level of recruitment of a cohort in a given year, and to do so as early in the life history of the species as possible. However, despite over three quarters of a century of research, recruitment variability remains the single least understood process in fisheries science (Houde 1987). Our lack of understanding is attributable to high inter-annual variability in recruitment of fish populations. Attempts to explain this high variability have invoked the influence of abiotic (e.g. temperature, wind, transport mechanisms, and retention mechanisms) and biotic (e.g. starvation, cannibalism, and predation) controls. Recently, research has focused on identifying characteristics of recruits as a guide to determine sources of mortality that may control survival (Crowder et al. 1989; Miller 1997).

Many important mortality sources controlling recruitment are size-dependent (Hunter 1981; Houde 1987; Miller et al. 1988; Bailey and Houde 1989). Larger and older larvae are commonly viewed as being less vulnerable to predation than their smaller counterparts (Bailey and Batty 1984; Blaxter 1986; Houde 1987; Hunter 1981; Miller et al. 1988; Pepin 1993). Yellow perch (*Perca flavescens*) is one species in which this view, commonly referred to as the 'bigger is better' hypothesis, has received support. Nielson (1980) observed that larger yellow perch individuals exhibited lower mortality to walleye (*Stizostedion vitreum*) predation than smaller individuals. Starvation can also be size-dependent (Hunter 1981; Miller et al. 1988). Post and Evans (1989) demonstrated that overwinter starvation mortality in yellow perch was also strongly size-dependent. We have shown previously that newly hatched yellow perch larvae with large yolk-sacs

are more resistant to starvation than their larger conspecifics that have smaller yolks (Chapter 3).

While differences in larval size at a given point in time may account for a considerable amount of mortality observed in fishes, recruitment is a dynamic process. Thus, differences in individual larval growth rates need also be considered. Differences in larval growth rates influence the duration of the larval stage and the time it takes larvae to pass through stages of high predation mortality (Houde 1987; Bertram et al. 1997). Faster growth rates can reduce the time that larvae spend in vulnerable size classes (Bailey and Houde 1989; Rice et al. 1993). Larvae exhibiting faster growth rates may experience a lower probability of mortality than slower growing larvae (Meekan and Fortier 1996). Furthermore, size-dependent predation mortality may select for survival of faster growing larvae to be favored and selected for over slower growing larvae (Rosenberg and Haugen 1982; Rice et al. 1987).

Differences in the size and growth rates observed among individuals within a cohort can vary substantially (Rosenberg and Haugen 1982; Meekan and Fortier 1996). One potential explanation for the high intra-cohort variability observed in larval size and growth rates is maternal effects. Previous research has shown that intra-cohort differences in growth rates can in part be attributed to maternal effects. Gall (1974) demonstrated that older rainbow trout *Oncorhynchus mykiss* females produced offspring that exhibited faster growth rates than larvae produced by younger females. Larger and older female striped bass *Morone saxatilis* produced larvae that exhibited faster growth rates than larvae produced by smaller and younger females (Monteleone and Houde 1990). Other studies have demonstrated similar findings for other species, including

chum salmon *Oncorhynchus keta* (Beacham and Murray 1985), Atlantic silversides *Menidia menidia* (Bengston et al. 1987) and winter flounder *Psuedopleuronectes americanus* (Buckley et al. 1991), among others.

In yellow perch, maternal effects were detected at hatching and were expressed most by differences in larval size and yolk volume (Chapter 2). Furthermore, these maternal effects observed at hatching contributed to significant differences in resistance to starvation (Chapter 3). However, the influence of maternal effects may be dampened during larval ontogeny as the influence individual experience becomes more important (Chambers and Leggett 1996). This raises the question of whether maternal effects can be of sufficient duration to influence mortality later in larval life. This study aimed to determine if the significant maternal effects observed at hatching in yellow perch were present throughout the first month of life. We tested whether maternal effects lead to significant differences in growth rate and survival of offspring under conditions of high food availability.

Materials and Methods

We investigated our objectives within a single experiment, which involved monitoring and quantifying several larval traits, and the survival of larval yellow perch to 32 days post hatch (dph). All larvae used were the offspring of the ten maternal lines described in Chapter 2. Experimental work was conducted at the University of Wisconsin's WATER Institute, Milwaukee, WI. The experiment was conducted as a randomized complete block design, consisting of three blocks of 20 10-gallon flow-through aquaria with two replicates per maternal line per block.

Full details of the collection and husbandry of eggs and larvae are given in Chapter 2, and are only summarized here. Male and female yellow perch were collected on 2 June and 4 June 1998 in the vicinity of Green Can Reef (42° 50' 00" N, 87° 50' 00" W) off Milwaukee, WI. Eggs were fertilized in the field and brought to the lab. Volumes of eggs sufficient to produce approximately 2000 larvae were incubated in each of 60 10-gallon flow-through, temperature-controlled aquaria.

Husbandry of Larvae

Hatching began on 14 June 1998 in all tanks. After the removal of the broken egg skeins and any dead eggs and larvae, the larvae in all 60 tanks were sampled for the presence of maternal effects at hatch. After sampling at hatch, one of two treatments, fed or starved, was randomly applied to one replicate tank of each maternal line within each block. The 30 fed treatment tanks were fed *ab libitum* throughout the duration of the experiment. The newly hatched yellow perch larvae were fed 'green tank water', a mixed batch culture of small-sized crustaceans, rotifers and protozoans. By two days post hatch (dph), larvae were introduced to *Artemia* nauplii in combination with continued feeding of green tank water, and by five dph their diet consisted solely of *Artemia* nauplii. A mixture of ground beef and liver was added to the larval diet by eight dph. By 12 dph, a commercially available dry pellet fish food was added to the diet. The combined diet of *Artemia* nauplii, beef liver mixture, and commercial fish food was maintained throughout the duration of the experiment. The food type given, the flow rate and temperature in the tanks were consistent with the laboratory's successfully established yellow perch rearing conditions. Mortalities in each tank were removed daily and enumerated.

Larval Sampling

At hatching, 15 larvae were sampled from each tank. Subsequently, five larvae were sampled every two days through 12 dph, and then every 4 days until 32 dph. However, in order to maintain some level of equal spacing of samples, only samples from 0, 2, 4, 6, 8, 10, 16, 24 and 32 dph were processed and used in the subsequent analyses. We quantified 9 larval traits (6 morphometric and 3 biochemical measures of condition - Table 1), for each larva from each tank on each sampling day. Larvae were first anesthetized with Tricaine Methansulfonate (MS-222), and then videotaped for analysis of morphometry. Individual larvae were placed into cryovials, flash-frozen in liquid nitrogen, and then stored at –80 °C for subsequent extraction of nucleic acids.

Post-Sampling Processing of Larvae

Morphometric analysis was conducted on videotaped images of larvae using Optimas (v 6.1, Media Cybernetics, Takoma Park, MD) as defined in Chapter 2. Larval total length, eye diameter, body depth at the insertion point of the pectoral fin, body depth at the insertion point of the first dorsal fin (inclusive of yolk-sac), body depth at the insertion point of the anus, and body area (calculated internally by Optimas from the outline of the larva) were measured for each individual larva sampled. From these primary measurements we also estimated dry weight using methods described in Chapter 2. We also determined yolk volume by methods described in Chapter 2. However, due to absorption of yolk very early on in the experiment it was omitted from these analyses. Extraction and determination of nucleic acids also was performed as described in Chapter 2, with two exceptions. Microplates were read on a fluorescence plate reader at Ex = 590

nm, Em = 625 nm (Spectra Max Gemini, Molecular Devices Corp., Sunnyvale, CA). Furthermore, larvae > 7 mm total length required an additional dilution of the extracted nucleic acid supernatant (Table 2). This additional dilution factor was used in the conversion of RNA and DNA concentration to RNA and DNA content (concentration*1.5 ml*additional dilution factor).

Statistics

Separate statistical analyses were conducted to assess each of our objectives. All statistical analyses were conducted using SAS (v 6.12, SAS Institute, Inc., Cary, NC). The persistence of significant maternal effects throughout the duration of the experiment (32 dph) was determined by multivariate analysis of variance (MANOVA; SAS Proc GLM) on all larval traits, except survival, for each sampling day. The MANOVA was based on the mean value of each larval trait within each fed tank, for each maternal line nested within the date of fertilization (2 June or 4 June 1998). Significance of MANOVA was determined based upon a Wilks' Lambda statistic with varying degrees of freedom.

Variation in survival over time and among maternal lines was evaluated by repeated measures analysis of variance (MANOVA; SAS Proc GLM). The proportion of larvae surviving on each day was arcsine transformed before analysis. The repeated measures analysis was based on the mean value of survival within each tank, for each maternal line on each sampling day. Repeated measures analysis was conducted on the sampling dates indicated above. Significance of the test of the null hypothesis of no difference in survival over time (i.e. time effect) was determined based upon a Wilks' Lambda statistic with 4 and 15 degrees of freedom. Significance of repeated measures

analysis for the test of the null hypothesis of no difference in survival among the offspring of the ten maternal lines over time (i.e. interaction between mother and time) was determined based upon a Wilks' Lambda statistic with 32 and 59.91 degrees of freedom. This test of significance examines whether the offspring of the maternal lines were dying at different rates.

Differences among the fed offspring of the maternal lines over time were determined by repeated measures analysis on each larval trait at each sampling day. The repeated measures analysis was based on the mean value of each larval trait within each tank, for each maternal line on each sampling day. Significance of repeated measures analysis for the test of the null hypothesis of no variation in the larval traits (Table 1) over time was determined based upon a Wilks' Lambda statistic with 8 and 11 degrees of freedom for the morphometric and biochemical traits. For all traits the repeated measures analysis was conducted on the sampling dates indicated above. Significance of repeated measures analysis for the test of the null hypothesis of no difference among the offspring of each maternal line for each larval trait over time was determined based upon a Wilks' Lambda statistic with 64 and 69.94 degrees of freedom for the morphometric and biochemical traits. These tests of significance examine whether the ten maternal lines have different rates of change for a given larval trait over time. Significance of repeated measures analysis was determined based upon an F statistic with 8 denominator degrees of freedom for the test of the null hypothesis of no difference among the offspring of each maternal line for a given larval trait (i.e. mother effect). This test of significance is analogous to differences in the absolute magnitude of a given larval trait among the offspring of the ten maternal lines.

Results

Maternal effects were detectable on six of the nine sampling days. Maternal effects were detected at hatching and at two and four dph (Table 3). At six dph, we were no longer able to detect significant maternal effects among the offspring of the nine maternal lines based upon MANOVA results ($F_{72, 68.41} = 1.15$, $p = 0.2825$) (Table 3). Tests for the presence of maternal effects were marginally non-significant on eight and ten dph. However, at 16 dph and through 32 dph significant maternal effects were once again detectable (Table 3).

Maternal $Line_2$ was the only maternal line that had no survival at the end of the experiment at 32 dph. Maternal $Line_2$ experienced 100% mortality, as a result of loss due to sampling in addition to daily mortality, by 13 dph in two replicates, and by 24 dph in the third replicate. Repeated measures analysis requires a balanced data set. Therefore, we analyzed differences in the change of larval traits over 32 days for only nine maternal lines. The results of the analyses presented here do not include those for Maternal $Line_2$, however, where appropriate, the results are presented in the tables.

Mortality rates and overall survival differed among the offspring of the maternal lines. Time to 50% mortality ranged from 2 to 21 dph among the offspring of the maternal lines (Table 4). The offspring of Maternal $Line_3$ exhibited the highest vulnerability to mortality, reaching 50% mean mortality by 4 ± 2.6 days, while offspring of Maternal $Line_7$ exhibited the lowest vulnerability to mortality, reaching 50% mean mortality by 15 ± 1.5 days (Table 4).

The lowest survival of 7 ± 1.2 % of the initial number of offspring surviving to 32 dph was exhibited by Maternal $Line_6$, while Maternal $Line_7$ had the highest survival with

23 ± 3 % of the initial number of offspring surviving to 32 dph (Table 5). We were able to reject the null hypothesis of no overall difference in the survivorship of the offspring of the nine maternal lines over time based upon repeated measures ANOVA results ($F_{32, 59.91} = 2.196$, $p = 0.0047$) (Table 6). This indicates that the rate of mortality was significantly different among the offspring of the maternal lines.

All larval traits varied significantly over time (Table 7). Furthermore, we were able to reject the null hypothesis of no overall significant difference in the DNA content of the offspring of the nine maternal lines over time based upon repeated measures ANOVA ($F_{64, 69.94} = 1.508$, $p = 0.0470$) (Table 7). This indicates that DNA content was significantly different among the offspring of the nine maternal lines over time. We also detected significant differences among the offspring of the nine maternal lines in body depth at the insertion of the pectoral fin, RNA content and the ratio of RNA:DNA, but not among the maternal lines over time (Table 7). No significant differences were detected among the offspring of the maternal lines for larval total length, body depth at the insertion of the first dorsal fin, body depth at the insertion of the anus, or larval dry weight (Table 7).

Changes in the traits of the offspring from the maternal lines observed between hatching and the end of the experiment were highly variable. Between hatching and 32 dph, larval total length increased by as little as 25 ± 7.1 % (Maternal $Line_3$) and by as much as 38 ± 1.8 % (Maternal $Line_5$) (Fig. 1, Table 8). Changes in body depth at the insertion of the pectoral fin among the maternal lines were less variable than those observed for larval total length. Body depth at the insertion of the pectoral fin varied over the duration of the experiment by as little as 15 ± 2.9 % (Maternal $Line_6$) and by as

much as 19 $\pm$ 2.4 % (Maternal Line$_5$) (Table 8). Changes in body depth at the insertion of the first dorsal fin and body depth at the insertion of the anus were higher among the offspring of the maternal lines than those observed in body depth at the insertion of the pectoral fin. Body depth at the insertion point of the first dorsal fin varied by as little as 14 $\pm$ 5.4 % (Maternal Line$_3$) and by as much as 23 $\pm$ 3.3 % (Maternal Line$_{10}$) while body depth at the insertion of the anus varied by as little as 16 $\pm$ 5.0 % (Maternal Line$_8$) and by as much as 24 $\pm$ 4.7 % (Maternal Line$_4$) among the offspring of the maternal lines (Table 8). A four-fold variation in the change of dry weight among the nine maternal lines was observed over the duration of the experiment with Maternal Line$_3$ exhibiting the smallest change (1 $\pm$ 1.3 %) and Maternal Line$_{10}$ exhibiting the largest change (4 $\pm$1.3 %) (Fig. 2, Table 8). Large variation among the maternal lines in the change of RNA content, DNA content and the ratio of RNA:DNA were observed over the duration of the experiment. The quantity of RNA varied by as little as 2 $\pm$ 1.5 % (Maternal Line$_3$) and by as much as 5 $\pm$ 1.2 % (Maternal Line$_7$) among the maternal lines, while DNA content varied by as little as 5 $\pm$ 1.1 % (Maternal Line$_5$) and by as much as 11 $\pm$ 4.8 % (Maternal Line$_3$) (Table 8). The ratio of RNA:DNA exhibited over five-fold variation in percent change among the maternal lines over the duration of the experiment with Maternal Line$_8$ exhibiting the smallest change (19 $\pm$ 5.7 %) and Maternal Line$_{10}$ exhibiting the largest change (120 $\pm$ 72.8 %) (Fig. 3, Table 8). Correlation analysis of larval total length and dry weight over time indicates that size at hatching becomes less important to size at later points in time after eight dph in total length and 10 dph in dry weight (Table 9, 10).

Differences in larval total length, yolk volume and dry weight among the maternal lines begin to increase beyond 16 dph (Figs. 1, 2 and 3). Daily growth rate as measured

in total length (mm/day) increased in all maternal lines between hatching and 16 dph (Fig. 4). Daily growth rates declined between 16 and 24 dph in all maternal lines except Maternal $Line_2$ and Maternal $Line_3$, after 24 dph daily growth rates again increased. Beyond 24 dph, differences in daily growth rate increase (Fig. 4).

All maternal lines exhibited increased instantaneous growth rates (G) between hatching and 6 to 8 dph, followed by declines in G (Fig. 5). Beyond 16 dph G either increased or remained relatively stable in all maternal lines except Maternal Line1. Maternal $Line_1$ exhibited increased G to 24 dph, followed by decreased G. Instantaneous natural mortality rates (M) decreased between hatching and 4 dph, followed by increases at 6 and 8 dph in all maternal lines (Fig. 5). Beyond 10 dph M remained relatively constant for all maternal lines.

Discussion

Significant maternal effects were present among the ten maternal lines in six of the nine samples between hatching and 32 dph. We were able to detect maternal effects consistently between hatching and 4 dph and between 16 and 32 dph. Tests conducted on samples collected on 8 and 10 dph were only marginally non-significant, suggesting the potential for maternal effects at these times also. However, analysis of samples collected at 6 dph did not indicate significant maternal effects. Overall, we rejected the null hypothesis that maternal effects were not detectable up to 32 dph, and we conclude that maternal effects are likely widespread through early life history. Furthermore, we conclude that the maternal effects observed at hatching and to 32 dph in yellow perch larvae, result in differences in survival.

The loss of all offspring of Maternal Line$_2$ by 13 dph in two tanks and by 24 dph in the third tank may be a direct result of the poor quality of the egg skein of Female$_2$ (Chapter 2). For whatever reasons, be they handling of the egg skein or perhaps premature expression of the eggs, the egg skein of Female$_2$ did not maintain its matrix-like gelatinous structure. Undoubtedly, the reduced quality of the egg skein is directly responsible for the low hatching success observed (Chapter 2) and the dramatically reduced survival of the subsequent offspring. However, even when larvae from this female were removed from the analysis, we were still able to detect significant maternal effects in survival, both expressed as the time to 50% mortality and in overall survival to 32 dph.

Why were maternal effects not detectable at 6 dph in the offspring of the ten maternal lines? An initial evaluation is that differences among the ten maternal lines in the slopes of the larval traits results in an intersection at some point between 6 and 10 dph and a divergence at 16, 24 and 32 dph. However, significant interactions between mother and time in the repeated measures analysis indicates that the slopes of the larval traits are only significantly different among the offspring of the ten maternal lines for DNA content. A more parsimonious explanation may be that the loss of detectable maternal effects is due to increased variability among larval traits within each maternal line associated with yolk absorption and the onset of feeding. This increase in variability may be due to differential increases in growth associated with both the onset of feeding and the first initial days of feeding. Such differences in feeding ability would account for differences in growth within a maternal line, and would thus increase size variability that could potentially mask previously detected maternally influenced differences in size.

Subsequently, we might expect to see growth compensation by, or selective mortality on smaller individuals, which may then reduce variability within maternal lines (Rice et al. 1993). As the variability within maternal lines is reduced, we would expect to again see significant differences among maternal lines as maximum size achieved within maternal lines may vary among maternal lines. Growth compensation has been demonstrated for other species used in maternal effects studies. Compensatory growth in rainbow trout was demonstrated to occur by 4 weeks post hatching (Springate and Bromage 1985) and after 25 dph in channel catfish *Ictalurus punctatus* (Reagan and Conley 1977).

The correlation analysis for larval total length and dry weight supports increased within maternal line size variation as a potential cause of the disappearance and reappearance of detectable maternal effects. Size, as measured by total length and dry weight, at later sampling days (i.e. after 10 dph) was not correlated with size at earlier, adjacent sampling days. Furthermore, correlation analysis between paired larval traits over time showed that correlations between morphometric measures strengthened as the offspring of the ten maternal lines aged. This suggests that reductions in the variation within larval traits occurred as the larvae approached 32 dph.

The large changes observed in the larval traits throughout the duration of the experiment were expected due to the high abundance of food given to the larvae on a daily basis. However, the variation in those changes among the ten maternal lines suggests that mechanisms aside from food availability and quality may be influencing the growth of individuals. We note that while all maternal lines were given identical quantities and quality of food, DNA content was significantly different among the offspring of the maternal lines over time. Given that quantity of DNA per cell is

relatively constant within somatic tissues of a species (Clemmesen 1994), we would conclude that the offspring of the different maternal lines were developing, at least at the cellular level, at different rates.

The abrupt changes in larval total length, dry weight, and RNA:DNA ratio observed beyond 16 dph are a little surprising. If growth rates vary among maternal lines, then differences in size and condition would be expected to be extenuated over time. The projections of each maternal line for each of these traits over time would be expected to gradually diverge from one another if growth rates differed. However, the projections of each maternal line for larval total length, dry weight and the ratio of RNA:DNA over time do not gradually diverge from one another, rather there are sudden divergences after 24 dph. This pattern carries over to the daily growth rate measured in total length where large divergences in daily growth rate are observed beyond 24 dph. It is not clear what may explain this sudden divergence in growth rate, size and condition among the maternal lines. One speculation would be that the observed divergences are attributable to the density dependent effects that are a consequence of mortality differences within each maternal line.

The expected change in instantaneous growth rate would have been a slightly sigmoidal response over time (i.e. with age) while instantaneous mortality would have been expected to decline in a curvilinear manner (Houde 1997). Furthermore, the ratio of M/G would be expected to decrease through the point at which M = G (M/G = 1) as mortality decreases and growth increases, then eventually begin to increase again as mortality increases and growth decreases (Houde 1997). In the yellow perch maternal lines, instantaneous growth rate declined in all maternal lines until about 10 dph, beyond

which point it slowly began to increase or in some instances remain stable. It is not clear why this response was observed. Instantaneous mortality rate of the maternal lines did behave in the expected manner. The decline in the ratio of M/G observed among the maternal lines indicates that biomass is declining over the period from hatching to 32 dph, and this is a combination of natural mortality and sampling mortality.

In the laboratory, in the absence of predators and in the presence of *ad libitum* food, one would expect larvae to have a relatively equal probability of survival. Yet, even under these conditions maternal effects on survival were detected. In the field, sources of selective mortality are prevalent (Miller 1997); thus, it is likely that maternal effects on larval survival may be even more important in the field. Some offspring may survive not because they are lucky (Pepin et al. 1999), but because their mother was more fit. While these results clearly support the significance of maternal effects on survival, the results regarding the significance of maternal effects on individual morphometric and biochemical traits do not clearly suggest a mechanism. Furthermore, no particular combination of the maternal characteristics that we measured best explains the differences observed in offspring survival. While the mechanisms for the differential survival observed among the offspring of the yellow perch maternal lines are not understood, the end result is clear. Differences among mothers, most likely in traits that were not measured in this study, account for differences in the survival of their offspring. Much future work on this topic is still needed, particularly research that can evaluate differences between mothers in more detail than we have here, perhaps even at the cellular level.

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References

Bailey, K.M. and R.S. Batty. 1984. Laboratory study of predation by *Aurelia aurita* on larvae of cod, flounder, plaice and herring: development and vulnerability to capture. Mar. Biol. **83**: 287-291.

Bailey, K.M. and E.D. Houde. 1989. Predation on egg and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1-83.

Beacham, T.D. and C.B. Murray. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhyncus keta*) from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. **42**: 1755-1765.

Bengston, D.A., R.C. Barkman, and W.J. Berry. 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. J. Fish. Biol. **31**: 697-704.

Bertram, D.F., T.J. Miller and W.C. Leggett. 1997. Individual variation in growth and development during early life stages of winter flounder *Pleuronectes americanus*. Fish. Bull. **95**: 1-10.

Blaxter, J.H.S. 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. Trans. Am. Fish. Soc. **115**: 96-114.

Blaxter, J.H.S. and G. Hempel. 1963. The influence of egg size on herring larvae (*Clupea harengus* L.). Journal du Conseil, Conseil International pour l'Exploration de la Mer. **28**: 211-240.

Buckley, L.J., A.S. Smigielski, T.A. Halavik, E.M. Caldarone, B.R. Burns, and G.C. Laurence. 1991. Winter flounder *Pseudoplueronectes americanus* reproductive success. II. Effects of spawning time and female size on size, composition and viability of eggs and larvae. Mar. Ecol. Prog. Ser. **74**: 125-135.

Chambers, R.C. and W.C. Leggett. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. Amer. Zool. **36**: 180-196.

Clemmesen, C. 1994. The effect of food availability, age or size on RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Mar. Biol. **118**: 377-382.

Crowder, L.B., J.A. Rice, T.J. Miller, and E.A. Marschall. 1989. Empirical and theoretical approaches to size-based interactions and recruitment variability in fishes, p. 237-255. In: DeAngelis, D.L. and L.J. Gross (eds.). Individual-Based Models and Approaches in Ecology. Chapman and Hall. New York, New York.

Gall, G.A.E. 1974. Influence of size of eggs and age of female on the hatchability and growth in rainbow trout. Calif. Fish and Game. **00(1)**: 26-35.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Amer. Fish. Soc. Symp. **2**: 17-29.

Houde, E.D. 1997. Patterns and consequences of selective processes in teleost early life histories. pp. 173-196. In: R.C. Chambers and E.A. Trippel (eds.) Early Life History and Recruitment in Fish Populations. Chapman and Hall, New York, New York.

Hunter, J.R. 1981. Feeding ecology and predation of marine fish larvae, p. 33-79. In. R. Lasker (ed.) Marine Fish Larvae: Morphology, Ecology, and Relation to Fisheries. University of Washington Press, Seattle, Washington.

Meekan, M.G. and L. Fortier. 1996. Selection for fast growth during the larval life of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. Mar. Ecol. Prog. Ser. **137**: 25-37.

Miller, T.J. 1997. The use of field studies to investigate selective processes in fish early life history. In: R.C. Chambers and E.A. Trippel (eds.). Early Life History and Recruitment in Fish Populations. Chapman and Hall. New York, New York.

Miller, T.J., L.B. Crowder, J.A. Rice, E.A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657-1670.

Monteleone, D.M. and E.D. Houde. 1990. Influence of maternal size on survival and growth of striped bas *Morone saxatilis* Walbaum eggs and larvae. J. Exp. Mar. Biol. Ecol. **140**: 1-11.

Nielson, L.A. 1980. Effect of walleye (*Stizostedion vitreum vitreum*) predation on juvenile mortality and recruitment of yellow perch (*Perca flavescens*) in Oneida Lake, New York. Can. J. Fish. Aquat. Sci. **37**: 11-19.

Pepin, P. 1993. An appraisal of the size-dependent mortality hypothesis for larval fish: comparison of a multispecies study with an empirical review. Can. J. Fish. Aquat. Sci. **50**: 2166-2174.

Pepin, P., G.T. Evans, and T.H. Shears. 1999. Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field. ICES J. Mar. Sci. **56**: 697-706.

Post, J.R. and D.O. Evans. 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, *in situ* enclosure, and field experiments. Can. J. Fish. Aquat. Sci. **46(11)**: 1958-1968.

Reagan, R.E. Jr. and C.M. Conley. 1977. Effect of egg diameter on growth of channel catfish. Prog. Fish. Cult. **39**: 133-134.

Rice, J.A., L.B. Crowder, and F.P. Binkowski. 1987. Exploration of mechanisms regulating survival in Lake Michigan bloater: a recruitment analysis based on characteristics of individual larvae. Trans. Am. Fish. Soc. **116**: 703-718.

Rice, J.A., T.J. Miller, K.A. Rose, L.B. Crowder, E.A. Marschall, A.S. Trebitz, and D.L. DeAngelis. 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. **50**: 133-142.

Rosenberg, A.A. and A.S. Haugen. 1982. Individual growth and size-selective mortality of larval turbot reared in enclosures. Mar. Biol. **72**: 73-77.

Springate, J.R.C. and N.R. Bromage. 1985. Effects of egg size on early growth and survival in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture. **47**: 163-172.

Table 1. Larval traits measured in each of the yellow perch (*Perca flavescens*) offspring of the ten maternal lines.

Linear measures
Total Length (mm)
Eye Diameter (mm)
Body Depth at the Insertion Point of the Pectoral Fin (mm) (BDIP)
Body Depth at the Insertion Point of the First Dorsal Fin (mm) (BDID)
Body Depth at the Insertion Point of the Anus (mm) (BDIA)
Biochemical measures
RNA Content (μg) (RNA)
DNA Content (μg) (DNA)
Volume measures
Yolk Volume (mm^3)
Derived measures
Dry Weight (μg or mg)
RNA:DNA Ratio (Ratio)

Table 2. Dilution factors used in the fluorometric determination of RNA content and DNA content in the offspring of the ten yellow perch (*Perca flavescens*) maternal lines.

Age (dph)	Mean Total Length (mm)	Dilution Factor	Supernatant (µl)	0.1% Sarcosyl (µl)
0	5.9	0	1000	0
2	6.1	0	1000	0
4	7.8	2	500	500
6	7.4	2	500	500
8	8.2	4	250	750
10	8.9	4	250	750
16	11.4	8	125	875
24	12.7	12.5	80	920
32	18.5	16	62.5	937.5

Table 3. MANOVA results for tests of significance for all morphometric and biochemical larval traits among the offspring of the ten yellow perch (*Perca flavescens*) maternal lines.

Day	Df	F	Pr>F
0	80, 255.92	2.67	0.0001
2	80, 65.60	1.69	0.0142
4	72, 68.41	1.90	0.0040
6	72, 68.41	1.15	0.2825
8	72, 68.41	1.43	0.0678
10	72, 68.41	1.37	0.0979
16	72, 56.24	2.11	0.0021
24	72, 56.24	1.58	0.0372
32	63, 51.16	1.79	0.0164

Table 4. Mean time to 50% mortality (± SD) (n = 3) of the offspring of the ten yellow perch (*Perca flavescens*) maternal lines over time, based upon the mean value for each tank per female on each sampling day.

Maternal Line	Minimum (days post hatch)	Maximum (days post hatch)	Mean (days post hatch)
1	4	13	9 ± 4.7
2	12.5[1]	23.5[2]	16 ± 6.4
3	2	7	4 ± 2.6
4	10	15	12 ± 2.6
5	12	18	15 ± 3.1
6	6	15	12 ± 4.9
7	13	16	15 ± 1.5
8	3	21	12 ± 9.0
9	13	17	15 ± 2.1
10	14	16	15 ± 1

[1]The offspring had not yet reached 50% mortality by 12 dph, however, by 13 dph 100% mortality had ensued.

[2]The offspring had not yet reached 50% mortality by 23 dph, however, by 24 dph 100% mortality had ensued.

Table 5. Mean survival (± SD) (n=3) to 32 dph in the yellow perch (*Perca flavescens*) offspring of the ten maternal lines sampled over nine days, based upon the mean value for each tank per female on each sampling day.

Maternal Line	Initial Number (0 dph)	Surviving Number (32 dph)	% Survival
1	1566 ± 433	167 ± 58	11 ± 2.7
2	84 ± 23	0	0
3	875 ± 214	91 ± 30	10.3 ± 0.87
4	1485 ± 112	168 ± 37	11 ± 2.3
5	1133 ± 156	159 ± 43	14 ± 3.0
6	1175 ± 265	76 ± 6	7 ± 1.2
7	1468 ± 344	331 ± 81	23 ± 3
8	896 ± 529	206 ± 185	22 ± 8.6
9	1378 ± 208	201 ± 24	14.7 ± 0.56
10	1283 ± 202	115 ± 26	9 ± 3.7

Table 6. Repeated measures ANOVA results for tests of significance among the yellow perch (*Perca flavescens*) offspring of the ten maternal lines (n = 263). Tests of significant differences among the offspring of the ten maternal lines were performed with 8 degrees of freedom (Mother). Tests of significant time effects were performed with 4 and 15 degrees of freedom (Time). Tests of significant differences among the offspring of the ten maternal lines over time (Time*Mother) were performed with 32 and 59.91 degrees of freedom.

	Mother at Age (dph)											
	0	**2**	**4**	**6**	**8**	**10**	**16**	**24**	**32**	**Mother**	**Time**	**Time*Mother**
Survival		**	*								***	**

Table 7. Repeated measures ANOVA results for tests of significance among the yellow perch (*Perca flavescens*) offspring of the ten maternal lines. Tests of significant differences among the offspring of the ten maternal lines were conducted with 8 degrees of freedom (Mother). Tests of significant time effects (Time) were conducted with 8 and 11 degrees of freedom for all morphometric and biochemical larval traits (n = 263). Tests of significant differences among the offspring of the ten maternal lines over time (Time*Mother) were conducted with 64 and 69.94 degrees of freedom for all morphometric and biochemical larval traits. All trait abbreviations are as defined in Table 1.

	Mother at Age (dph)											
	0	**2**	**4**	**6**	**8**	**10**	**16**	**24**	**32**	**Mother**	**Time**	**Time*Mother**
Larval Total Length	*	***			**						***	
BDIP	*				**		**			*	***	
BDID	**				**	*	*				***	
BDIA		*			*						***	
Larval Dry Weight	***	**		*	**						***	
RNA		*					*	*		*	***	
DNA	***	*							**	**	***	*
Ratio									*	*	***	

Table 8. Mean percent change (± SD) (n = 3) in the larval traits of the yellow perch (*Perca flavescens*) offspring of the ten maternal lines sampled between 0 and 32 dph[a], based upon the mean value for each tank per female on each sampling day. All trait abbreviations are as defined in Table 1.

	Maternal Line									
Larval Trait	**1**	**2[a]**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Total Length	35 ± 2.0	52 ± 15.0	25 ± 7.1	37 ± 5.9	38 ± 1.8	27 ± 5.0	35.0 ± 0.66	29 ± 8.5	36 ± 3.4	36 ± 3.6
BDIP	18.0 ± 0.60	34 ± 13.5	15 ± 3.2	19 ± 2.7	19 ± 2.4	15 ± 2.9	18 ± 1.9	15 ± 4.0	17.9 ± 0.29	20 ± 2.0
BDID	16 ± 1.3	35 ± 14.3	14 ± 5.4	18 ± 2.7	18 ± 3.4	17 ± 5.0	21 ± 3.4	18 ± 5.7	20.0 ± 0.56	23 ± 3.3
BDIA	21.5 ± 0.67	31 ± 9.9	17 ± 5.6	24 ± 4.7	22 ± 3.0	17 ± 3.1	20 ± 2.2	16 ± 5.0	20.9 ± 0.50	22 ± 3.0
Dry Weight	3.2 ± 0.14	16 ± 11.6	1 ± 1.3	4 ± 1.8	3.9 ± 0.83	2 ± 1.5	3.4 ± 0.41	2 ± 1.8	3.9 ± 0.76	4 ± 1.3
RNA	4.4 ± 0.28	10 ± 6.5	2 ± 1.5	4.5 ± 0.93	4 ± 1.0	3 ± 1.8	5 ± 1.2	3 ± 1.5	4.4 ± 0.59	5 ± 1.4
DNA	5.6 ± 0.88	18 ± 8.2	11 ± 4.8	8 ± 1.8	5 ± 1.1	8 ± 2.0	5.8 ± 0.74	7 ± 1.2	5 ± 1.6	4.2 ± 0.84
RNA:DNA Ratio	42 ± 7.9	63 ± 50.4	23 ± 27.3	39 ± 28.3	69 ± 45.1	34 ± 32.3	76 ± 13.4	19 ± 5.7	66 ± 46.2	120 ± 72.8

[a]Changes in the larval traits of Maternal Line2 occurred only over 5 and 7 sampling days due to the occurrence of 100% mortality in all tanks by those days.

Table 9. Correlations of larval total length between sampling days for the offspring of the ten maternal lines of yellow perch (*Perca flavescens*). Shaded correlation values represent significant correlations.

	0 dph	2 dph	4 dph	6 dph	8 dph	10 dph	16 dph	24 dph	32 dph
0 dph	1	0.6485	0.7697	0.6242	0.7212	0.2970	0.2727	0.3697	-0.0333
2 dph		1	0.7212	0.6606	0.5152	0.3091	0.3576	0.3939	0.0667
4 dph			1	0.6242	0.6970	0.3939	0.2364	0.2849	-0.1333
6 dph				1	0.8182	0.8061	0.8061	0.7939	0.4167
8 dph					1	0.5515	0.5152	0.5152	-0.0333
10 dph						1	0.9394	0.6000	0.4833
16 dph							1	0.6121	0.5833
24 dph								1	0.4667
32 dph									1

Table 10. Correlations of larval dry weight between sampling days for the offspring of the ten maternal lines of yellow perch (*Perca flavescens*). Shaded correlation values represent significant correlations.

	0 dph	2 dph	4 dph	6 dph	8 dph	10 dph	16 dph	24 dph	32 dph
0 dph	1	**0.8667**	**0.7333**	**0.6970**	**0.7455**	**0.6606**	0.4182	0.4667	0.0667
2 dph		1	**0.6970**	0.3818	0.5636	0.3939	0.2606	0.1152	-0.4667
4 dph			1	0.6242	**0.6849**	**0.6485**	0.6000	0.2000	0.0667
6 dph				1	**0.8788**	**0.9273**	**0.7333**	0.7697	0.6333
8 dph					1	**0.9394**	**0.7455**	0.5515	0.2667
10 dph						1	**0.8788**	**0.7333**	0.5500
16 dph							1	0.5879	0.5500
24 dph								1	**0.6667**
32 dph									1

Figure Legend

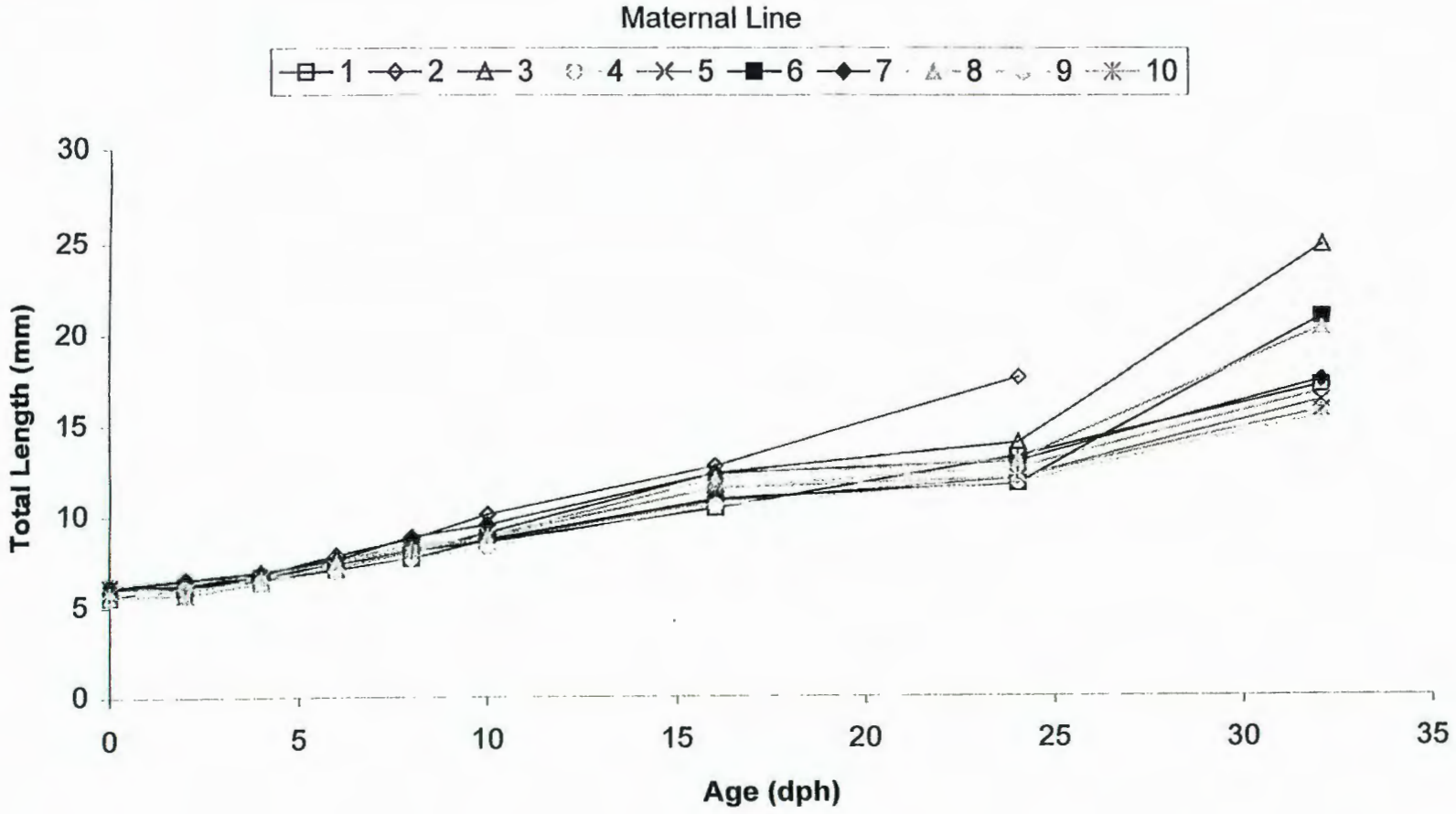
Figure 1. Change in the total length of the fed offspring of the yellow perch (*Perca flavescens*) ten maternal lines between hatching and 32 days post hatch (dph). Values on each sampling day for each maternal line are based upon the mean value of total length from a total of 15 larvae (45 larvae on 0 dph) from 3 replicate tanks.

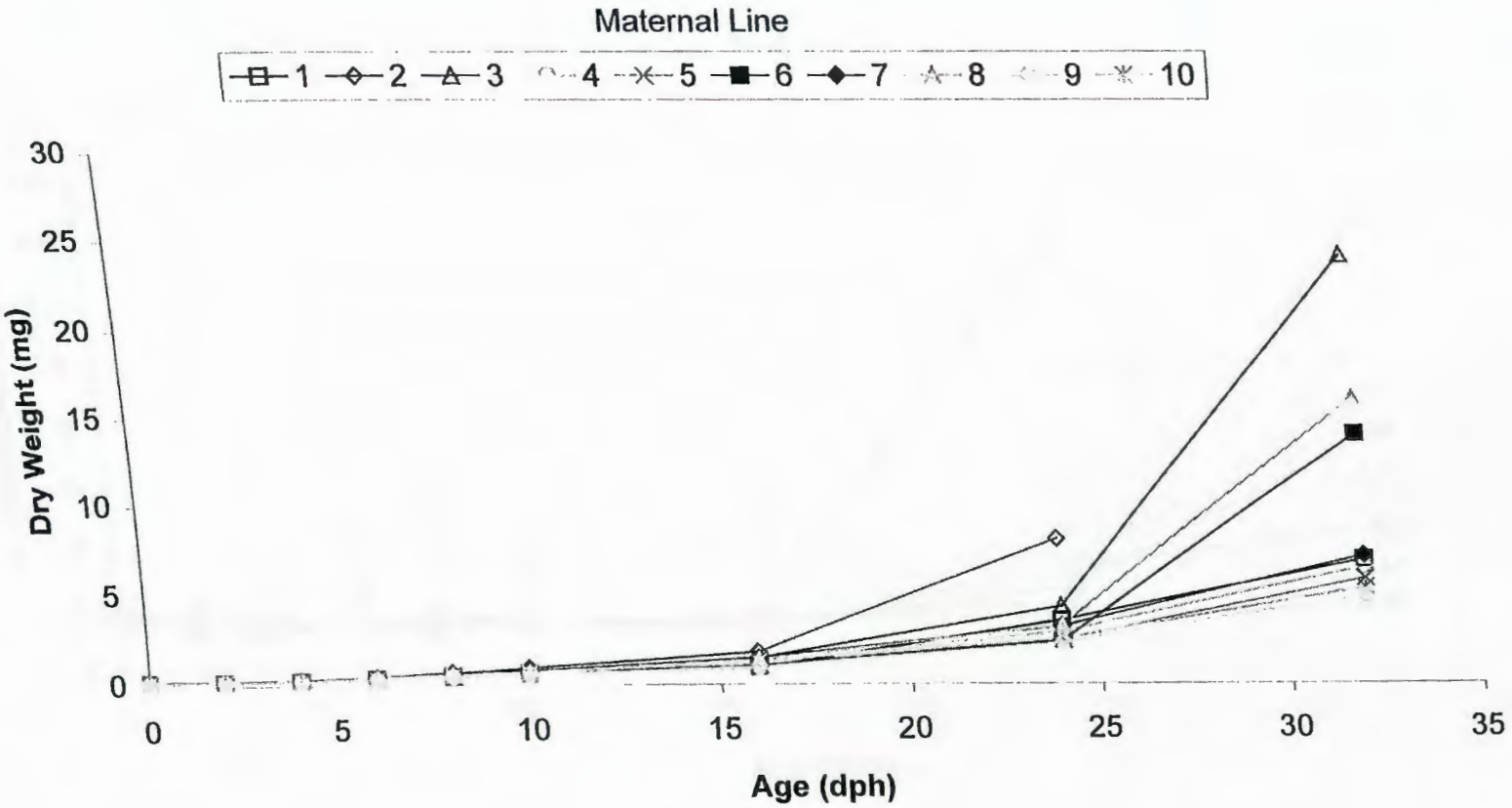
Figure 2. Change in the dry weight of the fed offspring of the yellow perch (*Perca flavescens*) ten maternal lines between hatching and 32 days post hatch (dph). Values on each sampling day for each maternal line are based upon the mean value of dry weight from a total of 15 larvae (45 larvae on 0 dph) from 3 replicate tanks.

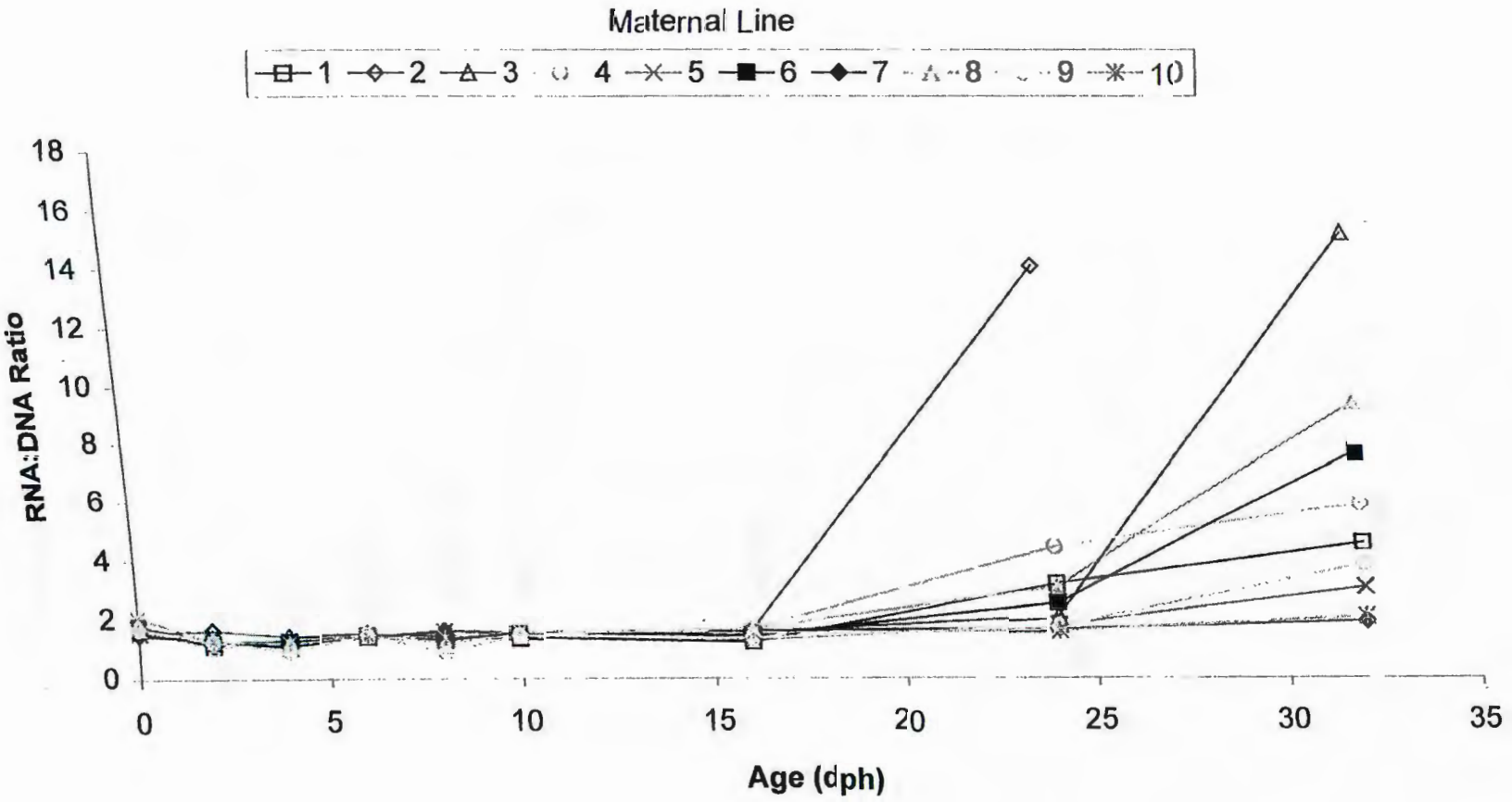
Figure 3. Change in the RNA:DNA ratio of the fed offspring of the yellow perch (*Perca flavescens*) ten maternal lines between hatching and 32 days post hatch (dph). Values on each sampling day for each maternal line are based upon the mean value of RNA:DNA ratio from a total of 15 larvae (45 larvae on 0 dph) from 3 replicate tanks.

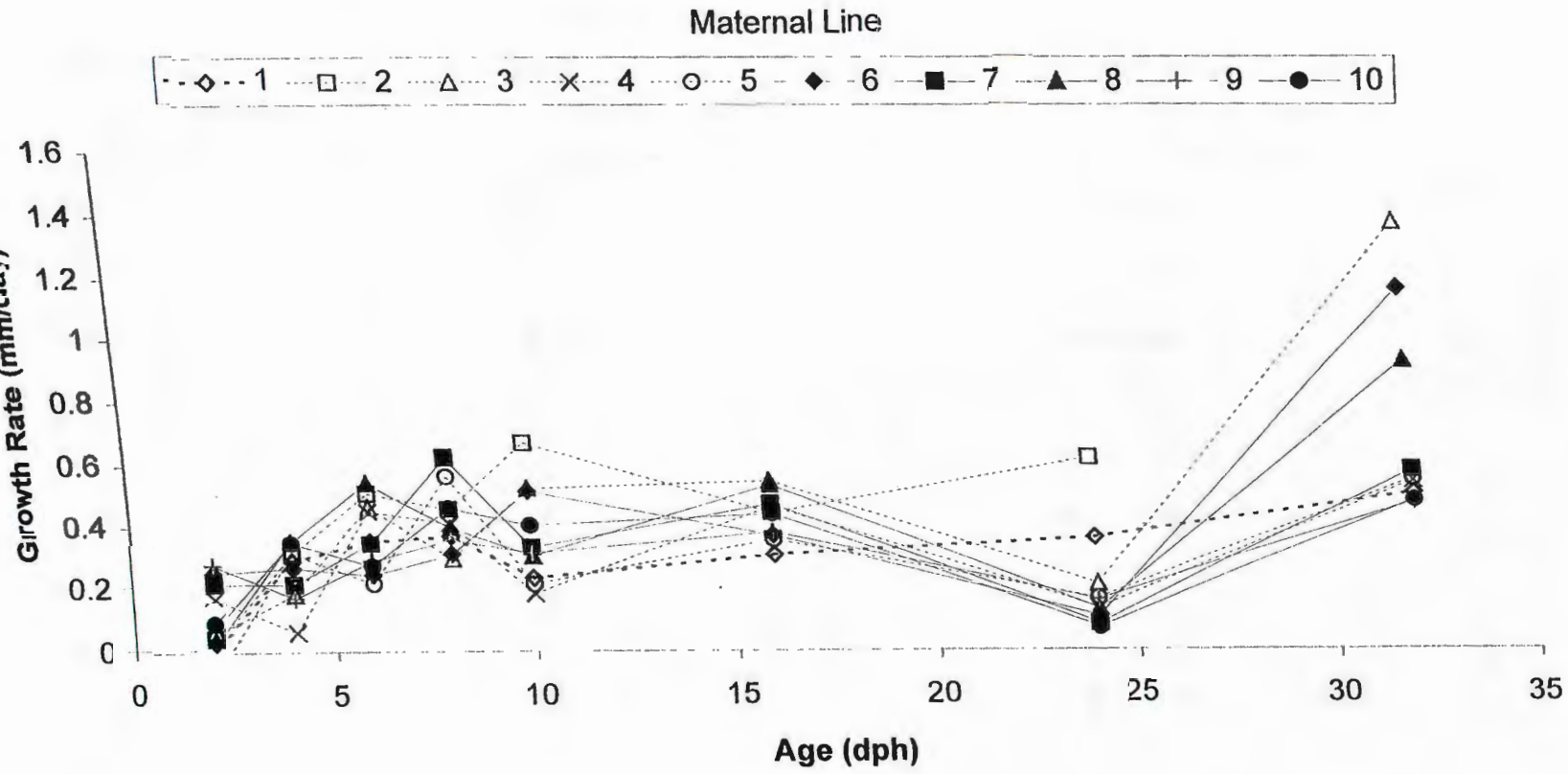
Figure 4. Daily growth rate measured in total length (mm/day) of the offspring of the ten maternal lines of yellow perch (*Perca flavescens*) from hatching to 32 days post hatch.

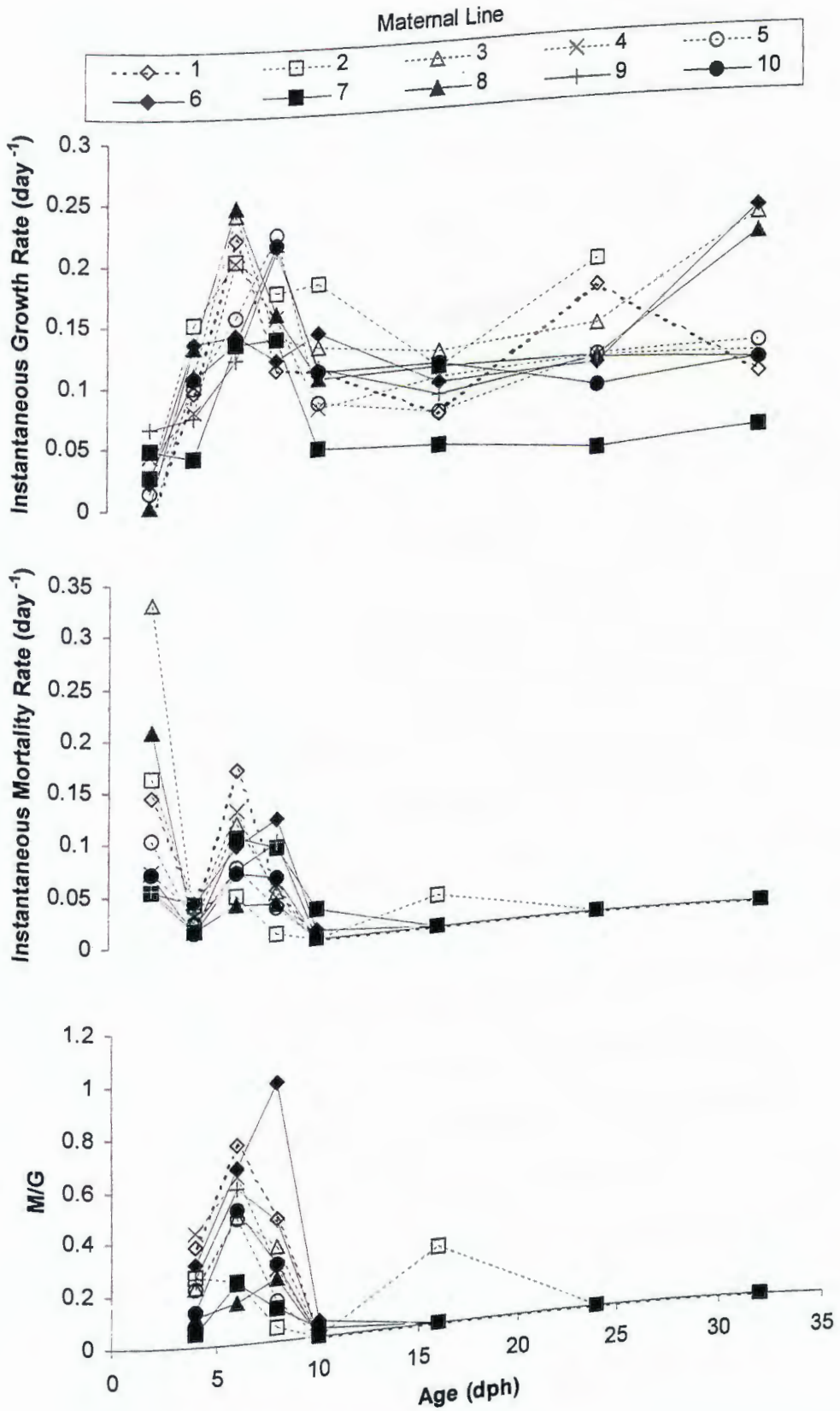
Figure 5. Instantaneous growth rate (G), instantaneous mortality rate (M) and the ratio of M/G for ten yellow perch (*Perca flavescens*) maternal lines from hatching to 32 days post hatch.


Maternal Line
1
2
3
4
5
6
7
8
9
10
Total Length (mm)
0
5
10
15
20
25
30
Age (dph)
0
5
10
15
20
25
30
35


Maternal Line
1
2
3
4
5
6
7
8
9
10
Dry Weight (mg)
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
Age (dph)


Maternal Line
1
2
3
4
5
6
7
8
9
10
RNA:DNA Ratio
18
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
Age (dph)


Maternal Line
1
2
3
4
5
6
7
8
9
10
Growth Rate (mm/day)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
Age (dph)


Maternal Line
1
2
3
4
5
6
7
8
9
10
Instantaneous Growth Rate (day-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
Instantaneous Mortality Rate (day-1)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
M/G
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
Age (dph)

Chapter 5

Synopsis

Maternal effects likely exist to some degree in all species due to the female's energetic investment in the production of eggs. It is the female that provides the initial 'survival kit' that a larva has to survive on until it can locate and begin feeding on exogenous food sources. This initial 'survival kit' includes not only the quantity of yolk in the yolk-sac, but also the quality of that yolk. Differences among individuals at hatching would be expected to result in differences in survival under sub-optimal environmental conditions. Beyond hatching, maternally derived differences in size and growth may remain important among larvae. If differences in hatchling traits are directly attributable to maternal contributions, the question remains, "For how long are such maternal effects important in early life history?"

Maternal effects were detected in Lake Michigan yellow perch (*Perca flavescens*) among the offspring of ten maternal lines at hatching. The differences among the offspring resulted from differences among their mothers with respect to size, age, gonadal somatic index (GSI), and egg production (Chapter 2). These maternal effects were expressed most by differences among the hatchlings of the maternal lines in larval total length, yolk volume, dry weight and the quantity of DNA. Furthermore, trade-offs in both the maternal and hatchling traits were observed. A trade-off between female GSI and all other female traits indicated that old, large females were characterized by high egg production and low GSI, while young, small females were characterized by low egg production and high GSI. In the hatchlings, a trade-off between larval total length and yolk-volume indicated that long larvae were characterized by small yolk-sacs, and short larvae were characterized by large yolk-sacs. The differences in larval traits of the hatchlings and the observed trade-offs suggest that the contribution of different females

to year class strength of yellow perch in Lake Michigan will vary with changes in either the biotic or abiotic environment.

For example, maternal effects were found to persist through six dph under conditions of food-limitation (Chapter 3). The maternal effects resulted in differences among maternal lines in their resistance to starvation by a factor of two. The offspring that exhibited the lowest resistance to starvation were characterized by high total length and small yolk-sacs at hatching. The offspring that were most resistant to starvation were characterized by low total length and large yolk-sacs at hatching. This suggests that it is not the size of the larvae that determines starvation resistance, but rather the amount of yolk the larvae were provided with by their respective mothers. The characteristics of the least and most starvation resistant larvae provide further evidence for a trade-off between larval total length and yolk-volume, and insights into the importance of such a trade-off in terms of starvation resistance. The observed differences in resistance to starvation among the maternal lines suggest that maternal effects do influence survival under conditions of food-limitation.

Moreover, maternal effects were found to persist through 32 dph when food was not a limiting factor (Chapter 4). The maternal effects result in differences among maternal lines in overall survival by a factor of three. Three to four fold differences among the maternal lines in the rate of mortality, as measured by time to 50% mortality, were also attributed to maternal effects. However, neither differences in the maternal characteristics nor the larval characteristics account for the observed differences in survival among the maternal lines. While differences in survival are attributable to maternal effects, the mechanisms responsible for those differences are not clear, and may

be due to differences in maternal and larval characteristics not measured in this study, such as feeding ability, sensory ability or genetics.

Clearly, maternal effects in yellow perch result in morphometric differences among hatchlings. Furthermore, those differences in morphometry result in distinct differences in resistance to starvation. And, even though the mechanisms are not clear, maternal effects also result in differences in survival among maternal lines when food is not limited. While these maternal effects would be expected to have substantial implications on recruitment under environmental uncertainty, they may only be important when high fishing pressure has reduced the variation in maternal characteristics in the spawning population (through selective harvest) at low or declining population levels. The larval traits that confer a survival advantage likely vary from year to year according to the biotic and abiotic conditions in the lake. When the distribution of spawning stock traits is wide, a wide distribution of larval traits would be produced within a cohort. Thus it is probable that a portion of the larval cohort possess the appropriate characteristics. Thus, the importance of maternal effects would be small. However, when high levels of selective fishing remove a portion of the spawning stock and alter the distribution of spawning stock traits, a narrow or even skewed distribution of larval traits would be produced within a cohort. Under this scenario it is less likely that larvae will possess the appropriate characteristics to ensure strong recruitment. Under environmental uncertainty, the distribution of larval traits artificially selected for through high fishing pressure may not be sufficient for adequate survival and successful recruitment.

The changes that have occurred in the Lake Michigan yellow perch population indicate that there are fewer yellow perch than in past decades, and moreover, lower

proportions of females than in the past. Females that are mostly greater than four years of age, and that are historically large for their age characterize the female population that does exist. These changes in the female portion of the population suggest that the importance of maternal effects may have increased during the population decline in the early 1990s, and may be most important now as the population attempts to recover. The collapse of the Lake Michigan yellow perch fishery that occurred in the last decade of the 20th century is likely not directly attributable to changes in the characteristics of the female spawning stock nor maternal effects. In fact, the initial cause or causes of the population decline may not ever be fully understood. However, the role maternal effects play in the rebuilding of the population may be very important.

The results of this study suggest that the large females currently dominating the spawning population are likely producing a substantial portion of offspring that would be characterized as relatively long and heavy with small yolk-sacs. If food were limited, these larvae would be expected to exhibit a lowered resistance to starvation than their smaller conspecifics with large yolk-sacs. In the past decade, Lake Michigan has undergone dramatic changes as a result of the introduction and rapid population booms of exotic species, particularly the zebra mussel (*Dreissena polymorpha*). The introduction of this species has potentially resulted in a degradation of not only the food quantity, but also quality, that yellow perch larvae depend on as first feeding begins.

If food were not limited, than the larvae being produced by the current spawning population would be expected to have a lowered vulnerability to predation than their smaller conspecifics. The opposed potential outcomes of being long with a small yolk versus being short with a large yolk, coupled with the environmental uncertainty that

exists in nature offer an indication of the difficulty the yellow perch population will face as it continues to recover. Several consecutive years of environmental conditions that are unfavorable to the types of offspring produced each year could result in high levels of embryonic and larval mortality that would substantially inhibit recovery. The Lake Michigan yellow perch population will remain at the mercy of the environment as it continues to rebuild itself, until it reaches a point where a wide distribution of traits are once again being produced each year.

As the population recovers, years when the environmental conditions favor recruitment of several phenotypes will result in the replacement of "missing" young yellow perch with new recruits that exhibit a wide distribution of traits. If enough favorable years occur consecutively, the age and size distribution of the population will begin to widen. Eventually the spawning population will include relatively high numbers of young, small spawning females in addition to the high numbers of old, large spawning females that are currently present. The subsequent diversity in the age and size structure of the population will result in the production of diverse year classes. The production of offspring that display a wide distribution of traits ensures that in the face of environmental uncertainty and size-selective mortality, some larvae will survive and recruit, and the population will endure.

High exploitation of a species in a size-selective manner will, over time, force shifts and truncation on the distribution of traits within that population. If we are constantly removing individuals from a population that exhibit some set of desired traits, we can naturally expect to see fewer and fewer individuals that exhibit those desired traits within the population. This presents a substantial risk if maternal effects within that

species have effects on the survival of offspring. Dramatic shifts or truncations in spawning population trait distributions may precipitate severe population declines and potential recruitment failures. The best protection against the inherent environmental uncertainty that exists in nature, and to ensure an adequate level of recruitment each year, is to ensure a wide distribution of characteristics within the spawning population. This will result in the production of offspring that exhibit a wide distribution of traits and will ensure some level of larvae survive, no matter what nature throws at them.

The results of this research identify several issues that need to be addressed by the managers of Lake Michigan yellow perch in the near future. The commercial fishery was completely shut down in 1997. There is considerable public pressure for the fishery to be reopened. Several key policies should be implemented before reopening occurs. Previously, the entire month of June was closed to harvest to protect the perch during spawning. However, in recent years the spawning season has actually begun in May as a result of earlier warming of the lake each year. Therefore, managers may better protect the spawning population through extending this closure to include the month of May. Furthermore, protection of female yellow perch through the rest of the year may best be accomplished through the use of formal, quantitative stock-assessments to determine the sustainable amount of yellow perch that can be taken each year. Before the closure of the Lake Michigan yellow perch fishery, detailed stock-assessments had not been used by the management agencies, and undoubtedly, the lack of assessment lead to excessive harvest, an overall reduction in the population level and a truncation of the spawning population's trait distribution. If managers chose to explore stock assessments as a management

option, they will want to ensure that a substantial monitoring effort is put in place to track the trait distribution of the spawning population throughout the year.

Future Directions

Phenotypes are the expression of genotypes and the interaction between those genotypes and the environment. This definition of phenotype strongly suggests that research on maternal effects need focus some effort on genetic components. Secondly, many teleost species are known to exhibit a high phenotypic plasticity. This allows individuals to quickly adapt to change. For example, density-dependent factors often bring about rapid changes in growth rate and age at maturity. It is conceivable that maternal effects may also influence an individual's scope for plasticity, which could have implications on survival and reproductive success. Bearing these two points in mind, future maternal effects research should begin to explore such areas. Are there maternal influences on resistance to starvation and survival that are not detectable at the phenotypic level? Are there maternal influences on phenotypic plasticity that result in differential adaptability and survival under changing conditions? These deeper insights will require research that employs genetic, physiological, biochemical, and ecological approaches simultaneously to determine fully the role of maternal effects on the dynamics of fish populations.

Appendix I

Relationship Between Larval Yellow Perch (*Perca flavescens*) Two-dimensional Body Area and Dry Weight

Overview

In addition to the ratio of RNA:DNA, the dry weight of a larva is a measure that yields insight into that individual's overall health and condition. However, in order to measure the dry weight of a larva and then extract nucleic acids from that larva for the determination of the ratio of RNA:DNA, one must freeze-dry the larva over night. If the drying process were performed in a drying oven, the high heat and duration of the drying process would increase enzyme activity substantially, resulting in the breakdown of RNA and DNA. Unfortunately, we were not privy to a freeze-dryer at the time of experimentation, therefore we opted to develop a relationship between the two-dimensional body area of a larva and the dry weight of that larvae.

Materials and Methods

Larval yellow perch (*Perca flavescens*) were sampled from a mass-rearing tank for each sampling age. Eighty-nine, one day post hatch (dph) yellow perch larvae were sampled from a mass rearing tank on 15 June 1998, and Eighty-one, 23 dph yellow perch larvae were sampled from a mass rearing tank on 7 July 1998. On each sampling day, the larvae were sampled five at a time to prevent mortality during sample processing. The larvae were first anesthetized with Tricaine Methansulfonate (MS-222), and then videotaped for analysis of morphometry. The larvae were then placed into individual pre-weighed aluminum foil envelopes and dried in a drying oven at 60° C to a constant weight (reached after about 60 hours). After drying, the individual larvae in the pre-weighed envelopes were re-weighed. The dry weight of each larva was determined as the

difference between the final weight of the larva in the aluminum envelope after drying minus the original weight of the aluminum envelope.

Imaging analysis on the videotaped larval images was performed using Optimas imaging analysis system (v 6.1, Media Cybernetics, Takoma Park, MD) at the Chesapeake Biological Laboratory, Solomons, MD. The two-dimensional body area of each larva (calculated internally by Optimas from the outline of each larva) was measured.

Results

The relationship between two-dimensional body area and dry weight was determined to be curvilinear and can be described by the equation (Dry weight (μg) = [(2 x 10^{-6}) x (Body Area)^2 + (6 x 10^{-5}) x (Body Area) + 3 x 10^{-5}] x 1000, n = 171, r^2 = 0.9836, p = 0.0001).

Discussion

The calibration between larval two-dimensional body area and dry weight accurately allows the researcher to determine dry weight from videotaped images of larvae. Furthermore, this technique allows the researcher to obtain accurate dry weights of larvae without the increased risk of RNA and DNA digestion that occurs when larvae are handled for long periods of time after death, before nucleic acid extraction. This technique also reduces the amount of processing time required for each individual larva. The time required to analyze videotape images for a group of larvae is far less than the time required to obtain their dry weights.

Table 1. Two-dimensional body area and dry weights of individual yellow perch (*Perca flavescens*) larvae sampled at 1 day post hatch (dph) and 23 dph. Consists of 4 pages.

Age (dph)	Sample Number	Body Area (mm^2)	Dry Weight (g)
1	1	2.00	0.0002
1	2	2.16	0.00013
1	3	2.21	0.00009
1	4	2.22	0.00013
1	5	2.22	0.00013
1	6	2.22	0.00034
1	7	2.28	0.00006
1	8	2.29	0.00015
1	9	2.34	0.00013
1	10	2.40	0.00017
1	11	2.40	0.00024
1	12	2.42	0.00017
1	13	2.45	0.00033
1	14	2.45	0.00022
1	15	2.48	0.00025
1	16	2.49	0.00018
1	17	2.51	0.00006
1	18	2.52	0.0003
1	19	2.53	0.00025
1	20	2.54	0.00023
1	21	2.56	0.00012
1	22	2.56	0.00019
1	23	2.61	0.00017
1	24	2.61	0.00024
1	25	2.62	0.00125
1	26	2.62	0.0002
1	27	2.63	0.00013
1	28	2.63	0.00018
1	29	2.63	0.00018
1	30	2.64	0.00005
1	31	2.65	0.00017
1	32	2.66	0.0003
1	33	2.74	0.0002
1	34	2.75	0.00035
1	35	2.75	0.00038
1	36	2.76	0.0004
1	37	2.76	0.00006
1	38	2.78	0.00031
1	39	2.78	0.00038
1	40	2.79	0.00031
1	41	2.81	0.0003
1	42	2.82	0.00029
1	43	2.82	0.0003
1	44	2.83	0.00025
1	45	2.84	0.00016
1	46	2.84	0.00013
1	47	2.84	0.00001
1	48	2.85	0.00036
1	49	2.87	0.0003
1	50	2.89	0.00013
1	51	2.89	0.00016

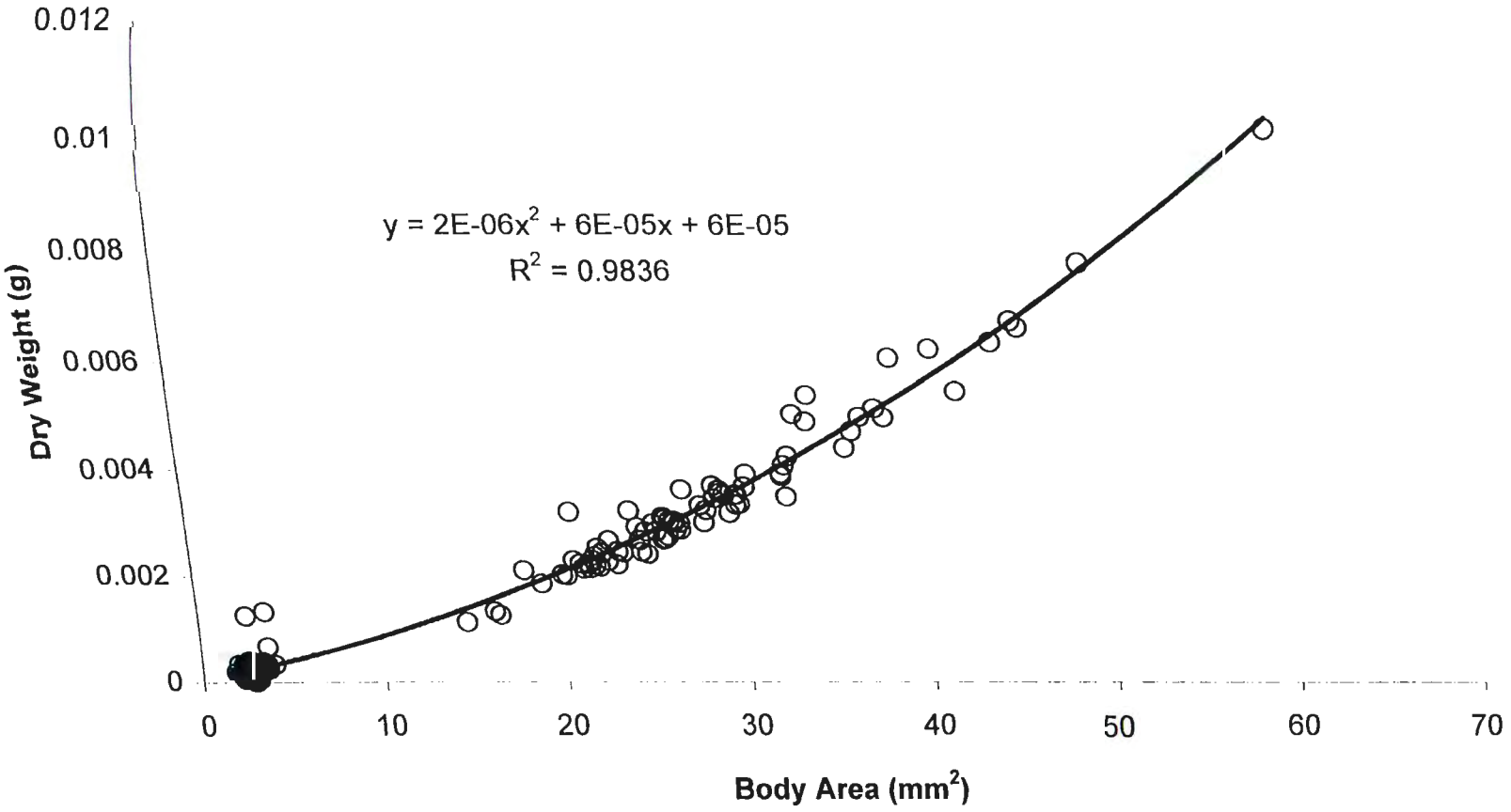
Age (dph)	Sample Number	Body Area (mm^2)	Dry Weight (g)
1	52	2.89	0.00024
1	53	2.90	0.00023
1	54	2.90	0.00002
1	55	2.91	0.00012
1	56	2.91	0.00026
1	57	2.93	0.00025
1	58	2.95	0.00007
1	59	2.96	0.0003
1	60	2.96	0.00031
1	61	2.97	0.0003
1	62	3.00	0.00034
1	63	3.03	0.00029
1	64	3.03	0.00023
1	65	3.03	0.00016
1	66	3.04	0.00026
1	67	3.07	0.00006
1	68	3.07	0.00022
1	69	3.07	0.00026
1	70	3.07	0.00025
1	71	3.07	0.00028
1	72	3.11	0.00026
1	73	3.12	0.00025
1	74	3.18	0.00022
1	75	3.21	0.00028
1	76	3.21	0.00032
1	77	3.22	0.0003
1	78	3.30	0.00031
1	79	3.30	0.00031
1	80	3.31	0.0003
1	81	3.32	0.00036
1	82	3.33	0.00039
1	83	3.39	0.00022
1	84	3.48	0.00025
1	85	3.55	0.00034
1	86	3.58	0.00032
1	87	3.61	0.00026
1	88	3.62	0.00025
1	89	3.66	0.00067
1	90	3.66	0.00133
1	91	3.97	0.00034
23	92	14.66	0.00113
23	93	16.24	0.00133
23	94	16.53	0.00126
23	95	18.04	0.0021
23	96	18.96	0.00185
23	97	20.12	0.00202
23	98	20.36	0.002
23	99	20.80	0.00229
23	100	20.80	0.0032
23	101	21.15	0.00223
23	102	21.30	0.00213
23	103	21.60	0.00221
23	104	21.65	0.00214
23	105	21.82	0.0023
23	106	21.96	0.00237

Age (dph)	Sample Number	Body Area (mm^2)	Dry Weight (g)
23	107	21.98	0.0022
23	108	22.13	0.00252
23	109	22.21	0.00217
23	110	22.39	0.00243
23	111	22.69	0.00228
23	112	22.80	0.00266
23	113	23.18	0.00221
23	114	23.26	0.00246
23	115	23.53	0.00243
23	116	24.02	0.00322
23	117	24.39	0.00291
23	118	24.49	0.00267
23	119	24.54	0.00245
23	120	24.84	0.00283
23	121	24.86	0.00241
23	122	25.27	0.00298
23	123	25.39	0.00283
23	124	25.76	0.00268
23	125	25.80	0.0031
23	126	25.88	0.0027
23	127	25.91	0.00306
23	128	26.03	0.00274
23	129	26.13	0.00302
23	130	26.37	0.00303
23	131	26.42	0.003
23	132	26.50	0.00288
23	133	26.68	0.00299
23	134	26.72	0.00287
23	135	26.94	0.00362
23	136	27.86	0.00332
23	137	28.01	0.003
23	138	28.17	0.00323
23	139	28.62	0.00369
23	140	28.64	0.00345
23	141	28.96	0.00361
23	142	28.97	0.00355
23	143	29.20	0.00352
23	144	29.41	0.00318
23	145	29.78	0.00352
23	146	29.79	0.00333
23	147	29.87	0.00352
23	148	29.99	0.00335
23	149	30.32	0.00367
23	150	30.47	0.00391
23	151	32.34	0.00392
23	152	32.35	0.00387
23	153	32.52	0.00407
23	154	32.54	0.00349
23	155	32.76	0.00424
23	156	33.18	0.00503
23	157	33.87	0.00489
23	158	34.03	0.00539
23	159	35.89	0.0044
23	160	36.29	0.0047
23	161	36.77	0.00498

Age (dph)	Sample Number	Body Area (mm^2)	Dry Weight (g)
23	162	37.61	0.00514
23	163	38.06	0.00497
23	164	38.57	0.00609
23	165	40.75	0.00626
23	166	42.02	0.00547
23	167	44.03	0.00638
23	168	45.12	0.00677
23	169	45.49	0.00665
23	170	48.89	0.0078
23	171	58.96	0.01019

Figure Legend

Figure 1. Nonlinear relationship between larval yellow perch (*Perca flavescens*) two-dimensional body area and dry weight for 1 day post hatch (dph) and 23 dph larvae.


0.012
0.01
0.008
0.006
0.004
0.002
0
Dry Weight (g)
y = 2E-06x² + 6E-05x + 6E-05
R² = 0.9836
0
10
20
30
40
50
60
70
Body Area (mm²)

Appendix II

Determination of the Effect of Post Death Condition, Preservation, and Age on the Shrinkage of Larval Yellow Perch (*Perca flavescens*)

Emily A. Kircher and Christopher J. Heyer

The following paper represents work that was conducted in the summer of 1998 by Emily Kircher, a student from Marquette University working at the WATER Institute under the Research Experience for Undergraduates program. Emily conducted this research under my direct supervision. This research will serve as a future reference for total length corrections of field-captured yellow perch larvae preserved in liquid nitrogen for subsequent nucleic acid extractions and analysis.

Abstract

Many studies have assessed the performance of larval fish as a function of size. However, it has been demonstrated that larval fish shrink upon death and preservation. If this shrinkage is not considered it is possible to overestimate or underestimate the performance and condition of larvae. The amount of shrinkage due to the effects of post death condition, preservation, and age was determined in yellow perch larvae (*Perca flavescens*). The difference in length was first taken from live yellow perch larvae and again after a post death condition. Computer image analysis tools were used to determine the lengths of the larvae. Results show that shrinkage does occur due to both death and preservation. Also, it was determined that the main effects of post death condition and age were significant, but there was no detectable interaction between death and age. An estimate of shrinkage in larval yellow perch is 14% on preservation in liquid nitrogen and 5% on death. Because many studies assess the performance of larval fish as a function of length, it is imperative to have an accurate measure of the fish. Therefore, it is necessary to take these shrinkage amounts into consideration when studying field-collected and lab-reared larvae so as to not overestimate or underestimate the performance or condition of the larvae.

Introduction

The survival of fish larvae is dependent upon size. The bigger the larvae, the less likely it is to be eaten by a predator, swept away in a current that will take it out of its optimal habitat, or starve to death (Miller et. al. 1988). Many researchers have assessed performance and condition of larval fish as a function of length. Performance measures such as burst swimming speed (Webb and Corolla 1981) and prey selectivity (Mills et. al. 1989) have been measured in laboratory experiments relative to length. The condition of wild larvae has been assessed in field studies by using RNA:DNA ratios also as a function of length (Suthers et. al. 1996; Rooker et al., 1997).

Because the size of larvae is linked so closely to its performance, it is imperative to have an accurate measure of the larval length. Inferences about the performance of larvae in the field are often made from lab experiments on live fish. Often, the lengths of fish in the lab experiments are measured from live fish while the lengths of fish collected in the field are measured from preserved fish. Ichthyoplankton sampling requires towing a net through the water to collect larvae, typically these tows can last anywhere from 1-10 minutes or longer depending on the type of research. During a tow, larvae will often die due to the stress on their fragile bodies, and subsequently, their body functions will shut down. Upon death, larvae lose the ability to osmoregulate and they experience a loss of water from their body and tissues, leading to shrinkage. After the duration of the net tow the larvae are brought on board the research vessel and preserved. With preservation the larvae shrink again, this time due to the chemical fixation process of the preservative that draws the water out of their bodies. In the lab and the field, the shrinkage due to

preservation is accounted for, however, for the fish collected in the field, the shrinkage due to death before preservation is neglected.

It has been demonstrated that fish larvae shrink upon preservation (Theilacker and Porter 1995). This amount of shrinkage is often accounted for by adding the amount the larvae shrink from death to preservation, typically ten percent (Theilacker and Porter 1995), however, this may not be adequate. Evidence from larvae of marine fish species suggests that fish may shrink up to twenty percent on death prior to any additional shrinkage due to preservation (McGurk 1985; Jennings 1991; Theilacker and Porter 1995). Not correcting for the full amount of shrinkage of fish larvae due to death and preservation could result in a substantial underestimation or overestimation of the relationship between fish length and measures of performance of larvae in the field.

The objectives of this study were to quantify shrinkage in different ages of yellow perch (*Perca flavescens*) larvae under three post death conditions in an attempt to demonstrate that accounting merely for shrinkage due to preservation is not adequate, and that the shrinkage due to death must also be considered.

Methods

Yellow perch larvae from multiple brood stocks, maintained using standard hatchery methods, were used to evaluate the effects of post death condition, age, and preservation on shrinkage. The potential interaction between post death condition and age was examined. Eight ages of larvae (1 day post hatch (dph), 6 dph, 11 dph, 16 dph, 21 dph, 27 dph, 31 dph, and 36 dph) were treated with three post death conditions. The post death conditions consisted of placing the larvae in one of three conditions: sitting in

open air in a Tricaine methansulfonate (MS-222) solution, sitting on ice in a MS-222 solution, or placed in liquid nitrogen. The liquid nitrogen treatment combined death and the post death condition. Larvae were collected from the larval stock tank, anesthetized with 1 mL of a 24.25 mg/mL MS-222 solution, and videotaped for later imaging analysis. To ensure death, larvae in the air and ice death treatments were treated with a lethal dosage of MS-222, and then left in the solution of MS-222 to prevent total drying and to mimic the conditions of field collection. The post death condition was applied for one half hour and the larvae were videotaped a second time.

The videotape was used to determine the amount of shrinkage by difference. A SNAPPY™ frame grabber was used to capture the images from the videotape. UTHSCSA *Image Tool* (developed at the University of Texas Health Science Center at San Antonio, Texas and available from the Internet by anonymous FTP from maxrad6.uthscsa.edu) was used to measure the total length of each larva. The shrinkage amounts determined from the larvae in open air and on ice demonstrated the shrinkage upon death, while the shrinkage amount of the larvae placed in liquid nitrogen showed the shrinkage upon liquid nitrogen preservation. The amount of shrinkage due to preservation with liquid nitrogen was examined because it related to the corresponding field study that was being conducted.

Results

Larval yellow perch did shrink in the air and ice post death conditions and preservation in liquid nitrogen. The larvae in the open-air post death treatment shrunk an average of 5 ± 0.65 % (range: 0.14 - 12.63 %), those on ice shrunk an average of 3 ± 0.61 % (range: 0.15 - 10.95 %), and the larvae preserved in liquid nitrogen shrunk an average of 14 ± 0.64 % (range: 5.84 - 26.84 %).

A significant effect of age on shrinkage was observed (Table 1). For air and ice, the amount of shrinkage increased with age (Fig. 1 and 2), while it decreased with age for liquid nitrogen (Fig. 3). There was no significant interaction determined between post death condition and age.

There was no significant difference determined between the air post death condition and the ice post death condition. However, a significant difference was determined between the air post death condition and the liquid nitrogen post death condition and also a significant difference was determined between the ice post death condition and the liquid nitrogen post death condition. (Fig. 4, Table 2).

Discussion

This study shows that larval yellow perch do shrink upon death and preservation. Larvae left on ice shrink less than larvae left in the open air indicating that a cooler post death condition can diminish the amount of shrinkage upon death. An estimate of the shrinkage of larval yellow perch is 14% on preservation in liquid nitrogen and 5% on death. However, the experience of the fish must be considered. Larval fish in laboratory studies are directly preserved, and therefore only shrinkage due to preservation must be

corrected for. Larval fish collected in field studies often die due to the stress on their bodies due to net towing and shrink upon death even before they are brought aboard the research vessel and preserved. Therefore, shrinkage due to death and preservation must be taken into consideration.

While no significant interaction effect was found between post death condition and age at the alpha = 0.05 level, there was a significant interaction at the alpha = 0.10 level. Based on the variability of data and the significant main effect of age coupled with the significant main effect of death, we feel there should have been a significant interaction between death and age. Looking at the air and ice post death conditions in Figure 4, it is evident that there is no noticeable interaction between post death condition and age. However, Figure 4 does indicate that a potential interaction between post death condition and age does exist if you include the liquid nitrogen post death condition. This interaction is most likely confounded by the large variability observed in the data.

Although this data does show that larval yellow perch do shrink upon death with the air and ice post death conditions, it is surprising when considering basic fish physiology. Many shrinkage studies have been performed on marine fish species (McGurk 1985; Theilacker and Porter 1994). At first, one would expect that if these larval fish shrank upon death, than larval yellow perch would shrink as well. However, marine fish have an internal concentration that is one-third that of their surrounding salt water environment (Moyle and Cech 1982). When they die, and their body functions (including osmoregulation) shut down, the laws of chemistry take over. Water in the hypotonic internal environment of the fish diffuses out to the external hypertonic environment. Therefore, the fish shrinks upon death. By contrast, while fresh water fish

also have an internal concentration that is one-third that of seawater (Moyle and Cech 1982), the external environment is not saline. Therefore, when fresh water fish die and their body functions shut down, one would expect that water from the external hypotonic environment would diffuse into the internal hypertonic environment of the fish, and the fish would bloat. This study, however, did show that these larval yellow perch did shrink upon death. There are a few possible explanations for this. One is that the MS-222 solution that the larvae were left to sit in for one half hour was a higher ion concentration than the internal environment of the fish and therefore, they shrunk. Another possible explanation is that water did enter the larvae's bodies but they were bloated in a vertical manner or in their width, therefore, since the larvae have a fixed amount of epidermis, their lengths shrunk.

The next step in this investigation is to review the videotape used in this study and remeasure the larvae. Perhaps the results of shrinkage were due to reader error. Also this experiment should be run again with a slightly different method. The larvae should be placed in a lethal dosage of MS-222 and then placed in water for the remainder of the half hour of post death condition. This way, it can be determined if MS-222 skewed the results of this study. Either way, shrinking or bloating, it essential to have a correct measure of larval fish when studying them to accurately estimate the performance or condition. After this experiment is refined, a critical review of previously published data that assess performance as a function of length will be conducted. It will be determined if their results are skewed because of neglecting to account for the effects of death upon larval fish length.

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References

Jennnings, S. 1991. The effects of capture, net retention and preservation upon lengths of larval and juvenile bass, *Dicentrarchus labrax* (L.). J. Fish. Biol. **38**: 349-357.

McGurk, M. 1985. Effects of net capture on the post preservation morphometry, dry weight, and condition factor of Pacific herring larvae. Trans. Am. Fish. Soc. **114**: 348-355.

Miller, T.J., L.B. Crowder, J.A. Rice, and E.A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657-1670.

Mills, E.L., M.V. Pol, R.E. Sherman, and T.B. Culver. 1989. Interrelationships Between Prey Body Size and Growth of Age-0 Yellow Perch. Trans. Am. Fish. Soc. **118**: 1-10.

Moyle, P.B. and J.J. Cech, Jr. 1982. Fishes: An Introduction to Ichthyology. Prentice Hall. New York, New York. p 72-73.

Rooker, J.R., G.J. Holt, and S.A. Holt. 1997. Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. Mr. Biol. **127**: 387-394.

Suthers, I.M., J.J. Cleary, S.C. Battaglene, and R. Evans. 1996. Relative RNA Content as a Measure of Condition in Larval and Juvenile Fish. Mar. Freshwater Res. **47**: 301-7.

Theilacker, G.H. and S.M. Porter. 1994. Condition of larval walleye pollock, *Theragra chalcogramma*, in the western Gulf of Alaska assessed with histological and shrinkage indices. Fish. Bull. **93**: 333-344.

Webb, P.W. and R.T. Corolla. 1981. Burst swimming performance of Northern Anchovy, *Engraulis mordax*, larvae. Fish. Bull. **79**: 143-150.

Table 1. ANOVA table. Significant effect of post death condition and age ($\alpha = 0.05$). No significant interaction between post death conditionand age ($\alpha = 0.05$)

Source	df	F	p
Death	2	85.20	<0.0001
Age	7	2.85	<0.05
Death*Age	14	1.62	0.09
Error	83		

Table 2. ANOVA table. Main effects of death. No significant difference between air and ice. Significant difference between air and liquid nitrogen and between ice and liquid nitrogen.

Source	p
Air vs. Ice	.15
Air vs. Liquid Nitrogen	<0.0001
Ice vs. Liquid Nitrogen	<0.0001

Figure Legend

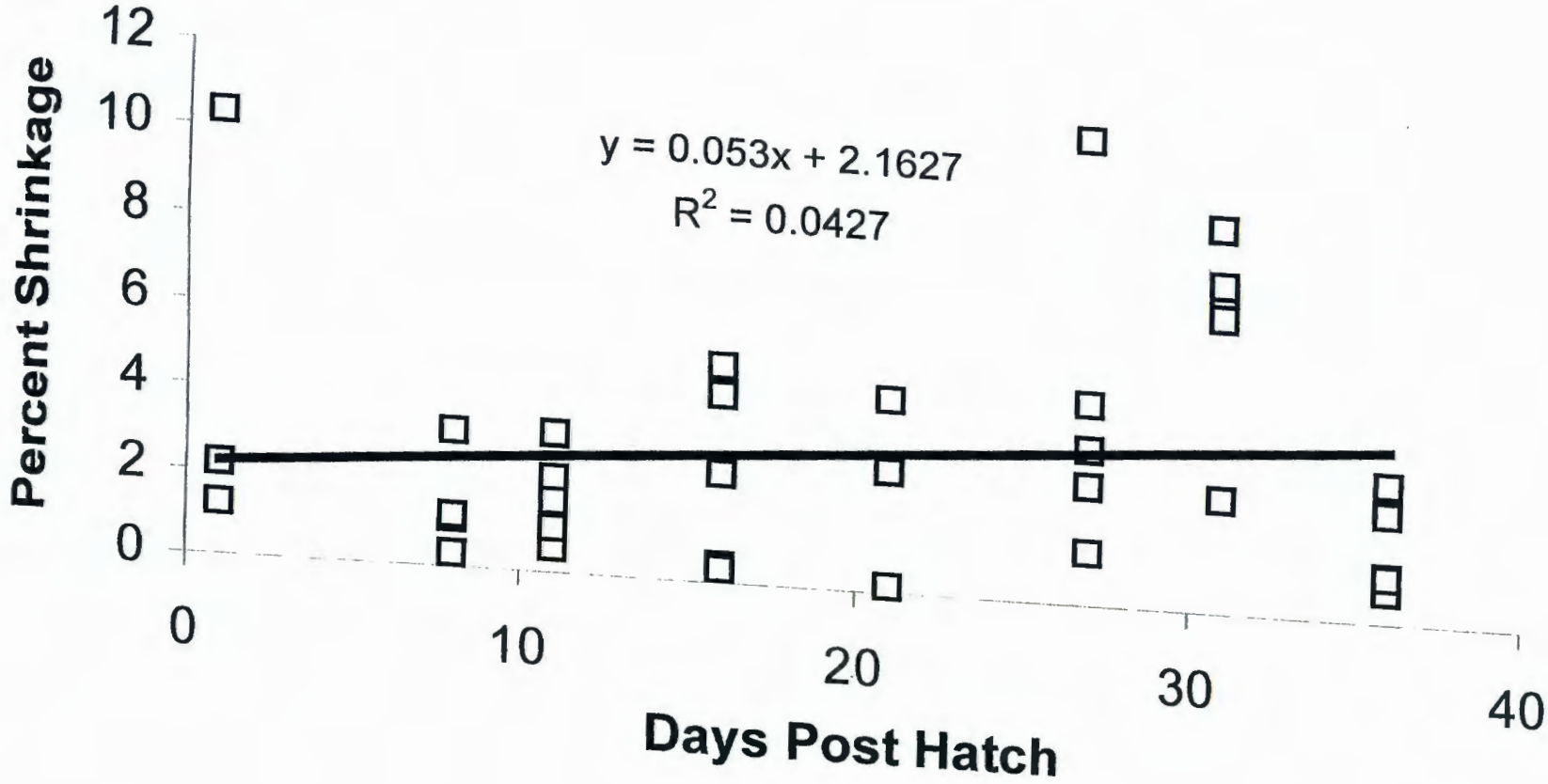
Figure 1. Percent shrinkage of larval yellow perch (*Perca flavescens*) in air as a function of age.

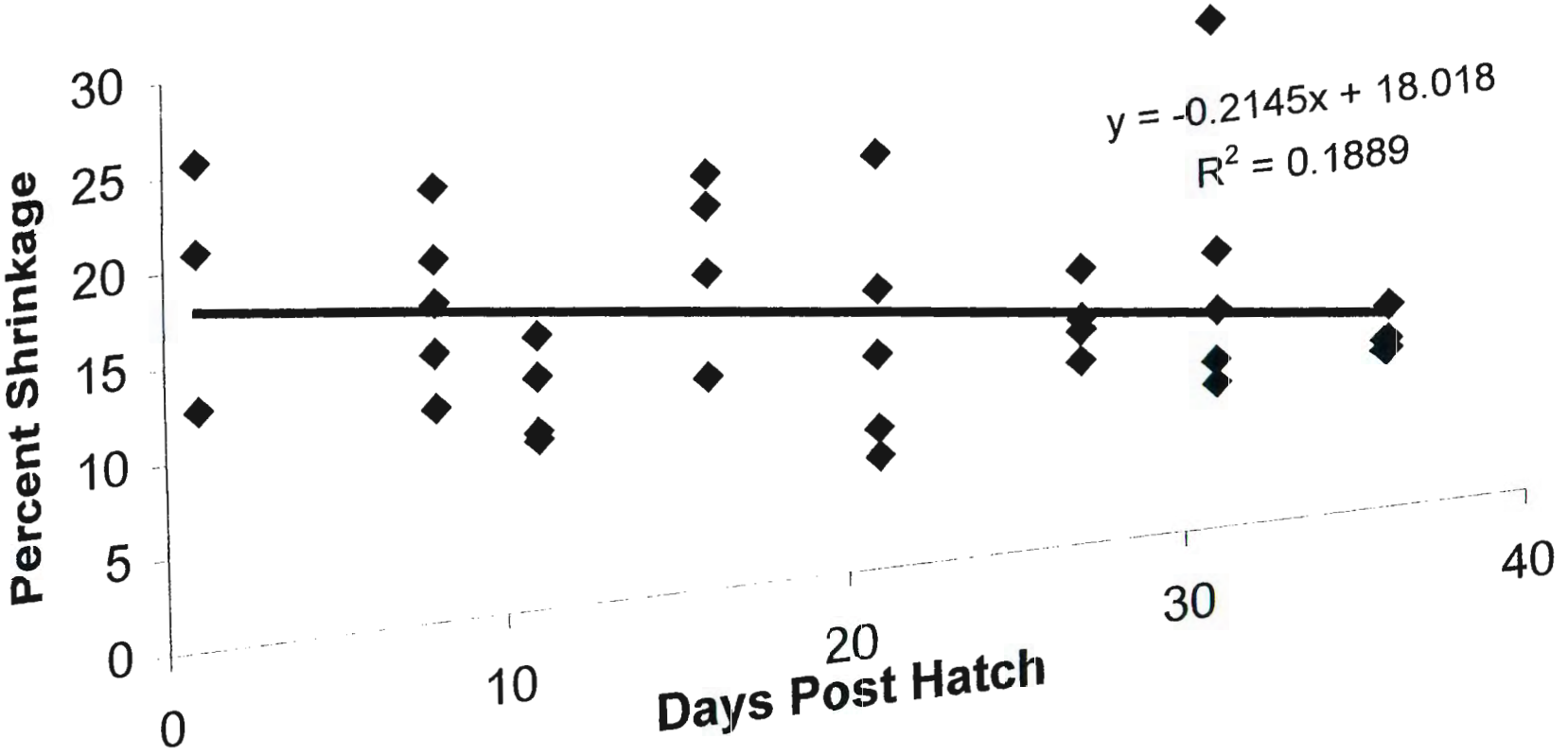
Figure 2. Percent shrinkage of larval yellow perch (*Perca flavescens*) on ice as a function of age.

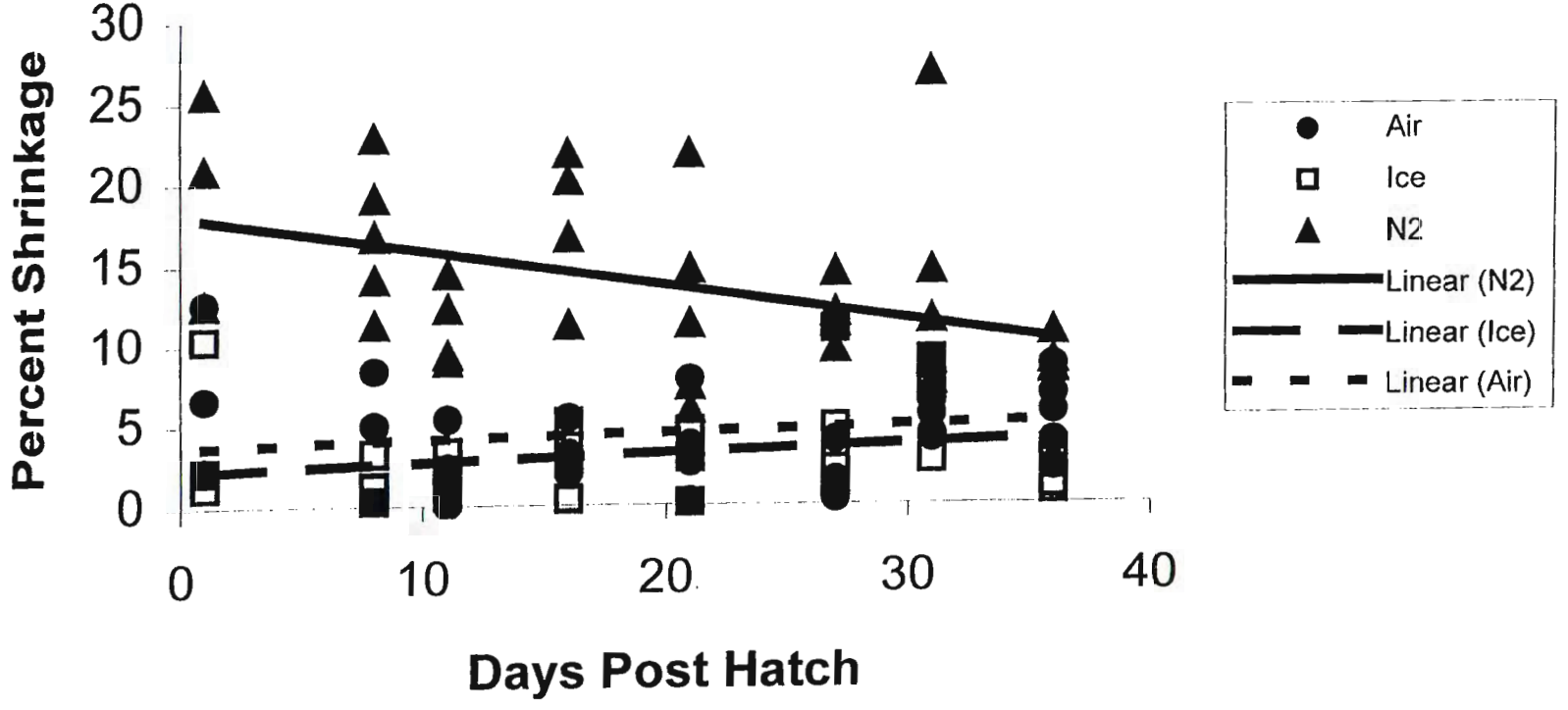
Figure 3. Percent shrinkage of larval yellow perch (*Perca flavescens*) in liquid nitrogen as a function of age.

Figure 4. Percent shrinkage of larval yellow perch (*Perca flavescens*) by death treatment as a function of age.

y = 0.0402x + 3.6474
R2 = 0.0168
Percent Shrinkage
14
12
10
8
6
4
2
0
0
10
20
30
40
Days Post Hatch


Percent Shrinkage
12
10
8
6
4
2
0
y = 0.053x + 2.1627
R2 = 0.0427
0
10
20
30
40
Days Post Hatch


Percent Shrinkage
30
25
20
15
10
5
0
0
10
20
30
40
Days Post Hatch
y = -0.2145x + 18.018
R2 = 0.1889


Percent Shrinkage
30
25
20
15
10
5
0
0
10
20
30
40
Days Post Hatch
Air
Ice
N2
Linear (N2)
Linear (Ice)
Linear (Air)