Food limitation during the early life history of walleye, *Stizostedion vitreum*

by

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FOOD LIMITATION DURING THE EARLY LIFE HISTORY OF WALLEYE, *Stizostedion vitreum*

BY

THOMAS A. JOHNSTON

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Abstract

I examined the feeding ecology, growth, energetics, and mortality of postlarval walleye, *Stizostedion vitreum*, using laboratory and field (extensive culture pond) experiments to assess the importance of food limitation to this period of the walleye's life history. Field studies revealed no period of intense mortality at the onset of exogenous feeding, and no significant relationship between early postlarval survival and zooplankton abundance (range 49-159·L^{-1}). Mortality rates in culture ponds were highest when predators were present. When predation pressure was low, mortality rates were an order of magnitude lower than those previously reported for natural lakes. In short-term, laboratory feeding studies, maximum consumption rate increased exponentially with walleye length and was not affected by prey size. Zooplankton abundances necessary to feed at near-maximal rates increased initially and then declined with walleye length, and declined with increasing prey size. Capture success increased with walleye length. Temperature affected attack rates but not capture success. Selection for relatively larger prey and selection for large cladocerans relative to cyclopoid copepods increased significantly with walleye length. Walleye selected most strongly for relatively uncommon prey. Effects of temperature and total prey abundance on prey selection differed between laboratory and field studies. Gut evacuation rate, R, of dry matter in non-feeding larvae varied with time since cessation of feeding, walleye weight, gut fullness, and temperature. The nature of these effects depended on how R was calculated. Apparent digestibility, AD, of crustacean zooplankton increased with gut retention time (maximum 78% at 6 h) but was not affected by walleye weight. First-feeding larvae required maintenance rations, R_{maint}, of 7-11%·d^{-1}. Estimates of R_{maint} increased with water temperature. Estimated prey abundances necessary to allow postlarvae to attain R_{maint} declined from 10-27 prey·L^{-1} for larvae of < 1 mg dry weight to < 2 prey·L^{-1} for larvae of ≥ 1.5 mg dry weight. Short-term (38-64 h) food deprivation caused the greatest weight loss and mortality in 1.5-3.5 mg dry weight larvae. Mortality rates during food deprivation were related to rates of weight loss but not energy densities. Maximum daily food consumption estimated from field data was considerably lower than the maximum observed in short-term, laboratory experiments. The use of laboratory-derived gut evacuation rates underestimated food consumption of larvae in the culture ponds. Published parameterizations of the Kitchell bioenergetics model underestimated the scope for consumption and/or overestimated respiration and waste losses of the pond larvae. Zooplankton abundance explained little variation in walleye food consumption over the range of zooplankton abundances observed in the ponds. Growth was primarily affected by temperature. Growth increased with food consumption but was always positive, suggesting that starvation was negligible in the ponds. Maintenance consumption derived from the bioenergetics model and field data was 49-73% higher than laboratory-derived values. Similarly, model-derived estimates of maximum growth rate, G_{max}, were much higher than laboratory-derived values. My results suggest that the feeding success of walleye larvae may be limited by both the abundance and size composition of zooplankton communities in natural environments. But, the effects of food limitation would only be evident at far lower zooplankton abundances than was previously thought. Starvation would only occur in very

oligotrophic systems. Food limitation probably exerts its greatest effect on survival by limiting growth and prolonging the period of vulnerability to predation.

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Foreword

This thesis was written in manuscript format. Chapters 2 through 7 were written as papers which have been or will be submitted for publication (Johnston and Mathias 1993b; Johnston and Mathias 1994a, b). Each contains an introduction, materials and methods, results, and discussion section. Chapters 1 and 8 present a general introduction and a general discussion of the thesis, respectively. All references in the thesis are contained in a common literature cited section following Chapter 8. Appendices A through E contain results obtained through research which was peripheral to the main objectives of the thesis, or results generated from unsuccessful experiments. Some of this work has also been published (Johnston et al. 1992; Johnston and Mathias 1993a). Figures, tables, and equations were numbered using a format such that Fig. 4.2 refers to the second figure of Chapter 4, Table A.1 refers to the first table of Appendix A, [7.3] refers to the third equation of Chapter 7, etc. Manuscripts from the thesis have been or will be sent to different journals but to maintain consistency I have chosen to follow the format of the Canadian Journal of Fisheries and Aquatic Sciences throughout the thesis, including the appendices. Journal abbreviations in the literature cited follow the format of the BIOSIS serial listings. Writing the thesis in the manuscript format has resulted in some overlap of contents between chapters and appendices, particularly in the introduction and materials and methods sections. However, I feel that the self-contained nature of each section makes the thesis easier to read and understand, and more than compensates for the overlap.

Chapter 1:
General Introduction

The Recruitment Problem

Much of fisheries research has the objective of understanding the processes which influence fish abundance. Many commercially exploited fish populations show dramatic fluctuations in abundance and this is usually attributed to variation in recruitment. Recruitment is the number or biomass of fish in a cohort which survive to a size which is exploitable by the fishery (Ricker 1975; Everhart and Youngs 1981). The term recruitment is also used to define the point in the life history where fish become susceptible to the fishery. Year-class strength can be defined as the relative contribution (landed numbers or biomass) to the fishery of a cohort during its lifetime. It is not synonymous with recruitment but is often used interchangeably to describe the strength of a cohort. Year-class strength is strongly correlated with recruitment if the post-recruitment mortality rate is fairly constant between cohorts. The recruitment problem for fisheries scientists is to determine the factors responsible for year to year fluctuations in recruitment, and to determine the causes of less frequent very high levels of recruitment occurring every 4 to 11 years (Anderson 1988). Because effective management relies on estimates of stock abundance and predictions of future year-class strength, understanding recruitment variability has become the central issue of fisheries science.

Research on recruitment variability has primarily focussed on economically valuable marine species such as Atlantic cod (*Gadus morhua*), Atlantic herring (*Clupea harengus*), and northern anchovy (*Engraulis mordax*). Most have high fecundities, small eggs and pelagic larvae, and exhibit little or no parental care. Compared to other vertebrate populations these fish species exhibit a strong type III survivorship pattern in their life history. This is characterized by a high mortality rate during the early life and a much lower rate thereafter (Krebs 1978). It has been generally accepted that the year-class strength of such species is determined during the high mortality period of a cohort's first year of life, probably in the larval or early juvenile periods (Gulland 1965; Larkin 1978). Studies of the early life history of fishes have thus become an essential part of understanding recruitment (Alderdice 1985).

To state that the year-class strength of a species is "determined" at a certain point in the life history implies that the normal variation in survival rate at that point has more influence on year-class strength than the normal variation at any other point. This concept that recruitment depends on a limiting or critical life stage is common in the fisheries literature (Everhart and Youngs 1981). Such stages are often referred to as bottlenecks in a cohort's life history. A limiting life stage is somewhat difficult to conceptualize. A logical approach to determining the position of a bottleneck is to examine the correlations between recruitment density and cohort density at various other life stages with the expectation that recruitment density will be strongly correlated with cohort density after but not before the bottleneck (as per Bradford 1992)(Fig. 1.1). Demonstrating this from field data would be difficult given the differences in distribution of the various life stages and the different gears required to sample them effectively. In the search for a unifying, general theory of fish recruitment it often appears that the limiting life stage is assumed to be the same for all species. However, it is not unreasonable to expect that the position of bottlenecks in the life history could vary between species, between stocks of a given species, or even between cohorts of a population.

Causes of Recruitment Variability

Recruitment variability could result from both biotic and abiotic factors. The number of eggs produced annually by a stock is dependent upon adult stock size. However, few studies have demonstrated any significant relationship between egg abundance and recruitment strength. Despite the development of theoretical stock-recruitment relationships (Ricker 1954), there is little empirical evidence to demonstrate that recruitment variability is dependent upon adult stock size. Physical factors are often cited as the primary cause of recruitment variability. The synchrony of strong year-classes between discrete stocks over wide geographic ranges suggests the importance of large-scale meteorological conditions (Cushing 1982; Koslow 1984). Numerous studies have found significant correlations between year-class strength and various climatic factors such as temperature, wind, and salinity (reviewed by Cushing (1982)). Though many of these empirical models explain significant amounts of variation in recruitment they often have very poor predictive capability (Sissenwine 1984). This is probably because physical variables do not directly influence survival but act indirectly by influencing one or more biotic factors.

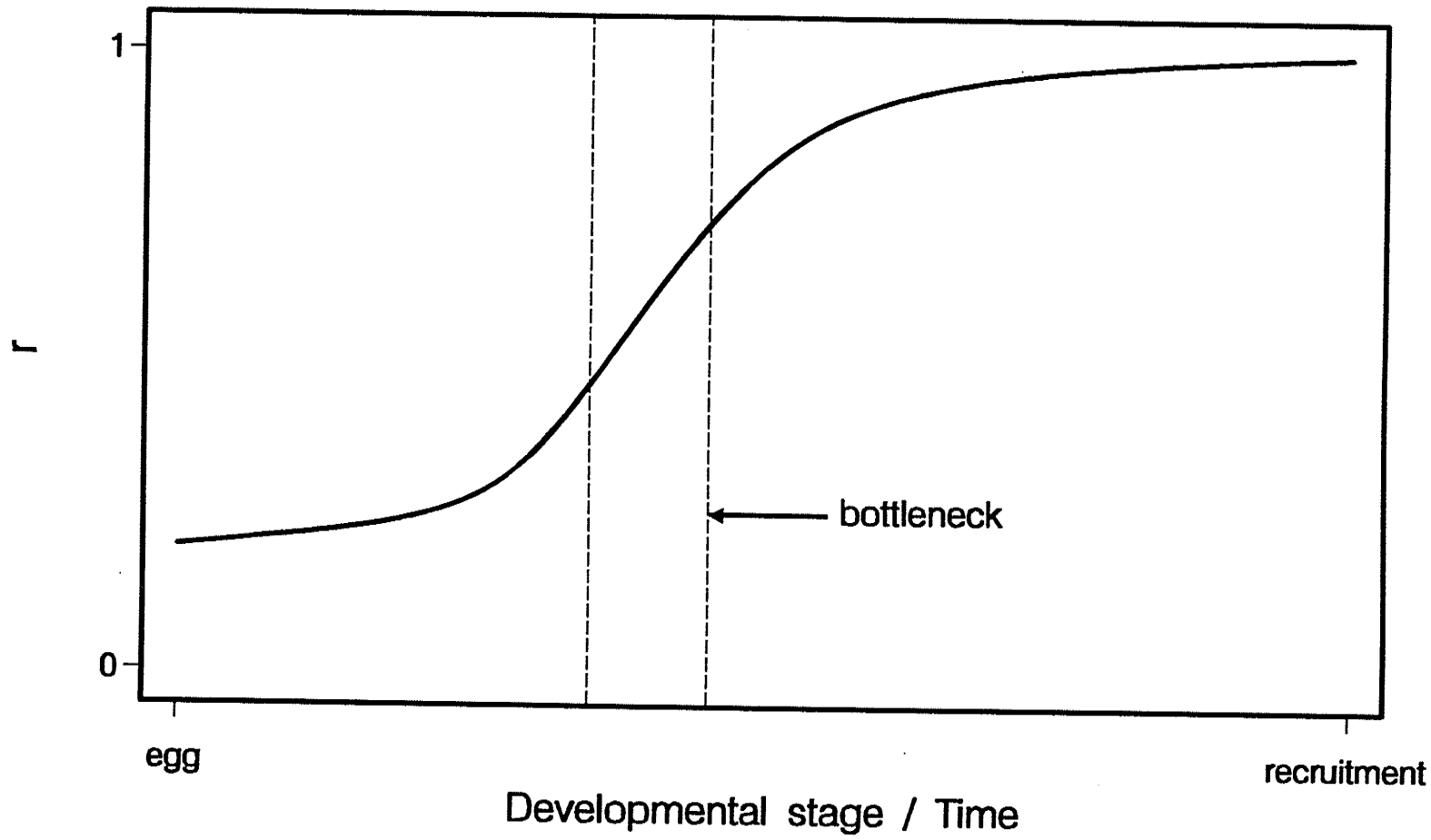

Figure 1.1. Theoretical relationship of correlation coefficients (r) between recruitment density and density at earlier developmental stages vs. developmental stage for a fish stock. Initial values of r at egg deposition would depend on stock but by definition r = 1 at recruitment. Between these two stages r should increase monotonically. A limiting life stage or bottleneck, if it exists, would be expected to coincide with or precede a sharp increase in r.

Biotic factors influencing early life survival can be broadly categorized as either food limitation (bottom-up effects from lower trophic levels) or predation (top-down effects from higher trophic levels). Disagreement over the relative influence of these two factors on population dynamics is a common theme in both aquatic and terrestrial ecology. Growth and mortality during the larval and early juvenile periods are usually assumed to be density-independent (Houde 1987). Cushing (1983) concluded that marine fish larvae were too dilute in their environment to reduce zooplankton densities but that their influence increased with size and density-dependent growth could appear at later stages. Similarly, predation in early life probably depends more on the density of other species than the density of the cohort or its parent stock.

One of the most enduring concepts in fisheries research is that of the critical period. The term critical period was first applied by Fabre-Domergue and Biétrix (1897) to describe the period of high mortality at the beginning of exogenous feeding in laboratory-reared marine fish larvae. Subsequently, the term has been used to describe any period of severe larval mortality or the life history period during which a cohort's year-class strength is determined (May 1974). However, the most commonly accepted definition of the critical period is that of Hjort (1914). He hypothesized that the year-class strength of North Sea cod and herring stocks was determined by the intensity of starvation mortality during the early stages of exogenous feeding. He believed that inadequate densities of zooplankton at first feeding would result in mass starvation and poor recruitment.

Zooplankton abundances may be limiting to fish larvae for various reasons. Unfavourable water currents may carry larvae away from the most productive areas. The larvae may also be temporally separated from their food source if the onset of first feeding does not coincide with the zooplankton production cycle (match-mismatch hypothesis) (Cushing 1982). Winds may disperse zooplankton so that high density patches do not form (Lasker 1981; Peterman and Bradford 1987). Low primary production and intensive grazing by other planktivores may also reduce zooplankton densities. Even when densities are sufficient for feeding, the nutritional composition of the zooplankton may be limiting because of inadequate nutrient content (Watanabe et al. 1983).

The critical period hypothesis has dominated the field of recruitment biology for many years but firm evidence of starvation mortality at first feeding remains elusive (May 1974; Leggett 1986). Recent reviews of early life history dynamics have pointed to predation as a more important determinant of survival (Sissenwine 1984; Bailey and Houde 1989). However, the lack of firm evidence for critical period mortality does not negate the importance of food availability and feeding during the early life history of fish. The critical period concept considers only a direct link between feeding and larval mortality. In aquatic environments body size is a strong determinant of both the potential food availability and the predation risk for an individual (Werner and Gilliam 1984; Miller et al. 1988). As individuals grow they can consume a wider range of food sizes and they have fewer potential predators. Even for similarly-sized larvae, individuals which have recently fed are often less susceptible to predation than those subjected to food deprivation (Rice et al. 1987). Therefore, feeding success and the factors which influence it may exert a strong indirect effect on larval survival through their effect on growth and susceptibility to predation.

The Walleye

The walleye (*Stizostedion vitreum*) is probably the most economically valuable freshwater fish in Canada. It is the most popular game fish among anglers in central Canada and is an important component of the commercial fisheries of Ontario and Manitoba (Scott and Crossman 1973). Because of this status, it is intensively studied from a management perspective. Recent declines in stocks which traditionally supported very productive fisheries have emphasized the need for research into the factors determining walleye year-class strength.

Ecologically the walleye is a piscivore and one of the top predators in the ecosystems it inhabits. It is thus an important species in controlling community structure. The walleye is considered a eurybiont and is tolerant of a wide range of biotic and abiotic conditions (Colby et al. 1979). Its range extends from the Mackenzie River delta in the north to the Mississippi River delta in the south (Scott and Crossman 1973). Walleye spawn in early spring generally over gravel or rock substrates in lakes or rivers. They are broadcast spawners with a relatively high fecundity and exhibit no parental care. The life history can be divided into the egg, prolarval, postlarval, juvenile and adult periods (Table 1.1). Previous studies have defined

Table 1.1. Life history periods of young-of-the-year walleye.

Developmental period	Approximate duration (d)	Habitat / Ecology
Egg	25	Deposited over rock or gravel beds in lakes or rivers during early spring
Prolarva	5	Drifts from spawning site; feeds endogenously on yolk and oil; negatively buoyant, positively phototactic
Postlarva	35	Inhabits pelagic zone; feeds primarily on zooplankton; development of air bladder and buoyancy regulation
Juvenile	100+	Becomes demersal and moves to littoral zone; diet shifts to larger benthic invertebrates and fish; becomes negatively phototactic

these periods based on larval morphology (Norden 1961; Nelson 1968) but for this study I felt that ecological definitions were more appropriate. The prolarval period extends from hatching until exogenous feeding commences. During this time the larvae drift from the spawning sites to the pelagic zone and rely on their endogenous supplies of yolk and oil. Postlarvae inhabit the limnetic zone and feed on zooplankton (Faber 1967). As the season progresses the walleye move to the littoral zone and begin to include benthos and fish in their diets. This marks the beginning of the juvenile period. The duration of each of these periods is variable and probably depends on a combination of abiotic and biotic factors.

Recruitment Variability in Walleye

As with many marine fishes, walleye stocks often show great variability in recruitment. Year-class abundances have shown fluctuations of 12 to 74-fold between the weakest and strongest cohorts of a stock (Koonce et al. 1977). Recruitment is usually more variable in heavily exploited stocks (Colby et al. 1979). Autumn abundance of young-of-the-year (YOY) fish is often used as an index of year-class stength. This reflects the general opinion that year-class strength is determined during the first spring or summer.

Examining recruitment variability in walleye in the framework of current theory should account for differences between freshwater and marine habitats since recruitment theory is largely based on marine species. Freshwater habitats, lakes, are analogous to islands, being difficult to recolonize and consequently more affected by local environmental extremes (Magnuson 1988). Both biotic and abiotic conditions, as well as exploitation rates, may differ greatly between lakes within a freshwater species' range. Factors which produce strong or weak year-classes are therefore more likely to differ between stocks of freshwater species than between stocks of marine species. Ultimately, a general theory of recruitment may not be possible for walleye and specific models may have to be developed to match specific stocks and their environments.

Few studies have examined the relationship between walleye recruitment variability and adult stock size despite the fact that walleye populations are often heavily exploited. Adult stock size may be a limiting factor in populations which have been overfished (Lysack 1986). Correlations between various physical factors and year-class strength have been found for many stocks but overall patterns are inconsistent. In most cases the physical factors are

assumed to influence spawning success. Temperature is the most commonly cited factor (Koonce et al. 1977). The rate and/or regularity of spring water warming was found to be a significant factor in some cases (Busch et al. 1975; Lysack 1986), but not others (Carlander and Payne 1977; Kempinger and Carline 1977; Ritchie and Colby 1988). Similarly, spring water level in lakes or flow rate of inflowing rivers were strong indicators of year-class strength in some waters (Chevalier 1977; Lysack 1986; Kallemeyn 1987) but not in others (Carlander and Payne 1977; Serns 1982). These studies illustrate the potential variability between factors affecting walleye recruitment in different lakes and the difficulty in using physical variables for general theories of recruitment. Temperature is undoubtedly a major influence but it can affect all life stages, and each in a variety of ways. A more useful approach would be to examine specific mechanisms which incorporate these physical factors.

Various life stages and mechanisms have been implicated as critical in determining walleye year-class strength. Egg mortality is often very high (Johnson 1961; Forney 1976). Busch et al. (1975) suggested that strong year-classes in Lake Erie coincided with years of rapid spring warming because egg incubation times were reduced and hatching success improved. This hypothesis implies that year-class strength is related to prolarval abundance, and thus, the stocking of prolarvae should enhance recruitment. However, in reviewing stocking programs, Laarman (1978) concluded that such introductions were generally ineffective in lakes where natural reproduction was good. In such lakes factors affecting survival after the prolarval period may be more important. Predation on juvenile walleye has been suggested as the major determinant of year-class strength in Oneida Lake, New York (Chevalier 1973; Forney 1976) but this hypothesis has rarely been proposed for other lakes. Mortality rates of postlarvae in natural lakes appear to be very high (95-96%, Noble 1972) but the exact causes of mortality are not clear. In contrast to studies of marine species, feeding and growth during the postlarval period has received little attention in terms of its effect on walleye survival and subsequent year-class strength.

Ecologically, the walleye exhibits several life history traits which could predispose it to food limitation during the postlarval period. First, the early spring spawning of this species results in larvae entering the pelagic zone very near the beginning of the spring plankton bloom. Thus the timing of hatching could have a major impact on food availability. Second,

first-feeding postlarvae are small with limited endogenous reserves and may not be able to withstand prolonged periods of food shortage. Third, the ontogenetic niche of the postlarva as a planktivore differs greatly from that of the adult as a piscivore and the postlarvae may be constrained by adult characteristics which reduce their effectiveness as planktivores (Werner and Gilliam 1984). Postlarvae may therefore be easily outcompeted by obligate planktivores. Laboratory studies of postlarvae have indicated that both feeding rate and survival increase with increasing zooplankton density (Li and Mathias 1982; Mathias and Li 1982). Further research into the ecology of walleye postlarvae could improve our understanding of the factors controlling feeding, growth, and ultimately survival during this period.

Objectives of this Study

The overall objective of my research is to examine the effects of food limitation on the feeding and growth of walleye postlarvae, and to assess the importance of food limitation to postlarval survival. I intend to meet this objective through six research projects which form six thesis chapters. Apart from characteristics of the biotic community in which a fish lives, the two most important factors governing early life history are body size and temperature (Pepin 1991). Whenever possible I will try to conduct my experiments and present my results with respect to these two variables. References to the developmental state of a larva will be based solely on body size not age. In addition, laboratory studies will be conducted in association with comparative field studies whenever possible.

My first step will be to look for direct evidence of critical period mortality. I will examine postlarval survival under semi-natural, predator-free conditions to determine if a period of severe mortality coincides with the onset of first feeding as predicted by the critical period hypothesis (Chapter 2). Second, I will examine the influence of zooplankton abundance and size on food consumption rate and prey selection (Chapter 3). Third, I will examine how temperature influences the feeding response in terms of capture success, food consumption, and prey selection (Chapter 4). The results of Chapters 3 and 4 will give information on food intake under varying biotic and abiotic conditions. Fourth, to understand how ingested zooplankton is utilized, I will examine the gut evacuation and assimilation processes in walleye postlarvae (Chapter 5). Next, I will try to determine the level of food intake necessary to prevent starvation, and to assess the resistance of postlarvae to food deprivation (Chapter 6).

Finally, in Chapter 7, I will examine postlarval consumption and growth in the field (culture ponds) using direct estimation and a bioenergetics model (both using Chapter 5 results), and compare my results with those of the laboratory studies (Chapters 3, 4 and 6). The overall implications of this research will be discussed in Chapter 8.

Chapter 2:

Mortality of walleye larvae in extensive culture ponds

Introduction

Walleye year-class strengths vary widely but are thought to be determined during a cohort's first year of life (Kempinger and Churchill 1972). However, the exact life stage where year-class strength is determined and the mechanisms involved are not well understood. Weak year-classes have been attributed to poor egg hatching (Busch et al. 1975) and predation on juveniles (Chevalier 1973; Forney 1976), but few studies have considered larval mortality as an important determinant of year-class strength.

Starvation and predation have been considered the principal causes of larval mortality in most fish species (Hunter 1980). The critical period hypothesis of Hjort (1914) stated that starvation resulting from inadequate zooplankton densities at the onset of exogenous feeding was the primary cause of larval mortality. Li and Mathias (1982) suggested that laboratory-reared postlarval walleye may also experience critical period (starvation) mortality under conditions of low zooplankton density. Starvation resulting from inadequate zooplankton densities has also been implicated as a cause of low and variable walleye survival in extensive culture ponds (Li and Ayles 1981). However, to my knowledge, no studies have demonstrated a period of high mortality at the onset of exogenous feeding in pond-reared walleye, and none has conclusively linked postlarval mortality to starvation.

The objectives of this research were to examine the pattern in mortality at the onset of exogenous feeding in pond-reared postlarval walleye, and to determine if this mortality was related to food limitation as predicted by the critical period hypothesis. Walleye larvae typically decline in weight from hatching until roughly 10 mm in length (Fig. 2.1). Though endogenous food sources are usually exhausted and exogenous feeding is underway by the time walleye reach 9 mm in size, the larvae are initially unable to consume enough food to prevent a continued decline in weight. Because smaller individuals are more susceptible to starvation (Miller et al. 1988), I predicted that starvation mortality would be most intense between the 9- and 11-mm stages when body weight was at a minimum and the larvae were still relatively inefficient at prey capture (see Chapter 4). In addition, I predicted that mortality rates during

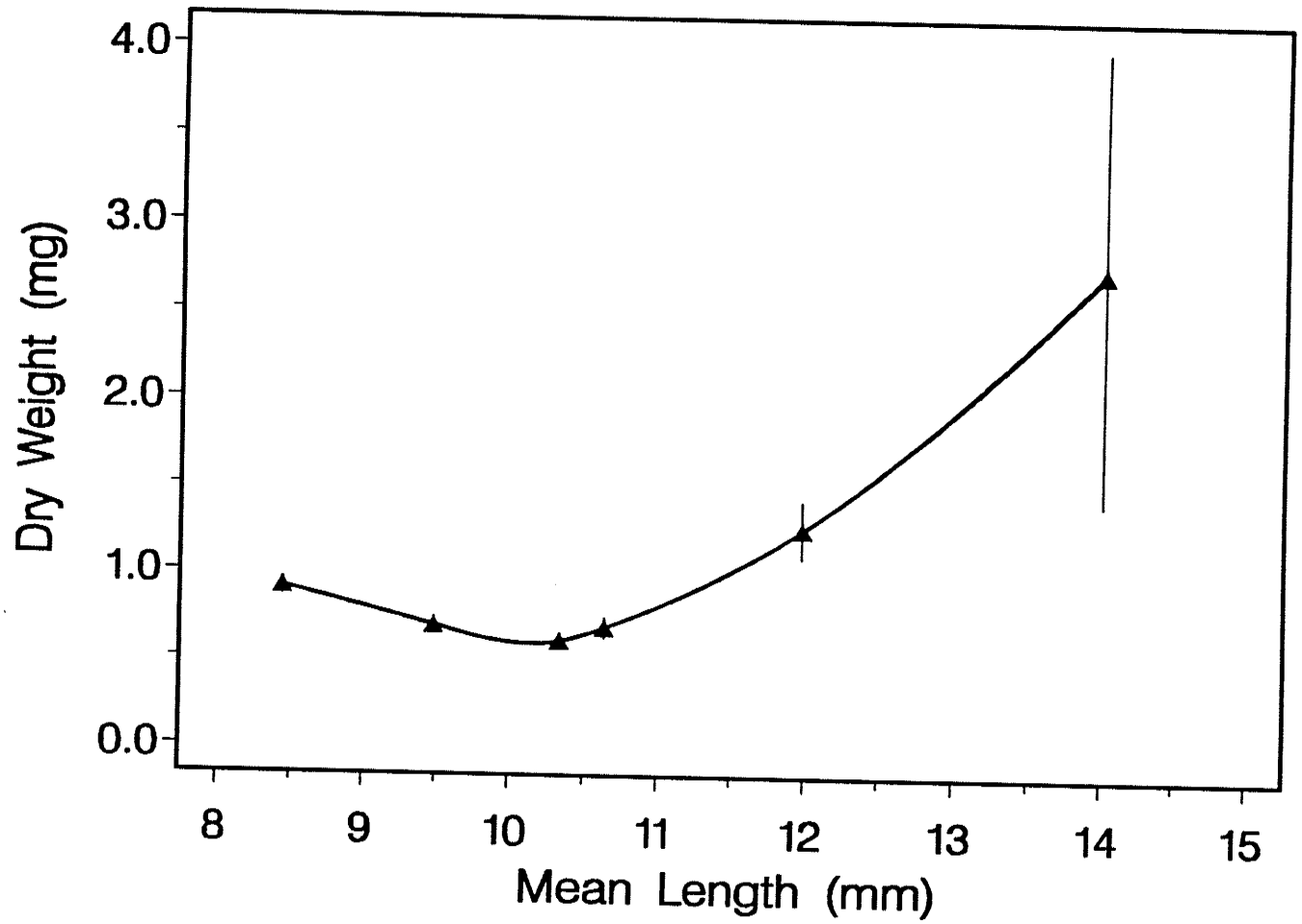

Figure 2.1. Weight-length relationship for walleye larvae (Lake Manitoba stock) raised in the laboratory at the Methley Beach field station in 1988. Larvae were maintained in 120-L aquaria at 20 °C and fed ad libitum on wild zooplankton. Symbols represent means ± 1 SD.

the early postlarval period would be higher than mortality rates calculated over the remainder of the culture period, and that postlarval mortality rate would be negatively correlated with zooplankton density. Finally, I looked for evidence of starvation by comparing the condition of pond-reared postlarvae to the condition of postlarvae deprived of food.

Materials and Methods

This study was carried out at the Department of Fisheries and Oceans' Dauphin Lake Walleye Rehabilitation and Research Station at Methley Beach, Manitoba (Fig. 2.2) during May and June of 1987, 1988, and 1989. The facility had four 1-ha, rectangular, earthen ponds for extensive walleye culture. A detailed description of the pond environments is provided in Appendix A. Prolarval walleye were stocked into the ponds within 1 to 3 d of hatching in each year. The number of fish introduced was estimated volumetrically. Stocking densities ranged from 40 000·ha^{-1} to 88 000·ha^{-1} (Table A.2). Each pond received a single introduction of prolarvae in 1987 and 1989, and two equal introductions one week apart in 1988 (Table A.2). The two stocks of walleye were introduced separately in 1988 as part of a concurrent research project on walleye stock differences (Brown 1990). In 1987 the ponds also contained assemblages of forage fish such as fathead minnow (*Pimephales promelas*), brook stickleback (*Culaea inconstans*), and Iowa darter (*Etheostoma exile*), but only walleye were present in 1988 and 1989.

The ponds were censused at roughly 4-d intervals following stocking by trawling paired bongo nets mounted on the front of an outboard boat. The bongo samplers were 315 μm mesh conical nets attached to aluminum mouth-reducing cones (15 cm diameter aperture) and were modified from the river drift samplers of Burton and Flannagan (1976). Each sample consisted of a 3- to 6-min horizontal tow. Each tow was divided into equal time intervals of towing at 0.25 m depth intervals from the bottom to the surface of the ponds. Tow speeds were approximately 1.4 m·s^{-1} in 1987 but were increased to 3.1 m·s^{-1} and 3.4 m·s^{-1} for 1988 and 1989 respectively. Census tows were unreplicated in 1987, but 4 and 6 replicate tows were taken at each sampling period in 1988 and 1989 respectively. A flowmeter (General Oceanics Inc., Model S2030-R) mounted inside one of the bongo cones was used to estimate volume sampled. The flowmeter was calibrated by making several tows over a known distance. Collected larvae were counted then frozen in 15 mL of pond water. Samples were

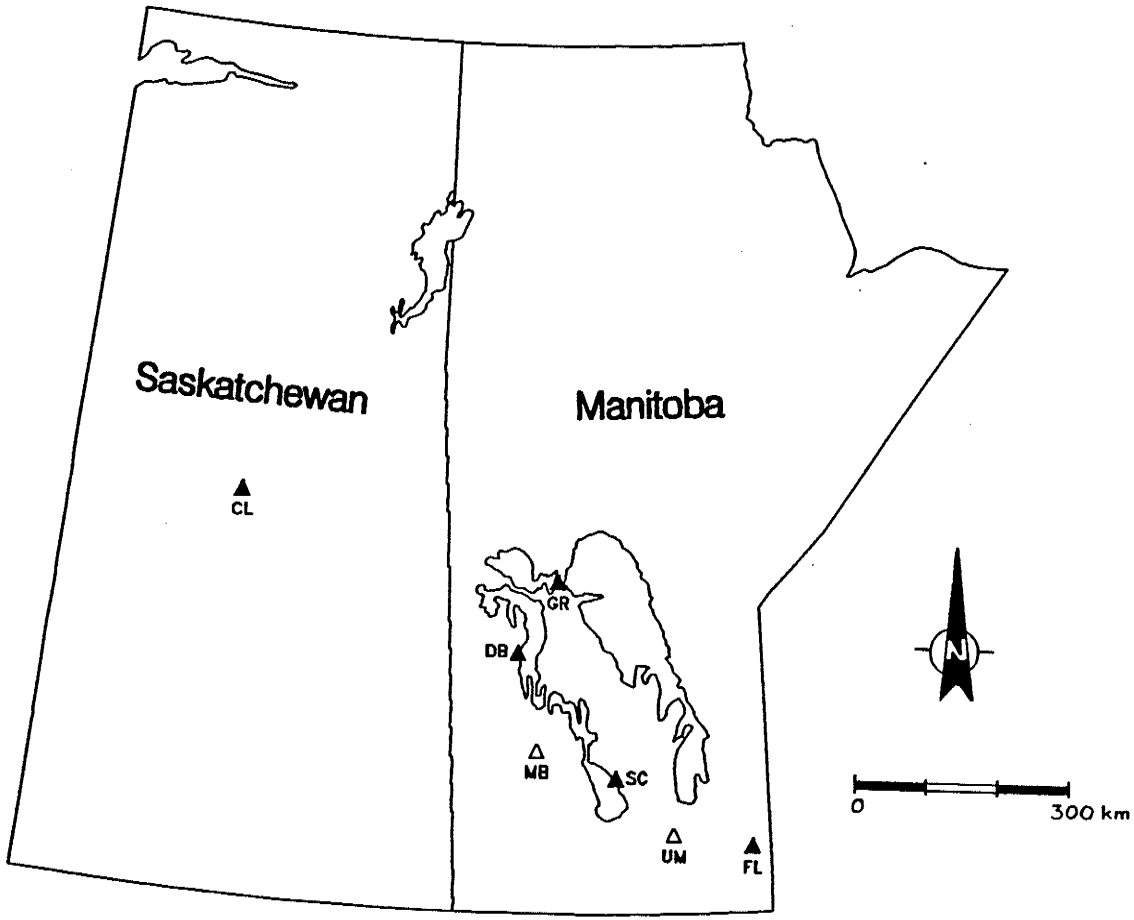

Figure 2.2. Study area, showing locations of research facilities (△) and sources of walleye stocks (▲) used in this study. MB = Methley Beach (Dauphin Lake) field station, UM = University of Manitoba, CL = Crean Lake, SC = Swan Creek (Lake Manitoba), GR = Grand Rapids (Lake Winnipeg), DB = Duck Bay (Lake Winnipegosis), FL = Falcon Lake.

later thawed and a minimum of 20 fish was measured. Lengths were measured as total length until a definite fork appeared in the tail and as fork length thereafter. These measurements were corrected for freezing shrinkage by using a fresh vs. preserved length conversion formula (Appendix B) and all results were expressed as fresh lengths. Walleye density (fish·m^{-3}) was estimated as the number of larvae captured in a tow divided by the volume sampled. Walleye population size was estimated as the walleye density multiplied by pond volume. Pond volumes were calculated separately for each sample date using depth measurements and the basin geometry. This method of estimating larval abundance assumed that the walleye were randomly distributed throughout the ponds.

Sampling was conducted at night between 23:00 and 04:00 with the exception of several sample dates in 1988 when diel sampling was conducted. Night sampling has been shown to be an effective means of preventing gear avoidance (Noble 1970). Comparative day-night sampling was carried out on pond 1 in 1989 to assess gear avoidance. Sampling efficiency was calculated as the ratio of the mean density based on daytime trawls to the mean density based on night trawls. Comparative day and night trawls were always conducted within 10 h of each other. The relationship between sampling efficiency and mean larval length was described by the equation $\log_e(\text{efficiency}) = 0.832 - 0.182 \cdot \text{length}$ ($r = -0.993$) (Fig. 2.3). Daytime population estimates in 1988 were converted to night estimates by dividing by the predicted sampling efficiency as estimated from the efficiency-length relationship. Gear avoidance may also depend on tow speed (Noble 1970). However, I felt that avoidance depended primarily on the ability of the larvae to see the sampling gear. Therefore, I assumed that gear avoidance would not depend on tow speed during night sampling and that population estimates from 1987 were directly comparable with those from 1988 and 1989 despite differences in tow speed. Because of differences in stocking densities, I converted all population estimates to percentages of stocking densities to allow comparisons between ponds and years.

I constructed survival curves by simply joining mean values on plots of estimated survival vs. mean length. Survival from stocking to a particular size was then estimated directly from the survival curves. I calculated instantaneous mortality rates, Z, using the formula

[2.1] $$Z = (\log_e(N_0) - \log_e(N_t)) / t$$

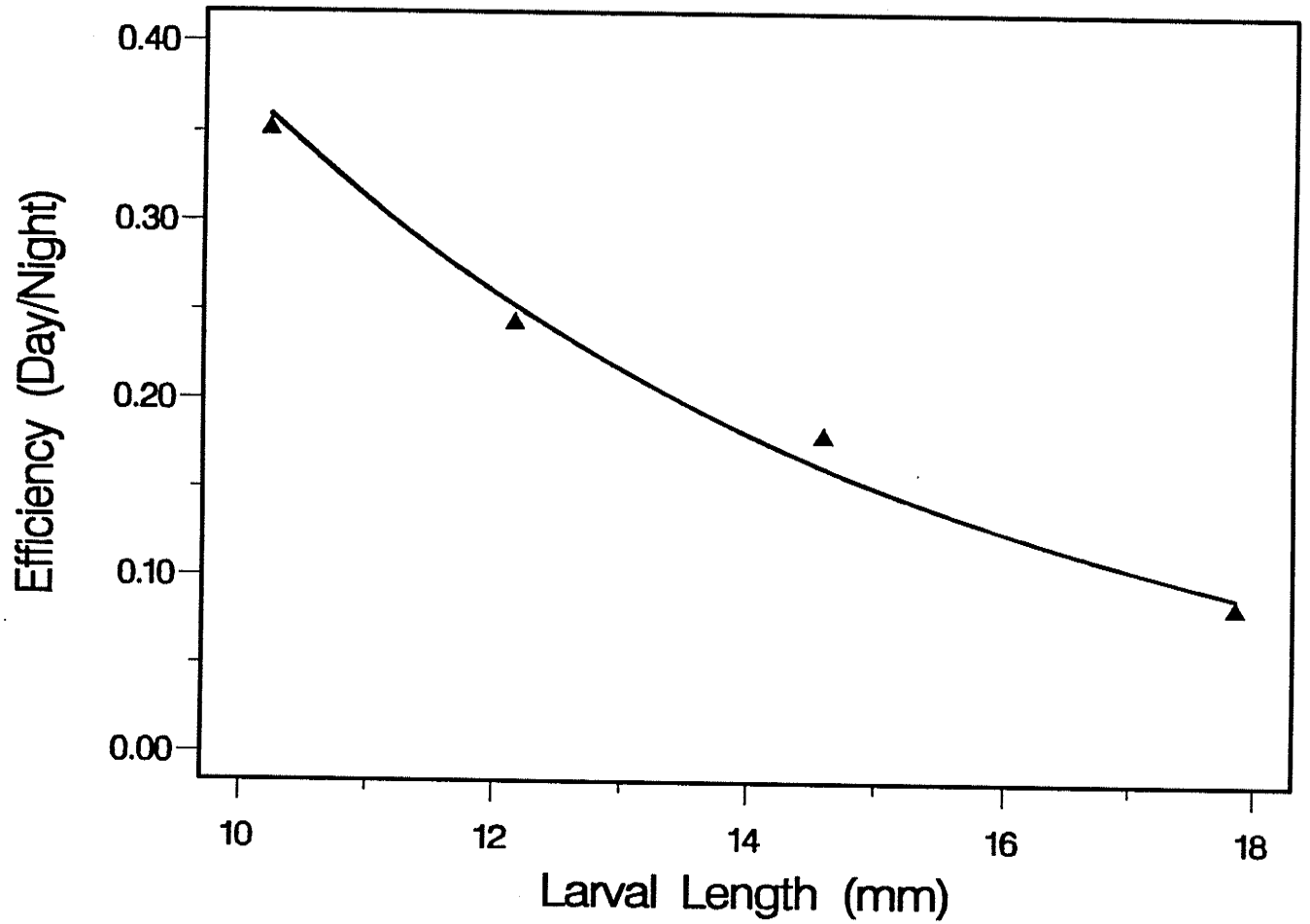

Figure 2.3. Relationship between sampling efficiency and larval length for walleye sampled from pond 1 of the Methley Beach walleye culture ponds in 1989. Sampling efficiency was calculated as the ratio of mean population densities estimated from day and night samples. The fitted relationship between the two variables was represented by the equation $\log_e(\text{efficiency}) = 0.832 - 0.182 \cdot \text{length}$ ($r = -0.993$).

where N_0 and N_t are population sizes at time 0 (stocking) and time t respectively (Ricker 1975). Mortality rates were calculated for the early postlarval period and for the remainder of the culture period (range 76-89 d). Data on survival to the end of the culture period were obtained from the pond harvest statistics in Johnston et al. (1992). Mortality rates for the postlarval period and for the entire culture period were arcsine-transformed. I then tested the hypothesis that postlarval mortality rates were higher than mortality rates over the entire culture period using a one-tailed, paired-observations *t*-test (Steel and Torrie 1980).

Crustacean zooplankton densities in the ponds were estimated as part of concurrent feeding studies (see Chapter 3). The sampling methodology and estimated densities are outlined in Appendix A. I examined the relationship between instantaneous mortality rate to a specific developmental stage and zooplankton density. Larval condition was estimated as

$$K_1 = W / L^3 \quad [2.2]$$

where K_1 is Fulton's condition factor, W is dry weight (mg) and L is length (cm). This ratio is generally considered the most appropriate index of fish condition (Ricker 1975; Bolger and Connolly 1989). Individual fish were measured, placed on pre-weighed aluminum trays, dried at 60°C for 24 h, moved to a desiccator for 1 h then weighed to within ± 1 μg on a Perkin-Elmer AD-6 Autobalance. Mean gut content dry weight was estimated for a subsample of these fish and subtracted from the dry weights for individual larvae (mean gut content dry weight was 8.4% of mean larval dry weight). I corrected both length and weight measurements for freezing effects following the formulae of Appendix B. Frequency distributions of larval condition were compared to those of similar-sized fish subjected to food deprivation for 48 h in the laboratory during 1990 experiments at the University of Manitoba (See Chapter 6). Differences in the distributions were tested using the Kolmogorov-Smirnov two sample test (Steel and Torrie 1980). All statistical analyses were conducted using SAS® software (SAS® Institute Inc. 1985).

Results

Survival curves were constructed for the individual ponds in 1988 and 1989 (Figs. 2.4 and 2.5 respectively), and for the combined pond data in 1987, 1988, and 1989 (Fig. 2.6). Because sampling in 1987 was unreplicated, I analyzed individual pond survival curves only

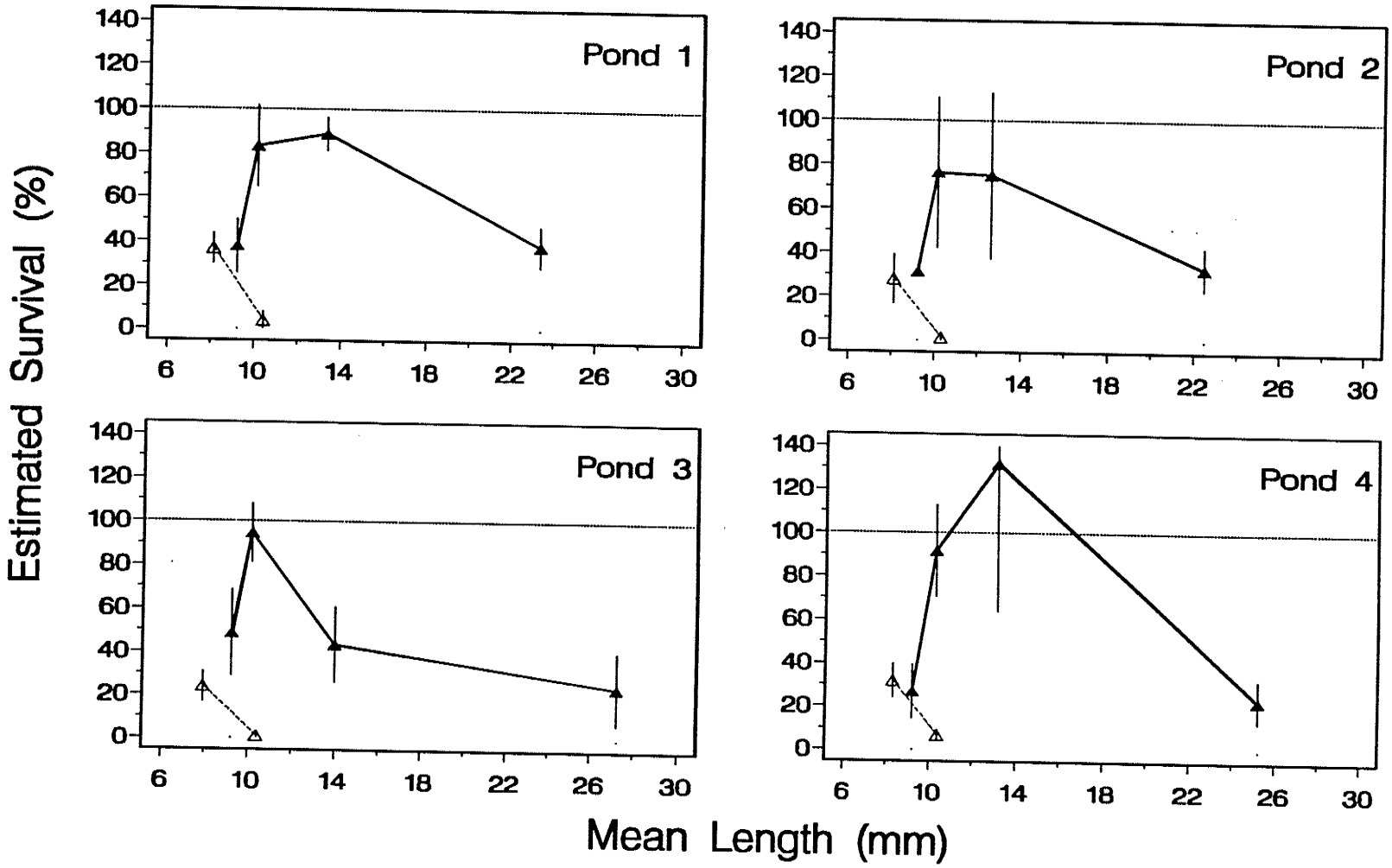

Figure 2.4. Survival curves for walleye larvae sampled from the Methley Beach culture ponds in 1988. Larvae of Crean Lake stock (△, - - - - - -) were introduced 1 wk after larvae of Lake Manitoba stock (▲, ————). Stocking dates and densities are listed in Table 2 of Appendix A. Reference lines indicate 100% survival. Symbols are means ± 1 SD.

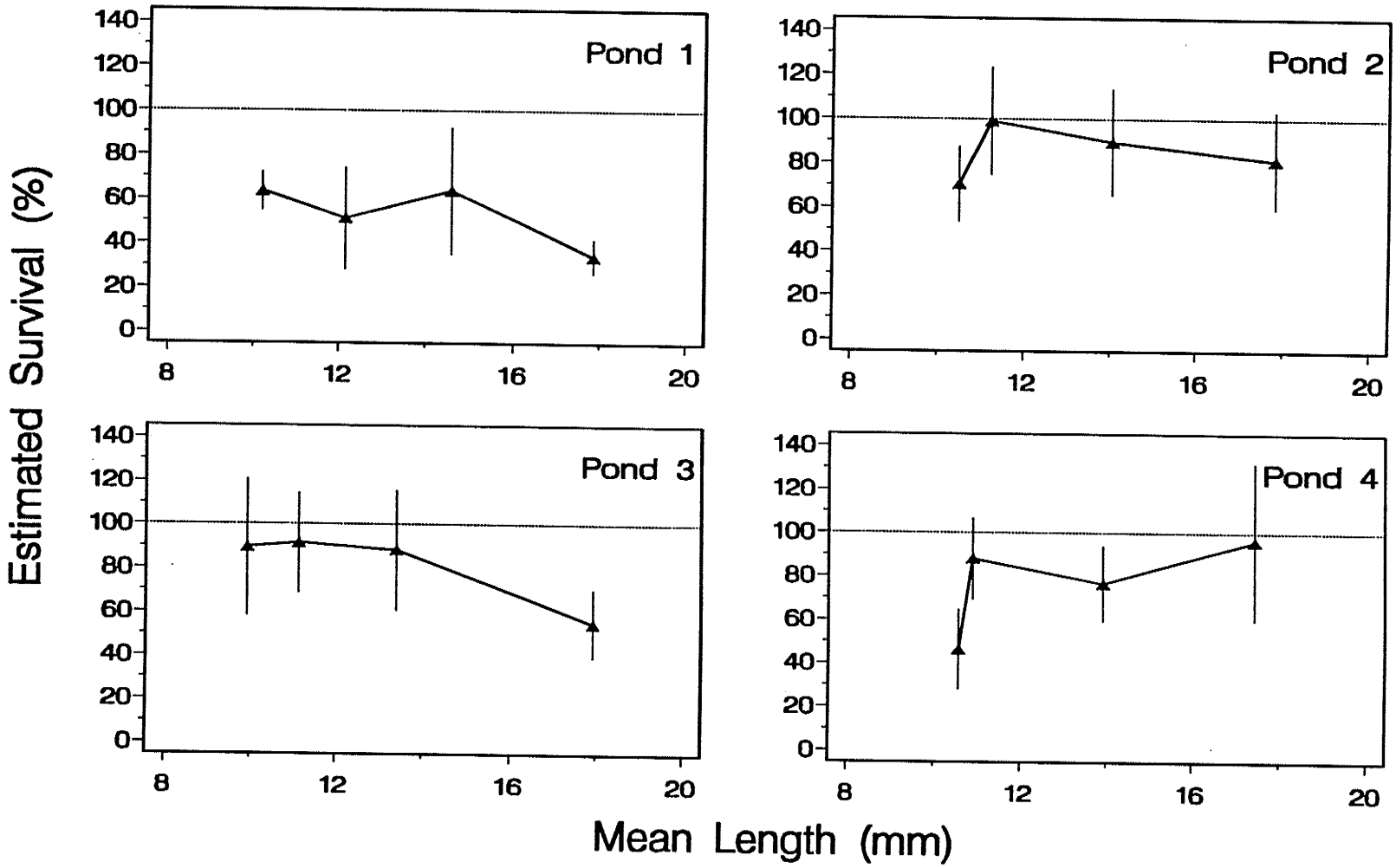

Figure 2.5. Survival curves for walleye larvae sampled from the Methley Beach walleye culture ponds in 1989. Stocking dates and densities are listed in Table A.2. Reference lines indicate 100% survival. Symbols are means ± 1 SD. Estimates for pond 1 may be biased low (see text).

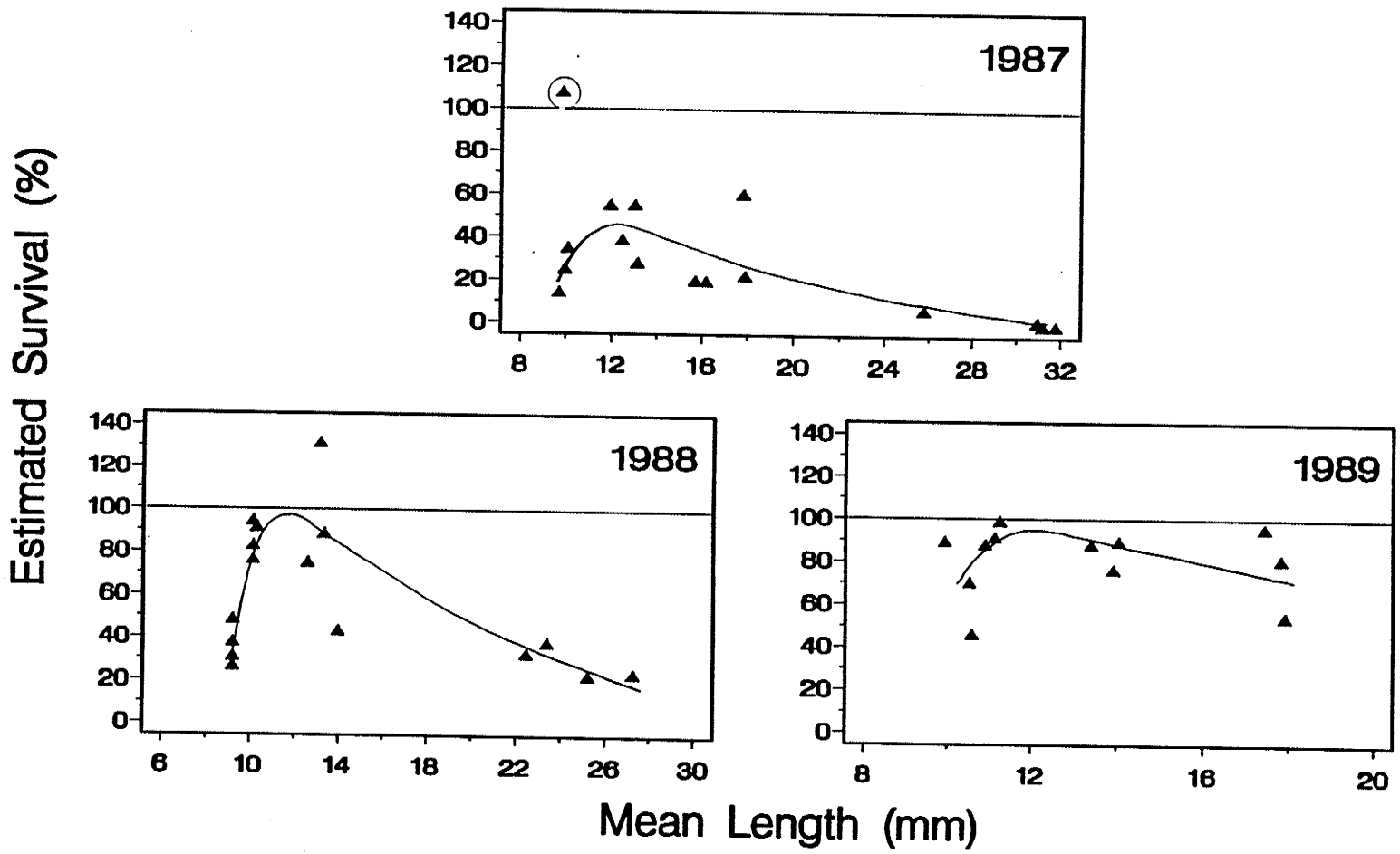

Figure 2.6. Combined survival data from the Methley Beach walleye culture ponds in 1987, 1988, and 1989. Symbols represent single samples in 1987, means of four samples in 1988, and means of six samples in 1989. Survival data from the second stocking in 1988 and pond 1 in 1989 were not included. Circled value in 1987 was considered an outlier because of an unusually low flowmeter reading. Curves were eye-fitted. Note differences in mean length axes among years. Stocking dates and densities are listed in Table A.2. Reference lines indicate 100% survival.

for the latter two years of the study. Some larvae were accidentally lost during the stocking of pond 1 in 1989 and consequently estimates of initial stocking density were biased high. I have included the survival curve for pond 1 in 1989 in Fig. 2.5 but I have excluded this pond's survival data from further analyses.

Larvae were fully recruited to the sampling gear by 12 mm in length (Figs. 2.4-2.6). At this length, larvae had been feeding for 4-5 d in 1987 and 1988, and 6-8 d in 1989. Though sampling lasted roughly three weeks in all years, the range of developmental stages covered was much narrower in 1989 than in 1987 or 1988. Lower spring temperatures in 1989 resulted in slower growth for this year (Appendix A). By the last sample periods in 1987 and 1988 some larvae may have become demersal (based on diet composition; Table A.7) and thus population estimates may be biased low at these stages as well. Walleye generally switch from a pelagic to a demersal existence at 25-30 mm in length (Colby et al. 1979). Therefore, the most reliable population estimates in all years were probably in the size range of 12-20 mm.

Survival of the separate introductions in 1988 differed greatly. Walleye from the initial introduction (Lake Manitoba stock) had a mean survival of 87% to the 12-mm stage (Fig. 2.4). The mean instantaneous mortality rate from stocking to the 12-mm stage for the four ponds was $0.019 \cdot d^{-1}$. However, walleye from the second introduction (Crean Lake stock) were reduced to negligible densities by the 12-mm stage (Fig. 2.4) probably due to predation by the Manitoba stock. Gut analysis of Manitoba larvae collected 24 h after the second introduction revealed that 4 of 169 (2.4%) contained Crean larvae. Assuming that 87% of the Lake Manitoba stock survived to this point, that 2.4% of them were actively cannibalizing at any one time, that they digested the smaller larvae in 5 h, and that light levels permitted 15 h of feeding each day I estimated that Manitoba larvae could have consumed Crean larvae at a rate of approximately $2500 \cdot d^{-1}$ ($40\,000 \times 0.87 \times 0.024 \times 3 \cdot d^{-1}$). There was no evidence of Manitoba larvae consuming each other in 1988, or of cannibalism in 1987 or 1989. In 1989, mean survival to the 12-mm stage (excluding pond 1) was 90% (Fig. 2.5). The mean instantaneous mortality rate over this same period was $0.012 \cdot d^{-1}$. Plots of the combined data over all ponds for each of the three years indicated a much lower survival in 1987 than in 1988 or 1989 (Fig.

2.6). Survival to the 12 mm stage was roughly 45% in 1987. This corresponded to an instantaneous mortality rate of $0.13 \cdot d^{-1}$.

Mean mortality rates calculated over the entire culture period were $0.037 \cdot d^{-1}$ in 1987, $0.016 \cdot d^{-1}$ in 1988 (Lake Manitoba stock only), and $0.046 \cdot d^{-1}$ in 1989. I compared postlarval mortality rates (stocking to 12-mm stage) with mortality rates over the remaining culture period (12-mm stage to harvest) using data from the individual ponds in 1988 (Lake Manitoba stock only) and 1989. Mortality rates over the early postlarval period were not significantly higher than those over the remainder of the culture period (one-tailed, paired-observations *t*-test, $t = -1.13$, df = 6, P = 0.85). I estimated the detection limit of this test using power analysis formulae and tables provided by Dixon and Massey (1969). Accepting a type I error rate of 5%, this test would have a power of 80% to detect a difference of $0.030 \cdot d^{-1}$ between postlarval and later mortality rates. Given the mean observed mortality rate of $0.030 \cdot d^{-1}$ during the remainder of the culture period, there would be a ≥ 80% probability of rejecting the null in this test at observed mean postlarval mortality rates ≥ $0.060 \cdot d^{-1}$. Accepting a slightly higher type I error rate would reduce this detection limit. However, I felt that this was a reasonable limit based on larval walleye mortality rates observed in natural systems (Table 2.1).

Mean crustacean zooplankton densities over the larvae's 9-12 mm stage ranged from 44-$388 \cdot L^{-1}$, but exceeded $100 \cdot L^{-1}$ in only two cases (Table A.5). I examined the survival vs. prey abundance and mortality vs. prey abundance relationships using data from the individual ponds in 1988 (Lake Manitoba stock only) and 1989. The regression of survival to the 12-mm stage on mean zooplankton density was not significant ($F = 4.14$, df = 1,5 , $R^2 = 0.45$, P = 0.097). Similarly, the regression of instantaneous mortality rate to the 12-mm stage on mean zooplankton density was also not significant ($F = 2.82$, df = 1,5 , $R^2 = 0.36$, P = 0.154). Scatter plots indicated that weak relationships may exist but they were strongly dependent on a single data point representing the highest zooplankton density (Pond 2, 1988).

Condition was examined for larvae captured from pond 3 on 5 June and 9 June in 1989. This pond had the second lowest mean zooplankton density ($49 \cdot L^{-1}$) over the 9-12-mm stage during this study. The lowest mean zooplankton density ($44 \cdot L^{-1}$) was observed in pond 2 in 1987 but insufficient numbers of larvae were collected to analyze condition. Mean condition of pond postlarvae on both dates was higher than that of laboratory-starved postlarvae of

similar mean length (Fig. 2.7). Tests for differences between the distributions of pond and lab fish condition (Fig. 2.7: 5 June (pond) vs. 30 May (lab), and 9 June (pond) vs. 2 June (lab)) were significant (Kolmogorov-Smirnov two-sample test, $D > 0.50$, $n \geq 30$, $P < 0.01$). Differences in condition between the pond fish and food-deprived fish may have been even greater than this analysis would suggest. For a given feeding regime, condition increases with length and the laboratory-starved fish used for comparison were slightly larger than the pond fish in both cases (mean lengths 10.2 mm vs. 9.8 mm and 11.0 mm vs. 10.4 mm). Also, the fish collected from the pond were subjected to rougher treatment than the laboratory fish and many were attacked by copepods and aquatic insects in the bongo nets before they were preserved. This may have reduced their weight somewhat. For these reasons, I feel that the tests were conservative.

Discussion

The survival curve pattern seen in this study agrees qualitatively with the results of previous studies. Prolarvae have been found to concentrate on or near bottom whereas postlarvae concentrate in the upper 3 m of water (Houde and Forney 1970). My results indicate that walleye larvae are not fully recruited to the bongo gear until they attain a length of 12 mm. The shift from a demersal to a pelagic existence is probably related to the development of the swim bladder and buoyancy regulation. Larvae with incomplete swim bladder inflation are negatively buoyant and spend a greater amount of time on or near bottom out of the path of the sampling gear. Thus neither prolarvae nor early postlarvae can be accurately sampled by bongo trawls. Alternative procedures may have to be employed to effectively sample this period of the walleye's life. I suspect this may also be true for postlarvae > 20 mm which adopt a more demersal existence.

In the absence of piscine predators, the mortality rate of walleye larvae from stocking to the 12-mm stage was quite low. Larval walleye in lakes often experience mortality rates which are an order of magnitude higher (Table 2.1). Mortality rates estimated from studies of extensive culture systems are comparable to those estimated in this study in 1988 and 1989 (Table 2.1). Mortality rates in culture pond populations are usually calculated from stocking to harvest. Variable returns from culture ponds have been attributed to variable survival during the early postlarval period (Li and Ayles 1981). My results indicated that mortality rates from

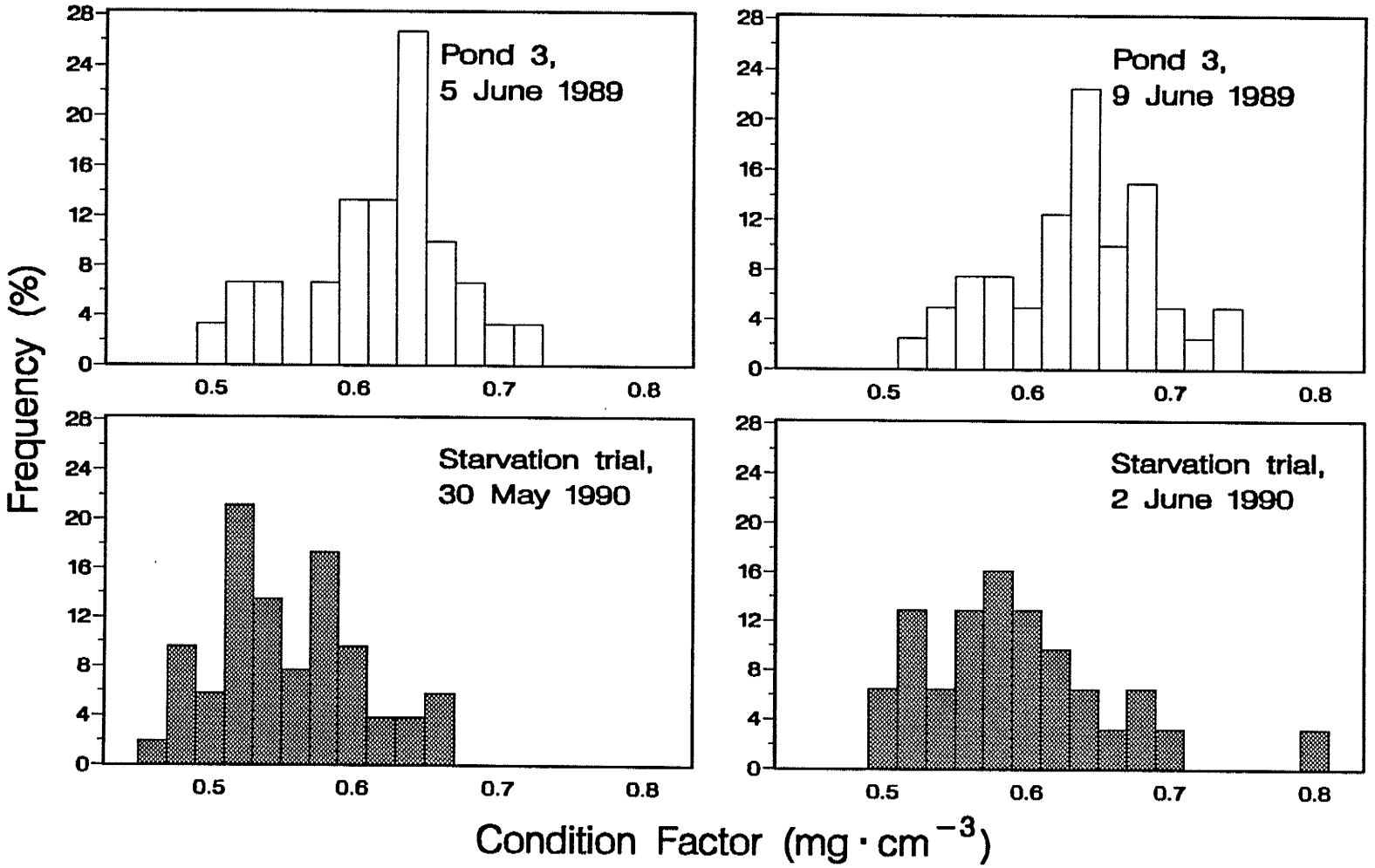

Figure 2.7. Frequency distributions of condition factor, K_1, for walleye postlarvae sampled from Methley Beach walleye culture pond 3 in 1989 and walleye postlarvae subjected to 48 h of starvation in the laboratory in 1990 (see Chapter 6). Mean lengths for the pond fish were 9.8 mm (n = 30) on 5 June and 10.4 mm (n = 40) on 9 June. Mean lengths for the laboratory fish were 10.2 mm (n = 52) on 30 May and 11.0 mm (n = 31) on 2 June.

Table 2.1. Instantaneous mortality rates, Z, of walleye larvae estimated from data of various studies.

Source	Location / Conditions	Methods	Interval	Z (d^{-1})
Noble (1972)	Billington Bay, Oneida Lake, New York, 1966, 1967; initial densities of 1 larva·m^{-3}, primarily of hatchery origin	Miller high speed samplers towed at 3.58 $m \cdot s^{-1}$; day sampling corrected for gear avoidance	10-18 mm mean length; 15 d	0.20-0.25
Forney (1975)	Oneida Lake, New York, 1968, 1970, 1972; initial densities of 0.03-0.05 larvae·m^{-3}, primarily of hatchery origin	Miller high speed samplers towed at 3.58 $m \cdot s^{-1}$; presumably day sampling	stocking to 9-10 mm mean length; 7 d	0.21-0.30
	Ten lakes, New York, 1969, 1971, 1973; mean initial density of 0.012 larvae·m^{-3}, primarily of hatchery origin	50 cm dia bridled ring net towed at 1.03 $m \cdot s^{-1}$; presumably day sampling	8-16 d post-stocking	0.35
Fox (1989)	Six 440 m^3 culture ponds near White Lake, Ontario, 1987; initial density of 40 larvae·m^{-3}; predators absent	Total count at harvest	stocking to 46.3 mm mean length; 57 d	0.0055-0.087
Te Brugge and McQueen (1991)	Three 1000 m^3 enclosures in Lake St. George, Ontario, 1986; initial densities of 8-32 larvae·m^{-3}; predators absent	Total count at harvest	9.3-30 mm mean length; 50 d	0.016-0.028

Table 2.1. Continued.

Source	Location / Conditions	Methods	Interval	Z (d^{-1})
Mitzner (1992)	Rathbun Lake, Iowa, 1984-1989; initial densities of 0.03-0.04 larvae·m^{-3}, primarily of hatchery origin	Bridled ring net towed at 1.55 $m \cdot s^{-1}$; day sampling	Stocking to 20-30 d post-stocking	0.067-0.26
Johnston et al. (1992)	Four 15 000 m^3 (max) culture ponds near Dauphin Lake, Manitoba, 1985, 1987, 1988, 1989; initial densities of 4-8 larvae·m^{-3}; predators absent (except 1987)	Total count at harvest	Stocking to 41-130 mm mean length; 88-107 d	0.022 (1985) 0.037 (1987) 0.016 (1988)[1] 0.046 (1989)
this study	same as above minus 1985 data	Paired bongo trawls towed at 1.4-3.4 $m \cdot s^{-1}$; night sampling or day sampling corrected for gear avoidance	Stocking to 12 mm; 9-11 d	0.134 (1987) 0.026 (1988)[1] 0.012 (1989)

[1] based on first introduction only (Lake Manitoba stock)

stocking to the 12-mm stage were not significantly higher than mortality rates over the entire culture period. Thus the early postlarval period may be no more critical than any other developmental period when piscine predators are absent and zooplankton densities are ≥ $50 \cdot L^{-1}$. Under these conditions walleye mortality may be fairly constant over the larval and early juvenile periods. The mortality rate to the 12-mm stage in 1987, when piscine predators were present, was intermediate between those observed in lakes and in extensive culture environments (Table 2.1). A high larval survival rate in the absence of predators has also been observed in mesocosm studies of both marine (Øiestad 1985) and freshwater (Karjalainen 1991) fish larvae.

Though my results indicate mortality between stocking and the 12-mm stage, I cannot determine whether the mortalities occurred during the prolarval or postlarval periods. Similarly, in most cases I cannot definitively state the causes of mortality. However, my results indicate that starvation was probably not a major source of mortality. No clear relationship existed between mortality and zooplankton densities. Survival was high even at zooplankton densities of $49 \cdot L^{-1}$ and the condition of larvae at these food densities was significantly higher than larvae subjected to food deprivation. This suggests that most larvae captured sufficient food to prevent starvation at zooplankton densities well below the optimum density of $100 \cdot L^{-1}$ suggested by Li and Mathias (1982). Though growth may be reduced at zooplankton densities < $100 \cdot L^{-1}$ (Fig. A.7), starvation would probably not occur at densities > $50 \cdot L^{-1}$. Fertilization of extensive culture ponds usually ensures that zooplankton densities are > $50 \cdot L^{-1}$ at first feeding, and thus critical period mortality would be unlikely. In natural environments, mean spring zooplankton densities are frequently < $50 \cdot L^{-1}$ (Chapter 8) though much denser patches undoubtedly exist in specific regions of lakes or rivers.

Survival was lowest in 1987 and for the Crean Lake stock in 1988. In both cases, predation appears to have been the most likely cause of mortality. The evidence for predation in 1987 was largely indirect. Mean zooplankton densities during the early postlarval period were similar to those in 1988 and 1989 and thus a higher starvation rate seemed unlikely. In addition, the forage species cohabiting the ponds in 1987 were known predators of walleye larvae (Franzin and Harbicht 1992). Ingested larvae provided direct evidence of intraspecific predation in 1988, but the impact on the population was probably much stronger than the

number of ingested larvae alone would suggest. Unsuccessful cannibalistic attacks resulting in escape of the victim may be ten times as frequent as successful attacks and the victims of unsuccessful attacks may experience a mortality rate of 19% over the following 24 h (Loadman et al. 1986). Thus attempted cannibalism may be as important or even more important than successful cannibalism as a source of mortality. The ratio of successful to unsuccessful attacks probably depends on the size difference between the attackers and the victims. This ratio was probably relatively high for Manitoba larvae attacking Crean larvae in 1988 and relatively low for attacks between similar-sized siblings in all years. Though I suspect that unsuccessful attacks are a major cause of mortality in culture ponds when only similar-sized siblings are present, such mortality would be difficult to quantify. The number of Crean larvae ingested by Manitoba larvae and the subsequent collapse of the Crean introduction in 1988 demonstrates that encounters, and consequently attacks, between larvae may be frequent even at larval densities < $8 \cdot m^{-3}$. Postlarval cannibalism is probably a relatively minor source of mortality in the natural environment (Loadman et al. 1986). The low density of walleye larvae relative to the densities of other fish larvae in the spring ichthyoplankton (Faber 1967; Mizera et al. 1981; Leslie and Timmins 1992) would reduce the probability of attacks between walleye.

I was unable to detect a critical period of high starvation mortality associated with the onset of first feeding in pond-reared larval walleye. I conclude that postlarval starvation would only be a major source of postlarval mortality when mean zooplankton densities are $<49 \cdot L^{-1}$. Thus, a critical period of starvation mortality would be unlikely in well-fertilized culture systems. These findings do not provide conclusive evidence that critical period mortality does not occur in natural populations of walleye. However, my results do indicate that zooplankton densities must be much lower than was previously thought for starvation to occur. These results also provide some evidence that both interspecific and intraspecific predation can be major sources of mortality for larval walleye. Though the composition and spatial distribution of the predator community in natural waters would differ greatly from that of the Methley ponds, my results do demonstrate the potential susceptibility of larval walleye to piscine predators. For this reason, I feel that the role of predation in postlarval mortality should be investigated further.

Chapter 3:

Feeding ecology of walleye larvae: the effects of body size, zooplankton abundance, and zooplankton community composition

Introduction

The larval period is considered critical in the life history of many fish species (Blaxter 1988) and starvation and predation are believed to be the major causes of larval mortality (Hunter 1981). Successful feeding during the larval period reduces the risk of starvation and ensures rapid growth through the size-classes most vulnerable to predation. Studies of the feeding ecology of fish larvae are thus essential to understanding the factors influencing the feeding success, and ultimately the survival, of fish larvae.

Feeding in fish may be affected by both the quantity and quality of available prey. Fish, like most animals, demonstrate a functional response to prey abundance (Ivlev 1961). The rate of prey consumption increases with increasing prey abundance with the most rapid change occurring at low prey abundances (Holling 1959). Among the qualitative characteristics of prey, size is one of the most important. The size of a prey item often determines how easily it can be detected, captured, and ingested. Size may also be an index of the nutritive value of a prey item. In addition, both the quantitative and qualitative effects of prey on feeding will vary with the development of the fish. The ecology of fishes changes during ontogeny (Werner and Gilliam 1984) and thus prey assemblages which are optimal for feeding by young larvae may not be optimal for older larvae and vice versa.

Li and Mathias (1982) noted a critical period of high mortality at the onset of exogenous feeding in postlarval walleye and hypothesized that food limitation during this period may be the major cause of larval mortality. However, since the early work of Mathias and Li (1982) few detailed studies on the feeding ecology of postlarval walleye have been carried out. No studies, to my knowledge, have examined the effects of prey size on the functional response or how the functional response changes during larval development. Though several studies have examined ontogenetic changes in prey selection by walleye (Raisanen and Applegate 1983; Graham and Sprules 1992) none has examined the influence of prey abundance or prey community composition on prey selection.

The purpose of this study was to conduct a detailed examination of the factors influencing the feeding ecology of postlarval walleye. My objectives were i) to examine the effects of walleye body size and prey size on the functional response of walleye larvae, and ii) to examine the effects of walleye body size, prey community composition and prey abundance on prey selection by walleye larvae. The results of these studies are interpreted in relation to the structure of zooplankton communities and the implications for walleye feeding success in the natural environment are discussed.

Materials and Methods

Experimental procedure

This research was carried out at the Department of Fisheries and Oceans' Dauphin Lake Walleye Rehabilitation and Research Station at Methley Beach, Manitoba (Fig. 2.2). Laboratory experiments were conducted in May and June of 1988. Field data were collected in May and June of 1987, 1988, and 1989 from culture ponds at the field station. Details of the pond environments and their management are provided in Appendix A.

Hatchery-raised walleye prolarvae from two stocks (Table 3.1) were brought into the laboratory within 1-3 d after hatching and raised in 120-L glass stock aquaria at 20 °C. The sides of the stock aquaria were covered with translucent green plastic sheeting. Wild zooplankton swept from the field station culture ponds were provided at least 1 d prior to the onset of feeding and maintained at densities > 200·L^{-1} thereafter. Experimental units were 13-L rectangular glass aquaria covered in green plastic sheeting and filled with 10 L of 20 °C (range 19-21 °C) pond water filtered through 45-µm Nitex® mesh. Light levels at the water surface of the experimental chambers were measured with a Li-Cor Quantum (Model LI-185) and ranged from 0.6 to 0.9 $\mu E \cdot m^{-2} \cdot s^{-1}$. Low light levels prevented glare at the water surface and thus allowed the larvae to feed in an apparently normal fashion.

Laboratory experiments were conducted on seven dates corresponding to walleye mean lengths of 9.45 to 14.8 mm (Table 3.1). Twenty-one aquaria were used in each experimental run and 2-3 runs were performed in a given day (Table 3.1). Within a run, each aquarium was randomly assigned a different zooplankton treatment and the runs represented replicates blocked for the time of day. Prior to each run, larvae were removed from the stock aquaria, randomly assigned to the experimental aquaria and left in total darkness for 4 h to allow their

Table 3.1. Summary of laboratory feeding experiments conducted in 1988. Walleye larvae were obtained from Crean Lake, Saskatchewan, and from the Swan Creek stock of Lake Manitoba. Each experimental run involved 21 experimental aquaria (see text and Table 2). Experimental runs were replicated (blocked) 2-3 times on each experimental date. Feeding time represents a mean calculated for all experimental aquaria. Dry weights for individual larvae were estimated from length-dry weight relationships.

Date	Walleye stock	Walleye size ($\bar{X}$ ± 1 SD)		Replicates	Larvae·aquarium^{-1}	Feeding time (min)
		Length (mm)	Dry weight (mg)			
27 May	Crean Lake	9.45 ± 0.06	0.50 ± 0.02	2	15	81
28 May	Crean Lake	9.67 ± 0.08	0.55 ± 0.02	3	18	65
19 May	Lake Manitoba	10.1 ± 0.15	0.71 ± 0.05	2	12	67
31 May	Crean Lake	10.9 ± 0.28	0.95 ± 0.11	3	12	55
22 May	Lake Manitoba	11.2 ± 0.19	1.13 ± 0.12	3	10	69
29 May	Lake Manitoba	13.9 ± 0.47	2.88 ± 0.42	3	6	44
6 June	Crean Lake	14.8 ± 0.93	3.64 ± 0.90	2	4	18

guts to clear. The number of larvae added to each experimental aquarium ranged from 18 for experiments with small larvae to 4 for experiments with the largest larvae (Table 3.1). Zooplankton treatments were added to the aquaria during the pre-experimental period. Zooplankton were swept from the Methley culture ponds and passed through a series of Nitex® sieves to separate them into three size fractions hereafter referred to as S (small, 183-300 µm), M (medium, 300-509 µm), and L (large, 509-1050 µm). Samples of each fraction were preserved in 5% buffered formalin for each experimental run to determine size and species composition. Zooplankton additions to the experimental aquaria are summarized in Table 3.2. One aquarium received no zooplankton addition. Twelve aquaria were randomly assigned one of the three size fractions at one of four abundances (15, 30, 60, or 120 prey·L^{-1}). The eight remaining aquaria were given assemblages of these three fractions in one of two ratios; A_{EQ} (equal proportions, S:M:L = 1:1:1) or A_{UNEQ} (unequal proportions, S:M:L = 9:3:1) at one of four abundances (40, 60, 100, or 180 prey·L^{-1}). I commenced experiments by turning on the lights and terminated experiments by removing the fish, sacrificing them in MS-222 (*m*-aminobenzoate methanesulfonate) and preserving them in 5% buffered formalin. The larvae were allowed to feed for a long enough duration to ingest a minimum of 10 prey per aquarium but for a short enough duration to prevent satiation. Total feeding time ranged from 81 min for first-feeding larvae to 18 min for the largest larvae (Table 3.1).

Preserved larvae were measured to the nearest 0.1 mm using an ocular micrometer for small larvae and a scientific ruler for larger larvae. Length was measured as total length until a definite fork appeared in the tail and as fork length thereafter. Preserved lengths were converted to fresh lengths using the formula of Appendix B. Gut contents were identified to genus, enumerated, and measured to the nearest 0.02 mm with an ocular micrometer. Zooplankter length was measured from the anteriormost point of the body to the base of the tail spine in Cladocera and to the end of the caudal rami in Copepoda. Zooplankter width was measured as the second longest body dimension. This corresponded to body width in Copepoda and to dorso-ventral depth in Cladocera. Zooplankter dry weight was estimated using length-dry weight formulae developed for zooplankton of the Methley culture ponds (Table A.3). Food consumption rate was calculated for each fish as the dry weight of stomach contents (µg) divided by the total foraging time (h).

Table 3.2. Experimental design for laboratory feeding experiments in 1988. Zooplankton treatments of various size compositions (C = control (no addition), S = small, M = medium, L = large, A_{EQ} = equal assemblage, A_{UNEQ} = unequal assemblage (see text)) were added in varying abundances to 21 experimental aquaria as indicated. This design was replicated in 2-3 blocks on each experimental date (see Table 3.1).

	Zooplankton treatment					
Abundance (prey·L^{-1})	C	S	M	L	A_{EQ}	A_{UNEQ}
0	✓					
15		✓	✓	✓		
30		✓	✓	✓		
40					✓	✓
60		✓	✓	✓	✓	✓
100					✓	✓
120		✓	✓	✓		
180					✓	✓

Samples of walleye larvae were trawled or seined from each of the four Methley culture ponds between 0900 and 2000 h at roughly 4-d intervals following stocking. Subsamples of ~20 fish (range 15-38) from each trawl or seining were processed and analyzed in the same manner as the laboratory experimental fish. Quantitative zooplankton samples were collected within 24 h of the fish samples using oblique tows (1987) or vertical tows (1988 and 1989) of Wisconsin nets (25 cm diameter; 73 μm mesh). Mean zooplankton abundances and species and size distributions were calculated from these samples. Detailed descriptions of the zooplankton and larval fish sampling procedures, and the pond zooplankton community are given in Appendix A.

Statistical analyses were divided into two parts. The first examined the functional response of walleye larvae using only laboratory data. The second examined prey selection of walleye larvae using both laboratory and field data.

Functional Response Analysis

The functional response was analyzed separately for each of the seven experimental dates (Table 3.1). The percent of fish feeding increased asymptotically with zooplankton abundance and the asymptote was below 100% for all but the largest fish. This suggested that some non-feeders were limited by the zooplankton treatment whereas others would not have fed regardless of the zooplankton treatment. It was this latter group of fish which I wished to eliminate from further analyses. However, because it was impossible to determine why each individual non-feeder did not feed, I decided to randomly eliminate a portion of non-feeders equal to (total number of experimental fish) x (1 - asymptotic percent feeding) from each experimental run.

Scatter plots of consumption rate versus zooplankton abundance indicated that a Holling type II functional response was the most appropriate model for most zooplankton treatments and fish sizes. I used a reparameterized version of the Holling disc equation (Holling 1959) of the form

[3.1] $$C = 9pC_{max} / (D_{90} + 9p)$$

where C is consumption rate ($\mu g \cdot h^{-1}$), p is prey abundance ($prey \cdot L^{-1}$), and C_{max} and D_{90} are fitted parameters representing maximum consumption rate and the prey abundance at which

90% of the maximum consumption rate is attained, respectively. These model parameters can be expressed in terms of the original model parameters as

[3.2] $$C_{max} = 1 / f$$

and

[3.3] $$D_{90} = 9 / af$$

where f is handling time ($h \cdot \mu g^{-1}$) and a is the attack coefficient ($\mu g \cdot L \cdot h^{-1}$). This model assumes that prey abundance remains constant or declines negligibly during the experiment, an assumption frequently violated in experiments with the larger larvae. Thus, I chose to use the geometric mean of initial and final prey abundances rather than initial prey abundance as the independent variable. I felt that this variable more accurately reflected the feeding conditions corresponding to the observed consumption. Data from experimental aquaria in which prey depletion was severe (> 80%) were eliminated from further analyses. Mean consumption rate was calculated for each experimental aquarium and the functional response model was fit to the aquarium means using non-linear least-squares (NLIN procedure, SAS® Institute Inc. 1985) and the method of iterative reweighting (Holland and Welsch 1977). Consumption rate means were weighted by the inverse of their predicted standard errors as estimated from the empirical variance-mean relationship (Taylor power plot; Elliott 1977). For each experimental date, the functional response model was fit to each of the individual zooplankton treatments (S, M, L, A_{EQ}, and A_{UNEQ}). The NLIN procedure provided estimates for the means and asymptotic standard errors of C_{max} and D_{90}. Changes in the functional response with respect to walleye size and prey size were examined by conducting univariate tests on the model parameters.

I tested the hypothesis that C_{max} varied with respect to walleye size by regressing $\log_e(C_{max})$ against $\log_e$(mean length) (GLM procedure, SAS® Institute Inc. 1985). Because the relationship between D_{90} and walleye mean length appeared complex, I tested the hypothesis that D_{90} varied with respect to experimental date using ANOVA (GLM procedure, SAS® Institute Inc. 1985) with experimental date treated as a class varaiable. In this latter analysis, variation in D_{90} due to experimental date included the effects of walleye mean length but also included variation due to other effects such as fish stock. Parameter estimates from

all zooplankton treatments (S, M, L, A_{EQ}, and A_{UNEQ}) were used in both analyses and D_{90} estimates were weighted by the inverse of their standard errors in the latter analysis. Tests of significance were based on the partial (Type III) sums of squares to account for the effect of zooplankton treatment.

I tested the hypothesis that C_{max} varied with respect to prey size using ANCOVA (Freund and Littell 1981) where walleye mean length was treated as a covariate. To test the hypothesis that D_{90} varied with respect to prey size I conducted an ANOVA (GLM procedure, SAS® Institute Inc. 1985) with experimental date treated as a block effect. In these analyses I compared parameter estimates from only the S, M, and L zooplankton treatments. My analysis of the effects of prey size on D_{90} required the further assumption that there was no interaction between the effects of experimental date and prey size on D_{90}.

Prey Selection Analysis

I examined prey selection using both laboratory and field data. From the laboratory experiments I used data from all zooplankton treatments, but primarily from the assemblage treatments (A_{EQ} and A_{UNEQ}). Ambient experimental conditions differed between laboratory and field studies (Table 3.3). Laboratory experiments provided more even distributions in the range of prey abundances and relative prey abundances whereas field data covered a wider range of fish sizes and water temperatures.

Prey selection was measured using an electivity index, $\mathcal{E}$, proposed by Chesson (1983). Electivity for the i^{th} prey category, $\mathcal{E}_i$, was calculated from the formulae

[3.4] $$\mathcal{E}_i = (m\alpha_i - 1) / ((m - 2)\alpha_i + 1)$$

and

[3.5] $$\alpha_i = (r_i / n_i) / \sum (r_i / n_i)$$

where α_i is Chesson's (Manly's) preference index, r_i is the proportion of consumed prey which were of category i, n_i is the proportion of available prey which were of category i, and m is the number of prey categories available. $\mathcal{E}_i$ can take values of -1 to 1 with 0 representing neutral selection. The above formula for α_i assumes that the prey community composition changes negligibly during the experiment. For this reason, I eliminated all laboratory data from aquaria in which > 10% of available particles were consumed during the course of an experiment.

Table 3.3. Comparison of environmental and experimental conditions of laboratory and field studies examining prey selection by walleye postlarvae. Field studies were carried out in the Methley Beach walleye culture ponds. Prey size and prey species categories are defined in the text.

Variable	Range of observed conditions	
	Laboratory	Field
Walleye		
Mean Length (mm)	9.45 - 14.8	8.90 - 26.1
Feeding state	guts rarely full	guts usually full
Environment		
Temperature (°C)	19 - 21	12.9 - 24.3
Light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)	0.6 - 0.9	100 - 1000[1]
Prey abundance ($prey \cdot L^{-1}$)	15 - 180	14 - 388
Prey size relative abundances (%)		
Small	1 - 76	15 - 96
Medium	5 - 94	3 - 78
Large	1 - 89	1 - 55
Prey species relative abundances (%)		
Cyclopoids	4 - 96	12 - 93
Large Cladocera	2 - 90	3 - 82
Others	1 - 13	1 - 42

[1] measured between the surface and a depth of 150 cm at noon on an overcast day when pond Secchi depth was > 200 cm.

Prey community composition was assumed constant within each sampling period for all field data. Electivity indices may sometimes be strongly influenced by prey items which are rare in the environment (Lechowicz 1982; Confer and Moore 1987). To reduce this possibility, I eliminated all data where $n_i < 0.005$ in field studies or $n_i < 0.01$ in laboratory studies. A slightly more conservative criterion was used for the laboratory studies because of the greater risk of prey depletion in small volumes.

Electivity indices were calculated using both prey size and prey species categorizations. In both cases food items were divided into three categories. To examine size selection I classified prey by maximum body width; < 0.3 mm, ≥ 0.3 - 0.6 mm, and ≥ 0.6 mm. This classification roughly corresponded to the zooplankton size treatments S, M, and L, respectively. I used prey width rather than length as it is a more consistent selection criterion across a wide range of prey types for gape-limited fishes (Hunter 1981; Hambright 1991). To examine species selection, zooplankton were categorized as cyclopoids (mostly *Diacyclops bicuspidatus thomasi*), large cladocerans (*Daphnia pulex*, *Ceriodaphnia quadrangula*, *Simocephalus vetulus*), and others (*Bosmina longirostris*, *Chydorus sphaericus*, *Diaptomus siciloides*). Means and standard errors of $\mathcal{E}$ were estimated for each experimental aquarium in laboratory studies and for each pond and sampling date in field studies by jackknifing (Krebs 1989) using the FORTRAN subroutine of Matloff (1980).

I tested the hypotheses that prey selection by walleye larvae varied with respect to walleye size, relative prey abundance, total prey abundance, and water temperature using regression analysis (SAS® GLM procedure, Freund and Littell 1981). Because the electivity indices were highly correlated, I chose to use the principal components of the electivity index vectors as dependent variables rather than the electivity indices themselves. This allowed me to examine most of the variability in prey electivity without repeating identical univariate analyses on highly correlated dependent variables. Principal components for the prey size and prey species electivity index vectors for both laboratory and field data (four vectors in total) were calculated from the variance-covariance matrices using the SAS® PRINCOMP procedure (SAS® Institute Inc. 1985). Generally, PC1 compared one electivity index against the combination of the other two in the vector whereas PC2 compared the latter two indices.

Thus, as dependent variables, the principal components represented the electivity for one group of prey relative to another (Table 3.4).

Eight separate regressions were conducted, one for each of the principal components of Table 3.4. The independent variables in each regression analysis were $\log_e$(mean length) (LEN), $\log_e$(relative prey abundance) (RA), total prey abundance (DENS, prey·L^{-1}), and water temperature (TEMP, °C). The variable TEMP was used only in analyses of field data. Relative prey abundance was calculated as the ratio of prey abundances as indicated by the interpretations of the principal components in Table 3.4. Thus, the calculation of RA was different for each regression. For example, when PC1 of the prey size electivity index for laboratory studies was used as a dependent variable, the relative prey abundance was calculated as the abundance of medium and large prey divided by the abundance of small prey (Table 3.4). The significance of an independent variable's effect on the principal component was determined by examining the partial (Type III) sums of squares. I plotted model residuals against the independent variables to examine the variable's influence on prey selection and to determine if transformation or weighting was required (Draper and Smith 1981).

Results

Functional Response

I examined trends in the functional response parameters with respect to walleye size by plotting means of C_{max} and D_{90} (adjusted for zooplankton treatment, LSMEANS option, GLM procedure, SAS® Institute Inc. 1985) for each experimental date against walleye mean length (Fig. 3.1). Maximum consumption rate, C_{max}, increased curvilinearly with walleye mean length over the size range of 9.45 to 14.8 mm (Fig. 3.1a). This relationship was defined by the equation $\log_e(C_{max}) = -16.7 + 8.38 \cdot \log_e$(mean length) (ANOVA, $F = 147$, $df = 1, 27$, $P < 0.001$, $R^2 = 0.85$). The prey abundance at which 90% of maximum consumption rate was attained, D_{90}, increased from 45·L^{-1} at first feeding to a high of 387·L^{-1} for 10.1-mm larvae then declined to 55·L^{-1} for the largest larvae examined (Fig. 3.1b). D_{90} differed significantly between experimental dates (ANOVA, $F = 3.47$, $df = 6, 22$, $P = 0.015$). Multiple comparisons of the means (TUKEY option, GLM procedure, SAS® Institute Inc. 1985) indicated significant differences ($P < 0.05$) between all means with the exception of two groupings. There were no significant differences in D_{90} among experimental dates corresponding to walleye mean lengths

Table 3.4. First (PC1) and second (PC2) principal components calculated for vectors of electivity indices (E_i). Values in brackets represent the percent of total variation in electivity accounted for by each principal component. Interpretations of the principal components in terms of the prey categories of the original indices were made in relation to the magnitude and sign of the coefficients. Prey size categories corresponding to E_1, E_2, and E_3 were small (< 0.3 mm body width), medium (≥ 0.3 - 0.6 mm), and large (≥ 0.6 mm) zooplankton. Prey species categories corresponding to E_1, E_2, and E_3 were cyclopoid copepods (mostly *Diacyclops bicuspidatus thomasi*), large cladocerans (*Daphnia pulex, Ceriodaphnia quadrangula, Simocephalus vetulus*), and other zooplankton (*Bosmina longirostris, Chydorus sphaericus, Diaptomus siciloides*).

Electivity Index Vector	Principal Component Formulae	Interpretation
Laboratory Studies		
Prey size	PC1 = $-0.77 \cdot E_1 + 0.53 \cdot E_2 + 0.36 \cdot E_3$ (74.2%)	medium and large relative to small
	PC2 = $-0.07 \cdot E_1 - 0.62 \cdot E_2 + 0.78 \cdot E_3$ (22.8%)	large relative to medium
Prey species	PC1 = $-0.76 \cdot E_1 + 0.47 \cdot E_2 + 0.44 \cdot E_3$ (58.8%)	large cladocerans and others relative to cyclopoids
	PC2 = $0.06 \cdot E_1 - 0.63 \cdot E_2 + 0.78 \cdot E_3$ (38.7%)	others relative to large cladocerans
Field studies		
Prey size	PC1 = $0.30 \cdot E_1 + 0.54 \cdot E_2 - 0.78 \cdot E_3$ (78.7%)	medium and small relative to large
	PC2 = $-0.76 \cdot E_1 + 0.63 \cdot E_2 + 0.15 \cdot E_3$ (19.4%)	medium relative to small
Prey species	PC1 = $0.56 \cdot E_1 - 0.70 \cdot E_2 + 0.45 \cdot E_3$ (62.9%)	cyclopoids and others relative to large cladocerans
	PC2 = $-0.67 \cdot E_1 - 0.05 \cdot E_2 + 0.74 \cdot E_3$ (35.4%)	others relative to cyclopoids

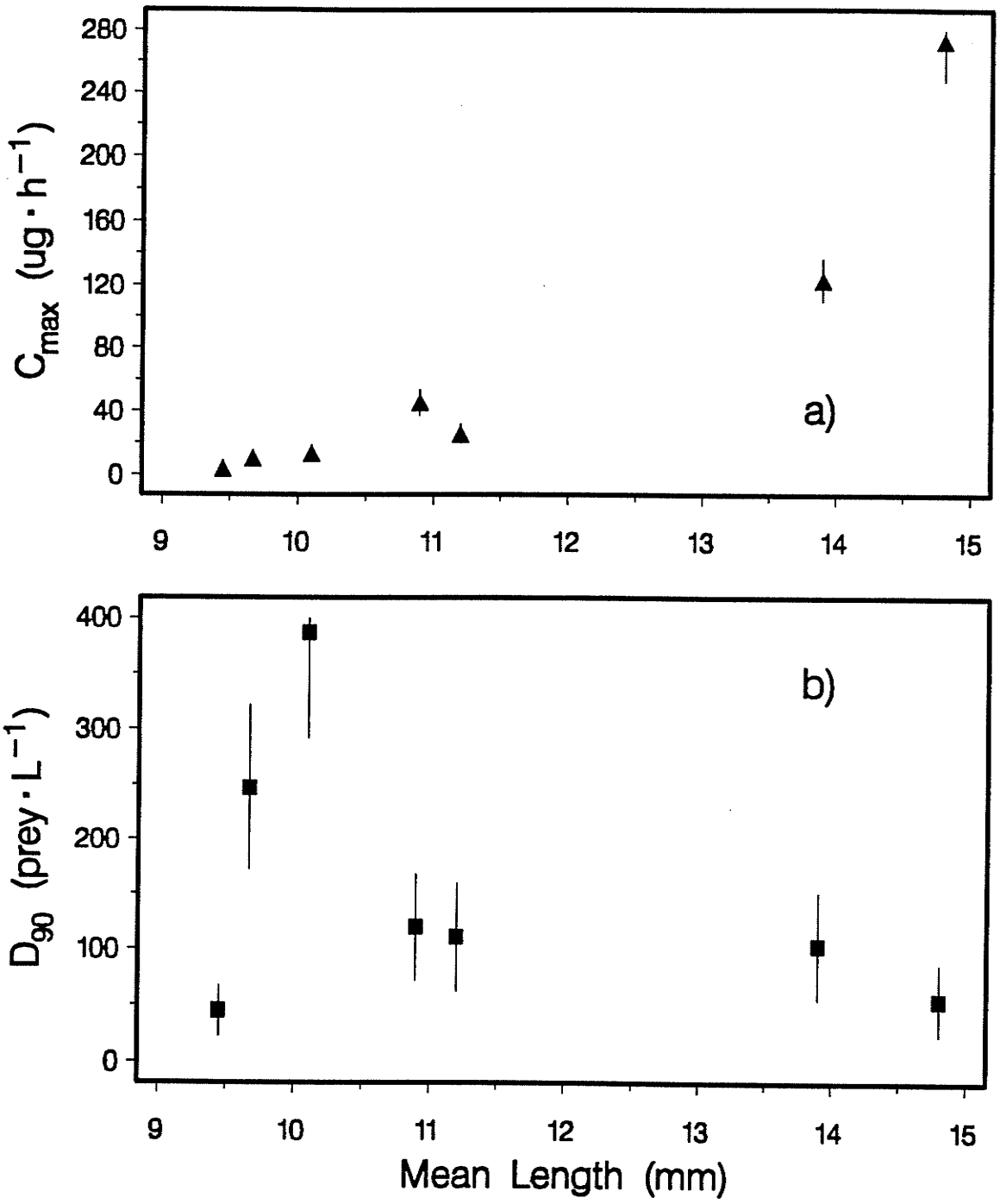

Figure 3.1. Relationships between functional response model parameters and walleye mean length for walleye postlarvae feeding on zooplankton in the laboratory. Parameters are a) maximum consumption rate, (C_{max}), and b) prey density at which 90% of maximum consumption rate was attained (D_{90}). The functional response model was a modified version of the Holling disc equation (equation [3.1], see text). Symbols represent means ± 1 SE. Means were adjusted for zooplankton size treatment.

of 10.9, 11.2, and 13.9 mm, and between experimental dates corresponding to walleye mean lengths of 9.45 and 14.8 mm.

I examined trends in the functional response parameters with respect to prey size by plotting estimates of C_{max} and D_{90} for each of the S, M, and L zooplankton treatments against walleye mean length (Fig. 3.2). For the smaller (9.45-9.67 mm) larvae, C_{max} was highest when feeding on small prey whereas for the larger (11.2-13.9 mm) larvae, C_{max} was lowest when feeding on small prey (Fig. 3.2a). However, this interactive effect of zooplankton size and fish size on C_{max} was not statistically significant. Slopes of the $\log_e(C_{max})$ vs $\log_e$(mean length) relationships were not significantly different between zooplankton size treatments (ANCOVA, $F = 0.97$, $df = 2,14$, $P = 0.40$) and following correction for the length covariate, there was no significant difference in C_{max} between zooplankton size treatments (ANCOVA, $F = 0.74$, $df = 1,16$, $P = 0.49$).

The relationship between D_{90} and walleye mean length was qualitatively similar for all zooplankton size treatments with the highest D_{90} estimates observed in 10.1-mm larvae (Fig. 3.2b). Generally, D_{90} was highest for small prey and lowest for large prey. The means of D_{90} adjusted for experimental date were 273, 146, and 112 $\cdot L^{-1}$ for S, M, and L treatments, respectively. An unusually high D_{90} value (1951 $\cdot L^{-1}$) was estimated for 10.1-mm larvae feeding on medium-sized zooplankton. I attributed this to unusually high mean consumption rates observed in two experimental aquaria which greatly influenced the fit of the model and, therefore, I excluded this point from further analyses. With this outlier eliminated, there was a significant effect of zooplankton size on D_{90} (ANOVA, $F = 5.61$, $df = 2, 10$, $P = 0.023$). Multiple comparisons of the treatment means (TUKEY option, GLM procedure, SAS® Institute Inc. 1985) indicated significant differences between all zooplankton sizes ($P < 0.05$). Thus, the zooplankton abundance necessary to attain 90% of C_{max} declined significantly with increasing zooplankton size.

These analyses indicated that the shape of the walleye functional response curve varies with prey size and with walleye body size (Fig. 3.3a, b). My models predict that 9.67-mm larvae can consume large prey at a faster rate than small prey if prey abundance is low (<40 prey $\cdot L^{-1}$) and consume small prey at a faster rate than large prey if prey abundance is high (Fig. 3.3a). Larger larvae (11.2 mm) can consume large prey at a faster rate than small prey

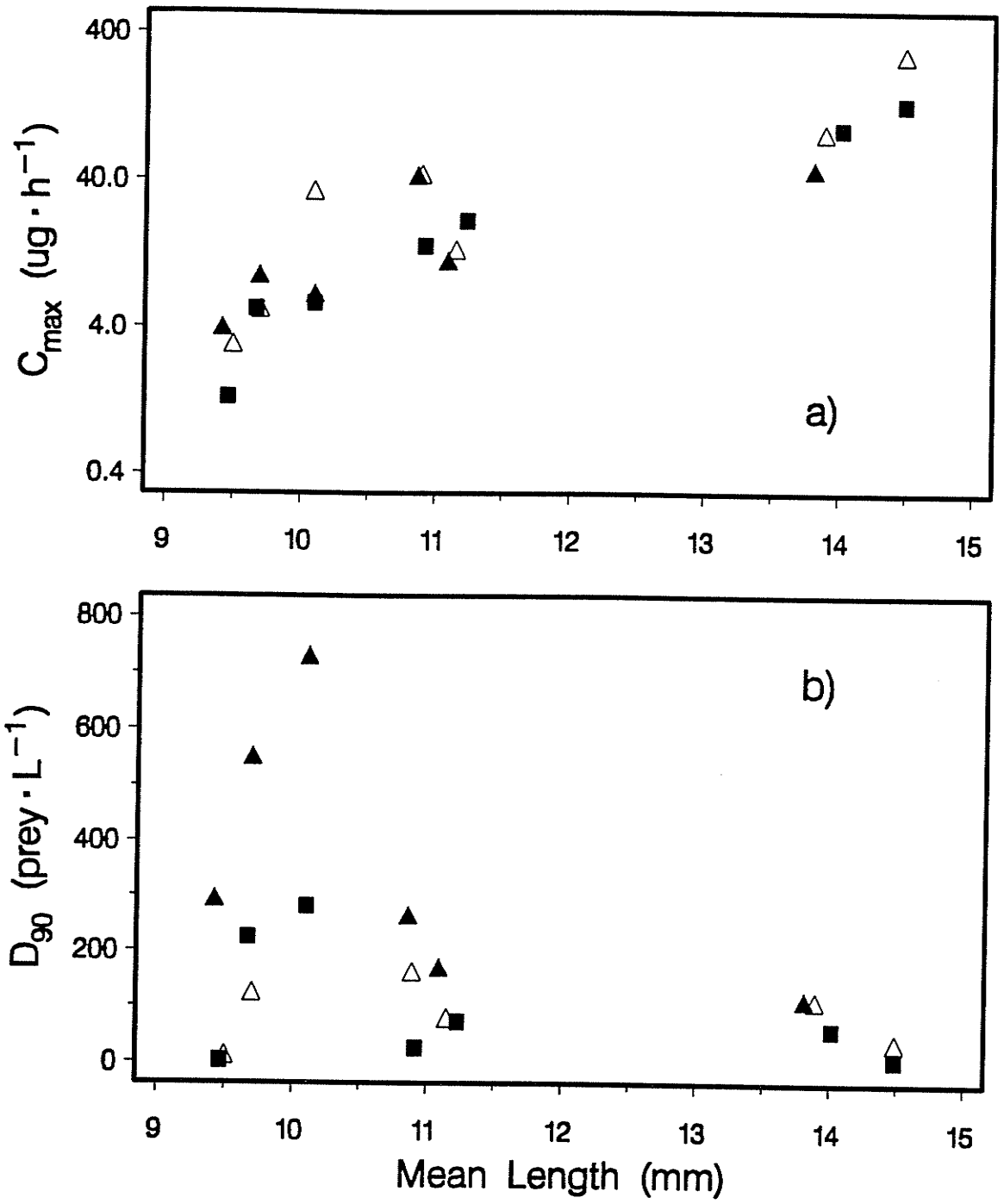

Figure 3.2. Relationships between functional response parameters and walleye mean length for walleye postlarvae feeding on small (183-300 μm, ▲), medium (300-509 μm, △), and large (509-1050 μm, ■) zooplankton in the laboratory. Parameters were a) maximum consumption rate (C_{max}), and b) prey density at which 90% of maximum consumption rate was attained (D_{90}). Symbols represent single fitted parameter estimates. The functional response model was a modified version of the Holling disc equation (equation [3.1], see text). A single observation representing the D_{90} value for 10.1-mm walleye feeding on medium-sized zooplankton (D_{90} = 1951 $\cdot L^{-1}$) is not shown. Note logarithmic scale in a).

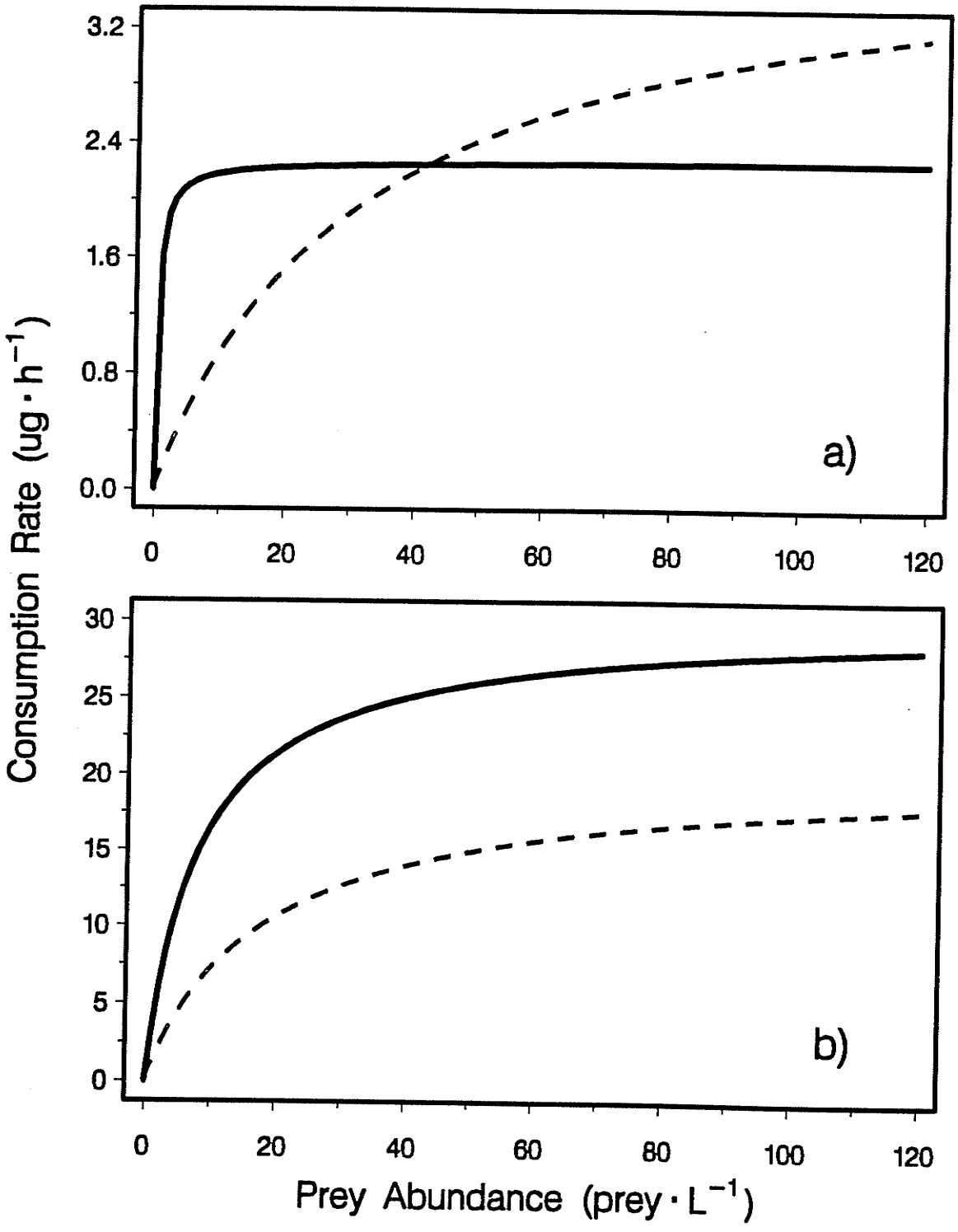

Figure. 3.3. Predicted functional responses of a) 9.67-mm mean length, and b) 11.2-mm mean length postlarval walleye feeding on small (183-300 µm, - - - - - -) or large (509-1050 µm, ————) zooplankton. The functional response model was a modified version of the Holling disc equation (equation [3.1], see text) and parameters were estimated from laboratory feeding experiments. Note difference in scales of consumption rate axes between a) and b).

over the entire range of prey abundances examined in this study (Fig. 3.3b). Based on these observations, I hypothesized that if walleye larvae selectively fed on prey sizes which maximized their consumption rate then selection for larger relative to smaller prey would decline with increasing prey abundance. Furthermore, I predicted that this trend would be most prominent among first-feeding walleye larvae. I examined these predictions in my analysis of prey selection.

Prey Selection

Prey selection was strongly influenced by walleye size and this was most apparent for the field data (Figs. 3.4 and 3.5). Electivity for small prey was positive only for the smallest walleye larvae and declined with increasing walleye length. Medium-sized prey were positively selected by walleye from first-feeding to a mean length of ~20 mm but declined in importance thereafter. Electivity for large prey was negative among first-feeders but increased steadily as the larvae grew. When electivity was calculated based on taxonomic rather than body-size prey categories the trends were less distinct (Fig. 3.5). The greatest variability in prey species electivity was seen in 8-12-mm larvae. In larger larvae, large cladocerans were generally selected over cyclopoids or other zooplankton. However, strong positive electivity for cyclopoids and negative or neutral electivity for large cladocerans by larger larvae (mean length > 20 mm) was observed on several occasions. Positive electivity for zooplankton other than cyclopoids or large cladocerans was observed only among the earliest larval stages. The 'other' prey species in these cases was primarily *Bosmina longirostris*.

Independent variables representing walleye size, relative prey abundance, total prey abundance, and water temperature accounted for significant amounts of variation in prey electivity by walleye larvae based on my regression analyses using principal components as dependent variables (Table 3.5). Coefficients of determination (R^2) for the complete regression models (all variables included) ranged from 2 to 19% for the laboratory studies and from 26 to 63% for the field studies. Residuals from some of the complete models (3 of 4 models using PC2 as the dependent variable) had non-normal distributions which I could not correct by transformation or weighting of the dependent variables. This may have reduced the power of these tests somewhat and they should therefore be considered conservative.

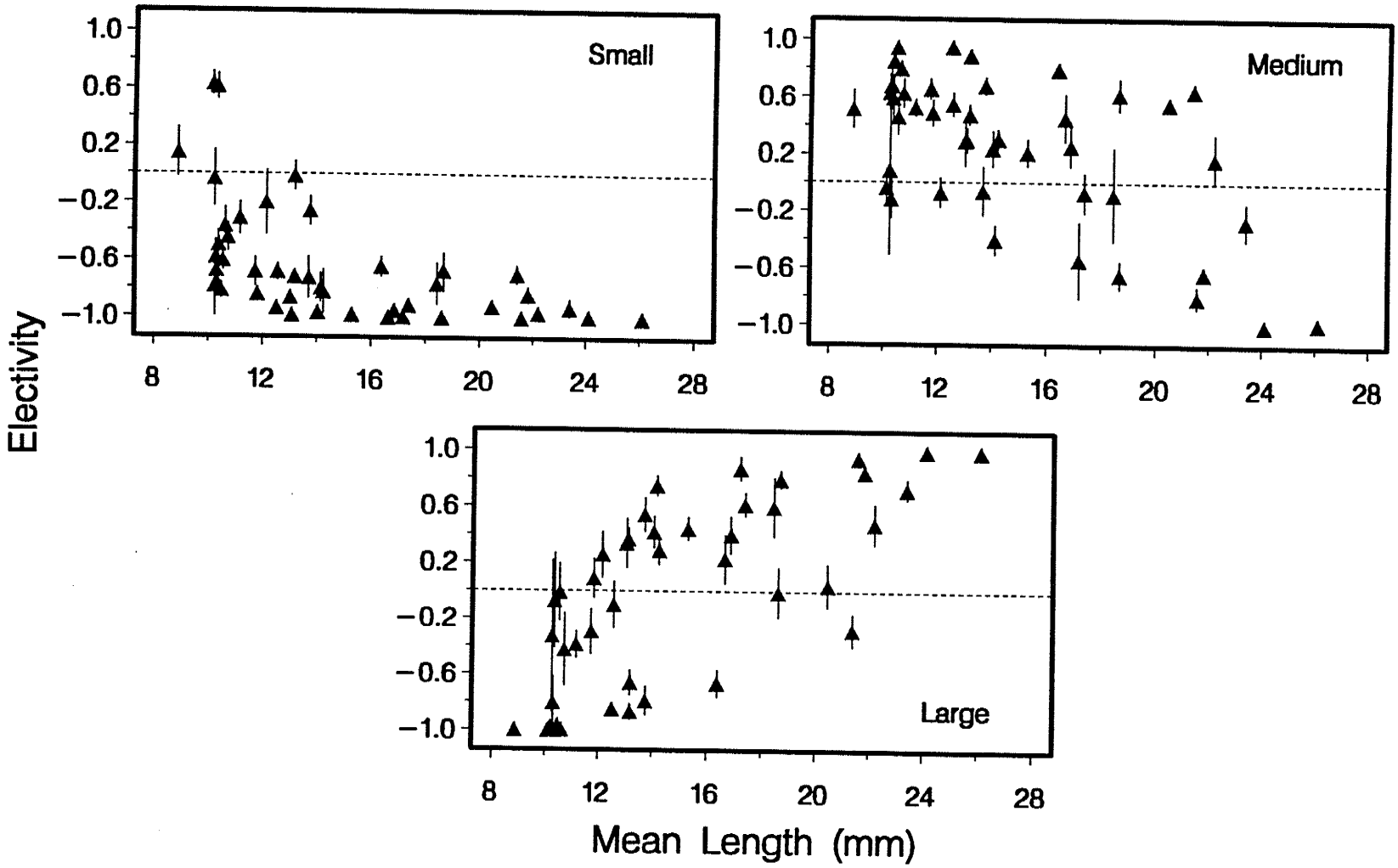

Figure 3.4. Scatter plots of electivity, E, for small (< 0.3 mm body width), medium (≥ 0.3 - 0.6 mm), and large (≥ 0.6 mm) prey items by walleye postlarvae vs. walleye mean length. Walleye were sampled from the Methley Beach culture ponds in 1987, 1988, and 1989. Symbols represent jackknifed means ± 1 SE. Reference line indicates neutral selection.

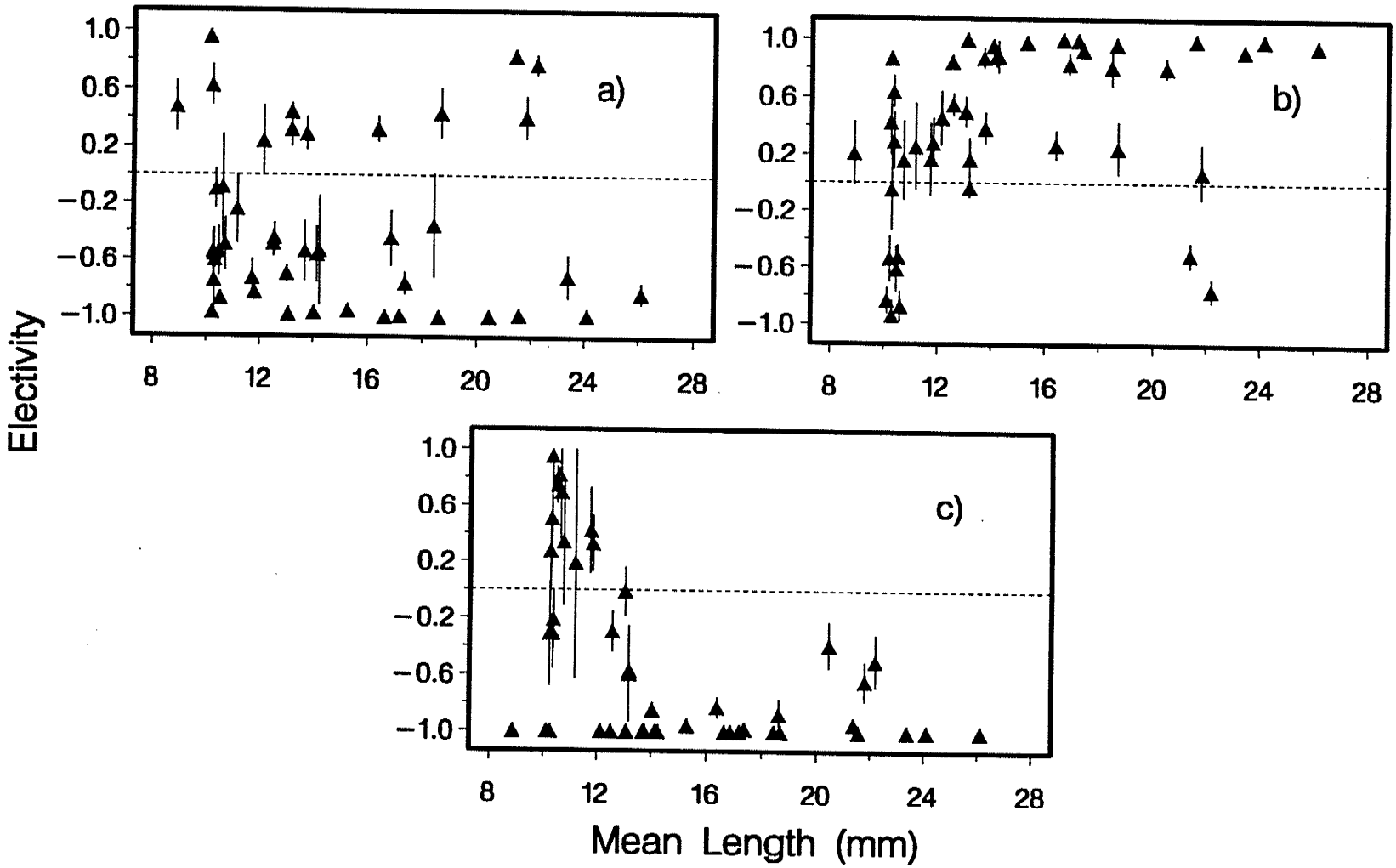

Figure 3.5. Scatter plots of electivity, ε, for a) cyclopoid copepods (mostly *Diacyclops bicuspidatus thomasi*), b) large cladocerans (*Daphnia pulex*, *Ceriodaphnia quadrangula*, *Simocephalus vetulus*), and c) other crustacean zooplankton (*Bosmina longirostris*, *Chydorus sphaericus*, *Diaptomus siciloides*) by walleye postlarvae vs. walleye mean length. Walleye were sampled from the Methley Beach culture ponds in 1987, 1988, and 1989. Symbols represent jackknifed means ± 1 SE. Reference line indicates neutral selection.

Table 3.5. Results of regression analyses using principal components (PC1 and PC2) of electivity index vectors as dependent variables (Y) and $\log_e$(mean walleye length) (LEN), $\log_e$(relative prey abundance) (RA, see text), total prey abundance (DENS, prey·L^{-1}), and water temperature (TEMP, °C) as independent variables (X_i). Principal component formulae and interpretations are listed in Table 3.4. Parameter estimates are indicated by b_i. F-statistics were calculated based on the partial (Type III) sums of squares. Degrees of freedom for the test statistics were 1 and n - 4 for the laboratory studies, and 1 and n - 5 for the field studies, where n is the number of observations (means).

Electivity vector	n	Y	X_i	b_i	F	P
Laboratory studies						
Prey size	104	PC1	LEN	3.70	9.74	0.002
			RA	-0.213	13.00	< 0.001
			DENS	-3.51×10^{-4}	0.03	0.854
		PC2	LEN	1.20	2.56	0.113
			RA	-4.52×10^{-2}	1.36	0.246
			DENS	-1.03×10^{-4}	0.01	0.924
Prey species	78	PC1	LEN	3.63	8.11	0.006
			RA	-0.242	8.93	0.004
			DENS	-1.37×10^{-3}	0.59	0.447
		PC2	LEN	-0.374	0.10	0.751
			RA	-9.86×10^{-2}	1.21	0.275
			DENS	3.63×10^{-4}	0.05	0.822
Field studies						
Prey size	43	PC1	LEN	-2.33	31.1	< 0.001
			RA	-9.99×10^{-2}	2.50	0.122
			DENS	2.83×10^{-3}	5.92	0.020
			TEMP	3.74×10^{-2}	0.74	0.396
		PC2	LEN	0.970	9.56	0.004
			RA	-0.142	7.41	0.010
			DENS	5.78×10^{-4}	0.58	0.451
			TEMP	-7.34×10^{-2}	5.77	0.021

Table 3.5. Continued

Electivity vector	n	Y	X_i	b_i	F	P
Field studies						
Prey species	34	PC1	LEN	-1.86	8.61	0.007
			RA	-0.223	5.13	0.031
			DENS	4.09×10^{-3}	7.69	0.010
			TEMP	8.21×10^{-2}	2.24	0.145
		PC2	LEN	-1.35	7.63	0.010
			RA	-3.68×10^{-2}	0.21	0.653
			DENS	-1.75×10^{-3}	1.93	0.175
			TEMP	3.41×10^{-2}	0.65	0.426

Regression analyses confirmed the strong influence of walleye size on prey electivity (Table 3.5). The signs of the regression parameters (b_i) for the variable LEN indicated that selection for larger relative to smaller prey was an increasing function of walleye mean length (Table 3.5). The effect of LEN was significant in six of the eight regression models (Table 3.5). In laboratory studies, electivity for medium and large prey relative to small prey (prey size, PC1), and electivity for non-cyclopoid relative to cyclopoid prey (prey species, PC1) were both positively related to mean length. When differences in the interpretations of principal components were taken into account the trends seen in field data agreed well with the laboratory data (Table 3.5). For the pond walleye, electivity for medium and small relative to large prey (prey size, PC1) decreased significantly and electivity for medium relative to small prey (prey size, PC2) increased significantly with walleye mean length. Electivity for cyclopoids and others relative to large cladocerans (prey species, PC1) and for others relative to cyclopoids (prey species, PC2) both declined significantly with increasing walleye length. Thus, the regression analyses confirmed the qualitative patterns observed in scatter plots of the original electivity indices (Figs. 3.4 and 3.5).

Regression parameters for the variable RA indicated that electivity of one prey group relative to another was consistently negatively related to the relative abundance of the two groups (Table 3.5). The effect of RA was significant in four of the eight regression models. In laboratory studies, electivity for medium and large relative to small prey (prey size, PC1) and electivity for non-cyclopoids relative to cyclopoids (prey species, PC1) declined significantly with increasing relative prey abundance. In field studies, electivity for medium relative to small prey (prey size, PC2) and electivity for cyclopoids and other zooplankton relative to large cladocerans (prey species, PC1) showed significant, negative relationships with RA.

The effect of prey abundance (DENS) on electivity was negligible in the laboratory studies ($F \leq 0.59$, $P \geq 0.45$, Table 3.5). However, plots of residuals from the complete model vs. DENS indicated that error variance declined with increasing prey abundance. When the variance of the residuals was calculated at DENS intervals of $20 \cdot L^{-1}$ for each of the four principal component models and regressed against prey abundance, a significant, negative relationship was obtained (ANOVA, $F = 8.08$, $df = 1, 22$, $P = 0.0095$). Thus, the variation in

prey electivity declined significantly with increasing prey abundance. I reconducted the original regression analyses using a weighting function proportional to the inverse of the predicted residual variance to account for this pattern. However, in the weighted analysis the effects of DENS on electivity were still not significant ($F \leq 0.99$, $P \geq 0.32$).

Prey abundance had a stronger effect on prey selection under field conditions (Table 3.5). Electivity for medium and small relative to large prey (prey size, PC1) and electivity for cyclopoids and others relative to large cladocerans (prey species, PC1) were both significantly and positively related to prey abundance. Plots of complete model residuals vs. DENS were constructed as for the laboratory studies. The trend of decreasing residual variance with increasing prey abundance was apparent, but not as strong as the trend observed in the laboratory data. When residual variance was calculated as described above and regressed against DENS the relationship was not significant (ANOVA, $F = 0.41$, $df = 1, 22$, $P = 0.53$).

From my functional response analysis I predicted that when several prey sizes were available in equal proportions selection for small relative to larger prey would increase with increasing prey abundance, and that this trend would be strongest among first-feeding larvae. The observed effect of DENS on electivity in the above analyses was consistent with these predictions for the field studies but not for the laboratory studies. I thus decided to analyse the laboratory data more closely. The A_{EQ} treatment in the laboratory experiments provided larvae with equal proportions of the three zooplankton sizes. Using only data for small larvae (< 11 mm mean length) from the A_{EQ} treatment, I reconducted the regression of PC1 of the prey size electivity vector against LEN, RA, and DENS. In this analysis, DENS had a significant, negative effect on the electivity of medium and large relative to small prey (ANOVA, $F = 4.57$, $df = 1,43$, $P = 0.038$). This result was thus in agreement with the predictions arising from the functional response analysis.

The effects of temperature on electivity were examined only in the field studies (Table 3.5). Regression parameters indicated that selection for larger relative to smaller prey declined with increasing temperature. This effect was significant for the second prey size principal component. Temperature effects on prey species electivity were not significant (Table 3.5).

Discussion

Functional Response

My analysis and interpretations of the walleye's functional response must be put in perspective with the characteristics of the model that I used. The Holling functional response model predicts that search time (foraging time spent actively locating prey) declines relative to the handling time (foraging time spent in the pursuit, capture, and ingestion of prey plus a digestive pause before resumption of searching) with increasing prey abundance and that virtually all foraging time is spent in handling when prey are very abundant. The model parameters, C_{max} and D_{90} (or f and a, equations 3.2 and 3.3), are assumed constant. However, Abrams (1990) has argued that the parameters vary with prey abundance. Prey abundance may affect the model parameters indirectly through its influence on predator satiation. There is some evidence, for example, that the handling time per unit of prey increases with satiation (Werner 1974; Confer and O'Bryan 1989). If this is the case, then the reliability of my parameter estimates may depend upon the similarity in satiation levels among my experimental fish. I terminated laboratory trials well before the larval guts became full and the amount of food in the experimental fish guts was always much lower than that observed in the guts of the Methley pond walleye larvae, which presumably had been feeding continuously for several hours prior to capture. I feel that my results best represent the functional response of unsatiated larvae. My estimates of C_{max} and D_{90} are thus probably higher than would be expected for continuously feeding fish. Estimates of the consumption rate of walleye larvae based on feeding experiments of much shorter duration (Chapter 4) also suggest that my parameter estimates are lower than would be observed for fish which had just commenced feeding. Another factor that was not examined in this study, but may affect the interpretation of my results, is the number of fish used per aquarium in the trials. There is some evidence that larger groups of larvae feed at faster rates than individuals (Hartman and Brandt 1993). The reasons for this effect are not clear, but it should be studied further.

My results illustrated the changes in the walleye functional response which accompany increasing body size. C_{max} increased significantly with walleye mean length, a pattern observed in many other larval fishes (Houde and Schekter 1980; Miller et al. 1992). This corresponds with a rapid increase in the walleye's prey capture success during the same

period (Chapter 4). D_{90} increased initially then declined with respect to walleye mean length. Though my statistical analysis indicated that D_{90} differed significantly between experimental dates my design did not permit me to distinguish between the effects of mean length and other effects related to experimental date, such as fish stock. I feel that the observed trend between D_{90} and mean length does reflect the true relationship but suggest that it should be re-examined by future studies. If the observed D_{90}-mean length relationship is correct, then the ability of walleye larvae to feed at maximal rates is most limited by zooplankton abundance not at first-feeding but when larvae are ~ 10-10.5 mm mean length. D_{90} will increase in response to a reduced handling time but decrease in response to an improved searching ability (equation [3.3]). This suggests that size-related changes in the feeding ecology of walleye during early development initially favour improvements in handling prey (increasing C_{max}) over improvements in locating prey. Changes in feeding ability during early development may result from, or simply be correlated with, increasing body size. Certainly swimming speed increases with larval length (reviewed by Hunter (1981) and Blaxter (1986)) and has a major influence on both search rate and handling time. Physical changes in musculature, swim bladder inflation, and fin structure also improve swimming ability (reviewed by Blaxter 1986) and changes in eye structure increase the distance at which prey can be detected (Hairston et al. 1982; Wahl et al. 1993). Experience also increases over time and may be as important as physical changes in improving the searching and handling efficiencies of larval fish (reviewed by Hughes et al. 1992).

The functional response of walleye larvae also varied with respect to prey size. C_{max} for first-feeding walleye was highest when feeding on small prey and lowest when feeding on large prey and this trend reversed with increasing walleye size. My analysis indicated that this interaction between the effects of walleye size and prey size on C_{max} was not statistically significant. However, the trend is consistent with my expectations. Larger zooplankton should become easier to handle relative to smaller zooplankton as the walleye grow because increasing gape and gill-raker spacings favour the capture of increasingly larger prey. I suspect that this trend would be more pronounced, and perhaps statistically significant, when examined over a wider range of walleye body sizes. Prey size had a stronger effect on D_{90} than C_{max}. D_{90} was significantly lower for large relative to small prey. Larger prey are easier

to detect (Confer and Blades 1975; Confer et al. 1978) and thus the abundance necessary to allow near-maximal rates of consumption would be lower. I expected that walleye larvae of all sizes would be able to detect larger prey more easily than smaller prey and thus interaction between walleye size and prey size would be negligible. My results supported this expectation as D_{90} was higher for small prey than for large prey for almost all walleye mean lengths examined.

Because my zooplankton size treatments varied in species composition, observed differences in the functional response between zooplankton size-classes may also reflect differences in prey species among them. Zooplankton of the small size fraction were primarily cyclopoid copepods whereas zooplankton of the large size fraction were mostly daphnids. Copepods and cladocerans utilize quite different predator evasion tactics (Kerfoot et al. 1980) with the latter being much more susceptible to the suction-type intake of fish (Drenner et al. 1978). The capture success of planktivorous fish is thus usually higher when feeding on cladoceran prey (Confer and Blades 1975; Wanzenböck 1992). These and other interspecific differences in prey may have influenced the observed effect of my zooplankton size treatments on the functional response model parameters.

A practical value of functional response analysis has been to provide estimates of the prey abundances necessary to allow fish to feed at maximal rates. Mathias and Li (1982) estimated that the consumption rate of young walleye was maximized at zooplankton abundances of $\geq 100 \cdot L^{-1}$ based on eye-fitted interpretations of their functional response data. My results indicate that the threshold zooplankton abundance varies with both walleye and zooplankton size. Based on the fitted parameter D_{90}, 9.5-10.5-mm walleye larvae would require prey abundances of 200-800$\cdot L^{-1}$ for small zooplankton or 20-300$\cdot L^{-1}$ for large zooplankton to attain 90% of C_{max}. Beyond this walleye size range, threshold zooplankton abundances decline markedly and 13-15-mm larvae would probably only require prey abundances as high as $100 \cdot L^{-1}$ for small zooplankton. However, these very high estimates of D_{90} must be viewed with caution. My experiments were conducted using prey abundances $\leq 180 \cdot L^{-1}$ but D_{90} estimates were sometimes far above these levels. For zooplankton abundances $> 180 \cdot L^{-1}$ the functional response model may be inappropriate. For example, cyclopoid copepods (the main component of the S treatment) at abundances $\geq 500 \cdot L^{-1}$ will

consume walleye larvae (Hokanson and Lien 1986; pers. obs. this study) and under such conditions walleye consumption rate would obviously decline with increasing prey abundance. Consumption rate may also decline with increasing prey abundance if the fish are confused by seeing many prey items at once (Marcotte and Browman 1986). Such an effect has been observed for first-feeding walleye larvae preying on large zooplankton (J.A. Mathias, unpubl. data). I also observed a declining consumption rate with increasing zooplankton abundance at high zooplankton densities in my 1987 feeding experiments (Appendix D). The inability of the model to predict the functional response at very high prey abundances would likely only be important under artificial conditions such as in extensive or intensive culture systems. Zooplankton abundances encountered by most walleye larvae in natural lakes or rivers would probably be within the range of abundances used in this study. In prairie lakes such as Dauphin Lake or Lake Winnipeg, mean zooplankton abundances at the time of walleye first feeding are in the range of 20-70·L^{-1} and are usually composed of small zooplankters dominated by cyclopoid copepods (Patalas 1975; Friesen and Mathias 1990; Patalas and Salki 1992).

The application of my functional response results to the prediction of walleye feeding under natural conditions must also consider the prey distribution. The functional response model assumes a random distribution of prey organisms, a condition which is easily maintained in laboratory aquaria but which is probably uncommon in natural communities. However, the feeding of fish larvae may depend as much on the variance as the mean of zooplankton abundance. For a given mean zooplankton abundance, feeding success should improve with increasing prey patchiness, reach an optimum, and then decline under conditions of extreme clumping (Vlymen 1977). At low levels, clumping creates localized high density patches which allow a higher consumption rate. Minor prey clumping would reduce the value of D_{90} but would not affect C_{max}. This may explain the higher consumption rates observed in field-sampled fish relative to laboratory fish at similar prey densities (MacKenzie et al. 1990). However, extreme prey clumping creates very high density patches which may be confusing, or even dangerous for fish larvae (if predacious zooplankton are present), and which are too widely spaced to be easily found. Thus, walleye larvae in natural lakes may be able to feed

at higher rates at low prey abundances than would be predicted by my functional response model if prey were moderately aggregated.

Prey Selection

Prey selection by walleye larvae was strongly dependent on walleye body size. Selection for larger relative to smaller prey and selection for large cladocerans relative to cyclopoid copepods both increased significantly with walleye mean length. Similar trends have been observed in previous studies of walleye larvae (Raisanen and Applegate 1983; Graham and Sprules 1992) and of numerous other species (Miller et al. 1988; Schael et al. 1991). Cyclopoid copepods appear to be an important dietary component and a preferred prey of first-feeding walleye (Spykerman 1974; Fox 1989; Graham and Sprules 1992; this study) but selection shifts rapidly to larger prey. This shift is associated with an increasing mouth gape which allows larger prey to be ingested (Mathias and Li 1982; Graham and Sprules 1992). Walleye have one of the largest gapes relative to their body size among freshwater fish larvae (summarized by Ghan and Sprules 1993) and they will eat larger prey than other larval fishes in the spring ichthyoplankton community (Bulkley et al. 1976; Michaletz et al. 1987). Even walleye as small as 10 mm will consume fish larvae though they are usually only a minor dietary component (Priegel 1969; Bulkley et al. 1976; Li and Ayles 1981). It should be noted that this study did not attempt to separate the effects of walleye size and age. Hence, experience may have been as important as physical ability in explaining the observed dietary shift. Because cyclopoids are generally more difficult to capture than cladocerans, fish which have experience with both prey types may learn to selectively prey on cladocerans (Vinyard 1980).

The negative relationship observed between electivity and relative prey abundance indicates that walleye larvae select most strongly for prey which are relatively uncommon in the zooplankton community. This trend was observed in both laboratory and field studies. Emlen (1966) hypothesized that selection for a prey type would increase with its relative abundance. Foraging studies on guppies, *Poecilia reticulatus*, confirmed the positive influence of relative prey abundance and the pattern, termed prey switching, was considered to be a more efficient foraging strategy (Murdoch et al. 1975). The 'counter-switching' exhibited by walleye larvae in this study suggests a strategy to broaden the diet by selecting most strongly

for uncommon prey. Similar patterns have been observed in zooplanktivorous juvenile yellow perch, *Perca flavescens*, (Mills et al. 1987), and in threespine sticklebacks, *Gasterosteus aculeatus*, feeding on a mixed assemblage of fish larvae and zooplankton (Kean-Howie et al. 1988). Mills et al. (1987) hypothesized that selection for novel prey reflected nutrient requirements of the fish. Diets may be broader than expected and foraging strategies may deviate from what is considered energetically optimal for a variety of reasons (Westoby 1978; Hart 1986). It is not possible to say from my results whether this foraging pattern is an adaptation to meeting nutrient constraints or simply a part of the learning process. If the latter is true, counter-switching may be a characteristic of feeding in young fish and/or a characteristic of the early stages of each feeding bout for all fish.

Based on the results of my functional response analysis, I predicted that selection for smaller relative to larger prey would increase with total zooplankton abundance. This trend was significant in field studies but not in the laboratory studies, with the exception of a specific subset of the data. Optimal foraging theory predicts that selectivity increases with prey abundance (Emlen 1966; MacArthur and Pianka 1966). More recent studies stress predator satiation as the primary determinant of selectivity and consider prey abundance as a correlate of satiation (Bence and Murdoch 1986; Hart and Gill 1992). Changes in electivity with time spent foraging (e.g. Furnass 1979; Confer and O'Bryan 1989) may reflect a similar satiation mechanism. But at least one study has found no effect of prey abundance or predator satiation on prey selection (Mills et al. 1986). I suspect that the laboratory walleye which were food-deprived then allowed to feed for only a short time were not as strongly selective as the pond walleye whose guts were usually full. However, laboratory and field conditions differed in other respects (Table 3.3) which may have also influenced the effect of prey abundance on prey selection. Juvenile yellow perch show stronger selection for copepods relative to *Daphnia sp.* with increasing prey abundance (Confer et al. 1990), a trend consistent with my field results. Increasing prey abundance (or predator satiation) may reduce the variance in prey selection as well as change the magnitude of selection. Reductions in the variance of electivity with increasing prey abundance were observed in both laboratory and field components of this research and were statistically significant in the former.

Temperature has a major influence on food consumption rate in fishes and yet relatively little is known of the effects of temperature on prey selection. In this study, temperature had a significant effect on prey size electivity by walleye larvae under field conditions. Selection for medium relative to small prey declined with increasing water temperature. In more recent laboratory experiments I found no statistically significant effect of temperature on prey selection by walleye larvae (Chapter 4). However, these experiments were of short duration (< 10 min), used larvae which were deprived of food prior to the experiment, and examined a much narrower range of water temperatures (15-22 °C). Werner and Hall (1974) hypothesized that increasing diet breadth in response to declining temperature represented an optimal allocation of time given that a fish's search rate will decline with temperature. This suggests that prey selection will become stronger with increasing temperature and is consistent with the findings of this study. The mechanism behind selection for smaller prey by walleye at warmer temperatures may also be related to changes in search rate. An increasing search rate may increase encounter rates with small prey relative to encounter rates with large prey. An alternative is that temperature might simply be correlated with another variable affecting prey selection, such as light, time of day or barometric pressure. Light has been shown to influence prey selection. In laboratory feeding experiments with juvenile yellow perch, Mills et al. (1986) observed a decline in the median size of prey consumed and a decline in selection for daphnids relative to cyclopoid copepods with increasing light intensity. However, this trend was not observed in their field studies.

The pattern of prey selection exhibited by walleye larvae may be the result of both predator and prey characteristics. Walleye may actively choose specific prey sizes and/or species which optimize growth. Growth of larval and juvenile fishes has been linked to both prey size (Fox 1989) and prey species (Forney 1966; Hokanson and Lien 1986; Confer and Lake 1987). The importance of prey size alone has been demonstrated in lab experiments by feeding fish with pellets of different sizes but identical compositions (Wankowski and Thorpe 1979; Tabachek 1988). However, the effect of prey species on growth is often difficult to separate from the size effect as different species are naturally of different sizes. Patterns in prey selection may also reflect the relative ease with which different prey species can be found, captured, and ingested.

My results have several implications for understanding the feeding ecology of walleye larvae in the natural environment. Walleye show strong selection for relatively large prey early in their development. However, in many lakes, and particularly those with abundant zooplanktivorous fishes, the zooplankton community is dominated by smaller organisms such as cyclopoid copepods and bosminids, and large cladocerans are relatively rare (Friesen and Mathias 1990; Mazumder et al. 1992; Patalas and Salki 1992). A lack of the preferred, larger zooplankton in such lakes may force walleye to become benthivorous or piscivorous at a smaller size than they would in lakes with abundant large zooplankton. A scarcity of larger-bodied zooplankton has been hypothesized as a major factor limiting growth and survival of other larval fishes (Crowder et al. 1987). If the walleye's strong selection for relatively uncommon prey reflects a nutrient constraint, feeding and growth may be limited in zooplankton communities which lack specific prey types. However, at present, little is known of the nutrient requirements of freshwater fish larvae or the nutrient composition of their zooplankton prey (discussed further in Chapter 8). The significance of changes in prey selection with respect to prey abundance and water temperature is not readily apparent. Growth rates of walleye larvae in extensive culture systems increase with both water temperature and prey abundance (Appendix A), presumably because of increased food conversion efficiency and/or food consumption. Increased selection for relatively smaller prey may thus be an adaptation which increases food conversion efficiency when large volumes of food are passing through the digestive tract and/or allows a higher consumption rate.

Chapter 4:

The effects of temperature on the feeding ecology of walleye larvae

Introduction

The importance of successful feeding during the early life history of fish has long been stressed in the fisheries literature (Hunter 1981; Blaxter 1988). Studies on the feeding ecology of postlarval walleye have indicated that food limitation may be a determining factor in survival through this period (Li and Mathias 1982). Further studies to determine what factors limit food consumption could aid in understanding postlarval walleye survival.

Food intake in actively foraging fish can be considered as two steps: prey capture and gut processing. Consumption could be limited by either of these two processes. The former is affected by a variety of factors acting on both the predator and prey whereas the latter is affected solely by factors acting on the predator's digestive system. One of the most important environmental factors influencing fish activity is temperature. Though it is generally accepted that food consumption in fish increases with increasing temperature (below an optimum), the relative effects of temperature on prey capture and digestive processing rates are less clear. Numerous studies have demonstrated the effect of temperature on gut evacuation rate (e.g. Laurence 1971; Persson 1979; Karjalainen et al. 1991) but few have examined the effect of temperature on prey capture (e.g. Schmidt and O'Brien 1982; Persson 1986; Bergman 1987).

My objective in this study was to examine the development of feeding in postlarval walleye and to determine the effects of temperature on the prey capture process. My approach was to examine short-term consumption in starved larvae so that feeding was not limited by the digestive process. Because long-term food consumption in fish increases with increasing temperature, I hypothesized that increasing temperature could affect prey capture by causing an increased attack rate, an increased capture success, a stronger electivity for larger prey items or some combination of these responses.

Materials and Methods

Laboratory experiments were conducted at the Department of Zoology, University of Manitoba (Fig. 2.2) in May and June 1990. Prolarval walleye from the Swan Creek (Lake Manitoba) stock and the Grand Rapids (Lake Winnipeg) stock (Fig. 2.2) were brought into the

laboratory within 2 d of hatching and raised in 120-L glass stock aquaria at 18.5°C. The sides of the stock aquaria were covered with translucent green plastic sheeting. Wild zooplankton swept from a local pond were added to the stock aquaria 1 d prior to the onset of feeding and maintained at densities of > 200·L^{-1} thereafter. Experimental chambers were 13-L rectangular glass aquaria covered in green plastic sheeting and filled with 10 L of dechlorinated city water. Seven experimental aquaria were placed in each of three shallow, flow-through water baths at 15, 18.5, and 22°C. I considered this range to reflect the temperatures most commonly encountered by postlarval walleye in natural systems (Schaap 1987) and extensive culture ponds (Appendix A) in Manitoba. In each water bath, one aquarium was used as a feeding chamber whereas the other six were used as acclimation chambers and received no zooplankton additions. The laboratory was lit by overhead fluorescent lights covered in translucent green plastic sheeting. Light levels at the water surface of the experimental chambers ranged from 1.0 to 1.5 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Experiments were conducted on six dates corresponding to walleye mean lengths of 9.76 to 18.0 mm (Table 4.1). On the evening prior to each experimental date, 2-3 fish were transferred from the stock aquaria to each acclimation chamber. Fish were deprived of food for 8-18 h prior to experiments to ensure total clearance of the gut. Experimental zooplankton were size-graded using Nitex® sieves. The zooplankton composition was altered between experimental dates with larger larvae generally receiving a larger mean and range of particle sizes (Table 4.1). However, size and species composition were always identical to the zooplankton added to the stock aquaria at that developmental stage. Zooplankton from a common collection were added to each of the three feeding chambers at densities of 150 particles·L^{-1}. All zooplankton remaining at the end of feeding trials were preserved in 5% buffered formalin and later analyzed to verify density and to determine species and size composition.

An experimental run was commenced by transferring a single fish from an acclimation chamber into the feeding chamber using a ladle. Time was logged with a stopwatch starting from the first visible strike (bent body posture followed by a lunge) at a prey item. Fish which showed no prey strikes within 5 min were removed and recorded as non-feeders. Prey strikes were tallied on a hand counter. After 10 strikes or 10 min (whichever came first) the larva was

Table 4.1. Experimental date, walleye stock, larval length ($\bar{X} \pm 1$ SD), and numerical composition of zooplankton fed to postlarval walleye during laboratory feeding experiments conducted in 1990. Zooplankton size categories 1, 2, and 3 were < 0.3, ≥ 0.3 - 0.6, and ≥ 0.6 mm body width, respectively. Zooplankton species categories 1, 2, and 3 were cyclopoid copepods, *Ceriodaphnia quadrangula* and *Daphnia sp.*, respectively. Subsample sizes (n) indicate the number of fish (excluding non-feeders) used in each temperature treatment for each developmental size.

				Zooplankton composition (%)					
				Size category			Species category		
Date	Stock	Length (mm)	n	1	2	3	1	2	3
9 June	Grand Rapids	9.76 ± 0.11	3-4	48	51	1	5	85	10
15 June	Grand Rapids	10.9 ± 0.41	9-10	55	45	0	25	53	22
6 June	Swan Creek	11.5 ± 0.55	7-8	2	30	68	7	13	80
5 June	Swan Creek	13.7 ± 1.45	13	16	35	49	17	24	59
21 June	Grand Rapids	14.3 ± 0.82	9	7	71	22	1	21	78
16 June	Swan Creek	18.0 ± 1.66	5-6	10	57	33	1	38	61

removed, sacrificed in MS-222, and preserved in 5% buffered formalin.

Preserved larvae were measured using an ocular micrometer for small larvae and a scientific ruler for larger larvae. Length was measured as total length until a definite fork developed in the tail and as fork length thereafter. Preserved lengths were converted to fresh lengths using the formula of Appendix B. Gut contents were identified to genus, enumerated, and measured to the nearest 0.02 mm with an ocular micrometer. Zooplankter length was measured from the anteriormost point of the body to the base of the tail spine in Cladocera and to the end of the caudal rami in Copepoda. Zooplankter width was measured as the second longest body dimension. This corresponded to body width in Copepoda and to dorso-ventral depth in Cladocera. Zooplankter dry weights were estimated from lengths using the formulae of Appendix A.

Consumption was calculated as the total biomass consumed divided by the total foraging time. Attack rate was calculated as the number of strikes divided by foraging time if the trial ended by time elapsing, or by the number of strikes - 1 divided by foraging time if the trial ended on a strike. Capture success was calculated as the number of prey in the gut divided by the number of strikes. Individual fish were treated as subsamples (Table 4.1) and the means of larval length, consumption, attack rate and capture success for each temperature treatment on each of the six experimental dates were used in further analyses. Treatment effects were assessed by regressing corrected means of each of the response variables against temperature. Means were corrected for the covariate mean larval length when a linear relationship existed between the response variable and length. Otherwise, means were corrected for the block effect of developmental stage which entered the model as a class variable. The significance of treatment effects was examined following removal of the treatment-covariate (or treatment-block) interaction term from the model. All models were fit and analysed using the SAS® GLM procedure (Freund and Littell 1981). Dependent variables were either $\log_e$-transformed or weighted by the inverse of their standard errors.

I examined both size and species selection of prey using the electivity index, $\mathcal{E}$, proposed by Chesson (1983)(equations [3.4] and [3.5]). In both cases food items were divided into three categories. For size selection, prey were categorized as < 0.3 mm, $\geq 0.3 - 0.6$ mm, or ≥ 0.6 mm body width. For species selection, prey were categorized as cyclopoid copepods (mostly

Diacyclops bicuspidatus thomasi), *Ceriodaphnia quadrangula*, or *Daphnia sp.* Means and standard errors of $\mathcal{E}$ were estimated for each temperature treatment on each experimental date (tank means) by jackknifing (Krebs 1989) using the FORTRAN subroutine of Matloff (1980). Overall treatment means were then calculated (LSMEANS option of GLM procedure, Freund and Littell 1981) by treating experimental date as a blocking effect and weighting tank means by the inverse of their standard errors. By nature of the index, standard errors are maximum when electivity is neutral (i.e. $|\mathcal{E}|=0$) and equal 0 when acceptance or rejection of a food category is complete (i.e. $|\mathcal{E}|=1$). For observations where $|\mathcal{E}|=1$, I set the standard error to 0.05 (the lowest observed standard error when $|\mathcal{E}|<1$) to allow the calculation of weights.

I examined the effects of temperature on electivity using the principal components (PRINCOMP procedure, SAS® Institute Inc. 1985) from each vector of electivity indices as the dependent variables (as in Chapter 3). The effect of temperature on the principal components was examined by ANOVA (GLM procedure, Freund and Littell 1981) with experimental dates treated as blocks and temperature treated as a class variable. Power analysis, following the methods of Cohen (1988), was conducted on all tests in which the null hypothesis of no treatment effect was not rejected.

Results

Larval walleye commenced feeding at a mean length of 9 mm during this study. Larvae transferred from the acclimation chambers to the feeding chamber generally began search and strike behaviour within 30 s of transfer. Approximately 50% of walleye did not feed during the trial with the smallest larvae. However, the percentage of non-feeders declined with increasing larval size in subsequent trials. The percentage of non-feeders was not related to the temperature treatment but may have been related to the method of transfer. Some of the larvae which did not feed appeared stressed during transfer. Because of the short feeding times ingested plankters occupied only a small portion of the gut volume relative to the guts of larvae feeding for longer periods (cf. feeding studies of Chapter 3). Thus, the larvae were probably not satiated and were probably not limited in their feeding by gut processing rate.

Consumption increased exponentially with increasing larval length in all temperature treatments (Fig. 4.1). I transformed both length and consumption into natural logarithms to linearize this relationship. Slopes of the mean $\log_e$(consumption) vs mean $\log_e$(length)

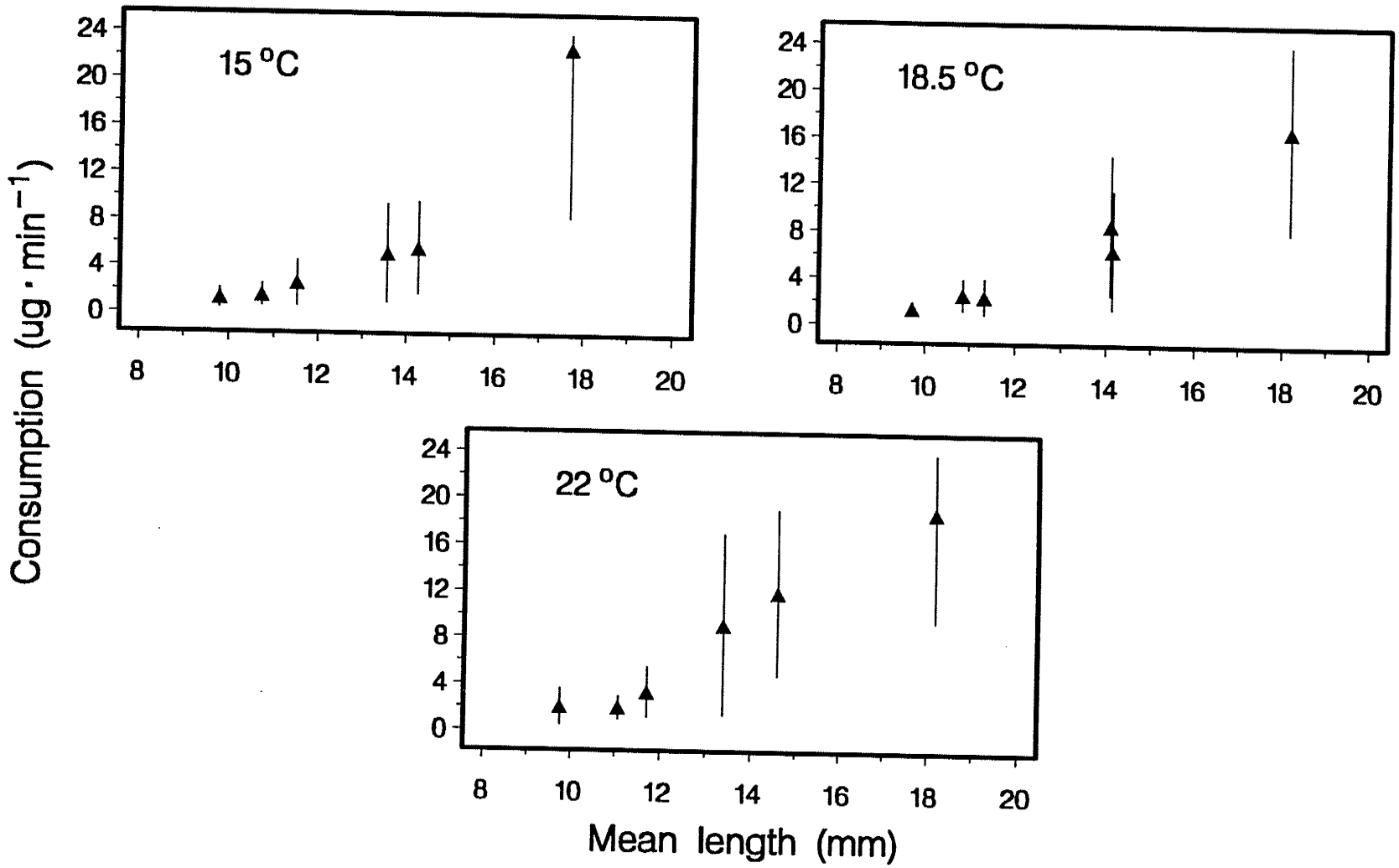

Figure 4.1. Scatter plots of prey consumption rate vs. mean fish length for walleye postlarvae feeding on zooplankton at a density of 150·L^{-1} in the laboratory at three temperatures. Symbols are means ± 1 SD.

relationships were not significantly different between temperature treatments (ANCOVA, F = 1.99, df = 1,14, P = 0.18). The treatment means, adjusted to a mean walleye length of 12.7 mm, were 3.09, 3.62, and 4.07 $\mu g \cdot min^{-1}$ for the 15, 18.5, and 22 °C treatments, respectively. There was a significant positive relationship between the adjusted consumption values for all experimental dates and temperature (ANOVA, F = 5.39, df = 1,15, P = 0.035).

Attack rate showed a declining trend with increasing fish length over the 9 to 15 mm length ranges but then rebounded in the largest larvae examined (Fig. 4.2). Because the relationship between attack rate and mean length appeared complex I chose to enter experimental date into the attack rate vs. temperature model as a block effect. The treatment means of attack rate, adjusted for block effects were 1.93, 2.02, and 2.38 strikes$\cdot min^{-1}$ for the 15, 18.5, and 22 °C treatments, respectively. There was a significant positive relationship between the adjusted attack rate values for all experimental dates and temperature (ANOVA, F = 5.74, df = 1,11, P = 0.035).

Capture success increased with walleye development and appeared to reach an asymptote at a success rate of 80-90% for the 18.5 and 22 °C treatments (Fig. 4.3). Natural logarithms of walleye length were taken to linearize the relationship between these two variables and allow length to be treated as a covariate. Means of capture success were weighted by the inverses of their standard error. Analysis of covariance indicated no significant difference in the slopes of the mean capture success vs mean $\log_e$(length) relationship between temperature treatments (F = 0.56, df = 1,14, P = 0.47). The treatment means of capture success, adjusted to a mean walleye length of 12.7 mm, were 56.1, 56.9, and 58.1% for the 15, 18.5, and 22 °C treatments, respectively. There was no significant relationship between the adjusted capture success values for all experimental dates and temperature (F = 0.34, df = 1,15, P = 0.57). This test had a power of 80% to detect differences between the largest and smallest capture success means of approximately 38-44%.

Treatment means of electivity adjusted for experimental date are summarized in Table 4.2. Generally, walleye larvae demonstrated positive electivity for medium-sized prey (≥ 0.3-0.6 mm body width) and *Ceriodaphnia quadrangula*, negative electivity for small prey (< 0.3 mm) and cyclopoid copepods, and neutral electivity for large prey (≥ 0.6 mm) and *Daphnia sp.*

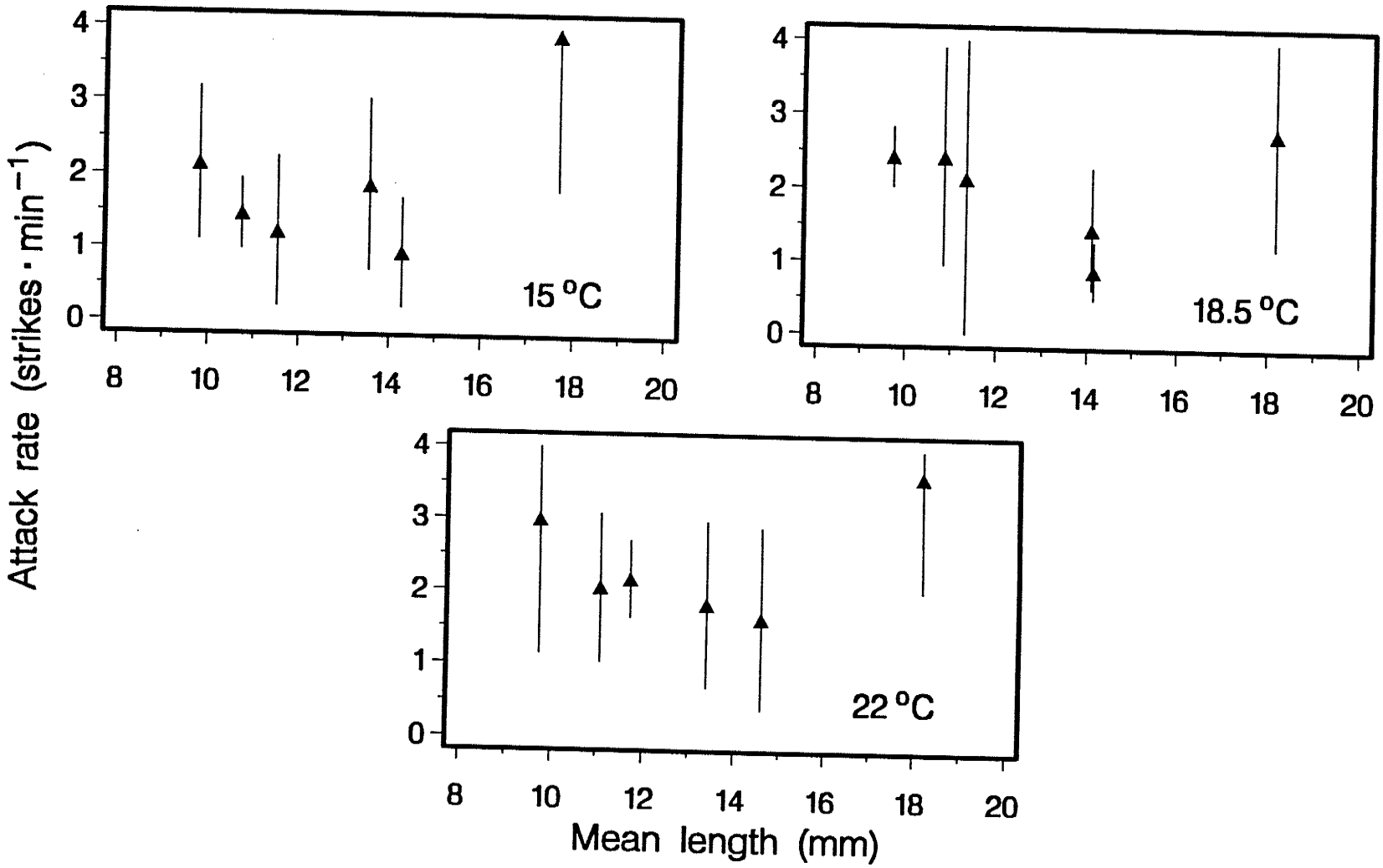

Figure 4.2. Scatter plots of prey attack rate vs. mean fish length for walleye postlarvae feeding on zooplankton at a density of 150·L^{-1} in the laboratory at three temperatures. Symbols are means ± 1 SD.

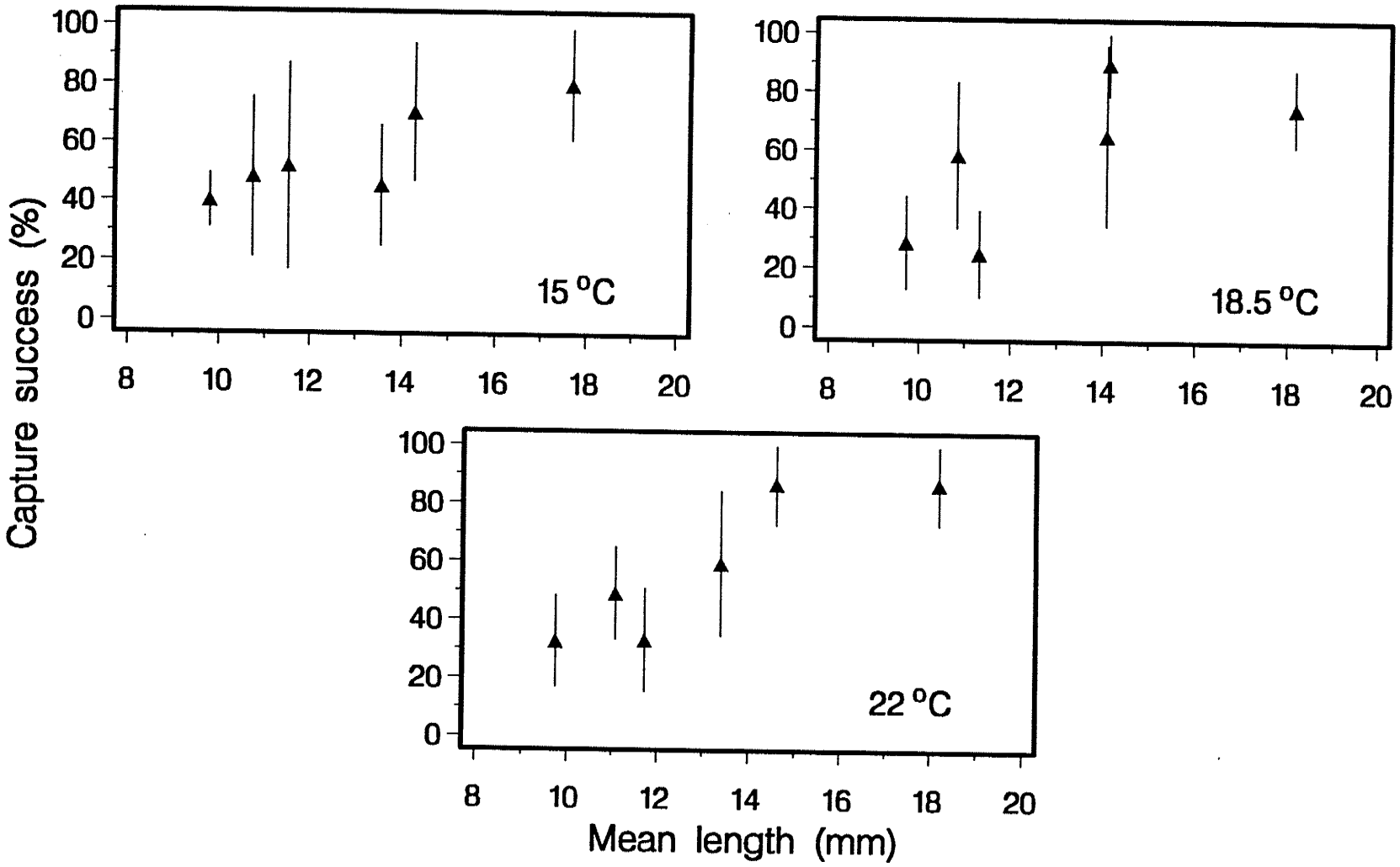

Figure 4.3. Scatter plots of prey capture success vs. mean fish length for walleye postlarvae feeding on zooplankton at a density of 150·L^{-1} in the laboratory at three temperatures. Symbols are means ± 1 SD.

Table 4.2. Prey electivities, $\mathcal{E}$ (mean ± 1 SE), for postlarval walleye feeding on zooplankton at three temperatures. Values represent means (n = 6, except n = 5 for prey size index $\mathcal{E}_3$) of experimental chamber means (3-13 fish) and were adjusted for experimental date. Zooplankton size categories represented by $\mathcal{E}_1$, $\mathcal{E}_2$, and $\mathcal{E}_3$ were < 0.3, ≥ 0.3 - 0.6, and ≥ 0.6 mm body width, respectively. Zooplankton species categories represented by $\mathcal{E}_1$, $\mathcal{E}_2$, and $\mathcal{E}_3$ were cyclopoid copepods, *Ceriodaphnia quadrangula*, and *Daphnia sp.*, respectively. Means which were significantly different from 0 (*t*-test) are indicated by * (0.01 < P < 0.05) or ** (P < 0.01).

		Temperature (°C)					
Electivity Index		15		18.5		22	
Prey size							
	$\mathcal{E}_1$	-0.42 ± 0.17	*	-0.72 ± 0.13	**	-0.38 ± 0.17	
	$\mathcal{E}_2$	0.23 ± 0.11		0.34 ± 0.11	*	0.39 ± 0.10	**
	$\mathcal{E}_3$	-0.17 ± 0.15		0.06 ± 0.15		-0.32 ± 0.14	*
Prey species							
	$\mathcal{E}_1$	-0.93 ± 0.11	**	-0.86 ± 0.10	**	-0.95 ± 0.10	**
	$\mathcal{E}_2$	0.37 ± 0.12	**	0.39 ± 0.12	**	0.48 ± 0.11	**
	$\mathcal{E}_3$	0.08 ± 0.15		0.06 ± 0.12		-0.01 ± 0.14	

Trends in prey selection with respect to temperature were inconsistent (Table 4.2). Maximum observed differences in the adjusted mean electivities between temperature treatments were in the ranges of 0.16-0.38 and 0.09-0.11 electivity units for the prey size and prey species electivity indices, respectively (Table 4.2). The effect of temperature on the principal components (PC_i) of electivity was not significant (Table 4.3). The power of these tests to detect the observed effect size was generally low (< 15%) with the exception of the test on PC_1 of the size electivity vector where the power of the test was 52%. This principal component represented the electivity of small relative to large prey.

Discussion

Temperature can have a strong influence on the early life history of fishes. Many freshwater species, including walleye, pass through the larval stage during the spring when water temperatures are changing rapidly. Water temperature influences egg incubation time (Koenst and Smith 1976), young-of-the-year growth (Forney 1966), and may ultimately influence future year-class strength (Busch et al. 1975). As with all poikilotherms, fish are subjected to fluctuating metabolic rates in response to changing temperature. This can affect all forms of activity from respiration rates to gut evacuation rates to swimming speeds. It therefore seems probable that temperature would also influence prey capture.

Though many studies have examined the effects of temperature on prey capture in poikilotherms, few have examined the effect of temperature on prey capture in fishes. The results of this study indicated that temperature does affect the prey capture process in larval walleye. Short-term consumption increased with temperature over the range of 15 to 22 °C. However, temperature affected the different components of the predation process to varying degrees.

The attack rate of larval walleye increased significantly with temperature. This suggests that walleye spend less time searching for and/or handling a given biomass of prey at higher temperatures. I suspect that the increased metabolism which accompanies a rise in temperature affects search time, and hence prey encounter rate, more than handling time for larval walleye. If this is true, the effect of temperature on attack rate may be more pronounced in natural environments where prey densities are lower because the ratio of search time to handling time increases with decreasing prey density. Functional response analysis of

Table 4.3. Principal component formulae for the electivity index vectors and results of ANOVA to test the effect of temperature on prey size and prey species selection (as represented by the principal components) by postlarval walleye. Experimental date was treated as a block effect. Interpretations of the principal components in terms of the prey categories of the original indices were made in relation to the magnitude and sign of the coefficients. Prey size categories represented by ε_1, ε_2, and ε_3 were < 0.3, ≥ 0.3-0.6, and ≥ 0.6 mm body width, respectively. Prey species categories represented by ε_1, ε_2, and ε_3 were cyclopoid copepods, *Ceriodaphnia quadrangula*, and *Daphnia sp.*, respectively.

Electivity vector	Principal component formulae	Interpretation	F	df	P
Prey size	$PC_1 = 0.72\ \varepsilon_1 - 0.03\ \varepsilon_2 - 0.69\ \varepsilon_3$	small relative to large	2.51	2,8	0.14
	$PC_2 = 0.44\ \varepsilon_1 - 0.75\ \varepsilon_2 + 0.49\ \varepsilon_3$	small and large relative to medium	0.52	2,8	0.61
Prey species	$PC_1 = -0.10\ \varepsilon_1 - 0.59\ \varepsilon_2 + 0.80\ \varepsilon_3$	*Daphnia* relative to *Ceriodaphnia*	0.05	2,10	0.95
	$PC_2 = 0.94\ \varepsilon_1 - 0.31\ \varepsilon_2 - 0.11\ \varepsilon_3$	cyclopoids relative to *Ceriodaphnia*	0.53	2,10	0.60

poikilothermic predators has generally shown that increasing temperature causes an increase in search rate (attack coefficient) and a decrease in handling time (Thompson 1978; Gresens et al. 1982; Chow et al. 1983; Spitze 1985; Persson 1986; Bailey 1989). The observed pattern of decline then increase in attack rate with walleye development was most likely related to concomitant changes in prey capture success. As capture success improved, walleye larvae attacked fewer prey. However, when prey capture success reached a plateau, further increases in consumption could only result from increases in attack rate and/or selection of larger prey.

Walleye prey capture success increased markedly with walleye development over this study. Similar patterns have been noted for the zooplanktivorous young of other species (Beyer 1980; Blaxter 1986 (review); Dabrowski et al. 1986; Meyer 1986; Drost 1987). Capture success in larval fish may depend upon mouth size, attack speed, and strike accuracy (Drost 1987), as well as the prey species (Wanzenböck 1992). I was unable to detect a significant change in walleye prey capture success with respect to temperature. However, the observed difference in $\log_e$(length)-adjusted mean capture success over the 15 to 22 °C range was only 2% and the biological significance of such a small effect seems questionable. These results suggest that walleye attack and prey evasive ability are equally influenced by the change in temperature from 15 to 22 °C. The effect of temperature on other predator-prey interactions appears to be quite varied. Arctic grayling, *Thymallus arcticus*, feeding on the calanoid copepod *Heterocope septentrionalis* showed a much higher capture success at 5 °C than at 15 °C apparently because of the slower copepod escape response at low temperatures (Schmidt and O'Brien 1982). In contrast, juvenile yellow perch feeding on lake whitefish, *Coregonus clupeaformis*, larvae had a significantly higher capture success at 15 and 18 °C than at 10 °C (Yocom and Edsall 1974). The capture success of roach, *Rutilus rutilus*, feeding on *Chaoborus obscuripes* also increased with increasing temperature (Persson 1986). However, temperature had no effect on the capture success of perch, *Perca fluviatilis*, or ruffe, *Gymnocephalus cernuus*, feeding on *Chaoborus obscuripes* (Persson 1986; Bergman 1987). Capture success of juvenile Atlantic cod and Atlantic herring feeding on herring and plaice, *Pleuronectes platessa*, larvae did not differ significantly between 8 and 13 °C (Fuiman and Batty 1994). Poulin and Fitzgerald (1988) found no significant increase in the attack rate or

attack success of the parasitic copepod *Argulus canadensis* on its stickleback (Gasterosteidae) hosts with increasing temperature.

My results indicated that larval walleye show a strong positive electivity for medium-sized cladocerans. In an examination of the feeding ontogeny of young Cyprinidae, Wanzenböck (1992) noted a much lower capture success for cyclopoid (*Eucyclops sp.*) prey than for *Ceriodaphnia sp.* prey. Variation in escape abilities of the prey species could explain much of this pattern (Heath 1993). Cladocerans are generally more susceptible than copepods to the suction-type intake of planktivorous fish (Drenner et al. 1978). Differential capture success may also explain the positive electivity for *Ceriodaphnia sp.* and strong negative electivity for cyclopoids exhibited by walleye larvae in this study.

Few studies have examined the effect of temperature on prey selection. Werner and Hall (1974) hypothesized that the diet breadth of a planktivorous fish should decline with increasing temperature. This implies that prey selection will become stronger with increasing temperature. Previous studies examining the effect of temperature on prey electivity have also shown variable results. Predaceous calanoid copepods have been observed to differentially increase their attacks on cladoceran prey relative to copepod prey with increasing temperature (Luecke and O'Brien 1983), but particle size selection in grazing calanoids has been observed to remain fairly constant with respect to seasonal temperature changes (Vanderploeg 1981). Swain and Lindsey (1984) found no significant change in prey selection with respect to temperature for pumpkinseed sunfish, *Lepomis gibbosus*, feeding on threespine sticklebacks, but examined a narrow temperature range (15 vs 17 or 20 °C). As with capture success, I did not detect a significant trend in walleye prey electivity with respect to temperature. However, the power of my tests to detect the observed temperature effects was generally low. In the case of prey species electivity, differences due to temperature were slight (0.09-0.11 electivity units). But, in the case of prey size electivity, differences due to temperature were fairly large (0.16-0.38). Though the observed temperature effect on species electivity was not very strong, I consider the effect on prey size electivity to be biologically meaningful. I conclude that my tests for temperature effects in this latter case were equivocal and suggest that future studies should re-examine the effect of temperature on prey size selection using more powerful tests. My analysis indicated that further increases in sample sizes would have to be large in order

to substantially increase the power of these tests. Utilizing a wider range of experimental temperatures and/or reducing the residual variance within treatments would probably be more efficient means of increasing power.

This study has demonstrated that temperature can affect prey capture independently of the digestive process in walleye larvae. Temperature appears to have the greatest influence on the rate of attack and less influence on the success of attack or the prey types attacked.

Chapter 5:

Gut evacuation and assimilation efficiency in walleye larvae

Introduction

The early stages of exogenous feeding are considered critical in the early life history of fishes and the probability of survival through this period is believed to be positively related to growth. Growth in fish may be limited both by the ability to locate and capture prey and by the ability to digest and utilize the prey once captured. In Chapters 3 and 4, I examined how various external factors, such as prey abundance, prey size, and temperature, influence the feeding success of walleye larvae. The next step in determining how this feeding success translates into growth is to examine the walleye larvae's ability to process and utilize their food once ingested.

Digestion in fishes can be considered as three processes; the movement of food through the gut from ingestion to egestion (gut evacuation), the movement of digestive enzymes and some waste products into the gut lumen, and the movement of food nutrients and recovered digestive enzymes out of the gut lumen (assimilation). In terms of the flow of matter, gut evacuation and assimilation are the two most important processes. The rate of gut evacuation is a key parameter in the estimation of food consumption (Eggers 1977; Elliott and Persson 1978). A rapid gut evacuation rate allows a fish to consume food at a faster rate. Assimilation efficiency, defined as the proportion of ingested matter that is assimilated, generally declines with increasing food consumption (Boehlert and Yoklavich 1984; Rösch 1987). Thus, for the purposes of understanding food intake and growth in fishes, both evacuation rate and assimilation efficiency must be understood.

Gut evacuation has been studied extensively in the adults (reviewed by Elliott and Persson 1978, Jobling 1981, and Bromely 1994) and larvae (reviewed by Govoni et al. 1986) of many species of fish. Both gut evacuation time (Mathias and Li 1982; Corazza and Nickum 1983) and gut evacuation rate (Fox 1991; Madon and Culver 1993) have been estimated for young walleye. Fox (1991) determined stomach evacuation rates of late larval and juvenile (17-45 d old) walleye using both field (culture ponds) and laboratory studies. Similarly, Madon and Culver (1993) estimated gut evacuation rates of larval and juvenile walleyes using pond

and enclosure studies. However, gastric evacuation of walleye larvae has not yet been examined under controlled laboratory conditions, and no study has attempted to estimate assimilation efficiency of walleye larvae.

The purpose of this chapter is twofold. First, I wish to examine the pattern in gut evacuation in walleye larvae and determine the influence of temperature, gut fullness, fish size, and time since cessation of feeding on gut evacuation rate. The results of this analysis will be used to build an empirical model which will allow the calculation of gut evacuation rates in the field. Second, I wish to determine the assimilation efficiency of walleye larvae consuming a crustacean zooplankton diet, and examine the effects of gut evacuation time and fish size on assimilation.

Materials and Methods

Gut evacuation

Laboratory experiments to determine the gut evacuation rate of walleye larvae on a zooplankton diet were conducted at the Department of Fisheries and Oceans' Dauphin Lake Walleye Rehabilitation and Research Station at Methley Beach, Manitoba (Fig. 2.2) during May and June of 1989. Walleye prolarvae from the Duck Bay (Lake Winnipegosis) and Swan Creek (Lake Manitoba) stocks (Fig. 2.2) were brought into the laboratory within 1 d of hatching and raised in 120-L glass stock aquaria in three climate-controlled rooms at 15, 20, and 25 °C (± 0.5 °C). The sides of the stock aquaria were covered with translucent green plastic sheeting. Wild zooplankton swept from the field station culture ponds were provided daily and maintained at densities > 200·L^{-1}. Experimental units in each temperature room were 13-L rectangular glass aquaria covered in green plastic sheeting and filled with 10 L of filtered (through 45-µm Nitex® mesh) pond water. Room temperatures were contolled by air conditioners and water temperatures in all aquaria were controlled passively by room temperature. Light levels over the aquaria ranged from 0.6 to 0.9 µE·m^{-2}·s^{-1}.

Experiments were conducted at various walleye sizes between 10.4 and 16.2 mm mean length and 0.53 and 3.53 mg mean dry weight (Table 5.1). Experimental fish were removed from the stock aquaria after 2 h of feeding following the daily addition of fresh zooplankton. Equal numbers of fish were randomly assigned to two experimental aquaria. Samples of 10-20 fish were removed from each unit at the time of transfer (time 0) and at 1-2 h intervals

Table 5.1. Water temperature, experimental date, number of runs (n), and walleye size for gut evacuation experiments conducted in 1989. All fish were of the Duck Bay (Lake Winnipegosis) stock except for a single experiment (15 °C, 10 June) which used walleye of the Swan Creek (Lake Manitoba) stock.

			Walleye size ($\bar{X}$ ± 1 SD)	
Temperature (°C)	Date	n	Length (mm)	Dry weight (mg)
15	24 May	2	10.4 ± 0.30	0.53 ± 0.06
	3 June	1	12.7 ± 0.66	1.27 ± 0.29
	5 June	2	15.8 ± 0.73	3.16 ± 0.63
	10 June	1	15.4 ± 1.70	2.98 ± 0.98
20	23 May	2	10.9 ± 0.32	0.63 ± 0.08
	28 May	2	12.8 ± 0.39	1.26 ± 0.16
	3 June	2	16.2 ± 0.70	3.53 ± 0.65
25	21 May	2	10.5 ± 0.22	0.54 ± 0.05
	25 May	2	12.4 ± 0.49	1.14 ± 0.19
	30 May	2	14.8 ± 1.06	2.49 ± 0.77

thereafter. Following removal, these fish were immediately sacrificed in MS-222 and frozen in water at -18 °C. At a later date, fish were thawed and their gut contents were weighed using the following procedure. The length of each fish was measured with an ocular micrometer. Lengths were recorded as total length until a definite fork appeared in the tail and as fork length thereafter. For each fish, I dissected out the entire gut from the throat to the anal sphincter, placed the gut on a pre-weighed aluminum tray (~ 1.3 mg), dried the gut at 60 °C for 8 hr, transferred the tray to a desiccator for 1 hr, and finally weighed the gut to the nearest µg on a Perkin-Elmer AD-6 Autobalance.

I corrected these digestive tract dry weight estimates for freezing effects using data from the 1993 assimilation experiments (see **Assimilation efficiency**, below). Fish collected during the 1993 experiments were divided into two groups for each collection. The first group (control) were sacrificed in MS-222, and their complete digestive tracts were immediately removed, dried, and weighed as described above for the fish of the 1989 gut evacuation experiments. The second group (treatment) were sacrificed in MS-222, frozen in water for 2 d at -18 °C, then thawed at room temperature before their digestive tracts were removed, dried, and weighed. Gut weights from control and treatment fish were each ranked and then matched by rank for each collection. Scatter plots of control vs. treatment weight indicated that freezing caused weight reductions only in digestive tracts of < 225 µg frozen dry weight. I estimated the percent change in dry weight (PC) due to freezing for digestive tracts < 225 µg as PC = (control weight - treatment weight) / (treatment weight), then regressed PC against treatment (frozen) gut weight and collection time. The resultant relationship was $PC = 3.8851 - 0.6521 \cdot \log_e(\text{frozen weight}) - 0.0853 \cdot \log_e(\text{time} + 1)$ (ANOVA, $F = 70.5$, $df = 2, 88$, $P < 0.001$, $R^2 = 0.62$). I used this empirical relationship to estimate PC for each digestive tract of < 225 µg frozen dry weight from the 1989 gut evacuation experiments. Fresh dry weight was then calculated as frozen dry weight x (PC + 1). Fish length was corrected for freezing shrinkage using the formula of Appendix B. Following correction for freezing effects, I developed empirical relationships between $\log_e$(fresh dry weight) of empty digestive tracts and $\log_e$(walleye length) for each experimental date. These relationships were used to estimate empty digestive tract weights for individual fish. The dry weight of gut contents was then

estimated by subtraction of estimated digestive tract weight from the measured weight of digestive tract + contents.

To determine the instantaneous gut evacuation rate, R, in each experiment I analysed the relationship between gut fullness, F_t, and time since cessation of feeding, t. Gut fullness was estimated as

[5.1] $$F_t = 100 \times (W_{food} / W_{fish})$$

where W_{food} is the dry weight (mg) of food in the fish's gut, and W_{fish} is the dry weight (mg) of the walleye larva. Walleye dry weight was estimated from fresh length using a regression developed from 1988 feeding studies (Chapter 3). For the dependent variable, F_t, I used the 75^{th} percentile from the distribution of F_t for each time series sample in an experiment. This reduced the problem of increasing constriction by the X-axis with increasing time during the experiments (Olson and Mullen 1986). The instantaneous gut evacuation rate, R, was estimated in two ways. First, I calculated R_1 for each time interval in each experiment as

[5.2] $$R_1 = (\log_e(F_0) - \log_e(F_t)) / t$$

where F_0 and F_t are gut fullness at the beginning and end of an interval, respectively, and t is the duration of the interval (h). Second, I calculated R_2 as the slope of the regression of $\log_e(F_t)$ against t. I multiplied R_2 by -1 prior to using it in further analyses. The method of calculating R_2 assumes a constant exponential decline in gut content weight over the entire experimental period. The exponential model has been considered the most appropriate evacuation model for fishes consuming zooplankton prey (Jobling 1986).

I examined the effects of time since cessation of feeding, mean gut fullness, water temperature, and walleye dry weight on R_1 and R_2 using multiple regression analysis (GLM and STEPWISE procedures, SAS® Institute Inc. 1985). The independent variables used in these analyses (Table 5.2) were all treated as continuous variables, including temperature. Scatter plots of model residuals vs. each independent variable were examined prior to each variable's entry into the model to determine the nature of the relationship. The significance of an independent variable's effect on R_i was assessed by its partial (type III) sums of squares. First, I tested the hypothesis that R was dependent upon the time since cessation of feeding

Table 5.2. Descriptions of independent variables used in analysis of instantaneous gut evacuation rate. Dependent variables, R_1 and R_2, are defined in the text.

Variable	Description
TIME	time since cessation of feeding (h); measured from the initiation of an experiment to the midpoint of the interval over which R was calculated; included only in models using R_1
$\bar{F}$	mean gut fullness (%); estimated as the geometric mean of F_0 and F_t for models using R_1, and as $\hat{F}$ at the midpoint of the time interval for models using R_2
TEMP	water temperature (°C)
$\bar{W}$	walleye mean dry weight (mg)

by examining the relationship between R_1 and TIME (Table 5.2). Second, I tested the hypothesis that R was dependent upon gut fullness by examining the relationships between R_i and $\bar{F}$ (Table 5.2). Because gut fullness was related to time since cessation of feeding, these two tests were similar though not identical to each other. Third, I tested the hypotheses that R varied with respect to water temperature and walleye dry weight. All dependent and some independent variables were transformed as $\log_e(X_i + 1)$ prior to analysis.

Assimilation efficiency

Laboratory experiments to determine the assimilation efficiency of walleye larvae on a zooplankton diet were conducted at the Department of Fisheries and Oceans' Freshwater Institute in June 1993. Fertilized eggs from the Swan Creek (Lake Manitoba) stock (Fig. 2.2) were incubated at the Freshwater Institute and newly-hatched prolarvae were held in a flow-through incubation trough at 11 °C. Three separate batches of larvae were brought into the laboratory on 1 June (Batch 1), 7 June (Batch 2), and 16 June (Batch 3) and raised in 60-L glass stock aquaria covered in translucent green plastic sheeting. Water temperature was maintained at 20 ± 1 °C. The laboratory was lit by overhead fluorescent lights covered in translucent green plastic sheeting. Zooplankton were swept from local ponds and added to the stock aquaria once daily to maintain densities > $200 \cdot L^{-1}$.

Experiments were conducted on ten dates corresponding to walleye mean lengths of 10.1-18.8 mm and mean dry weights of 0.46-6.80 mg (Table 5.3). On each date, walleye larvae were allowed to feed in the stock aquarium for 2 h following the daily addition of fresh zooplankton. During this period, a food sample was collected from the stock aquarium, transferred to a glass microscope slide and placed in a drying oven. A group of ~ 100-200 larvae was then transferred to the experimental chamber, an aquarium identical to the stock aquarium but containing 30 L of water filtered through 72-µm Nitex® mesh. Faeces were siphoned from the bottom of the experimental chamber and collected on a 72-µm screen at 2 and 4 h (and occasionally 3 and 6 h) following transfer. On six dates, subsamples of fish (~ 20) were sacrificed in MS-222 at these same time intervals, as well as at the beginning of the experiments (t = 0 h), and their total gut contents were removed. These were also termed faecal samples but were analyzed separately from the faeces siphoned from the bottom of the experimental chamber. Faecal samples were dried on glass slides as for the zooplankton food

Table 5.3. Experimental dates, walleye batch used (Swan Creek stock, batch 1, 2, or 3, see text), fish size, time intervals following cessation of feeding when faeces were collected, and method of faeces collection (G = faeces collected from posterior section of gut, T = faeces siphoned from bottom of experimental chamber (tank)) for assimilation experiments conducted in 1993. Time interval for tank-collected faeces represents end of faecal accumulation period. All trials were conducted at 20 ± 1 °C.

		Walleye size ($\bar{X}$ ± 1 SD)			
Date	Batch	Length (mm)	Dry weight (mg)	Interval (h)	Method
7 June	1	10.1 ± 0.46	0.46 ± 0.09	2, 4, 6	T
8 June	1	10.3 ± 0.30	0.50 ± 0.06	2, 4	T
9 June	1	10.4 ± 0.41	0.52 ± 0.08	0, 2, 4	G, T
23 June	3	10.5 ± 0.50	0.54 ± 0.08	0, 3, 6	G
15 June	2	11.2 ± 0.53	0.73 ± 0.15	2, 4	T
10 June	1	11.4 ± 0.57	0.78 ± 0.17	0, 2, 4	G, T
12 June	1	12.4 ± 0.70	1.12 ± 0.26	0, 2, 4	G, T
14 June	1	13.6 ± 0.98	1.72 ± 0.53	0, 2, 4	G, T
19 June	2	14.0 ± 0.72	1.89 ± 0.38	2, 4	T
16 June	1	15.4 ± 1.03	2.88 ± 0.80	0, 3, 4	G, T
25 June	2	18.8 ± 1.15	6.80 ± 1.68	2, 4	T

samples. After drying at 60 °C for 8 h, food and faecal samples were scraped from the glass slides with a scalpel and stored in glass vials at -18 °C. On six of the eleven experimental dates, a random sample of 20 fish was also removed from the experimental chamber at 2 h intervals, sacrificed in MS-222, and used to estimate preservation effects on gut weights. The analysis of these fish samples is described in the previous section (see **Gut evacuation**, above). All fish remaining in the experimental chamber after 6 h were returned to the stock aquarium. On every experimental date, a minimum of 10 fish were anaesthetized and measured for fresh length. Dry weights were then estimated from fresh length using a regression developed from 1988 feeding studies (Chapter 3).

Dried food and faecal samples were analysed for total dry weight and ash-free dry weight. Samples were placed on pre-combusted, pre-weighed, aluminum pans (~ 1.3 mg), dried for an additional 4 h at 60 °C, moved to a desiccator for 1 h then weighed to the nearest μg on a Perkin-Elmer AD-6 Autobalance to determine total dry weight. The pans with samples were placed in a muffle furnace for 16 h at 500 °C, moved to a desiccator for 1 h, then reweighed to determine ash weights. All weighings were duplicated to improve precision.

Assimilation efficiency was estimated from the qualitative food and faecal samples using the apparent digestibility equation of Talbot (1985). Apparent digestibility (AD) of a nutrient in the food was estimated as

$$AD = 1 - [(F_M / E_M) \times (E_N / F_N)] \quad [5.3]$$

where F_M and F_N are the dry weight fractions of the food composed of reference marker and nutrient, respectively, and E_M and E_N are the dry weight fractions of the faeces composed of reference marker and nutrient, respectively. I estimated AD using total organic matter, measured as ash-free dry weight, as the nutrient fraction. Initially, I intended to use chitin as the internal reference marker substance. However, difficulties in assaying chitin (Appendix E) led me to select another internal marker. I chose to use dietary ash following the approach of Conover (1966). This method simplifies equation [5.3] in that $F_N = 1 - F_M$ and $E_N = 1 - E_M$. However, some ash may be assimilated by the fish (Austreng 1978; Tacon and Rodrigues 1984) and some ash may leach from the faeces following egestion into the water (Brown et al. 1989). These losses would result in an underestimation of E_M, and consequently, an

underestimation of AD (equation [5.3]). I took the following approach in correcting E_M for these combined losses. Because the proportion of ash lost is probably dependent upon the proportion of ash in the diet, F_M, I first adjusted for this covariate. I regressed the proportion of ash in the faeces, E_M, against F_M. There was a significant, positive, linear relationship between E_M and F_M (ANOVA, $F = 54.7$, $df = 1,33$, $P < 0.001$). I used the slope of this relationship to adjust all E_M estimates to a common F_M value of 0.12 (Steel and Torrie 1980). For faeces collected from the bottom of the experimental chamber, I assumed that 20% of egested ash was lost prior to collection and I corrected for this by dividing all adjusted E_M values by 0.80. For faeces collected from both the gut and the experimental chamber, I assumed that 0% and 20% of dietary ash would be assimilated by the larvae at $t = 0$ and $t \geq 4$ h, respectively. I corrected for this by dividing all adjusted E_M values by $1 - 0.05 \cdot t$ for $t < 4$, and by 0.80 for $t \geq 4$ h. Finally, I estimated AD using the adjusted and corrected estimates of E_M and a fixed estimate of 0.12 and 0.88 for F_M and F_N, respectively.

I examined the effects of walleye mean dry weight and faecal collection time on AD using multiple regression analysis (GLM procedure, SAS® Institute Inc. 1985). I arcsine-transformed AD to stabilize residual variance. Collection time was the time from cessation of feeding (transfer to the experimental chamber) until the faeces were collected. For AD estimates based on faeces collected from the bottom of the experimental chamber, collection time was represented by the middle of the time interval (generally 2 h) over which the faeces accumulated. Because of the difference in the interpretation of the time variable between gut- and tank-collected faeces, I analyzed AD estimates based on these two collection methods separately. The significance of an independent variable in the regression model was assessed by its partial (Type III) sums of squares.

Results

Gut evacuation

Patterns in the gut evacuation of walleye larvae are illustrated in Fig. 5.1 (15 °C), Fig. 5.2 (20 °C), and Fig. 5.3 (25 °C). The overall patterns were quite variable between experiments. A decline in gut content weight over the first 2-3 h of food deprivation was fairly consistent among experiments. In several cases, a period of stability was observed during the middle of the evacuation trial (~ 3-5 h), and this was sometimes followed by another decline in gut

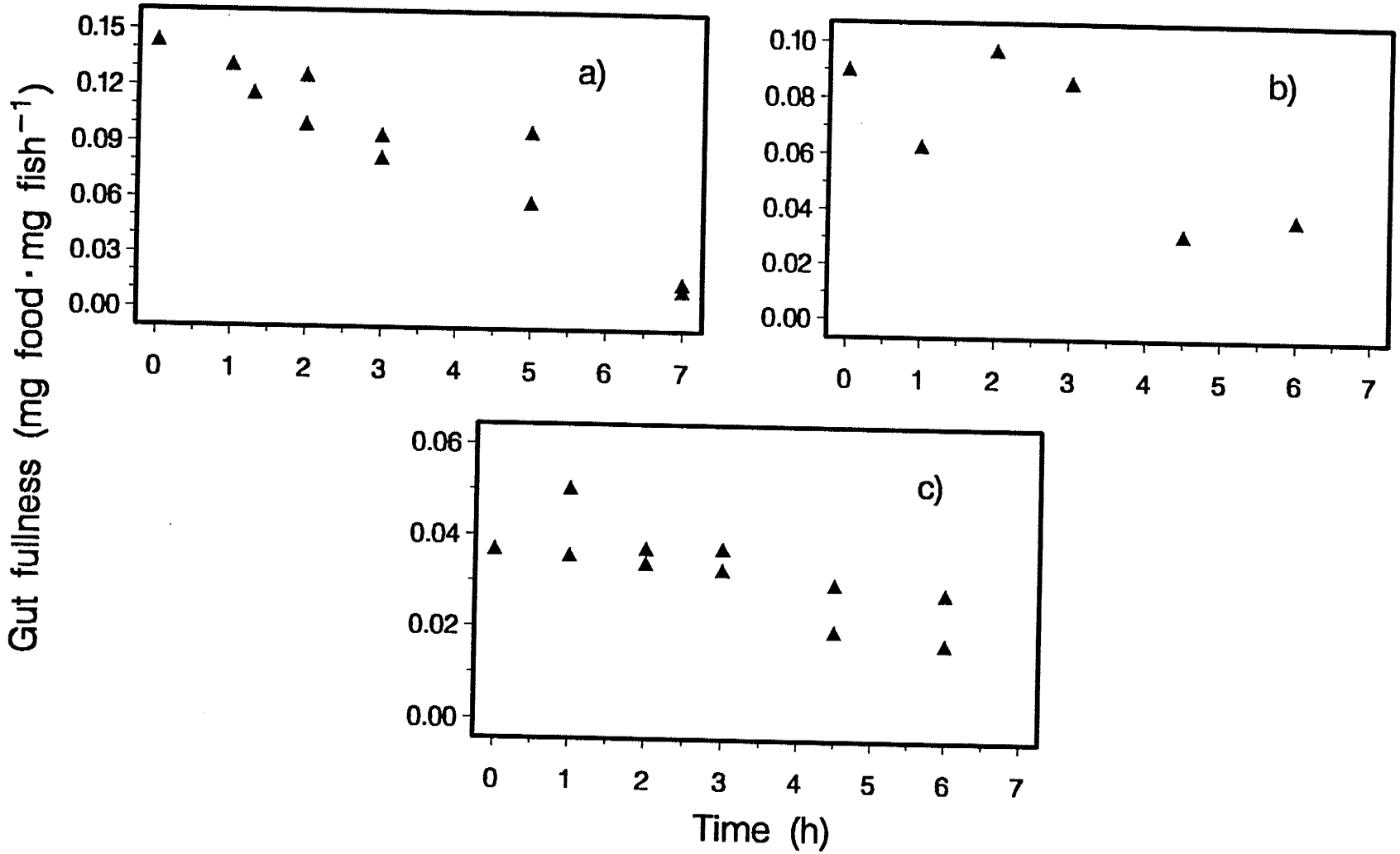

Figure 5.1. Scatter plots of gut fullness vs. time since cessation of feeding for walleye larvae consuming a zooplankton diet in the laboratory at 15 °C. Walleye mean dry weights were a) 0.53 mg, b) 1.27 mg, and c) 3.16 mg. All larvae were of the Duck Bay (Lake Winnipegosis) stock (Table 5.1). Symbols represent 75th percentiles. Note differences in scale of axes between plots.

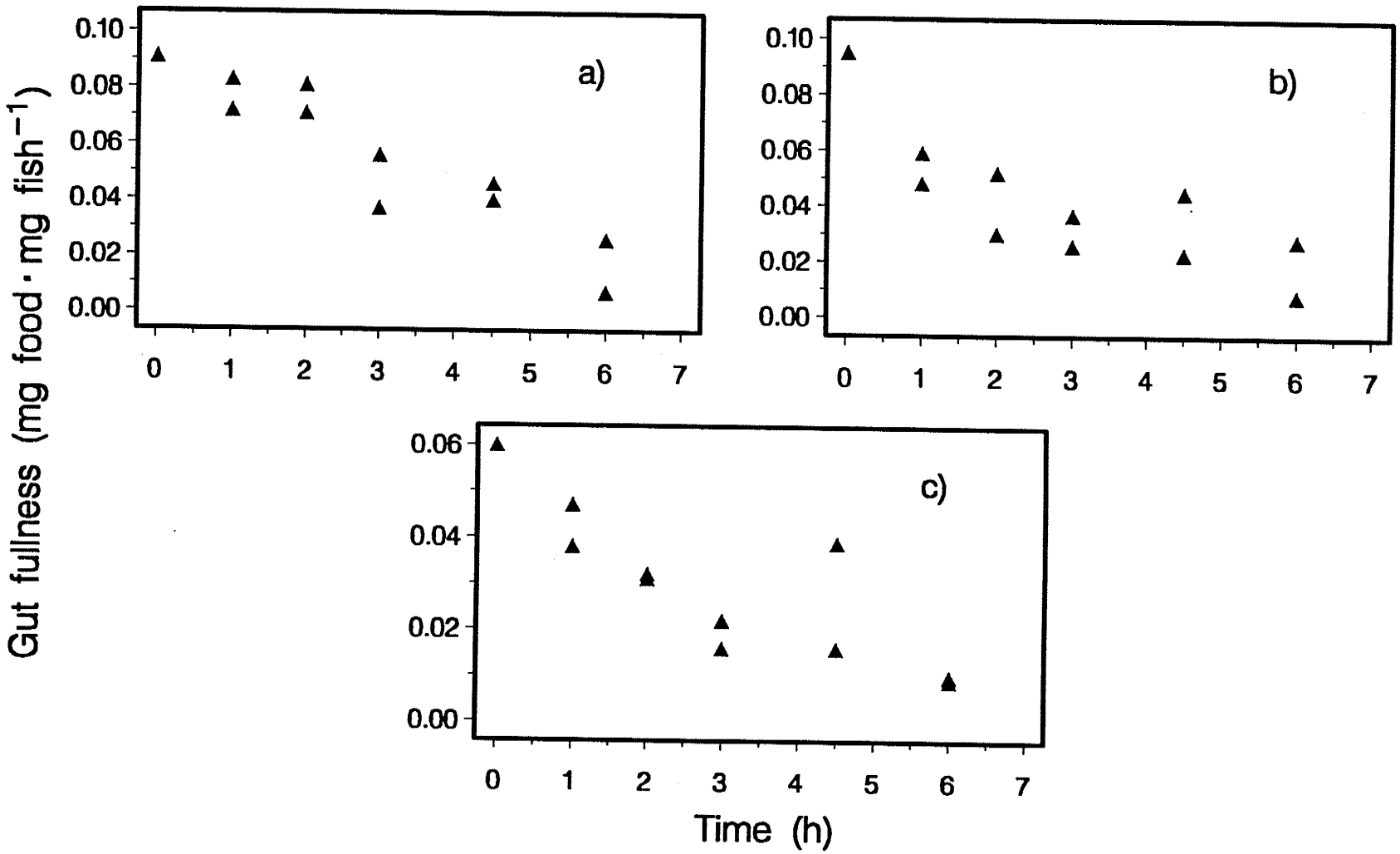

Figure 5.2. Scatter plots of gut fullness vs. time since cessation of feeding for walleye larvae consuming a zooplankton diet in the laboratory at 20 °C. Walleye mean dry weights were a) 0.63 mg, b) 1.26 mg, and c) 3.53 mg. All larvae were of the Duck Bay (Lake Winnipegosis) stock (Table 5.1). Symbols represent 75th percentiles. Note differences in scale of axes between plots.

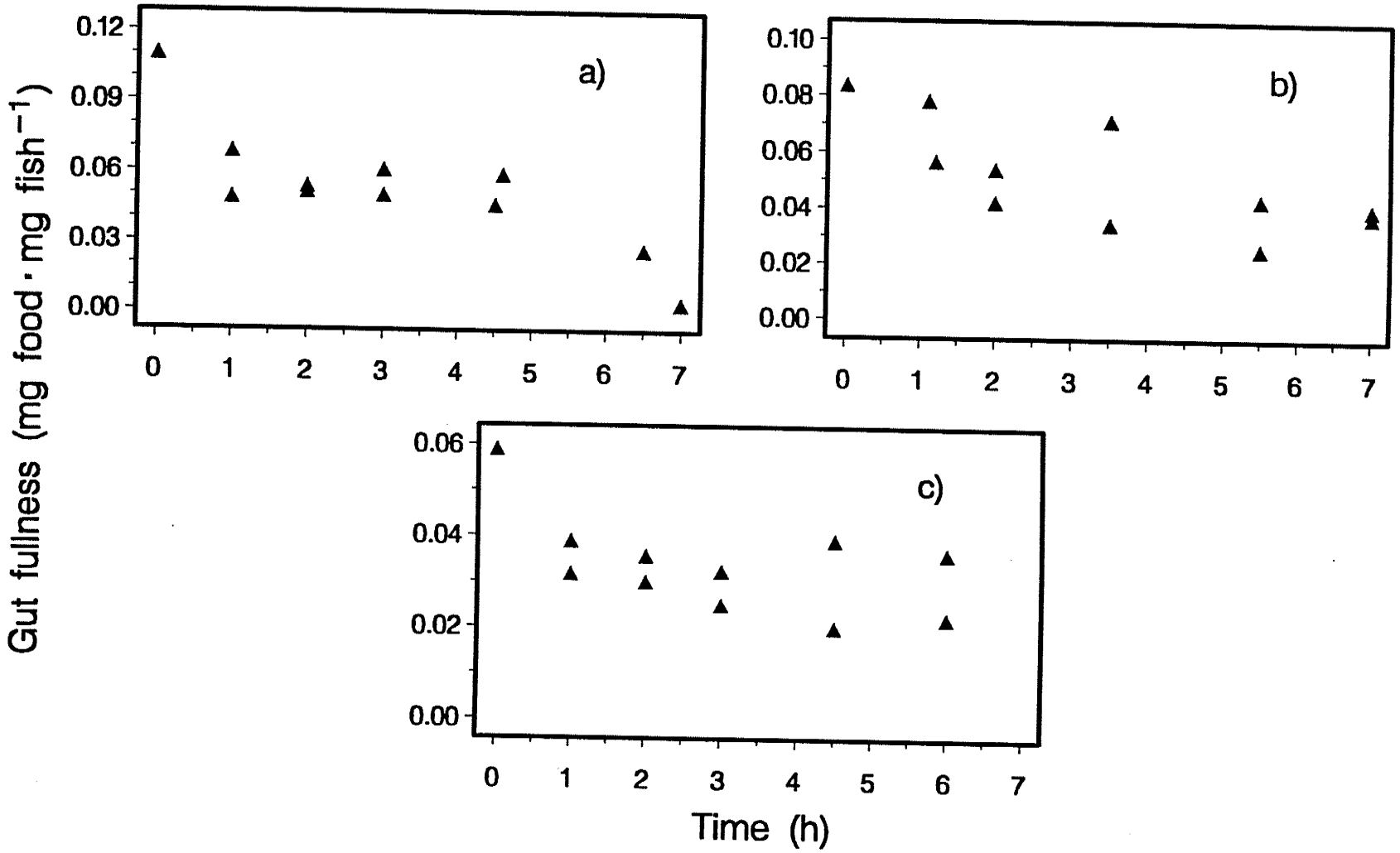

Figure 5.3. Scatter plots of gut fullness vs. time since cessation of feeding for walleye larvae consuming a zooplankton diet in the laboratory at 25 °C. Walleye mean dry weights were a) 0.54 mg, b) 1.14 mg, and c) 2.49 mg. All larvae were of the Duck Bay (Lake Winnipegosis) stock (Table 5.1). Symbols represent 75th percentiles. Note differences in scale of axes between plots.

content weight after 5 h thus creating a sigmoidal evacuation pattern (e.g. Fig. 5.2b,c, 5.3a). In all trials, some larvae contained visible food in the posterior section of the intestine even 6 h after the cessation of feeding. The pattern of decline in gut fullness did not appear to be related to water temperature or fish size.

Gut evacuation rate as estimated over short time intervals, R_1, varied with respect to time since cessation of feeding. When R_1 was adjusted for the effects of water temperature, walleye dry weight, and gut fullness there was a significant negative relationship between R_1 and TIME (Table 5.4). When TEMP was included in the model, the relationship between R_1 and TIME was best defined by a quadratic. Evacuation rate was faster at the beginning and end of the evacuation trials than during the middle. This agreed with the sigmoidal pattern observed in some evacuation trials. Regression analysis also indicated that R_1 declined significantly with increasing mean gut fullness and walleye dry weight (Table 5.4). The relationship between R_1 and temperature was also quadratic with a maximum in the mid-range of temperatures examined. However, the significance of the temperature effect was not as strong as for the other independent variables ($0.05 < P < 0.10$, Table 5.4). Eliminating temperature from this analysis changed the relationship between R_1 and TIME, but had little effect on the relationships between R_1 and $\bar{W}$ and $\bar{F}$. With TEMP and $TEMP^2$ eliminated from the model, there was a significant negative relationship between $\log_e(R_1 + 1)$ and $\log_e(TIME + 1)$ (ANOVA, $F = 4.05$, $df = 1, 94$, $P = 0.047$).

Because evacuation rate varied with respect to time in the evacuation trials, I chose to develop a predictive relationship for gut evacuation rate using a restricted data set. I calculated R_2 using data from only the first 2 h of each gut evacuation run as this interval showed the most consistent pattern between trials. First, I examined the relationship between R_2 and gut fullness, temperature, and walleye dry weight. Gut evacuation rate increased exponentially with water temperature (ANOVA, $F = 6.15$, $df = 1, 17$, $P = 0.024$) but showed no significant relationship with either gut fullness or walleye mean weight ($P > 0.50$). This yielded the predictive model $R = (0.421 \cdot (TEMP + 1)^{0.338}) - 1$ ($R^2 = 0.27$). Second, I examined the relationship between R_2 and all the simple variables along with their crossproducts (3 two-way, 1 three-way). All variables and cross-products were included in the original model and sequentially dropped based on the lowest contribution to explained variance in R_2

Table 5.4. Results of multiple regression analysis using instantaneous gut evacuation rate, R_1 (defined in formula [5.1] in text), as the dependent variable and time since cessation of feeding (TIME), mean gut fullness ($\bar{F}$), water temperature (TEMP), and mean walleye dry weight ($\bar{W}$) as independent variables (X_i). R_1, $\bar{F}$, and $\bar{W}$ were transformed by $\log_e(X_i + 1)$ prior to analysis. Independent variables are defined in Table 5.2. Parameter estimates are indicated by b_i. Tests of significance were based on partial (Type III) sums of squares.

X_i	b_i	F	df	P
TIME	-0.17	6.26	1, 91	0.014
$TIME^2$	0.021	4.44	1, 91	0.038
$\bar{F}$	-0.25	5.20	1, 91	0.025
TEMP	0.17	3.29	1, 91	0.073
$TEMP^2$	-0.0044	3.46	1, 91	0.066
$\bar{W}$	-0.27	6.58	1, 91	0.012

Table 5.5. Results of multiple regression analysis using instantaneous gut evacuation rate, R_2 (defined in text), as the dependent variable and mean gut fullness ($\bar{F}$), water temperature (TEMP), mean walleye dry weight ($\bar{W}$), and all of their crossproducts as independent variables (X_i). R_2, $\bar{F}$, and $\bar{W}$ were transformed by $\log_e(X_i + 1)$ prior to analysis. Independent variables are defined in Table 5.2. Parameter estimates are indicated by b_i. Tests of significance were based on partial (Type III) sums of squares.

X_i	b_i	F	df	P
$\bar{W}$	0.42	5.41	1, 15	0.034
$\bar{W} \times \bar{F}$	-0.99	12.3	1, 15	0.032
$\bar{W} \times \bar{F} \times$ TEMP	0.23	8.24	1, 15	0.012

(BACKWARD option, STEPWISE procedure, SAS® Institute Inc. 1985). The final model included one simple variable, $\bar{W}$, and two crossproducts, and explained 46% of the variation in R_2 (Table 5.5). The significance of the cross-product terms indicated that the effects of each of the independent variables on R_2 varied with respect to the levels of the others.

Assimilation efficiency

The relationship between AD and faecal collection time is illustrated in Fig. 5.4. Estimates of AD based on faecal samples collected from walleye guts were generally lower and more variable than those based on faecal samples siphoned from the bottom of the experimental chamber. Visual inspection of gut-collected faeces at the time of collection indicated that they contained a larger quantity of liquid organic matter, primarily oil droplets, than the tank-collected faeces. This liquid organic matter appeared to be more abundant in gut faeces collected at the initiation of the experiment (t = 0) than in gut faeces collected ≥ 4 h after the cessation of feeding. It was not possible to determine if this material was eventually assimilated by the larvae, egested as liquid waste, or egested within the solid waste but subsequently lost through leaching.

Regression analyses indicated that AD based on both gut- and tank-collected faeces increased significantly with time of collection but was not significantly affected by walleye dry weight (Table 5.6). However, walleye dry weight had a near-significant (P = 0.062), negative effect on AD based on gut-collected faeces. To ensure that the significant time effect was not caused by my use of a time-dependent correction factor for ash assimilation, I reconducted this analysis under the assumption that ash assimilation was 20% at all faecal collection times. The outcome of this reanalysis was similar to the initial analysis; AD increased significantly with time for estimates based on both gut- and tank-collected faeces. Mean estimates of AD adjusted for walleye mean weight are summarized in Table 5.7. Mean apparent digestibility ranged from 0.35 for gut samples at t = 0 h to 0.93 for tank samples at t = 5 h. The results suggested that further assimilation may occur beyond t = 4 h. However, data beyond t = 4 h were based on single experiments for both gut and tank samples.

I developed a relationship between AD and R using the following procedure. Time appeared to be the major factor affecting AD based on the gut- and tank-collected faeces. Therefore, I calculated simple linear relationships between AD and time for both sets of data.

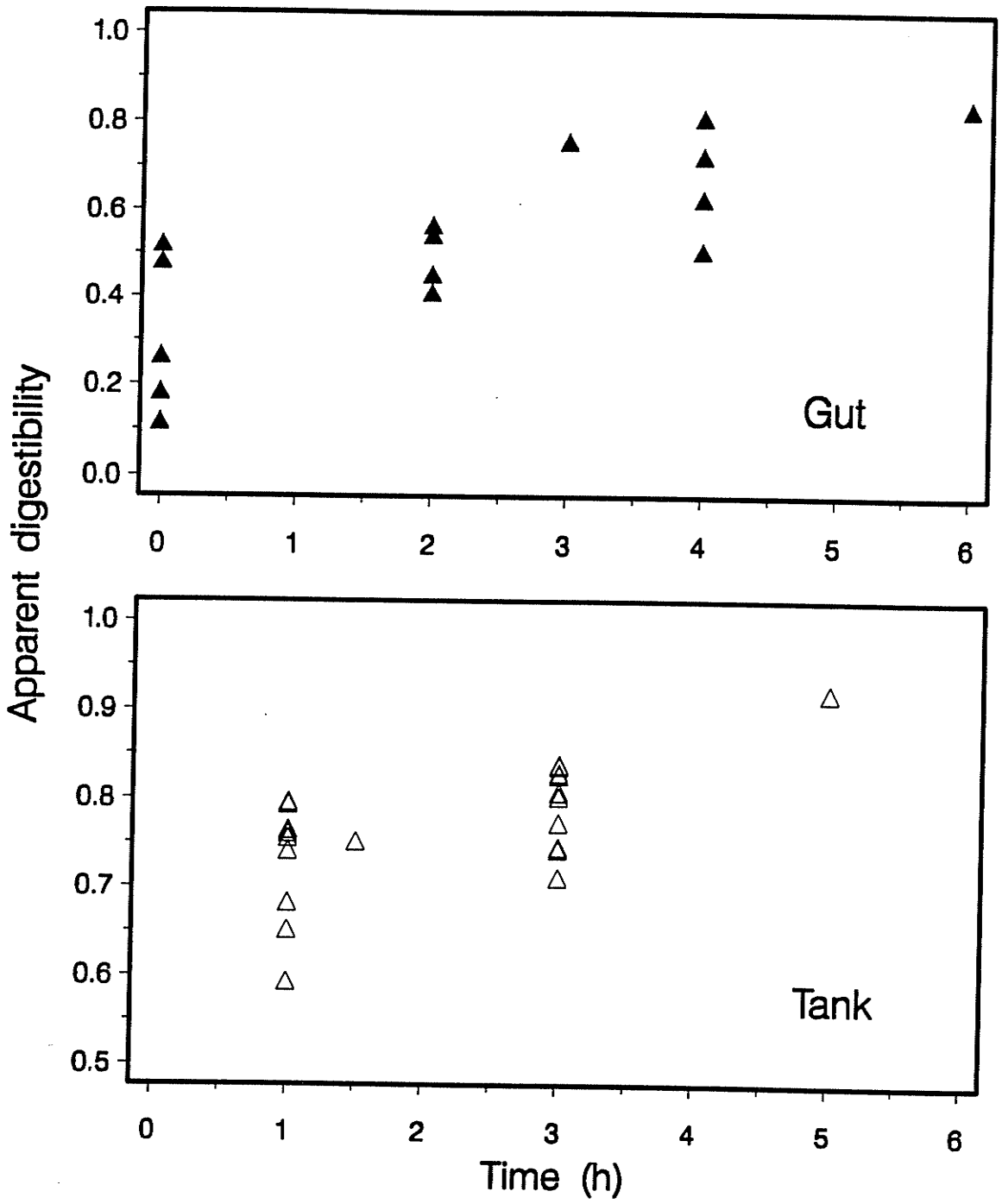

Figure 5.4. Apparent digestibility (AD, fraction of organic matter assimilated from food), vs. time since cessation of feeding for walleye larvae consuming crustacean zooplankton in the laboratory at 20 °C. Apparent digestibility was calculated using equation [5.3] and was based on faeces collected from walleye guts (top) and egested faeces collected from the bottom of the experimental chamber (bottom). Time represents the time from the cessation of feeding until the middle of the interval during which faeces accumulated for AD estimates based on tank-collected faeces. Note difference in scale of AD axis between plots.

Table 5.6. Results of multiple regression analyses examining the relationship between apparent digestibility (AD) of crustacean zooplankton by walleye larvae, and time since cessation of feeding (TIME, h), and walleye mean weight ($\bar{W}$, mg). Apparent digestibility estimates were arcsine-transformed and mean dry weights were transformed as $\log_e(\bar{W} + 1)$ prior to analysis. Regression coefficients are represented by b_i. Analyses were conducted separately for AD estimates based on faeces collected from the walleye guts and AD estimates based on egested faeces collected from the bottom of the experimental chamber (tank). Tests of significance were based on the partial (Type III) sums of squares.

Collection	X_i	b_i	F	df	P
Gut	TIME	0.095	24.4	1, 13	< 0.001
	$\bar{W}$	-0.28	4.19	1, 13	0.062
Tank	TIME	0.066	15.8	1, 18	< 0.001
	$\bar{W}$	0.029	0.55	1, 18	0.47

Table 5.7. Apparent digestibilities, AD ($\bar{X}$ ± 1 SD, where available), for walleye larvae consuming crustacean zooplankton in the laboratory at 20 °C. Apparent digestibility was calculated using equation [5.3] and was based on faeces collected from walleye guts and egested faeces collected from the bottom of the experimental chamber (tank). Individual AD estimates were adjusted for mean walleye dry weight prior to calculating means. Time represents the time from cessation of feeding until faeces were collected for gut estimates, and the time from cessation of feeding until the middle of the interval during which faeces accumulated for tank estimates. Sample sizes (n) represent the number of experiments conducted.

Collection	Time (h)	n	AD
Gut	0	6	0.35 ± 0.12
	2	4	0.48 ± 0.14
	3	1	0.64
	4	4	0.65 ± 0.13
	6	1	0.71
Tank	1	9	0.73 ± 0.06
	1.5	1	0.74
	3	10	0.79 ± 0.05
	5	1	0.93

I assumed that walleye could not assimilate further from food which had remained in their guts for ≥ 6 h, and estimated AD from the relationships for t = 6 h. These estimates were 0.78 and 0.92 for gut- and tank-collected faecal samples, respectively. Because organic matter may be lost from faeces egested into water (see **Discussion** below) I assumed that the former was more accurate. Thus, I assumed that the maximum AD attainable by walleye larvae feeding on zooplankton was 0.78 and that this required a gut retention time of ≥ 6 h. For shorter durations, I assumed a linear relationship between AD = 0.00 at t= 0 h and AD = 0.78 at t = 6 h. This can be defined as AD = 0.13·t (for t < 6 h). For a continuously feeding fish (assuming weight of gut contents constant) the residence time for food in the gut can be expressed as the inverse of the gut evacuation rate (i.e. t = 1 / R) and thus the relationship between AD and R can be expressed as AD = 0.13 / R, for R > 0.167, and AD = 0.78, for R ≤ 0.167.

Discussion

Comparisons between the results of gut evacuation studies are often difficult because of different methodologies. Gut contents may be measured from the stomach or the entire digestive tract, as corrected or uncorrected for preservation effects (if necessary), as volume, wet weight or dry weight, and as relative (to initial meal size or fish size) or absolute units. Furthermore, evacuation trials may be conducted in the laboratory or the field, in light or in darkness. The choice of experimental conditions and procedures may influence the interpretation of results. For example, Hopkins and Larson (1990) found that the shape of the evacuation curve for rockfish, *Sebastes chrysomelas*, varied from linear to strongly concave depending on whether volume, wet weight, or dry weight was used to represent gut contents. In this study, I examined the dry weight (corrected for freezing effects and standardized for body weight) of entire digestive tracts of walleye larvae maintained under lighted conditions in the laboratory. These factors should be considered when making comparisons with previous research.

In his review of gastric evacuation models, Jobling (1986) concluded that the exponential model would best describe gut evacuation for fish feeding on small, easily digested prey such as zooplankton. In this study, I found that the decline in gut fullness of walleye larvae resembled an exponential decline for only the early stages of food deprivation. Statistical

analyses confirmed that the evacuation rate varied with respect to time since cessation of feeding. A slower evacuation rate when fish are not feeding may allow the remaining food to be more effectively assimilated and would therefore seem to be a useful adaptation. Few studies have noted similar trends or interpreted their results in this manner. Hayward and Bushmann (1994) observed the fastest stomach evacuation rates during the first 2.5 h of 10-h food deprivation trials for juvenile largemouth bass, *Micropterus salmoides*. They concluded that the exponential model was inappropriate and fit their data to a reciprocal model. Scatter plots of larval walleye gut evacuation presented by Madon and Culver (1993)(their Fig.1, p. 802) suggested slower evacuation rates during the middle of their food deprivation trials. However, these researchers did not examine this time effect and fit the exponential model to their full time series of data. Variation in evacuation rate with time since cessation of feeding may have been more pronounced in this study because of my methodology. Previous research has primarily focussed on evacuation of the stomach. Relatively few studies have examined evacuation of the entire digestive tract (e.g. Boisclair and Marchand 1993; Madon and Culver 1993). The passage of matter from the stomach is primarily through evacuation to the intestine, and there is relatively little absorption other than water. By contrast, matter in the intestine may be assimilated or evacuated as waste. Thus, if the decline in evacuation rate during food deprivation is a strategy to improve assimilation efficiency, then it should be more evident when evacuation rate is examined for the whole gut rather than for just the stomach.

Gut evacuation rate is influenced by a variety of factors, of which temperature, fish size, prey type, and meal size are believed to be the most important (reviewed by Bromely 1994). Numerous studies have demonstrated that evacuation rate in fish increases with water temperature (e.g. Laurence 1971; Persson 1979, 1982; Karjalainen et al. 1991). Gut evacuation rate has been observed to vary with respect to fish size (Flowerdew and Grove 1979; Madon and Culver 1993) but the nature of the variation depends on whether evacuation is measured as an absolute or relative rate (Bromely 1994). In an interspecific analysis of 22 fish species, He and Wurtsbaugh (1993) found that evacuation rate was significantly affected by temperature and prey size, but not predator size. Smaller prey tend to be evacuated at a faster rate than larger prey (Swenson and Smith 1973; Karjalainen et al. 1991). Certain prey

species may also be evacuated more quickly than others (MacDonald et al. 1982). At present, the effect of meal size on evacuation rate is not clear and may depend on whether an absolute or relative rate is examined (Bromely 1994). Some of these factors influencing evacuation rate may be interrelated. For example, food consumption, and hence meal size, may vary with temperature and fish size (Chapter 4) as well as prey size (Chapter 3). Similarly, the size and type of prey consumed may change with fish size (Chapter 3).

In this study, I found significant effects of temperature, gut fullness, and fish size on the gut evacuation rate, R, of walleye larvae. However, the significance of the effects of these variables depended on how R was calculated. Analyses using R_1 demonstrated that gut fullness and fish size had significant, negative effects on evacuation rate. Analyses using R_2 indicated that temperature, gut fullness, and walleye dry weight had significant interacting effects on evacuation rate. In this latter analysis, temperature had a positive effect, and walleye weight and gut fullness had negative effects. Both the positive effect of temperature and the negative effect of fish size are in agreement with a previous study on walleye larvae (Madon and Culver 1993). To my knowledge, the negative effect of mean gut fullness has not been reported previously. A slower evacuation rate at higher gut fullness may be another mechanism to facilitate assimilation. For a given length of gut, the proportion of food in contact with the gut surface at any one time declines with gut fullness. Thus, reducing evacuation rate may allow more complete assimilation. The significant effect of cross-product terms in my analysis suggests that the influence of various factors on gut evacuation rate may be more complex than has been previously suggested. The nature or magnitude of the effects of gut fullness or temperature on gut evacuation may vary with fish weight.

The accuracy of my AD estimates is dependent upon my assumptions about the fate of dietary ash ingested by walleye. For rainbow trout, *Oncorhynchus mykiss*, assimilation of dietary ash may range from 8.7-16.8% for a high ash diet (14-17%)(Tacon and Rodrigues 1984) to as high as 37-40% for a low ash diet (6.9%)(Austreng 1978). Leaching may reduce the ash content of fish faeces by up to 21% after immersion in water for 1 h (Brown et al. 1989). Combined losses of ash from assimilation and leaching may be as high as 40-60% (Kelso 1972; Buddington 1980; Lambert 1985). Based on these previous studies, I feel my assumptions that walleye larvae assimilated 20% of the ash from their diet (standardized to

12% ash content), and that 20% of defecated ash was lost to leaching were reasonable. My AD estimates for tank-collected faeces may be biased high because of leaching of organic matter. Several studies have demonstrated a loss of organic matter, particularly nitrogenous wastes, from fish faeces egested into water (Windell et al. 1978; Smith et al. 1980). This leads to an underestimation of E_N (equation [5.3]) and an overestimation of AD. Windell et al. (1978) observed that AD estimates based on faeces collected from the water 1 h after egestion were 0.115 units greater than AD estimates based on faeces removed from the posterior intestine. Similarly, I estimated AD at t = 6 h to be 0.14 units greater when based on tank-collected faeces than when based on gut-collected faeces. Thus, my AD estimates calculated from gut collections may be more accurate than those calculated from tank collections.

Reported estimates of assimilation efficiency for larval fish feeding on zooplankton range from 0.44 to 0.99 (reviewed by Govoni et al. 1986). This variablity can be attributed to differences in fish species, prey species, experimental conditions, and methodologies for estimating assimilation efficiency. Using a similar procedure to that used in this study, Mills and Forney (1981) estimated an AD of 0.68 for young yellow perch consuming *Daphnia pulex*. The fish in their experiments were feeding continuously and prey were passing through the yellow perch guts in ~ 0.9-1.8 h. Their AD estimate may be biased low because ash losses due to assimilation and leaching were not accounted for. Using a quantitative methodology, Wissing (1974) estimated that juvenile (44-77 mm SL) white bass, *Morone chrysops*, assimilated 66-69% of the energy, and 61% of the dry matter from their zooplankton diet. Apart from these studies, I could not find previously published estimates of assimilation efficiency in zooplanktivorous freshwater YOY fish.

The results of this study indicate that the apparent digestibility of zooplankton by walleye is positively related to the length of time that the zooplankton is retained in the gut. Boehlert and Yoklavich (1984) found that assimilation efficiency in herring larvae declined with increasing ingestion, presumably because of a declining gut retention time. Elliott (1976) also observed a declining assimilation efficiency with increasing consumption in brown trout, *Salmo trutta*, consuming *Gammarus pulex*. However, other studies have found no effect of consumption (reviewed by Talbot 1985). I observed a near significant negative effect of walleye size on assimilation efficiency in one of my analyses. Previous studies have found

no clear relationship between assimilation efficiency and the age or size of zooplanktivorous fish larvae (Govoni et al. 1982; Conway et al. 1993). Because enzyme activity in walleye larvae increases with age (Mitchell et al. 1986), I had expected that assimilation efficiency would only improve, not decline, with increasing age (reviewed by Kamler 1992). Thus, the fairly strong negative effect of fish size on AD observed in this study is somewhat puzzling. However, Kelso (1972) also observed a declining assimilation efficiency with increasing fish size for sub-adult and adult walleye. I did not examine the effect of temperature on assimilation efficiency. Elliott (1976) noted an increase in assimilation efficiency with increasing temperature (range 4 - 20 °C) in brown trout. However, Rösch (1987) found no effect of temperature (range 8 - 20 °C) on the assimilation efficiency of juvenile vendace, *Coregonus lavaretus*, consuming *Daphnia sp.* I suspect that the major effect of temperature on assimilation is indirect through its influence on gut evacuation rate. I also did not examine the effect of prey type on assimilation efficiency. Conway et al. (1993) noted large variations in the assimilation efficiency of turbot, *Scophthalmus maximus*, larvae with respect to the type of planktonic prey consumed. Generally, they found that large copepods were the most efficiently digested prey. Sub-adult and adult walleye exhibit higher assimilation efficiencies on fish diets than on invertebrate diets (Kelso 1972). Walleye larvae may also be able to utilize certain prey types more efficiently than others but this remains to be tested.

The value of the assimilated matter to the walleye larvae depends on its composition. It would be interesting to know if walleye larvae selectively assimilate higher proportions of certain dietary components than others, or if certain dietary components are not assimilated at all. Walleye larvae can apparently assimilate free amino acids much more easily than amino acids forming parts of protein molecules (Rust et al. 1993). Many of the digestive enzymes found in the guts of adult fishes have also been found in the guts of larval fishes (Govoni et al. 1986). One exception may be lipase, though relatively few studies have assayed for its presence. Active lipolytic enzymes have been found in the guts of larval turbot (Cousin et al. 1987) but not in the guts of 18-d-old northern pike, *Esox lucius*, larvae (Szlaminska 1980). Lipase activity appeared much later in turbot development than other digestive enzymes and was not detected in food-deprived larvae (Cousin et al. 1987). Digestion studies on other species have suggested that fish larvae may be less efficient at

digesting and assimilating lipid than protein (Rösch 1987; Conway et al. 1993). Rösch (1987) noted that protein assimilation was consistently higher than lipid assimilation in zooplanktivorous vendace. He also noted that protein assimilation remained constant whereas lipid assimilation declined with increasing ration. Thus, protein appears to have been a more important dietary constituent to the young vendace. Conway et al. (1993) noted that some copepods egested by turbot larvae still contained oil globules. I also noted oil globules in the copepods digested by walleye larvae, though they were less evident in egested copepods than in those still in the fish guts. Some digestive enzymes, but not lipase, have been assayed in walleye larvae (Mitchell et al. 1986). However, the presence of an enzyme does not necessarily mean that its substrate is easily assimilated. Fish larvae may possess chitinase but are probably unable to assimilate chitin (Lindsay 1984, Appendix E) and thus, chitin probably forms a large percentage of the undigested organic matter in the faeces of zooplanktivorous fish. Curiously, the presence of chitin in the diet may enhance fish growth (Kono et al. 1987).

The total assimilation of matter by fish is a function of ingestion rate and assimilation efficiency. Generally, assimilation efficiency declines with ingestion rate but the ingestion more than compensates for this decline such that total assimilation increases steadily with increasing ingestion (Boehlert and Yoklavich 1984; Rösch 1987). I attempted to define how assimilation efficiency changes in relation to ingestion by developing a relationship between AD and R. However, this relationship is rather simplistic. For a given gut evacuation rate, R, it would be expected that AD would decline with gut fullness simply because at high gut fullness more food passes through the gut in a given unit of time and a smaller proportion of food is in contact with the gut surface at any one time. Thus, ideally, assimilation efficiency should be modelled as a function of ingestion rate rather than gut evacuation rate. However, the relationship between AD and R developed in this study will account for some of the variation in assimilation efficiency. Few previous studies have examined variation in assimilation efficiency and it is often incorporated into bioenergetics models as a constant (e.g. Post 1990; Madon and Culver 1993).

Chapter 6:
Maintenance food requirements and resistance to food deprivation of walleye larvae

Introduction

Starvation mortality resulting from scarce prey has been implicated as a cause of low and variable survival of walleye larvae in laboratory rearing studies (Li and Mathias 1982) and in some studies of extensive culture ponds (Li and Ayles 1981) but not others (Chapter 2). However, the susceptibility of walleye larvae to starvation under conditions of food deprivation has not been examined. Several studies have now examined the feeding ecology of walleye larvae and their developmental and functional responses are relatively well-known (Mathias and Li 1982; Chapters 3, 4). However, assessing the probability of starvation requires estimates of both food intake and maintenance food requirements and no study, to my knowledge, has estimated the latter for walleye larvae.

The objectives of this research were twofold. First, I wished to establish the maintenance food requirements of walleye larvae by examining walleye growth at various rations. Second, I wished to examine the weight loss, change in energy content, and mortality of walleye larvae which accompanied short periods of food deprivation. Because rates of vital processes in fish larvae are strongly size-dependent (Miller et al. 1988) and temperature-dependent (Pepin 1991), I conducted these experiments over a range of walleye body sizes and water temperatures. I predicted that weight loss and mortality of food-deprived larvae would be most intense among the smallest larvae examined and would decline with increasing walleye size. Furthermore, I predicted that if starvation was the primary cause of mortality, that mortality rate would be positively related to the rate of weight loss observed in the larvae. The implications of my results in terms of walleye survival in natural environments are discussed.

Materials and Methods

Experiments were conducted in the Department of Zoology, University of Manitoba during May and June 1990. Experimental conditions were similar to those described in Chapter 4. Hatchery-raised walleye larvae from two stocks (Table 6.1) were brought into the laboratory within 2 d after hatching, raised in 120-L glass stock aquaria at 18.5 °C, and fed ad libitum on

Table 6.1. Starting dates, walleye stock used (Swan Creek = Lake Manitoba stock, Grand Rapids = Lake Winnipeg stock), number of fish per aquarium (n = subsample size), duration of trial, initial fish sizes ($\bar{L}_0$ = mean initial length, $\bar{W}_0$ = mean initial dry weight), and ration treatments (S = no food addition, L = low ration, H = high ration) for experimental trials conducted in this study. All trials were conducted at 15, 18.5, and 22 °C.

				Initial Size		
Date	Stock	n	Duration (h)	$\bar{L}_0$ (mm)	$\bar{W}_0$ (mg)	Treatments
30 May	Swan Creek	10	48	10.01 ± 0.04	0.74 ± 0.01	S, L, H
2 June	Swan Creek	8	47	10.69 ± 0.07	0.82 ± 0.03	S, L, H
7 June	Swan Creek	1	38	11.65 ± 0.20	1.34 ± 0.08	S
10 June	Grand Rapids	4	39	9.71 ± 0.07	0.60 ± 0.01	S
12 June	Swan Creek	1	64	15.11 ± 0.27	4.14 ± 0.30	S
17 June	Grand Rapids	1	40	11.87 ± 0.18	1.49 ± 0.09	S
19 June	Grand Rapids	1	40	13.79 ± 0.13	2.72 ± 0.10	S
22 June	Grand Rapids	1	40	15.51 ± 0.16	4.46 ± 0.20	S
27 June	Grand Rapids	1	63	17.15 ± 0.45	6.96 ± 0.46	S

wild zooplankton collected from local ponds. Experiments were initiated on nine experimental dates corresponding to walleye mean sizes of 9.17-17.15 mm mean length and 0.60-6.96 mg mean dry weight (Table 6.1). Experimental units were rectangular glass aquaria containing 10 L of dechlorinated water and immersed in one of three water baths at 15, 18.5, or 22 °C. Each water bath contained six experimental aquaria. Light levels over the aquaria ranged from 1.0 to 1.5 $\mu E \cdot m^{-2} \cdot s^{-1}$ and the light:dark regime of the laboratory was maintained at 16:8. Larvae were removed from the stock aquaria and randomly assigned to the experimental aquaria on the evening prior to the start of each experiment. The following morning, an initial, pooled sample of larvae (control sample) was removed randomly from all the experimental aquaria, sacrificed in MS-222 and frozen in 15 mL of water at -18 °C. The number of larvae per experimental aquarium following removal of the control sample ranged from 10 for experiments with first-feeding walleye to 1 for the largest larvae (Table 6.1). I reduced walleye densities with increasing walleye size to reduce cannibalistic attacks.

On the first two experimental dates, experimental aquaria received one of three zooplankton treatments hereafter referred to as S (no food addition), L (low food addition), and H (high food addition). Each treatment was applied to two replicate aquaria within each water bath. Zooplankton (> 99% *Diacyclops bicuspidatus thomasi*) were swept from a local pond and brought to the laboratory where they were maintained in stock aquaria at 18.5 °C. Zooplankton were size-graded by sieving (passed through 300 µm sieve and retained on 183 µm sieve), and individually counted into each aquarium in two daily aliquots. The number of prey added was chosen to provide a prey density which allowed the larvae to consume 0%, 30%, and 75% of maximum daily ration for the S, L, and H treatments, respectively. Maximum daily ration and required prey densities were estimated from the functional response model for first-feeding walleye larvae (~ 0.50 mg mean dry weight; 9.5 mm mean length) feeding on small (183-300 µm) prey (Chapter 3). All zooplankton remaining at the end of the experiment were individually counted out of each aquarium and subtracted from the number added to estimate the number eaten. Non-predatory mortality of zooplankters in the experimental aquaria was negligible in all experiments. The mean fresh dry weight of the zooplankters was determined from frozen subsamples following the methods of Appendix C. The number of zooplankton consumed per aquarium was multiplied by mean plankter dry weight to calculate

total biomass consumed. I was unable to provide multiple ration levels to the larvae after the second experimental date, and for the seven remaining experimental dates, only the S treatment was used. The methodology for these experiments was identical to that of the first two experimental dates with the exception that six replicate aquaria were used in each of the three water baths. The duration of each experiment was generally 38-48 h but two experiments with larger larvae had durations of 63-64 h (Table 6.1). Larvae were sacrificed in MS-222 and frozen in 15 mL of water at -18 °C at the end of all experiments. For the first two experimental dates, experiments were terminated in the morning (> 10 h after the last food addition) to ensure that their guts contained little food.

Instantaneous mortality rates, Z, over each experiment were calculated as

[6.1] $$Z = (\log_e(N_t) - \log_e(N_0)) / t$$

where N_0 and N_t are the number of fish alive at the beginning and end of the experiment, respectively, and t is the duration of the experiment (d) (Ricker 1975). This rate was calculated for each aquarium when aquaria contained > 1 fish and for each temperature treatment otherwise. The frozen fish were later thawed, measured with an ocular micrometer, placed on pre-weighed aluminum trays (~ 1.3 mg), dried at 60 °C for a minimum of 8 hr, transferred to a desiccator for a minimum of 1 hr, weighed to the nearest µg on a Perkin-Elmer AD-6 Autobalance, then stored in the freezer for later energetic determinations. Lengths and dry weights were corrected for preservation effects following the formulae of Appendix B. Instantaneous growth rates, G, for individual fish were calculated as

[6.2] $$G = (\log_e(W_t) - \log_e(W_0)) / t$$

where W_0 is the estimated initial dry weight, W_t is the measured dry weight at the end of the experiment, and t is the duration of the experiment (d) (Ricker 1975). Fish which died during a trial were assumed to have not eaten any zooplankton and were omitted from the analysis. Total food consumed per individual was assumed to be equal for all survivors in each aquarium. Ration consumed was calculated for individual fish as the fraction of $\bar{W}$ consumed per day, where $\bar{W}$ is the arithmetic mean of W_0 and W_t.

Initial dry weights, W_0, were estimated as follows. First, I ranked fish of the control samples by $\log_e$(initial dry weight) ($\log_e(W_0)$) then eliminated a proportion of this sample, equal to the mortality rate observed during the experiment, from the lower end of the weight distribution. Second, the remaining fish were assigned normal scores based on the $\log_e(W_0)$ ranking (RANK procedure, SAS® Institute Inc. 1985) and the linear relationship between $\log_e(W_0)$ and normal score was determined (REG procedure, SAS® Institute Inc. 1985). Finally, experimental fish were ranked and assigned normal scores based on $\log_e$(final dry weight) ($\log_e(W_t)$) and their initial weights were estimated from their normal scores using the $\log_e(W_0)$-normal score relationship developed from the control fish sample. The entire procedure was conducted separately for each aquarium when aquaria contained > 1 fish and for each temperature treatment otherwise. This method of estimating W_0 assumed that the initial size ranking was maintained throughout the experiment and that the fish which had died during the experiment were most likely the smallest individuals. The former assumption has been supported by enclosure experiments on juvenile walleye (K.E. Broughton, Freshwater Institute, Winnipeg, Manitoba, unpubl. data) and the latter has been supported by the mortality patterns observed in interspecific studies of fish larvae (Pepin 1991). To further support this latter assumption, I compared lengths and dry weights of larvae which died during the experiment with larvae collected at the beginning (control) and end of food-deprivation trials. After adjusting for experimental date and temperature, the overall mean length and dry weight of mortalities were lower than those for both of the other groups of larvae. For the experiments with the largest larvae (12, 22, and 27 June; Table 6.1) all $\log_e(W_0)$ and $\log_e(W_t)$ values were adjusted to a common mean $\log_e$(length) (Steel and Torrie 1980) prior to ranking. This slight modification provided more precise estimates of G but required the further assumption that fish length did not change during the experiments, an assumption which was only valid for the largest larvae in this study.

Energy densities were determined for the combined larvae of each aquarium for the first two experimental dates and for the combined larvae of each temperature treatment for the remaining experiments. Groups of larvae were homogenized by pulverizing with a mortar and pestle and pressed into pellets of ~ 2-8 mg. The pellets were placed on pre-weighed platinum trays, dried again for a minimum of 8 h, desiccated for 1 h, weighed to the nearest 0.01 mg

on a Cahn Electrobalance®, then finally burned in a Phillipson microbomb calorimeter (Gentry Instruments, Inc., Aiken, S.C.) following standard calorimetry techniques (Prus 1975). Platinum trays were reweighed after bombing to determine the total weight of non-combustible residue. Energy density was calculated on both a total dry weight and a combustible dry weight basis and corrected for preservation changes according to Appendix C.

Experiments were divided into two components prior to analyses. The first part, termed ration experiments, was comprised of data from all ration treatments (S, H, and L) for the first two experimental dates. The second part, termed food-deprivation experiments, included data of the S ration treatment from all experimental dates. Data for the S treatment for the first two experimental dates were thus common to both components. For the ration experiments, I examined the effects of temperature and ration on G, energy density, and Z. These analyses used means of the response variables calculated for individual aquaria. For the food-deprivation experiments, I examined the effects of experimental date and temperature on G, energy density, and Z. Experimental date effects were assumed to be primarily due to differences in walleye body size (W_0). These analyses used means of the response variables calculated across experimental aquaria for each temperature x experimental date combination.

The effects of experimental date, temperature and ration on G, energy density, and Z were examined using analysis of variance (SAS® GLM Procedure, Freund and Littell 1981). Experimental date was treated as a class variable whereas temperature and ration were treated as continuous variables. Ration represented ration consumed, not food provided. All potential interaction terms between class and continuous variables were included in the original model then sequentially dropped if they were found to be non-significant. The significance of a variable or an interaction term to the model was assessed by its partial (Type III) sums of squares. Residual plots were examined to determine if weighting or transformation was necessary. A strong variance-mean dependence necessitated a weighted analysis for Z and I used a weighting function proportional to the inverse of the predicted standard error. No weighting was required for G or energy density. Power analysis, following the methods of Cohen (1988), was conducted for analyses which failed to reject the null hypothesis of no treatment effect. I assumed a type I error rate of 5% in all power calculations. Effect sizes were estimated and assessed using Cohen's **f** index and classification criteria (small effect,

f = 0.10; medium effect, f = 0.25; large effect, f = 0.40) for analyses of class variables, and using Cohen's f^2 index and classification criteria (small effect, f^2 = 0.02; medium effect, f^2 = 0.15; large effect, f^2 = 0.35) for continuous variables (Cohen 1988).

The preceding analysis assumed a linear relationship between G and ration. To estimate maintenance food requirements I used a more realistic model which considered G to increase at a decelerating rate with respect to ration (Brett et al. 1969). I modelled G as a function of ration for each temperature treatment using a segmented model (NLIN procedure, SAS Institute Inc. 1985). This model assumed that G increased quadratically with ration consumed up to a maximum ration (R_{max}, defined as the point at which further increases in ration do not result in further increases in growth), and that G was a constant, termed maximum growth rate (G_{max}), at higher rations. I then estimated maintenance rations, R_{maint} ($mg \cdot mg^{-1} \cdot d^{-1}$), by setting G = 0 and solving the quadratic equations for R. I used these R_{maint} estimates and functional response models from Chapter 3 to estimate the cyclopoid copepod abundance necessary to allow walleye larvae to attain R_{maint}. I first made the simplifying assumptions that R_{maint} was constant with respect to walleye body size for a given temperature, that the functional response was not affected by temperature, and that larvae fed continuously throughout 16 h of daylight each day. Thus, each larva had a mean maintenance consumption rate, C_{maint} ($mg \cdot h^{-1}$), equal to its body weight (mg) x R_{maint} / 16. I calculated C_{maint} for walleye dry body weights of 0.50 to 2.50 mg. I then estimated maintenance prey abundance, D_{maint} ($prey \cdot L^{-1}$), from the formula $D_{maint} = (C_{maint} \times D_{90}) / (9C_{max} - 9C_{maint})$, which was derived from the functional response model of Chapter 3. The functional response parameters, C_{max} and D_{90}, for walleye consuming small prey (183-300 µm body width, primarily cyclopoid copepods) were estimated for each walleye size-class from the parameter vs. dry weight relationships.

Results

Growth

In the ration experiments, the mean ration consumed increased with the level of ration provided (Table 6.2) and was positively correlated with water temperature (Table 6.3). The ranges of mean rations consumed were 0.00-0.12, 0.00-0.28, and 0.00-0.38 $mg \cdot mg^{-1} \cdot d^{-1}$ for the 15, 18.5, and 22 °C treatments, respectively. There was a significant, positive relationship between G and ration consumed (ANOVA, $F = 31.5$, $df = 1, 32$, $P < 0.001$) and a significant,

Table 6.2. Rations (mg (dry) zooplankton·mg (dry) fish^{-1}·d^{-1}) consumed by first-feeding walleye larvae during ration experiments (Trials 1 and 2, Table 6.1). Ration treatments S, L, and H corresponded to no food addition, 30% of estimated maximum ration, and 70% of estimated maximum ration, respectively. Values are means ± 1 SE (n = 6) calculated after adjustment for temperature effects.

	Ration treatment		
Trial	S	L	H
1	0	0.015 ± 0.014	0.020 ± 0.024
2	0	0.102 ± 0.055	0.228 ± 0.146

Table 6.3. Correlation coefficients (r) between ration (mg (dry) zooplankton·mg (dry) fish^{-1}·d^{-1}) consumed by first-feeding walleye larvae and water temperature for each ration treatment in the ration experiments (Trials 1 and 2, Table 6.1). Ration treatments S, L, and H corresponded to no food addition, 30% of estimated maximum ration, and 70% of estimated maximum ration, respectively. Values in parentheses represent the probability of obtaining a larger value of r (n = 6).

	Ration treatment		
Trial	S	L	H
1	-	0.92 (0.0099)	0.49 (0.32)
2	-	0.92 (0.010)	0.95 (0.0041)

negative relationship between G and temperature (ANOVA, $F = 6.66$, $df = 1, 32$, $P = 0.015$).

The observed relationship between growth and ration is illustrated in Fig. 6.1. There was considerable scatter in growth estimates, particularly at lower rations. The quadratic term of the segmented growth vs. ration model was not significantly different from 0 for any temperature treatment, suggesting that a simple linear model was sufficient to describe the trends. I considered the poor fit of the model to be the result of the narrow range of observed rations rather than the inadequacy of the model itself. Thus, despite the poor fit, I used the segmented model to estimate R_{maint}, R_{max}, and G_{max}. Estimates of R_{maint} were 0.074, 0.090, and 0.11 $mg \cdot mg^{-1} \cdot d^{-1}$ for the 15, 18.5, and 22 °C treatments, respectively. Because of the narrow range of rations, R_{max} and G_{max} were not estimable for the 15 °C treatment. I estimated R_{max} as 0.38 and 0.35 $mg \cdot mg^{-1} \cdot d^{-1}$ for the 18.5 and 22 °C treatments, respectively, and G_{max} as 0.13 $mg \cdot mg^{-1} \cdot d^{-1}$ for both the 18.5 and 22 °C treatments.

My estimates of the prey abundances, D_{maint}, necessary to allow walleye larvae to attain R_{maint} are summarized in Table 6.4. Under my assumptions that R_{maint} was similar for all sizes of walleye larvae and that the walleye functional response was temperature-independent, there was a general trend of declining D_{maint} with increasing walleye dry weight and decreasing water temperature. I estimated that larvae of < 1 mg (< 10.9 mm) would require cyclopoid copepod abundances of 10-27 $\cdot L^{-1}$ to prevent a decline in body weight whereas larvae of ≥ 1.5 mg (≥ 11.9 mm) would not require prey abundances > 2 $\cdot L^{-1}$ to attain R_{maint} (Table 6.4).

In the food-deprivation experiments, growth differed significantly between experimental dates (ANOVA, $F = 14.1$, $df = 8, 17$, $P < 0.001$) and decreased significantly with water temperature (ANOVA, $F = 6.93$, $df = 1, 17$, $P = 0.018$). When adjusted for temperature effects, mean G decreased from -7.6% $\cdot d^{-1}$ at first-feeding to ~ -20 % $\cdot d^{-1}$ in 1.5-3.5 mg (11.9-14.6 mm) larvae then increased to ~ -8 % $\cdot d^{-1}$ in the largest larvae examined (Fig. 6.2).

Energy Density

The percentage of walleye dry body weight which was combustible in the bomb calorimeter declined with walleye size for food-deprived walleye (Fig. 6.3). There was a significant interaction between the effects of experimental date and water temperature on combustible dry weight (ANOVA, $F = 4.33$, $df = 8, 9$, $P = 0.021$). But, scatter plots did not indicate any systematic pattern to the interaction. I subsequently pooled data from all

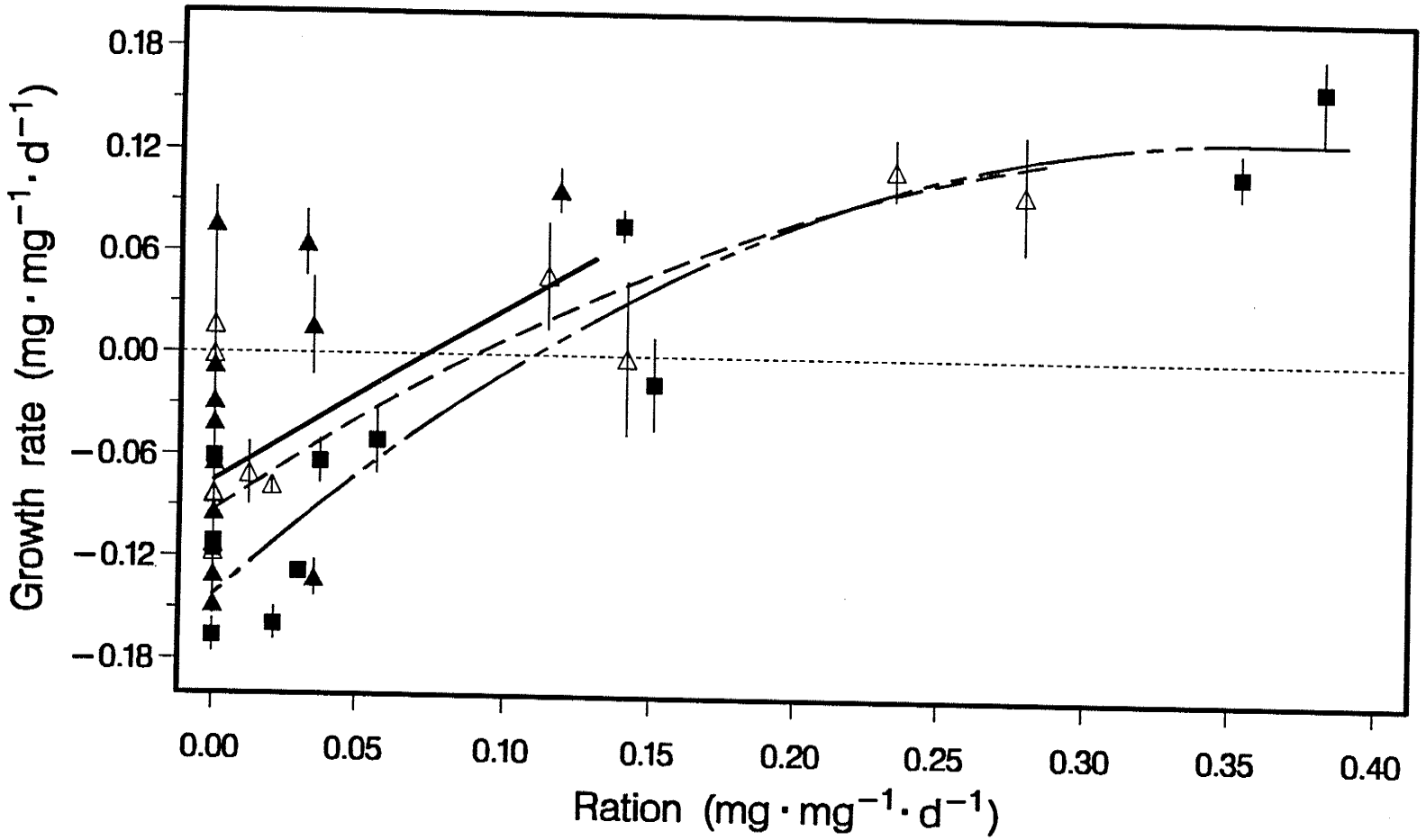

Figure 6.1. Scatter plot of instantaneous growth rate (G, mg fish·mg fish^{-1}·d^{-1}) vs. ration consumed (R, mg (dry) zooplankton·mg (dry) fish^{-1}·d^{-1}) for first-feeding walleye larvae (0.74-0.82 mg mean dry weight) raised in the laboratory on wild zooplankton at 15 (▲), 18.5 (△), and 22 (■) °C. Data points represent individual aquaria and symbols are means ± 1 SE. All data from experimental trials 1 and 2 (Table 6.1) are shown. Relationships between G and R were defined by a segmented model (see text) and were fit to individual temperature treatments (———— 15 °C, - - - - - - - - - 18.5 °C, ———- - ——— 22 °C).

Table 6.4. Estimates of maintenance prey abundances, D_{maint} (prey·L^{-1}), necessary to allow walleye larvae of various sizes to consume a maintenance ration, R_{maint} (mg·mg^{-1}·d^{-1}), at 15, 18.5, and 22 °C. Walleye larvae were assumed to feed 16 h each day, and R_{maint} was assumed to be constant over all walleye sizes for a given temperature. Maintenance consumption rate (C_{maint}, mg·h^{-1}) was calculated from larval dry weight and R_{maint}, and D_{maint} was estimated from C_{maint} and the functional response model of Chapter 3. Methods of calculation and R_{maint} estimates are outlined in text. C_{max} and D_{90} are functional response parameters representing maximum consumption rate (mg·h^{-1}) and the prey abundance at which 90% of C_{max} is attained (prey·L^{-1}). Model parameter estimates are for walleye larvae foraging on small prey (183-300 μm body width, primarily cyclopoid copepods, Chapter 3).

Larval dry weight (mg)	C_{max} (mg·h^{-1})	D_{90} (prey·L^{-1})	D_{maint} (prey·L^{-1})		
			15 °C	18.5 °C	22 °C
0.50	0.0099	300	10.0	13.2	17.6
0.70	0.019	690	16.1	20.7	26.8
1.00	0.030	252	5.07	6.49	8.32
1.50	0.046	55	1.09	1.40	1.79
2.00	0.058	33	0.69	0.88	1.13
2.50	0.069	14	0.31	0.40	0.52

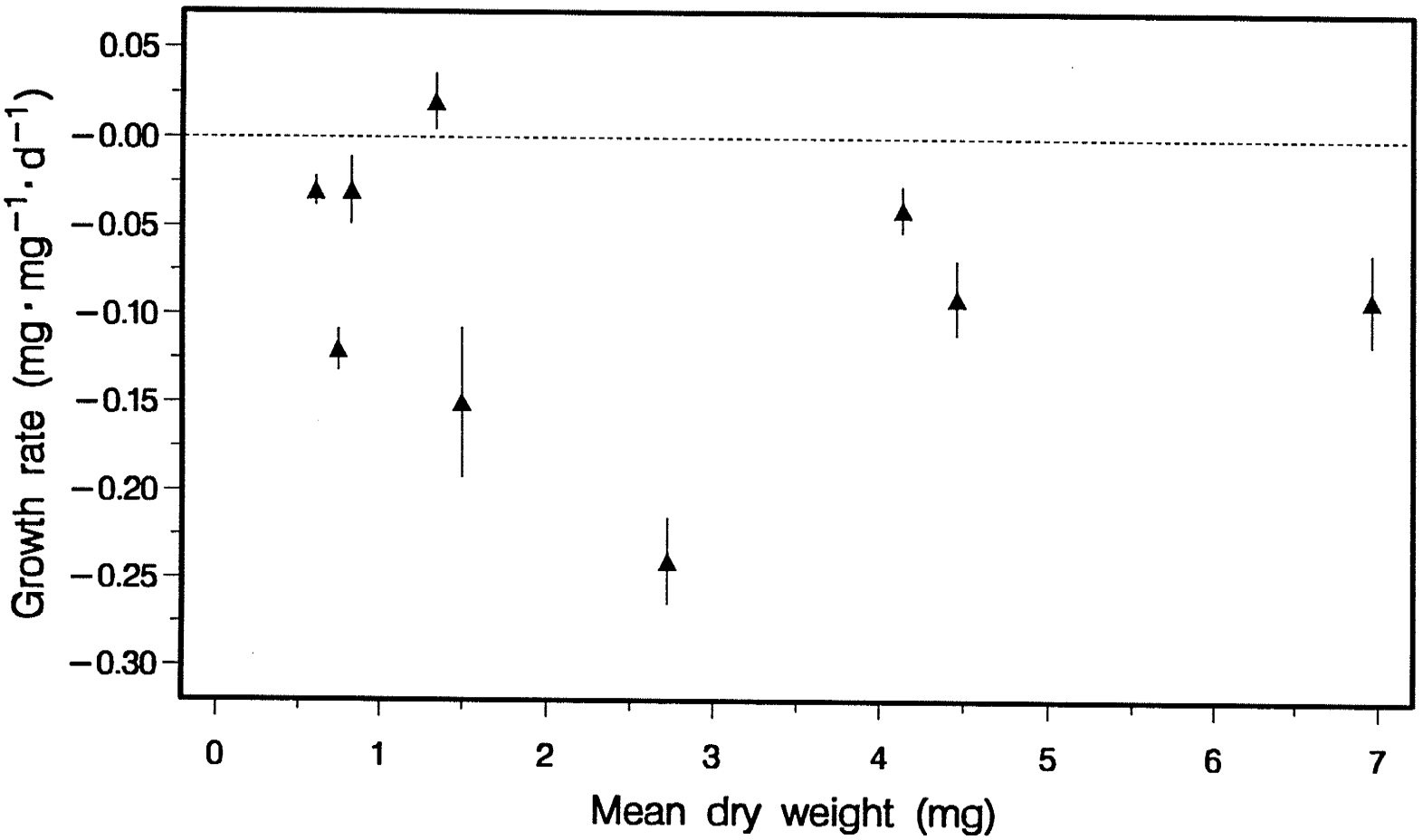

Figure 6.2. Scatter plot of instantaneous growth rate (G, $mg \cdot mg^{-1} \cdot d^{-1}$) vs. mean dry weight for walleye larvae held in laboratory aquaria for 38-64 h without food. Symbols are means ± 1 SE. Mean growth rates were calculated over all temperature treatments (15, 18.5, and 22 °C) for the food-deprivation treatment (S, See Table 6.1) after adjusting for temperature effects. Mean dry weights were calculated from fish samples taken at the initiation of the experiments (control samples).

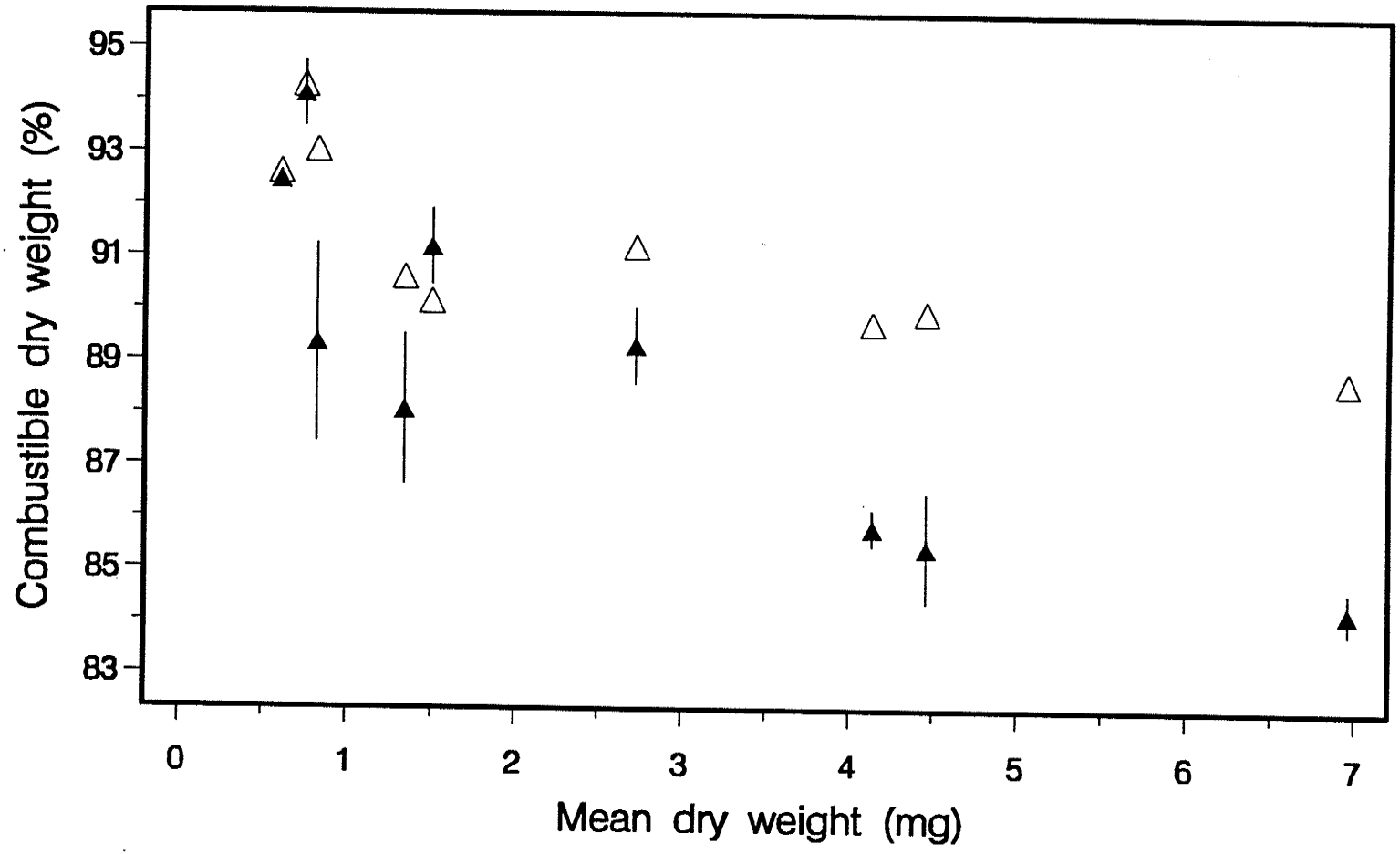

Figure 6.3. Scatter plot of combustible dry weight (as a percentage of total dry weight) vs. mean dry weight for walleye larvae raised in the laboratory. Combustible dry weights were calculated separately for fish of the initial (control, △) and final (experimental, ▲) samples. Symbols for the control samples represent single determinations whereas symbols for the experimental samples represent means calculated over all temperature and ration treatments ± 1 SE.

temperature treatments and compared combustible dry weight between larvae of the control sample and the pooled food-deprivation treatments. The mean combustible dry weights adjusted for experimental date were 91.2 and 88.9% for the control and food-deprivation treatments, respectively. Combustible dry weight content was significantly lower in fish of the food-deprivation treatment (paired-observations *t*-test, $|t| = 3.25$, df = 8, P = 0.012). I conducted all subsequent analyses using energy density calculated on a combustible dry weight rather than total dry weight basis.

In the ration experiments, there was no significant effect of ration (ANOVA, F = 0.06, df = 1, 31, P = 0.81) or temperature (ANOVA, F = 0.04, df = 1, 31, P = 0.84) on the energy density of first-feeding larvae. The effect sizes in both of these tests were very small ($f^2 \leq 0.0041$).

In the food-deprivation experiments, energy density of walleye larvae declined sharply from first-feeding to a mean weight of 1.5 mg (11.9 mm), reached a minimum of ~ 21 $J \cdot mg^{-1}$ for 2.7-mg (13.7-mm) larvae and increased slightly over the size range of 3-7 mg (14.1-17.2 mm) (Fig. 6.4a). However, energy density determinations were quite variable, particularly for the smaller larvae. I found no significant difference in walleye energy density between experimental dates (ANOVA, F = 2.30, df = 8, 17, P = 0.071). This test had a maximum observed difference in energy densities of 5.6 $J \cdot mg^{-1}$ and a correspondingly large observed effect size (f = 0.44). However, because of the relatively large residual variance, a maximum observed difference of > 10 $J \cdot mg^{-1}$ or sample sizes > 9 for each experimental date would be required to increase the power of this test to ~ 80%. In contrast to energy density, mean total energy content increased nearly monotonically with walleye mean weight (Fig. 6.4b). The energy density of walleye larvae deprived of food was not significantly related to water temperature (ANOVA, F = 0.00, df = 1,17, P = 0.99). I pooled energy density data from all temperature treatments into a single food-deprivation treatment and compared this with the energy density of larvae of the control sample. The mean energy densities adjusted for experimental date were 23.1 and 23.2 $J \cdot mg^{-1}$ for the control and food-deprivation treatments, respectively, and there was no significant difference in energy density between these two treatments (paired-observations *t*-test, $|t| = 0.17$, df = 8, P = 0.87). Thus, the energy density of the combustible matter of walleye larvae changed little during short periods of food

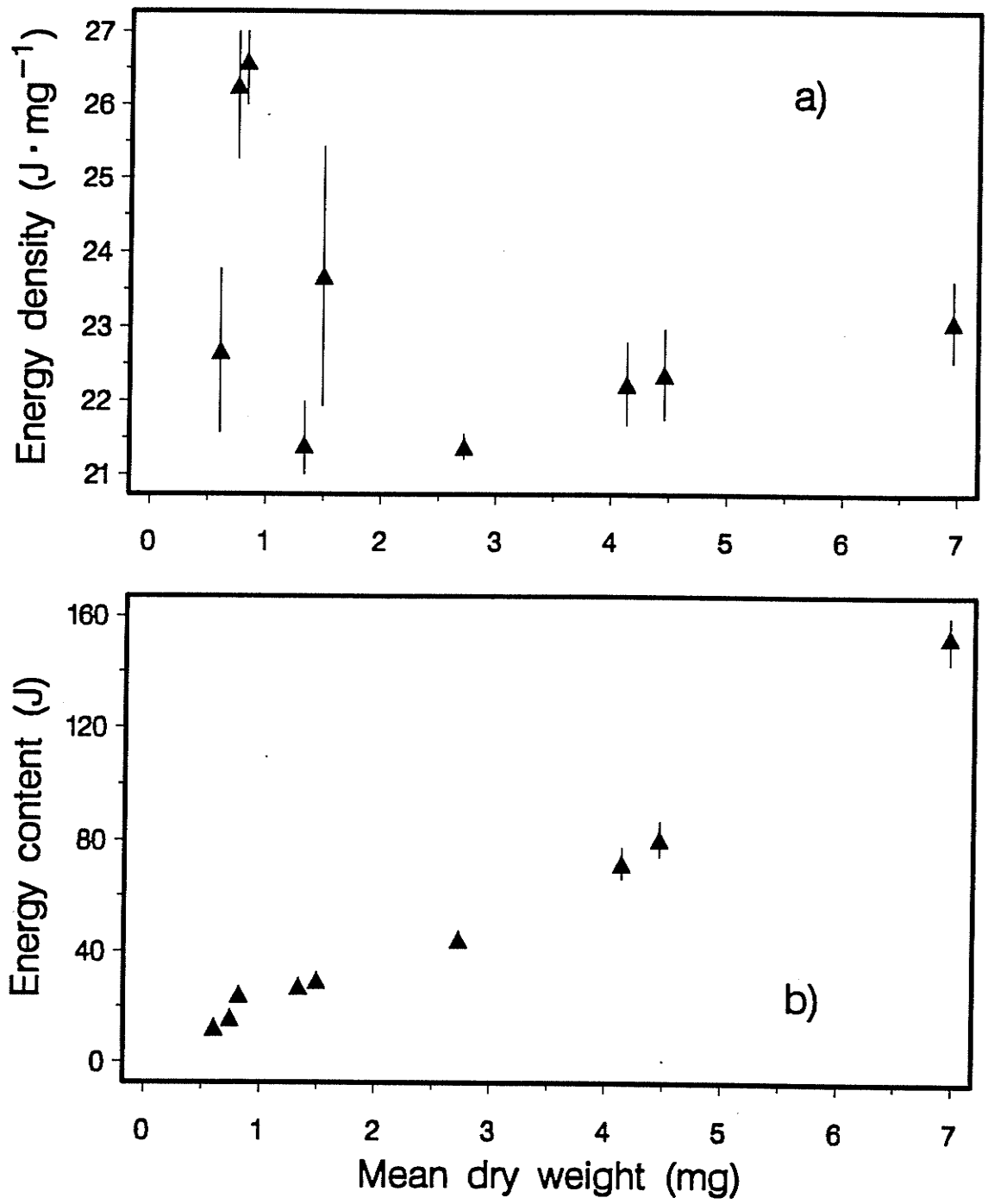

Figure 6.4. Scatter plots of a) energy density (J·mg combustible dry weight^{-1}) vs. mean dry weight (mg), and b) total energy content (J) of individual fish vs. mean dry weight (mg) for walleye larvae raised in the laboratory. Symbols represent means ± 1 SE. Means were calculated over determinations for control samples and determinations for all temperature and ration treatments.

deprivation regardless of temperature.

Mortality

For the ration experiments, I examined the effect of temperature and ration on Z for first-feeding larvae. There was a positive, but non-significant effect of temperature on Z (ANOVA, $F = 0.87$, $df = 1, 32$, $P = 0.36$). The relationship between Z and ration was negative, but also not significant (ANOVA, $F = 0.17$, $df = 1, 32$, $P = 0.68$). The observed effect size due to ration in this test was exceedingly small ($f^2 = 0.0055$).

Mortality rates varied greatly over the walleye size range examined in the food-deprivation experiments (Fig. 6.5). The overall trend in Z was a rapid increase from first-feeding to a mean weight of 1.5 mg (11.9 mm) followed by a peak, and a rapid decline over the 3-5 mg (14.1-15.9 mm) size range. Mortality rates differed significantly between experimental dates (ANOVA, $F = 11.1$, $df = 8, 17$, $P < 0.001$). The highest observed mean mortality rates were not associated with the lowest mean body weights, but rather with body weights in the range of 1.5-3.5 mg (11.9-14.6 mm). As with the ration experiments, the relationship between Z and water temperature was positive, but not significant (ANOVA, $F = 0.92$, $df = 1, 17$, $P = 0.35$) for the food-deprivation experiments. The observed effect size of temperature on Z was relatively small ($f^2 = 0.054$), but, this test had a power of ~ 65-70% to detect large effect sizes ($f^2 = 0.35$).

The observed trend in Z in relation to walleye mean weight roughly mirrored the observed trend in G (cf. Fig. 6.2 and Fig. 6.5). Following correction for experimental date and temperature treatment, I found a significant, negative relationship between Z and G for walleye larvae of the food-deprivation experiments (ANOVA, $F = 5.50$, $df = 1, 16$, $P = 0.032$). The highest observed mortality rates also appeared to coincide with the lowest observed energy densities. However, I found no significant relationship between Z and the energy density of walleye larvae (ANOVA, $F = 0.00$, $df = 1, 16$, $P = 0.98$).

Discussion

I estimated maintenance food requirements of walleye larvae using a model which assumed a quadratic relationship between growth and ration. Although my analysis indicated that a linear model would have sufficiently described this relationship over the range of rations

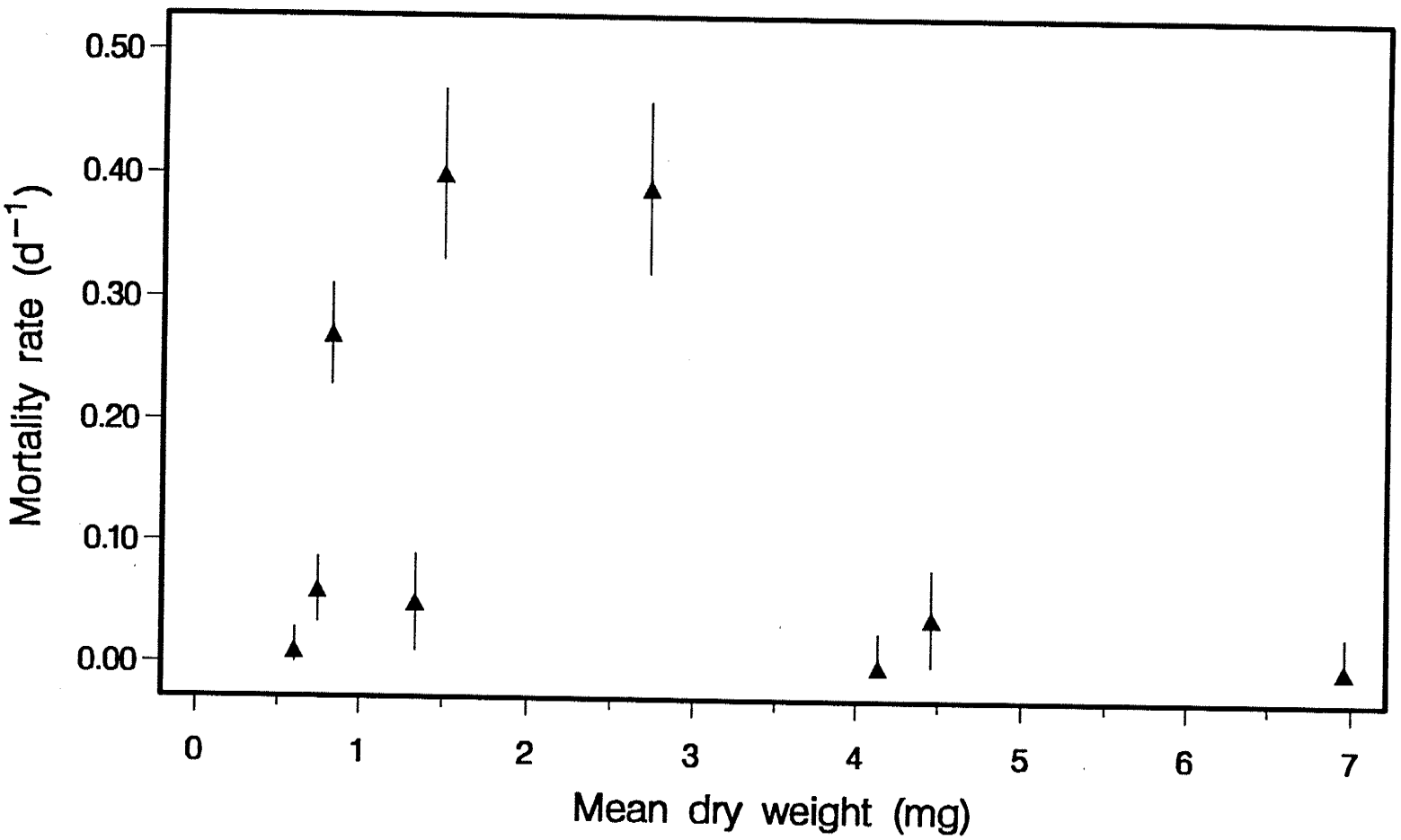

Figure 6.5. Scatter plot of instantaneous mortality rate (Z, %·d^{-1}) vs. mean dry weight for walleye larvae held in laboratory aquaria for 38-64 h. Symbols are means ± 1 SE. Mean mortality rates were calculated over all temperature treatments (15, 18.5, and 22 °C) for the food-deprivation experiments (S treatment, see Table 6.1). Mean dry weights were calculated from fish samples taken at the initiation of the experiments (control samples).

observed in this study, I believe that the quadratic model provided a more realistic description of the true relationship based on the shape of growth vs. ration relationships of other fish species (e.g. Brett et al. 1969; Freeberg et al. 1990; Brown and Taylor 1992). The rather poor fit of the segmented growth vs. ration model in this study was probably related to the lack of data at higher rations. Almost all observed rations were below the predicted maximum ration, R_{max}. Though I am confident in my R_{maint} estimates, I feel that the lack of data at higher rations may have influenced my estimates of G_{max} and R_{max}. Estimates reported in this study for these latter two parameters may be biased low. I observed considerable scatter about my growth vs. ration relationships, particularly at low rations. In field studies, variation about such relationships is often attributed to the effects of fish size, temperature, prey quality, or other factors (Hewett et al. 1991; Hewett and Kraft 1993). However, in this study I examined a restricted size range of fish, controlled water temperature, and fed all fish from the same food source. Much of the observed variation may have been due to my method of calculating growth rate. Because I used dry weight as a size criterion I could not measure the same fish at the beginning and end of experiments and thus my precision was affected by the randomness of my sampling for the control and experimental fish. Though I feel that dry weight is still the best measure of fish size for larval fish research, I suggest that future studies should examine larger subsamples of larvae to improve the precision of their results.

My results indicated that walleye larvae of 0.74-0.82 mg dry weight (10.0-10.7 mm) have a maintenance ration, R_{maint}, of 7-11% of their body weight per day. These estimates are somewhat lower than those for other freshwater fish larvae. Borgmann and Ralph (1985) estimated R_{maint} values of 0.19 and $0.13 \cdot d^{-1}$ for 1.1-2.2 mg dry weight white sucker, *Catostomus commersoni*, and common shiner, *Notropis cornutus*, larvae, respectively, raised on a zooplankton (primarily Cladocera) diet in the laboratory at 20 °C. Estimates of R_{maint} in this study increased with water temperature. My results also indicated that for a given ration consumed, growth declined with increasing temperature. These findings are consistent with observations for other species (Brett et al. 1969; Williams and Caldwell 1978). Previous studies have also indicated that R_{maint} declines with increasing body size (Boehlert and Yoklavich 1983). If R_{maint} declines with increasing body size for walleye as well, then my estimates for maintenance prey abundances (D_{maint}, Table 6.4) may be biased low for walleye

which are smaller than those examined in this study, and biased high for larger walleye. Thus, the negative relationship between D_{maint} and walleye dry weight may be even more pronounced than my results would suggest. Another important factor may be the influence of temperature on the functional response parameters used to calculate D_{maint}. The parameters used were derived from feeding experiments conducted at 20 °C (Chapter 3). However, temperature may affect the functional response such that for a given prey density, fish at higher temperatures will have higher consumption rates (Persson 1986; Chapter 4). This suggests that my estimates for D_{maint} may be biased low at 15 and 18.5 °C, and biased high at 22 °C, and thus the effect of temperature on D_{maint} may be less pronounced than my results would suggest.

In relation to zooplankton abundances observed in natural lakes, my estimates of D_{maint} indicate that first-feeding walleye larvae may have some difficulty attaining R_{maint} whereas larvae > 1 mg (10.9 mm) would probably always attain R_{maint}. Mean zooplankton abundances in large prairie lakes at the time of walleye first feeding are in the range of 20-70·L^{-1} and these zooplankton communities are usually dominated by cyclopoid copepods (Friesen and Mathias 1990; Patalas and Salki 1992). Walleye inhabit a wide range of lakes varying greatly in secondary production and some of the more oligotrophic lakes probably have mean spring zooplankton abundances < 20·L^{-1} (Chapter 8). To avoid weight loss under such conditions, first-feeding walleye larvae may have to actively locate or be carried into localized, higher-density patches of zooplankton. However, an initial decline in body weight would not necessarily lead to starvation mortality. Feeding success during early development may improve steadily with experience or body length despite short-term declines in body weight and these improvements would effectively lower D_{maint}.

The results of my analysis of weight loss during short bouts of food deprivation suggest that walleye larvae of 1.5-3.5 mg (11.9-14.6 mm) lose weight more rapidly than smaller or larger larvae. Larvae in this size range may, therefore, be more susceptible to starvation under conditions of food deprivation than first-feeding or larger larvae. I had expected that the rate of weight loss would be highest among the smallest larvae and decline steadily with increasing walleye size. The reasons for the observed trend may be related to changes in activity energy expenditures. First-feeding larvae still possess an oil globule which may

provide an endogenous energy reserve as well as some buoyancy. As this oil is gradually used up the larvae must spend more time searching for food and must expend more energy to swim. This would cause daily energy consumption, and thus weight loss, to increase. Also during this time, the air bladder would have been developing and larvae would have gradually become more energetically efficient at swimming, thus reducing their rates of energy consumption. The high rate of weight loss in the 1.5-3.5 mg (11.9-14.6 mm) size range may thus indicate the transition between different buoyancy mechanisms. During this period the larvae have lost their oil globules but have not fully benefitted from the development of their air bladders and thus energy expenditures are very high. More rapid weight loss at higher temperatures, as observed in this study, is consistent with the known effects of temperature on standard and active metabolism of larval fish (reviewed by Houde 1989). Because rates of energy expenditure decline over time in food-deprived fish (Beamish 1964; Wieser et al. 1992), walleye larvae may lose weight at progressively slower rates during prolonged food-deprivation. However, reduction in energy expenditure may come at the expense of reduced swimming speed (Laurence 1972; Rice et al. 1987) and overall activity. In natural environments, where the probability of finding food is related to the volume searched, reduced activity may increase the chances of starvation mortality. A reduced swimming performance may also lead to an increased susceptibility to predation (Rice et al. 1987).

The effects of short periods of food deprivation on growth and survival may be evident even after feeding resumes. Studies on juvenile Cyprinidae have indicated that initial growth rates following food deprivation are higher than those observed in continuously-fed fish, and that this initial growth rate increases with the length of the food-deprivation period (Wieser et al. 1992). They termed this phenomenon 'compensatory growth'. In contrast, Gadomski and Peterson (1988) noted a reduced growth rate and survival in marine fish larvae which began feeding after being food-deprived for 1-2 d following yolk absorption compared to larvae which fed immediately following yolk absorption. Similarly, Yúfera et al. (1993) noted a much reduced feeding ability in gilthead seabream, *Sparus aurata*, larvae which were initially fed 8 d after hatching compared to larvae which were initially fed 5 d after hatching. A 2-d delay in supplying food to first-feeding white perch, *Morone americana*, larvae has been shown to result in a significant decrease in survival (Margulies 1988). Thus the aftereffects of short periods

of food deprivation may vary with fish size, developmental stage and possibly species, and it would be difficult to speculate on the aftereffects of food deprivation on walleye larvae.

I observed a trend of declining combustible dry weight as a percentage of total dry weight with increasing walleye size. This trend probably reflects the increase in ash content of the larvae which accompanies skeletal development. I also observed a reduced combustible dry weight in larvae subjected to starvation. However, the energy density of the combustible matter appeared to be unaffected by ration consumed for first-feeding larvae, or by water temperature for all sizes of larvae subjected to starvation. I found no significant difference between energy densities of control fish and those subjected to starvation. This suggests that walleye larvae utilize their energy reserves roughly in proportion to their body composition during the early stages of food deprivation. I had expected a decline in energy density during food deprivation indicating a disproportionate use of lipids (Kamler 1992). Brett et al. (1969) observed that food-deprived salmonids had lower lipid:protein dry weight ratios than well-fed fish, suggesting that lipid was metabolized disproportionately more than protein during food deprivation. I was unable to detect a statistically significant difference in walleye energy density between experimental dates corresponding to different walleye mean weights. However, the effect size in this analysis was quite large and I feel that the observed trend is biologically significant. The decline in energy density with increasing dry weight during the early larval period corresponds with the utilization of the oil globule.

Based on the critical period hypothesis, the mortality of first-feeding fish larvae is primarily the result of starvation, and thus, larvae which feed more should be less likely to perish. This was not apparent in the ration experiments of this study. I found no significant relationship between mortality rate and ration consumed in first-feeding walleye larvae. This may have been due to the short duration of my experiments (47-48 h). In laboratory experiments of ~ 5 d duration, Li and Mathias (1982) noted an increase in the survival of walleye larvae (~ 8.5-10.5 mm) with increasing prey abundance up to 100 prey·L^{-1}. Yellow perch larvae, which are slightly smaller than walleye larvae, have been observed to survive food deprivation for up to 65 d after hatching at 5.3 °C and for up to 15 d after hatching at 19.8 °C (Hokanson and Kleiner 1974). Rice et al. (1987) observed a 50% survival of bloater, *Coregonus hoyi*, larvae deprived of food for 25 d after hatching at temperatures increasing from 8 to 14 °C. I had

expected a positive relationship between mortality rate and temperature because with increasing temperature walleye larvae lose weight more rapidly (see above) and thus, should be more susceptible to starvation mortality. Such a trend has been observed in laboratory studies on white perch larvae (Margulies 1988), and in interspecific studies of fish larvae (Pepin 1991). However, the indirect effect of temperature on the mortality of walleye larvae appeared to be rather weak in this study as I found no statistically significant effect in either my ration or food-deprivation experiments. My results did suggest that the mortality rate of walleye larvae during short bouts of food deprivation varies with respect to body size. I found a statistically significant difference in mortality rates between experimental dates corresponding to different walleye mean weights. Contrary to my predictions, the highest mortality rates observed were not associated with the smallest body weights but instead with body weights of 1.5-3.5 mg (11.9-14.6 mm).

Some walleye larvae did die when subjected to short-term food deprivation in this study. But why did they die? Some of the mortalities may have been the result of attacks by other larvae in the stock tanks prior to the experiment or in the experimental aquaria. The mortality rate of unsuccessfully attacked larvae may be as high as 19% over the following 24 h (Loadman et al. 1986). However, by maintaining adequate prey abundances I was able to keep cannibalistic activity low in the stock aquaria and I always selected only actively-feeding (i.e. apparently healthy) individuals for the experiments. Furthermore, attacks during experiments could only have occurred on dates where > 1 fish was placed in each experimental aquarium (30 May, 2 June, and 10 June, Table 6.1) and mortality rates were generally lower on these dates. For these reasons, I feel that injuries from cannibalistic attacks were a negligible source of mortality in this study. The significant, negative relationship between mortality rate and growth rate (or positive relationship between mortality rate and rate of weight loss) observed in my food-deprivation experiments suggests that mortality was related to the decline in body weight. It is not clear whether the larvae died after losing a certain percentage of their energy reserves or muscle mass, or after exhausting a particular substance in the body. If the latter is true, what body constituent limits the walleye's ability to resist starvation? Recent studies have emphasized the importance of essential fatty acids to the growth and survival of fish larvae (Chapter 8). Essential fatty acid deficiencies in egg yolks

has been linked with high incidences of body deformities and high mortality rates in walleye larvae (Moodie et al. 1989). However, no study, to my knowledge, has compared the fatty acid profiles of fed and food-deprived walleye larvae.

I did not design my experiments to test for stock effects. I assumed such effects to be negligible and raised two stocks of walleye simply to allow myself to conduct experiments over a prolonged period. However, some of my results suggested differences between the two stocks. Larvae of the Grand Rapids stock generally showed faster rates of weight loss during starvation, somewhat lower energy densities, and higher mortality rates. Not accounting for stock differences may have increased the residual variance and reduced the power of statistical tests in the food-deprivation experiments, where data from both stocks were utilized. Therefore, these tests may be conservative. Though I could not test for stock effects, I feel that such effects may be real and suggest that between-stock differences in both walleye eggs and larvae should be examined more closely in future studies.

Chapter 7:
Consumption and growth of walleye larvae in culture ponds

Introduction

Understanding food consumption and growth of larval fish is an important step towards understanding their survival. Larvae with a high rate of food consumption should be less susceptible to starvation mortality. Furthermore, because smaller fish are generally more vulnerable to predation, a cohort which grows rapidly through the most vulnerable size ranges of its life should have higher survival. Growth in turn depends upon the quantity and quality of food consumed. In previous chapters, I examined food consumption (Chapters 3 and 4), and growth (Chapter 6) of walleye larvae under laboratory conditions. In this chapter, I will examine consumption and growth of walleye larvae under semi-natural conditions in extensive culture ponds.

I had several objectives in this chapter. First, I wished to estimate the food consumption of walleye larvae in the field using direct and indirect (bioenergetics modelling) methodologies and compare my results with laboratory-derived estimates of food consumption (Chapters 3 and 4). In addition, I compared several parameterizations of bioenergetics models for larval percids and assessed their utility for estimating the food consumption of walleye larvae. Second, I wanted to examine the relationship between food consumption and prey abundance in the field for comparison with laboratory-derived relationships (Chapter 3). Finally, I examined the relationship between growth and food consumption under field conditions for comparison with the results of my laboratory growth vs. ration experiments (Chapter 6).

Materials and Methods

Food consumption: Field estimates

I examined the food consumption and growth of walleye larvae raised in the Methley Beach walleye culture ponds from 1987 to 1989. Food consumption and growth were estimated from the same samples of walleye larvae, and were monitored for 15-17 d following first-feeding in all years. Single samples were collected during daylight hours (0900-2200 h) on each of 4 sampling dates in 1987 and 1989, and on 3 sampling dates in 1988 (Appendix A). The number of fish collected for a given pond and sample date ranged from 15 to 34 but

was < 20 on only two dates. Water temperature was monitored continuously throughout the culture periods. Complete descriptions of the culture pond environments and sampling procedures for walleye and zooplankton are given in Appendix A. For comparison with the results of the bioenergetics modelling (see below), all field estimates of growth and consumption were calculated on a wet weight basis. This required the conversion of dry weight estimates of fish and invertebrates into wet weight. I assumed that fish larvae were 85% moisture (Madon and Culver 1993), zooplankton were 92% moisture (Yurkowski and Tabachek 1979; Luecke and Brandt 1993), and all benthic invertebrates were 85% moisture (Yurkowski and Tabachek 1979).

Contents of entire digestive tracts of individual fish were analyzed as outlined in Appendix A. Prey items were converted to fresh dry weights using the formulae of Table A.3. Total dry weight of gut contents was estimated by summing the dry weight of all prey items and subtracting estimated assimilation losses. I assumed that at any point in time during continuous feeding that 34% of organic matter and 0% of ash from gut contents would already have been assimilated (Chapter 5). I assumed that 6% of copepod dry weight and 15% of cladoceran, benthos, and fish dry weight was composed of ash (Tables A.6, C.1). The ash component of cladoceran dry weight is actually quite variable (Table A.6), but, I feel that 15% represents a good estimate for cladocerans in the size range consumed by larval walleye.

The most commonly employed method of estimating daily food consumption in the field involves collecting gut data at 3-h intervals over a 24-h period (Elliott and Persson 1978). Because gut data from the Methley ponds was collected only once on each sampling date, I used an alternative approach. If walleye show a consistent diel pattern in feeding, then daily ration should be estimable from a single daily sample (Hayward and Hiebert 1993). I first examined the daily pattern in gut fullness of walleye larvae using data from all ponds, years, and sampling dates. Gut fullness, F, was calculated as

$$[7.1] \qquad F = 100 \times (W_{food} / W_{fish})$$

where W_{food} is the dry weight (mg) of food in the fish's gut, and W_{fish} is the dry weight (mg) of the walleye larva. I calculated the mean and SE of F for each fish sample, and regressed $\bar{F}$ against $\log_e$(walleye mean dry weight), water temperature, $\log_e$(zooplankton abundance), and

time of day (t = 0 to 1, where 0 is midnight and 0.5 is noon) using a weighting function proportional to the inverse of the SE (GLM procedure, SAS® Institute Inc. 1985). Mean gut fullness showed significant, positive relationships with zooplankton biomass (ANOVA, F = 4.36, df = 1, 39, P = 0.043) and time of day (ANOVA, F = 5.69, df = 1, 39, P = 0.022), but no significant relationships with walleye mean dry weight or water temperature (P > 0.40). Thus, the mean gut fullness of walleye larvae increased linearly with time. I assumed that the slope of this relationship was similar for all walleye feeding in the ponds during daylight hours (0.5 h before sunrise to 0.5 h after sunset), and that gut fullness followed an exponential decline during the night. I estimated F at 0.5 h intervals throughout daylight hours on each sample date by assuming that the single direct estimate of $\bar{F}$ fell on the $\bar{F}$ vs. t relationship for that date. I also used the single direct estimate of $\bar{F}$ and the $\bar{F}$ vs. t relationship to estimate F at dusk (0.5 h after sunset). I then estimated F at 0.5 h intervals during the night using the dusk estimate of gut fullness and the instantaneous rate of gut evacuation as estimated from night-time water temperature (Chapter 5). Finally, I estimated the overall daily mean of gut fullness, $\bar{F}_d$, by taking the mean of the 48 half-hour estimates. Daily food consumption, C (g dry food·g dry fish^{-1}·d^{-1}), was then estimated as

[7.2] $$C_{field} = 24 \times \bar{F}_d \times R$$

where R is the instantaneous rate of gut evacuation as estimated from mean daily water temperature (Chapter 5) (Craig 1978). This estimate was then converted to a wet weight basis using the diet composition data of Table A.7, and the moisture contents of walleye and their prey as listed above. To obtain estimates of C_{field} corresponding to those of growth rate, G_{field} at $\bar{W}$ (see below), I calculated the arithmetic means of C_{field} at the beginning and C_{field} at the end of the sampling intervals.

Food consumption: Model estimates

I estimated the food consumption of postlarval walleye in the Methley culture ponds using the Hewett and Johnson (1987) version of Kitchell et al.'s (1977) bioenergetics model for percids. The basis of the model is the balanced energy equation of the form

[7.3] Growth = Consumption - (Respiration + Waste Losses)

All components are expressed as wet weight-specific rates (g wet food·g wet fish^{-1}·d^{-1}). Mathematical formulae for each of these components are presented in Hewett and Johnson (1987). Consumption, C_{model}, is modelled as the product of maximum consumption at optimum temperature, C_{max}, a water temperature function, F(T), and a proportionality constant, p (Kitchell et al. 1977; consumption model 2 of Hewett and Johnson (1987)). Maximum consumption is modelled as an allometric function of fish wet weight, W (g), and requires intercept (CA) and slope (CB) parameter estimates (Table 7.1). The temperature function describes the change in C_{max} with water temperature and requires parameter estimates for optimum temperature of consumption (CTO), maximum temperature of consumption (CTM), and slope (CQ) (Table 7.1). The proportionality constant, p, is used to scale C to C_{max} when fitting the model ($p = C / (F(T) \times C_{max})$). Respiration, R, is modelled as the product of standard metabolic rate at optimum temperature (SMR), a temperature function, and an activity coefficient, A (Kitchell et al. 1977; respiration model 2 of Hewett and Johnson (1987)). Standard metabolic rate is modelled as an allometric function of W, and requires intercept (RA) and slope (RB) parameter estimates (Table 7.1). The temperature function requires parameter estimates analogous to those of F(T) for consumption (RTO, RTM, and RQ, Table 7.1). The activity coefficient scales R to account for metabolic expenditures due to activity. Waste losses include specific dynamic action (S), egestion (F), and excretion (E). Specific dynamic action is modelled as a constant proportion of digestible energy (C - F). Egestion and excretion are both modelled as functions of water temperature and p (Kitchell et al. 1977; egestion and excretion model 2 of Hewett and Johnson 1987). Each of the egestion and excretion models requires three parameter estimates (Table 7.1).

In addition to parameter estimates, the model requires information on water temperature, diet composition, predator caloric density, and prey caloric density. Mean daily water temperatures were calculated from the Methley culture pond temperature data (Appendix A). Diet composition was estimated from stomach analyses of the Methley pond walleye (Appendix A). Proportions of copepods, cladocerans, benthos, and fish in the diet were initially calculated on a dry weight basis (Table A.7), but were converted to wet weight proportions using the assumed moisture contents listed above. Predator caloric density was determined on a dry weight basis for walleye larvae (Swan Creek stock) raised in the laboratory during 1990

Table 7.1. Parameter values used in this and earlier percid bioenergetics modelling studies. The parameter values for RA are expressed here as g fish·g $fish^{-1}·d^{-1}$. However, the Hewett and Johnson (1987) version of the bioenergetics model requires RA to be input as 0.0108 g O_2·g $fish^{-1}·d^{-1}$ for percids.

Symbol	Description	Kitchell et al. (1977)	Post (1990)	Madon and Culver (1993)	this study
Consumption					
CA	Intercept for maximum consumption	0.25	0.51	0.45	0.78
CB	Slope for maximum consumption	-0.27	-0.42	-0.27	-0.27
CTO	Optimum temperature for consumption (°C)	22	29	25	25
CTM	Maximum temperature for consumption (°C)	28	32	28	28
CQ	Slope for temperature dependence of consumption	2.3	2.3	2.3	2.3
Respiration					
RA	Intercept for maximum standard respiration	0.035	0.035	0.056	0.035
RB	Slope for maximum standard respiration	-0.20	-0.20	-0.22	-0.20
RTO	Optimum temperature for standard respiration (°C)	27	32	27	27
RTM	Maximum temperature for standard respiration (°C)	32	35	32	32
RQ	Slope for temperature dependence of standard respiration	2.1	2.1	2.1	2.1
ACT	Activity coefficient	1.0	4.4	3.0	1.0
S	Specific dynamic action coefficient	0.172	0.15	0.1	0.15
Waste losses					
FA	Intercept for proportion of consumed food egested	0.158	0.15	0.25	0.428
FB	Coefficient for egestion vs. temperature	-0.222	0	0	-0.222
FG	Coefficient for egestion vs. feeding level	0.631	0	0	0.631
UA	Intercept for proportion of consumed food excreted	0.0292	0.15	0.05	0.0292
UB	Coefficient for excretion vs. temperature	0.58	0	0	0.58
UG	Coefficient for excretion vs. feeding level	-0.299	0	0	-0.299

(Chapter 6; Fig. 6.4a) and 1993 (Appendix B; Table B.1) and converted to a wet weight basis. Walleye energy density was input as a declining fuction of mean wet weight from first feeding (~ 0.0030 g wet weight, ~ 880 cal·g wet^{-1}) to 0.030 g (760 cal·g wet^{-1}) and as a constant 760 cal·g wet^{-1} for larger fish. Prey caloric density was estimated directly only for crustacean zooplankton in 1988 (Appendix A; Table A.6). Because the energy density of zooplankton showed a seasonal trend in 1988 (Table A.6), I was reluctant to use these data for 1987 and 1989 when walleye were cultured over slightly different seasonal time periods. Instead, I assumed constant prey energy densities over all walleye culture periods in all years. I obtained mean estimates of 460, 400, and 797 cal·g wet^{-1} for cyclopoid copepods, Daphniidae, and Chironomidae from the dry weight energy densities of Cummins and Wuycheck (1971) and the assumed moisture contents listed above. These were used as prey caloric densities for the copepod, cladoceran, and benthic components of the diet, respectively. Prey fish were assumed to have the same energy density as walleye larvae (760 cal·g wet^{-1}).

I estimated food consumption from the bioenergetics model using walleye mean wet weights at age as input, and compared the model estimates of consumption with my field estimates of consumption. Running the model requires two steps. First, the consumption parameter p is estimated by iteration such that the estimated consumption matches the observed growth for the given model parameterization. Second, the estimates of p are input to the model and the various components of the energy budget are estimated at specified intervals over a simulation period. I modelled walleye consumption separately for each pond by year combination. This provided 32 growth intervals or cohorts (12 in 1987, 8 in 1988, and 12 in 1989). I used several different parameterizations of the bioenergetics model (Table 7.1). First, I used the parameter values suggested by Kitchell et al. (1977) for adult walleye. Parameter values were derived from various studies on the energetics of percids (see references in Kitchell et al. (1977) and Hewett and Johnson (1987)). This version will be referred to hereafter as the Kitchell parameterization. Second, I used the parameters suggested by Madon and Culver (1993) for YOY walleye. This model will be referred to hereafter as the Madon and Culver parameterization. Third, I used the Kitchell model with altered parameter estimates for consumption, egestion, and specific dynamic action based on the results of more recent studies. I used the CA and CB parameter estimates of Madon and

Culver (1993), and I increased CTO to 25 °C based on the results of intensive culture studies (J.A. Mathias, pers. comm.). I lowered the parameter S from 0.172 to 0.15. This value falls in the mid-range of estimates provided by Beamish and MacMahon (1988) for juvenile walleye. I increased FA to 0.428, the intercept value that allows walleye an assimilation efficiency of 78% (or egestion of 0.22) at 20 °C and minimum ration (p = 0). This was the maximum assimilation efficiency estimated in Chapter 5. This third version will be referred to hereafter as the Johnston parameterization.

Growth

Fresh lengths and dry weights of walleye larvae were determined from preserved measurements (Appendix A) and the conversion formulae of Appendix B. Instantaneous growth rates, G_{field} (g wet·g wet^{-1}·d^{-1}), of walleye larvae were estimated over each sampling interval in each pond as

[7.4] $$G_{field} = (\log_e(W_t) - \log_e(W_0)) / t$$

where W_t and W_0 are walleye mean weights (g) at the beginning and end of the sample interval, respectively, and t is the duration of the interval (d)(Ricker 1975). I assumed that G represented growth rate at the mid-point of the weight interval, $\bar{W}$, as estimated by the arithmetic mean of W_0 and W_t.

Analyses

I compared my C_{field} estimates with other direct estimates of consumption from laboratory feeding experiments (Chapters 3 and 4) and published field studies (Table 3 of Fox 1991), and my C_{model} estimates. To convert feeding rates of Chapters 3 and 4 into daily rates I assumed the larvae could feed at the observed rate for 17 h in a day. To facilitate comparison with previous studies, all consumption estimates were standardized to 25 °C using the temperature dependence function of Kitchell et al. (1977). I also examined the relationship between C_{field} and C_{model} and mean zooplankton biomass (mg dry·L^{-1}) over the sampling intervals. Methods of estimating zooplankton biomass are summarized in Appendix A. Zooplankton densities (prey·L^{-1}) in the Methley ponds are summarized in Table A.5. I modelled these relationships using the Holling functional response model of Chapter 3 (Equation [3.1]).

I examined the relationship between G_{field}, and both C_{field} and C_{model}. This analysis was analogous to the growth vs. ration analysis of Chapter 6. Note that consumption, C, in this chapter is the wet weight equivalent of ration, R, in Chapter 6. I modelled G_{field} as a function of C_{field} using a segmented model (NLIN procedure, SAS Institute Inc. 1985) and estimated maintenance consumption, C_{maint} (equivalent to maintenance ration, R_{maint}) and maximum growth rate, G_{max}, from this relationship as in Chapter 6. I also estimated C_{maint} and G_{max} directly from the bioenergetics model using the Johnston parameterization. I estimated C_{maint} by setting input weights constant over 2-d intervals at various temperatures and using the p-fit procedure to obtain estimates of food consumption at G = 0. To estimate G_{max}, I first set p = 1 and simulated growth over 2-d intervals at various temperatures. Maximum growth rates (g wet fish·g wet fish^{-1}·d^{-1}) were then estimated from the predicted weights at age in the model output and equation [7.3].

Results

Field estimates of food consumption were considerably lower than those determined for similar-sized walleye in laboratory feeding studies. Consumption was highest in short-term feeding experiments (Fig. 7.1b, data of Chapter 4), lower in slightly longer-duration feeding experiments (Fig. 7.1a, data of Chapter 3), and lowest from field studies (Fig. 7.1c,d). Field estimates in this study were generally in the same range as those reported by Fox (1991) for similar-sized walleye in culture ponds.

Estimates of relative consumption, p, derived from the Kitchell parameterization of the bioenergetics model were very high. This version predicted that the walleye of the Methley culture ponds would have had to eat above maximum consumption to attain the observed growth for 19 of the 32 growth intervals examined. In 2 cohorts, consumption would have had to exceed twice maximum consumption. This indicates that the Kitchell parameterization underestimated consumption, and/or overestimated respiration and waste losses for the Methley pond walleye. Using the Madon and Culver parameterization yielded similar results. However, when the Madon and Culver version was used with ACT = 1 rather than ACT = 3 (Table 7.1), the bioenergetics model predicted p values of 0.28 to 1.16 with only 3 of 32 values exceeding 1. My parameterization of the model incorporated both increased inputs (consumption) and increased outputs (egestion) relative to the original Kitchell version. The

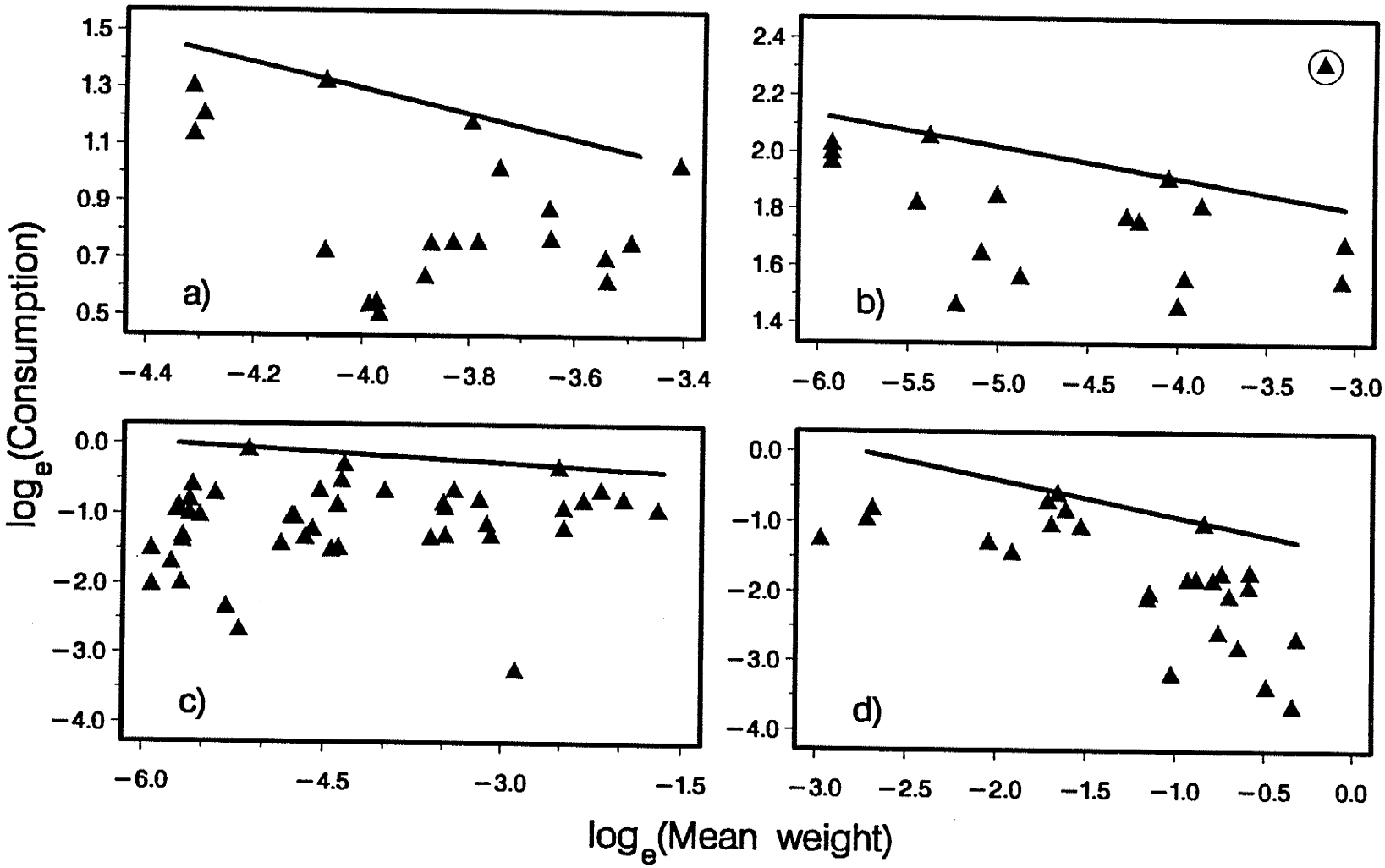

Figure 7.1. Consumption (g wet·g wet^{-1}·d^{-1}) vs. mean weight (g wet) of walleye larvae. Data are from a) Chapter 3 laboratory studies (not all data shown), b) Chapter 4 laboratory studies, c) Methley culture ponds, and d) White Lake (Ontario) culture ponds (Fox 1991). All data were adjusted to 25 °C using the temperature function of Kitchell et al. (1977). Symbols represent means of 2-13 fish in a) and b), and single determinations in c) and d). Lines represent relationships between maximum consumption and weight (see text). Axis scales differ between plots. Circled point was considered an outlier.

Johnston parameterization predicted relative consumption, p, to range from 0.31 to 1.33. Predicted consumption was higher than maximum ($p > 1$) in 5 of the 32 cohorts (16%).

I chose to further alter my version of the bioenergetics model by changing the maximum consumption function. To determine how this function should be changed, I used the relationships between consumption and body weight illustrated in Fig. 7.1. I defined the maximum consumption function for each of these data sets by the regression line with the lowest possible intercept, which had a slope between 0 and -1, and enclosed all data points (modified from the method of (Post 1990)). The maximum consumption functions, thus defined, varied greatly between data sets (Fig. 7.1). The allometric functions defining these relationships were $C = 0.63 \cdot W^{-0.44}$ (Fig. 7.1a), $C = 4.38 \cdot W^{-0.11}$ (Fig. 7.1b), $C = 0.59 \cdot W^{-0.092}$ (Fig. 7.1c), and $C = 0.24 \cdot W^{-0.52}$ (Fig. 7.1d). Comparison of these model parameters with the intercept and slope values (CA and CB, respectively) used in previous bioenergetics models for larval percids (Table 7.1) indicates that the maximum consumption observed in both this and Fox's (1991) field studies falls below that predicted by the models of Post (1990) and Madon and Culver (1993).

Though the preceding analysis was interesting, it did not provide any clear support for changing the consumption function of the bioenergetics model in any particular way. The mean of the slopes (CB) in the four allometric functions listed above was -0.29. This value was very close to the parameter value of -0.27 used by both Kitchell et al. (1977) and Madon and Culver (1993). I therefore chose to retain the parameter value of -0.27 for CB, and increased the intercept parameter, CA, until the highest predicted value of p was ≤ 1. This occurred at CA = 0.78. When this new value was substituted into the Johnston model, the predicted relative consumption, p, ranged from 0.18 to 0.99. I used these p estimates to run the model.

Model estimates of daily food consumption were much higher than field estimates (Fig. 7.2). Model estimates, C_{model}, averaged 4.3 times higher than C_{field} estimates for the same days. The greatest discrepancy was observed among smaller walleye larvae (< 0.08 g mean wet weight). The relationship between model and field estimates appeared to be fairly weak and resembled a logarithmic or quadratic relationship more than a linear relationship (Fig. 7.2).

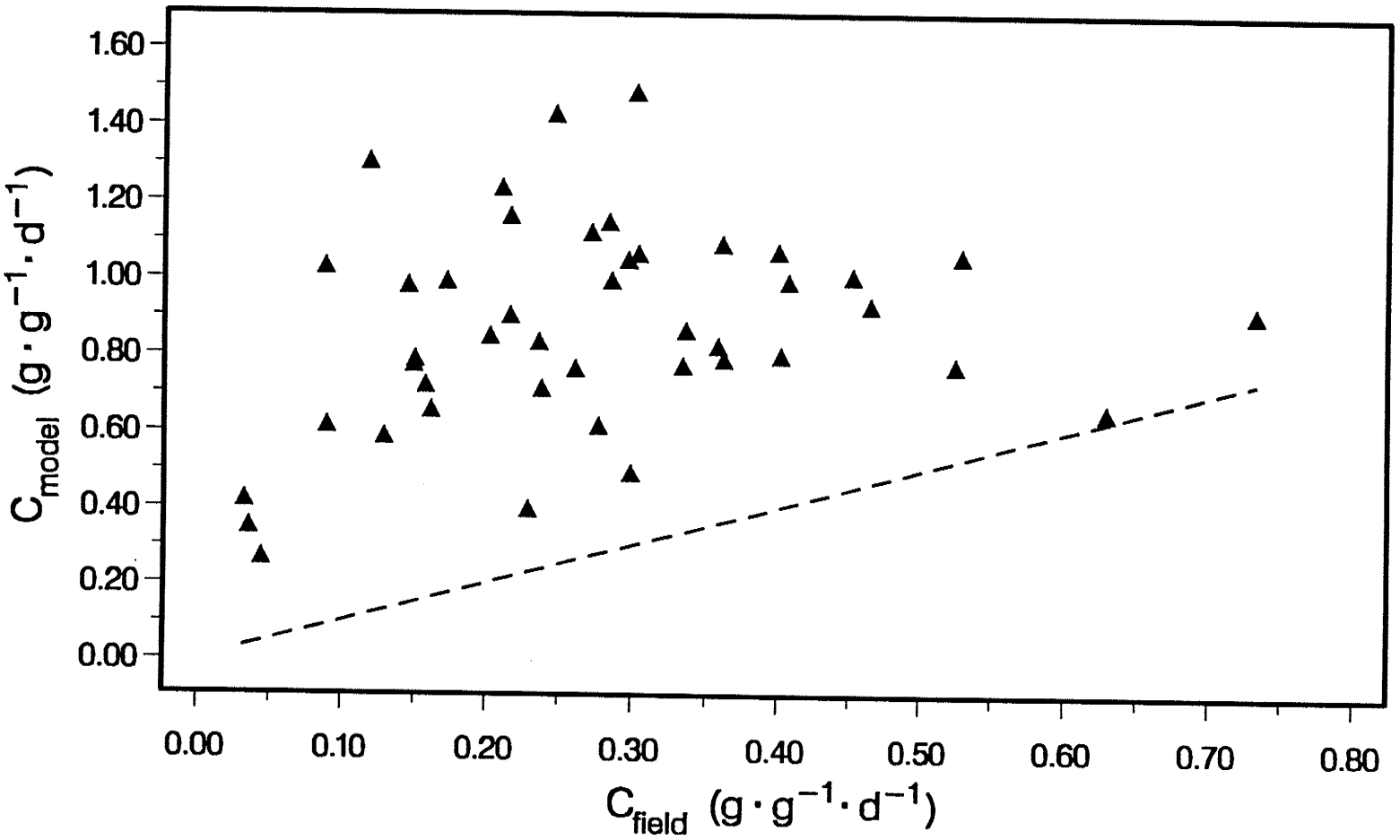

Figure 7.2. Relationship between food consumption (g wet food·g wet fish^{-1}·d^{-1}) estimated by a bioenergetics model (C_{model}) and food consumption estimated directly from field samples (C_{field}) for walleye larvae in the Methley culture ponds. Consumption was modelled from walleye growth using the Hewett and Johnson (1987) version of the Kitchell et al. (1977) bioenergetics model and the parameter estimates of Table 7.1. Walleye mean weights ranged from 0.003 to 0.180 g wet. Dashed line indicates 1:1 agreement between model and field estimates.

The relationship between food consumption and zooplankton abundance was highly variable both for field (Fig. 7.3a) and model (Fig. 7.3b) estimates of consumption. Some of this variation may have been due to the effects of temperature and body size. I adjusted the consumption estimates to 25 °C using Kitchell et al.'s (1977) temperature dependence function prior to conducting further analyses. However, I did not adjust for weight effects as I found no significant relationship between either C_{field} or C_{model} and walleye mean weight, and reducing the data set to a narrower size range of fish did not appear to clarify the functional response relationship. The functional response model did not account for a significant amount of variation in the consumption vs. zooplankton abundance relationship for C_{field} (ANOVA, $F = 3.49$, $df = 1, 42$, $P = 0.069$, $R^2 = 0.08$). Functional response analysis did reveal a significant relationship between C_{model} and zooplankton abundance, but the coefficient of determination for this model was still very low (ANOVA, $F = 6.59$, $df = 1, 42$, $P = 0.014$, $R^2 = 0.14$). Reducing the data set to include only those fish samples where > 90% of the diet was composed of zooplankton did not reduce the residual variation substantially. Maximum consumption, C_{max}, and the prey abundance at which 90% of C_{max} is attained, D_{90}, for the latter model were 1.26 g wet food·g wet fish^{-1}·d^{-1} and 0.0089 mg dry zooplankton·L^{-1}, respectively. Another approach to analyzing the relationship between consumption and food abundance has been to examine the relationship between relative food consumption, the p values generated by the bioenergetics model, and prey abundance (as per Madon and Culver 1993). Using data from all cohorts, I found no statistically significant relationship between p and zooplankton abundance (ANOVA, $F = 3.61$, $df = 1, 30$, $P = 0.067$, $R^2 = 0.11$). However, when the data set was reduced to include only those cohorts in which > 90% of the diet was zooplankton, the relationship was marginally significant (ANOVA, $F = 5.14$, $df = 1, 22$, $P = 0.034$, $R^2 = 0.19$)(Fig. 7.4).

Growth of the postlarvae varied considerably among the three years of the study. The most rapid growth was observed in 1988. Mean wet weights ranged from 0.0026 g to 0.18 g (Pond 3, 1988). The highest mean wet weights observed in 1987 and 1989 were 0.099 g (Pond 1) and 0.041 g (Pond 1), respectively. There was a positive, linear relationship between field estimates of growth, G_{field}, and the mean temperature during the growth intervals (ANOVA, $F = 18.5$, $df = 1, 38$, $P < 0.001$, $R^2 = 0.33$), but residuals did not show any clear

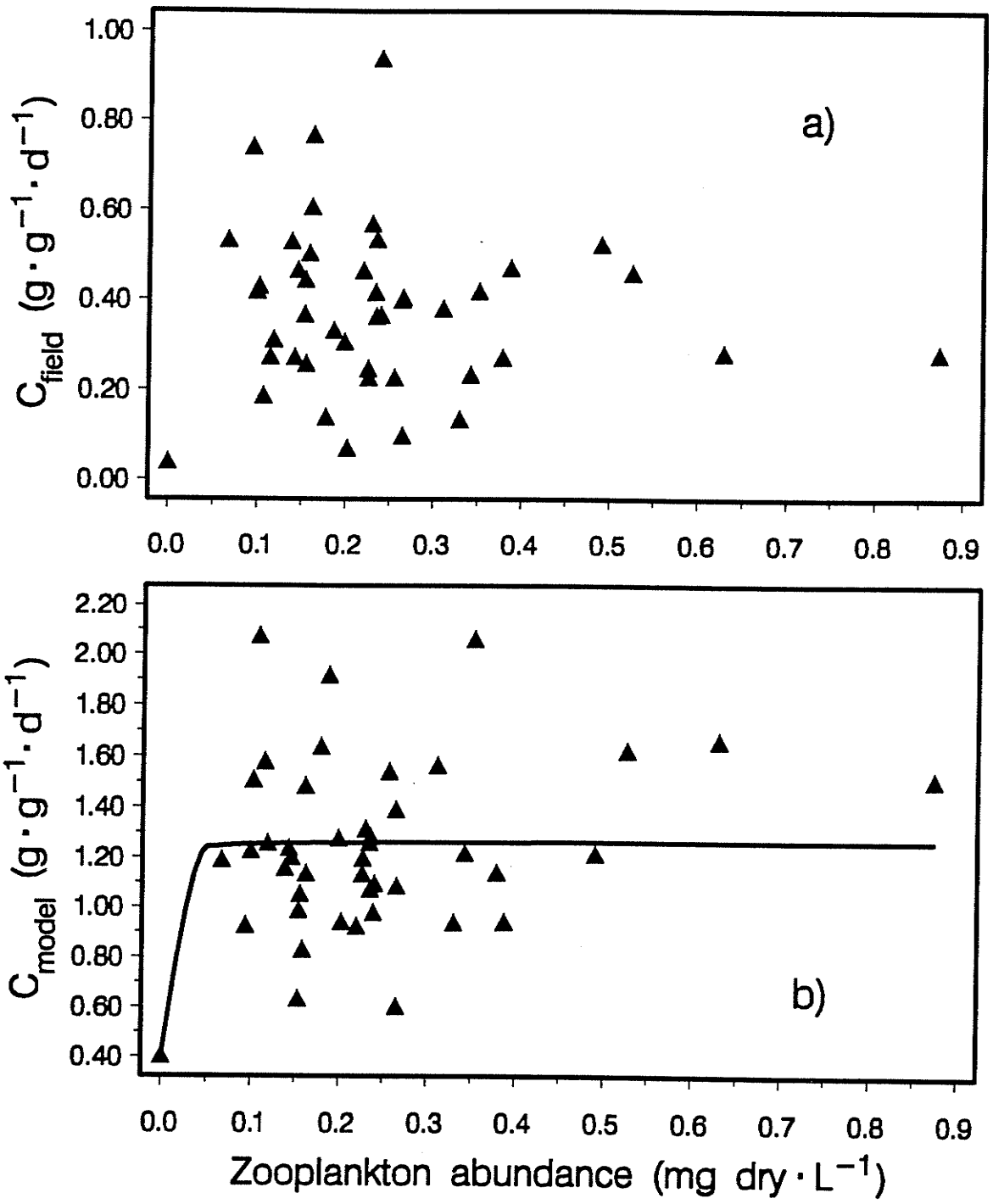

Figure 7.3. Scatter plots of consumption (g wet food·g wet fish^{-1}·d^{-1}) vs. zooplankton abundance (mg dry·L^{-1}) for a) consumption estimated directly from field samples (C_{field}), and b) consumption estimated from a bioenergetics model (C_{model}). Fitted relationship in b) is defined in text. Mean walleye wet weight ranged from 0.0026 to 0.18 g.

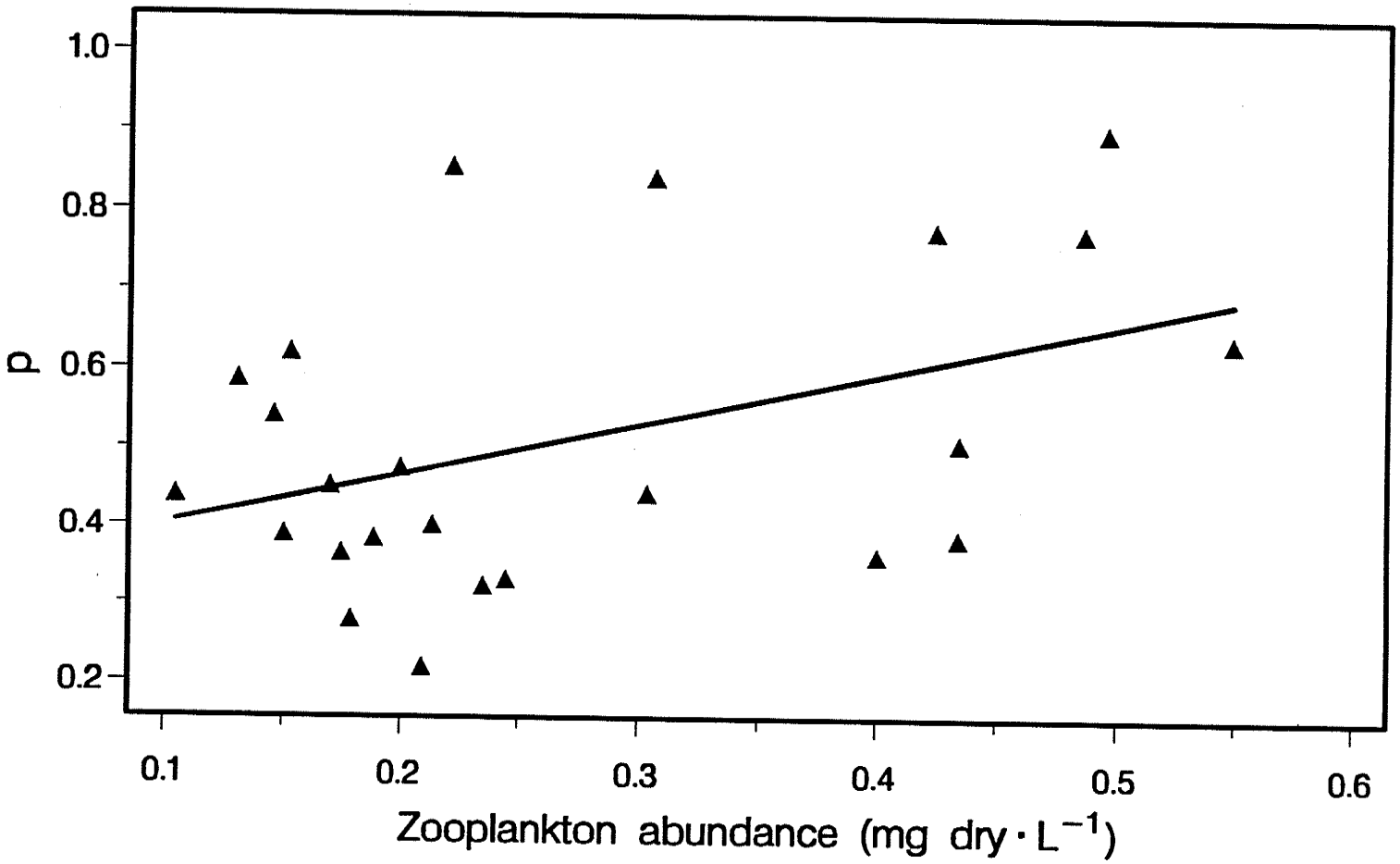

Figure 7.4. Relationship between the relative food consumption, p (defined in text), of walleye larvae and zooplankton abundance in the Methley culture ponds. Data from dates on which < 90% (wet weight basis) of the walleye's diet was composed of zooplankton were omitted from this plot. Line represents fitted least squares relationship. Relative consumption was modelled from walleye growth using the Hewett and Johnson (1987) version of the Kitchell et al. (1977) bioenergetics model and the parameter estimates of Table 7.1.

trends with respect to mean walleye weight or zooplankton abundance. The relationship between field estimates of growth, G_{field}, and consumption, C_{field}, is illustrated in Fig. 7.5. Growth rate increased then levelled off with respect to C_{field} (Fig. 7.5a). The relationship was described by the equation $G_{field} = -0.211 + 3.59 \cdot C_{field} - 7.60 \cdot C_{field}^2$ for $C_{field} < 0.24$ g wet food·g wet fish^{-1}·d^{-1}, and $G_{field} = 0.21$ g wet fish·g wet fish^{-1}·d^{-1} for $C_{field} \geq 0.24$ g wet food·g wet fish^{-1}·d^{-1} (ANOVA, $F = 3.67$, df = 2, 29, $P = 0.038$, $R^2 = 0.20$). Extrapolating this relationship back to $G_{field} = 0$ gave a maintenance consumption, C_{maint}, estimate of 0.069 g·g^{-1}·d^{-1} (0.037 mg dry·mg dry^{-1}·d^{-1}). In comparison with laboratory growth vs. ration studies (Chapter 6), growth and consumption in the ponds were quite high. Most estimates of C_{field} were near or above the plateau of the relationship and there were no instances of negative growth (cf. Fig. 6.1 and Fig. 7.1a). Adjusting G_{field} and C_{field} for the allometric effects of walleye mean weight, W, by multiplying both by $W^{0.2}$ (as per Hewett and Kraft 1993) did reduce the residual variance (Fig. 7.5b). There was a significant linear relationship between $G_{field} \times W^{0.2}$ and $C_{field} \times W^{0.2}$ (ANOVA, $F = 19.9$, df = 1, 30, $P < 0.001$, $R^2 = 0.40$). Residuals suggested that a linear relationship may not have been ideal. However, I was unable to find a more appropriate model. Gross growth efficiencies, estimated as G_{field} / C_{field}, for these data ranged from 0.16 to 1.43, and 9 of the 32 estimates were > 1. This suggests that field estimates of growth were overestimated and/or field estimates of food consumption were underestimated.

Predicted relationships between C_{maint} and weight and G_{max} and weight generated by the Johnston version of the bioenergetics model are illustrated in Fig. 7.6. Both C_{maint} and G_{max} declined exponentially with walleye weight and increased with temperature. Model estimates of C_{maint} were > 0.10 g wet·g wet^{-1}·d^{-1} over the entire range of temperatures and body sizes examined, and thus higher than the C_{maint} estimate derived from the G_{field} vs. C_{field} relationship. Laboratory-derived estimates of C_{maint} for walleye larvae of ~ 0.80 mg dry weight (0.0053 g wet) were 0.074, 0.090, and 0.11 mg dry zooplankton·mg dry fish^{-1}·d^{-1} at 15, 18.5, and 22 °C, respectively (Chapter 6). Model estimates were somewhat higher. After conversion to dry weights, the model estimates of C_{maint} for similar-sized larvae in the culture ponds were 0.11, 0.15, and 0.19 mg dry zooplankton·mg dry fish^{-1}·d^{-1} at 15, 18.5, and 22 °C, respectively. Similarly, my laboratory-derived estimate of G_{max} was substantially lower than that predicted by the bioenergetics model in this analysis. In Chapter 6, I estimated G_{max} to

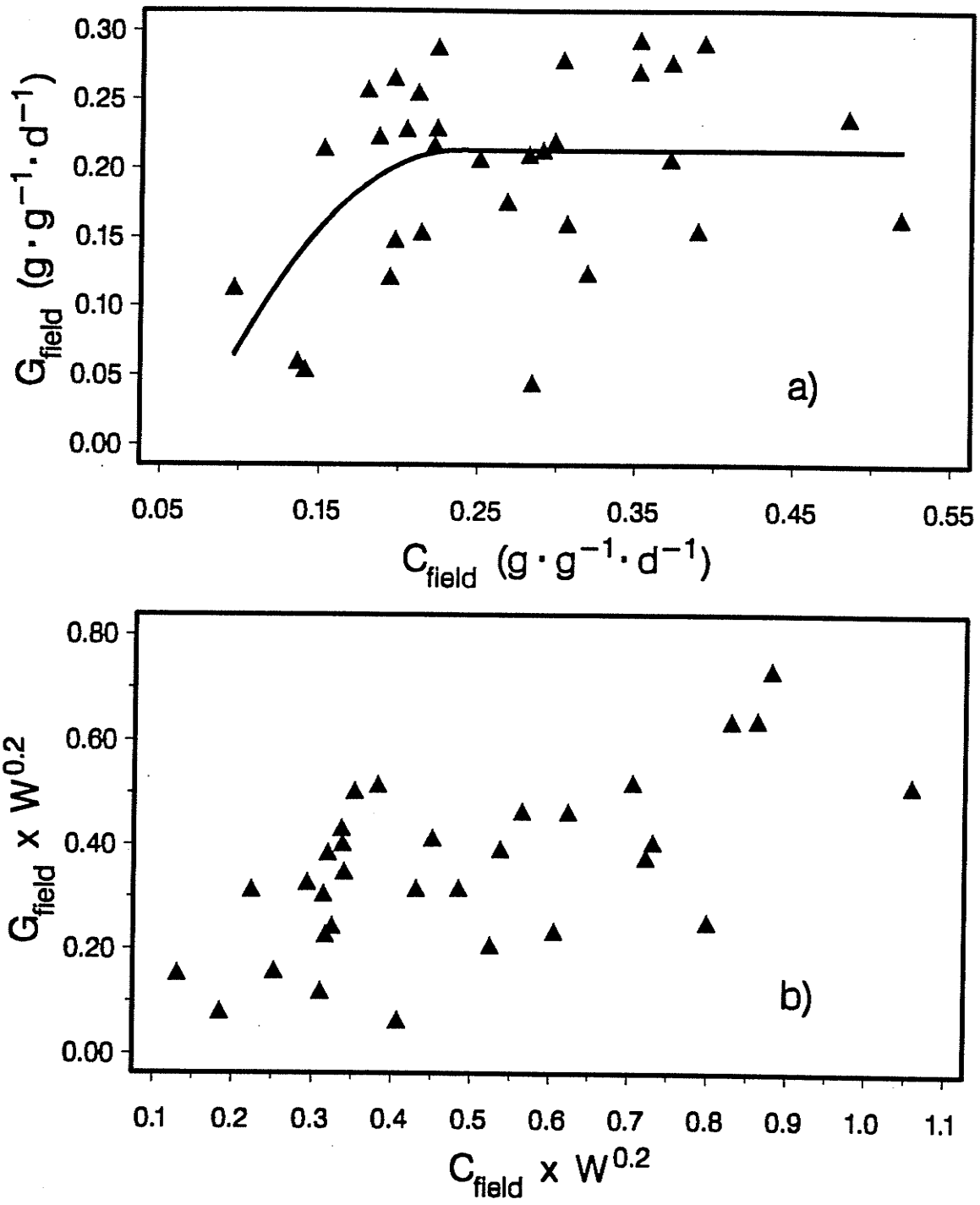

Figure 7.5. Scatter plots of a) growth in the field (G_{field}, g wet fish·g wet fish^{-1}·d^{-1}) vs. consumption in the field (C_{field}, g wet food·g wet food^{-1}·d^{-1}), and b) standardized growth vs. standardized consumption where the variables of plot a) were both multiplied by mean wet weight (W) raised to the power 0.2. Fitted relationship in a) is defined in text. Mean walleye wet weight ranged from 0.0026 to 0.18 g.

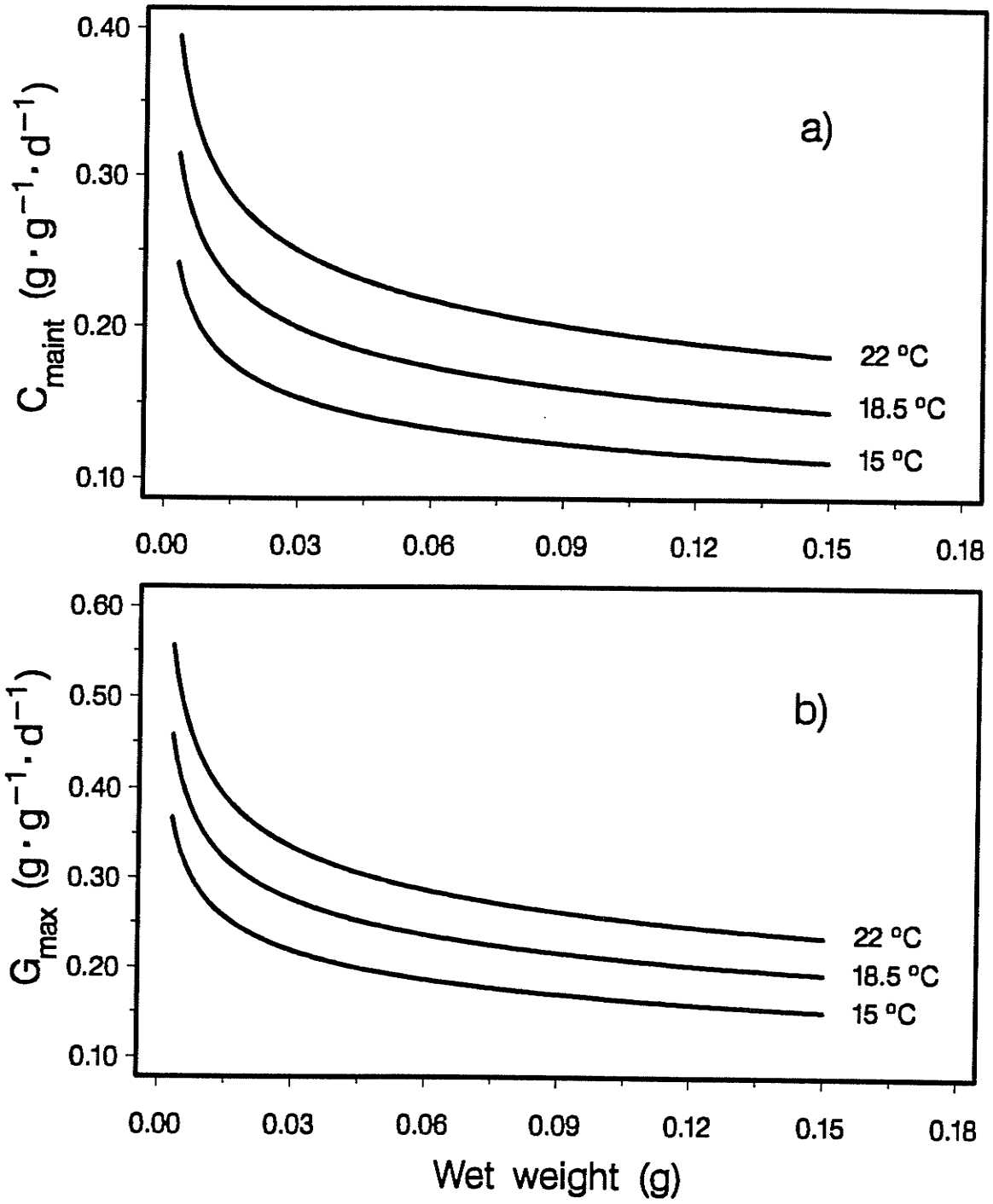

Figure 7.6. Relationships between a) maintenance consumption (C_{maint}, g wet food·g wet fish^{-1}·d^{-1}) and wet weight, and b) maximum growth rate (G_{max}, g wet fish·g wet fish^{-1}·d^{-1}) and wet weight at three temperatures. Values were generated from the Johnston parameterization of the Hewett and Johnson (1987) bioenergetics model (Table 7.1).

be 0.13 $mg \cdot mg^{-1} \cdot d^{-1}$ for 0.80 mg dry weight larvae at 22 °C. My version of the bioenergetics model predicts a G_{max} of 0.49 $mg \cdot mg^{-1} \cdot d^{-1}$ for the same fish size and temperature.

Discussion

Estimates of maximum daily food consumption derived in this study were lower than those estimated in my earlier laboratory studies (Chapters 3 and 4). This was true for both direct estimates of consumption, C_{field}, and those derived from bioenergetics modelling, C_{model}. Maximum feeding rates observed in the laboratory were higher than predicted by any of the maximum consumption functions of the larval percid bioenergetics models used in this and previous studies (Table 7.1). The difference between laboratory and field results may be partly explained by differences in zooplankton abundance. Zooplankton densities in laboratory aquaria were usually higher than mean densities in the culture ponds. However, I feel that the primary difference was the satiation level of the fish. In my laboratory studies of Chapters 3 and 4, consumption reflected the early stages of feeding before satiation. Walleye larvae were food-deprived prior to the experiment and allowed to feed for a relatively short time. Consumption estimates in this study represented feeding rates on a daily basis. The laboratory studies demonstrated that walleye larvae are capable of capturing and ingesting food at very high rates for short periods of time whereas this study demonstrated that they do not, or cannot, consume at these rates over the long-term. I suspect that long-term consumption rates in the culture ponds were primarily limited by the ability of the walleye's gut to digest, assimilate, and evacuate the ingested food, and the rates of these processes would increase with temperature (see Discussion of Chapter 5). The hypothesis that food consumption is limited by digestion rate, rather than food availability, has been suggested before (Boehlert and Yoklavich 1983). For a given temperature, there is probably an optimal gut evacuation rate that allows the highest assimilation per unit time. Though the larvae are capable of consuming above this rate, doing so would lead to reduced assimilation. Thus, consumption slows in response to increasing gut fullness.

The preceding discussion was not meant to imply that the long-term food consumption of walleye larvae is never food-limited. The functional response seen in short-duration feeding studies (Chapter 3) should also be evident for continuously-feeding fish in the field. However, the shape of the functional response may differ for two reasons. First, clumping in the

distribution of prey would tend to reduce the mean prey abundance at which maximum consumption is attained in the field (see Discussion of Chapter 3). Second, the maximum consumption rate would be lower in continuously-feeding fish because they are limited by their gut evacuation rate. Both of these changes to the functional response would reduce the range of prey abundances over which consumption increases in response to prey abundance. This was evident in the relationships between consumption and prey abundance derived from culture pond walleye in this study. The functional response model fit to the pond data indicated that most of the increase in consumption occurred at prey densities < 0.05 $mg \cdot L^{-1}$ (~ 37 $prey \cdot L^{-1}$ for cyclopoid copepods). Furthermore, this relationship was very weak both for C_{field} and C_{model}, even when adjusted for temperature. Similarly, the relationship between relative consumption, p, and prey abundance was very weak. Some of the residual variance about these relationships may have been due to differences in walleye size, prey size, or errors in the estimation of prey abundance. However, I feel that my results simply reflect the relatively high prey abundances observed in this study, and that the consumption vs. food abundance relationship would be stronger at lower food abundances. The reduction in the ascending arm of the functional response relationship under field conditions could explain why this relationship is generally weaker in field than laboratory studies (MacKenzie et al. 1990).

The relationship between growth and consumption observed in the culture ponds agreed qualitatively with the pattern observed in previous growth vs. ration studies (see Discussion of Chapter 6). A segmented model accounted for a significant amount of variation in growth rate though there was considerable scatter about the relationship. Variation could result from a variety of factors including measurement error (particularly in C_{field}), and differences in temperature, walleye mean weight, food availability, and food caloric density between point estimates (Hewett et al. 1991; Hewett and Kraft 1993). Adjusting for the allometric effects of walleye mean weight by $W^{0.2}$ (as per Hewett and Kraft 1993) did clarify the growth vs. consumption relationship. However, this may simply be a case of self-correlation. Using ever-larger adjustment factors such as $W^{0.4}$ (as suggested by Jobling 1983), or $W^{0.8}$ increase the strength of the correlation even further, and it is difficult to say whether or not this approach provides any real support to the growth vs. consumption relationship. The ranges of growth and consumption estimates from the ponds were much higher than those estimated in

laboratory growth vs. ration experiments (Chapter 6). Many treatment groups of walleye exhibited low consumption and negative growth in the laboratory studies whereas negative growth was never observed in the culture ponds. Walleye growth did increase in relation to food consumption in the culture ponds, but, it appeared that the greatest effect would have been at consumption levels below those observed in this study.

The growth versus consumption relationship derived directly from field data (G_{field} vs C_{field}) yielded a maintenance consumption, C_{maint}, estimate that was roughly half the estimate obtained at 15 °C in the laboratory experiments of Chapter 6. Maintenance consumption estimates from my parameterization of the bioenergetics model were 49-73% higher than those predicted from my laboratory studies at similar temperatures. I feel that the C_{maint} estimate from the G_{field} vs. C_{field} relationship was biased low because of the underestimation of C_{field} (see below). Explaining the higher C_{maint} estimates from the bioenergetics model is slightly more problematic. Compared to the laboratory fish, the culture pond fish may have consumed a zooplankton diet with a lower caloric density, or may have expended more in activity costs because they were not restricted to aquaria. I remain more confident with the laboratory-derived estimates of C_{maint} for two reasons. First, my method of estimating C_{maint} in the laboratory was more direct, and did not require assumptions about respiration and waste losses. Second, the range of consumption and growth estimates in the laboratory were closer to maintenance conditions than those in the ponds. For the same reason, I feel that field estimates of maximum growth rate, G_{max}, from this study are more accurate than those from my laboratory experiments (Chapter 6) because the pond fish generally consumed more. Maximum growth rate estimates derived from both the G_{field} vs. C_{field} relationship and the bioenergetics model were higher than those derived in the laboratory.

The interpretation of my analyses depends on the accuracy of my food consumption and growth estimates in the field. Growth is generally the easier of the two to estimate as it requires only lengths or weights at age, in addition to the usual assumptions of random sampling and size-independent mortality. Food consumption is more difficult, requiring estimates of gut evacuation rate and daily variation in gut fullness. Examination of the relationship between C_{model} and C_{field}, and gross growth efficiencies in this study suggested that my methods substantially underestimated C_{field}. This could be the result of inappropriate

assumptions about the periodicity of feeding, or poor estimates for gut evacuation rate, R. I assumed that walleye larvae fed continuously during the day. Examination of the daily trend in gut fullness seemed to confirm this. It is possible of course that walleye larvae exhibited a daily feeding periodicity which varied from day to day, depending on environmental conditions, or which varied with fish age. If this was the case, then my method of estimating mean daily gut fullness from single daily samples (as per Hayward and Hiebert 1993) may have been inappropriate. However, I suspect that most of the error in estimating C_{field} can be attributed to my method of estimating R. My examination of gut evacuation (Chapter 5) demonstrated that R declines during food deprivation. Because I estimated R from a model developed from food deprivation trials, my estimates of R in the pond fish may be low. Madon and Culver (1993) compared three methods of estimating R in pond walleye and chose to use the method that gave the largest R, and hence the highest food consumption estimates. Clearly, a better method of estimating food consumption of fish in the field is required.

Bioenergetics models appear to offer an alternative method of estimating energy budget components, including food consumption. However, the accuracy of estimating any energy budget component depends on the accuracy with which the remaining model components are estimated. Some are easier to measure accurately and precisely than others. The problems of estimating growth and consumption in the field have already been discussed. Estimation of the remaining components, respiration, egestion, and excretion, usually requires laboratory experiments. Few studies measure or examine all components directly, and one is usually estimated by difference. The problem with this approach is that cumulative errors in estimating the other components are shunted into the component measured by difference (Brett and Groves 1979). Because the energy budget is forced to balance, variation suppressed in one or more components will reappear in the one estimated by difference. This can lead to widely differing interpretations of results. Several studies have now used the Kitchell et al. (1977) bioenergetics model to examine the energy budget of larval percids (Post 1990; Fox 1991; Madon and Culver 1993; this study). Post (1990) and Madon and Culver (1993) estimated respiration costs by difference from field estimates of growth and consumption, and observed higher respiration costs than those predicted by the original Kitchell respiration function. Fox (1991) and this study estimated food consumption from growth and found that model

predictions of food consumption were higher than those estimated directly from field data. Fox (1991) interpreted this outcome as an overestimation of respiration costs by the Kitchell model and proposed using an alternative respiration function.

Many bioenergetics modelling studies have concluded that the discrepancy between observations and model predictions can be attributed to poor estimation of activity costs by the model (Boisclair and Leggett 1989; Post 1990; Madon and Culver 1993). Activity is represented by a constant multiplier in the respiration function of the Kitchell model (ACT in Table 7.1). However, it has been suggested that activity costs of actively-foraging fish in the field are highly variable, and positively related to food consumption or food abundance (Kerr 1982; Boisclair and Leggett 1989; Madon and Culver 1993). Numerous studies have shown that metabolic expenditures increase with food consumption (reviewed by Brett and Groves (1979)), and decrease during food deprivation (see Discussion of Chapter 6). However, it is not clear what forms of activity are involved in these changes. The activity component of the bioenergetics model would primarily include energy for swimming, capturing and digesting food, and anabolizing tissue. These latter two expenditures may be substantial. Respirometry of larval fish has indicated that the routine metabolism of fed larvae is more than twice that of unfed larvae (Giguère et al. 1988). Clearly, prey capture, digestion, and anabolism costs would be greater for a fish living in an environment where food was abundant. However, if most swimming is associated with searching for prey, then swimming costs may not increase monotonically with prey abundance. In fact, I would expect that swimming costs would decline as prey abundance increases and search time per unit of ingested prey decreases. Clarifying the relative importance of these various activities to total metabolic expenditures, and determining how they change in relation to food abundance could improve the calculation of the metabolism component of bioenergetics models.

Variability in the activity component may have been overemphasized in previous studies, primarily because variation in other components was suppressed. The Kitchell parameterization of the bioenergetics model estimates egestion and excretion as functions of relative consumption, p, (as per Elliott 1976) and temperature. However, combined egestion and excretion losses at 20 °C only range from 26% of consumption, C, at p = 0 to 30% of C at p = 1, and variation in the egestion and excretion components seems to have little effect

on the variability of model predictions (Bartell et al. 1986). Accordingly, many studies have estimated egestion and excretion losses as constant proportions of consumption (e.g. Kerr 1982; Rice et al. 1983; Dabrowski et al. 1988; Post 1990; Hartman and Brandt 1993; Madon and Culver 1993). I feel that some of the variability in activity costs observed in previous studies actually represents misallocation of other expenditures, primarily waste losses. Using results from my assimilation experiments (Chapter 5), I modified the egestion component of the Kitchell model such that combined losses from egestion and excretion at 20 °C range from 43% of C at p = 0 to 62% of C at p = 1. These values are based on weight, not energy, and variation with respect to p may differ if the larvae change the selectivity (lipid vs. protein) of their assimilation with respect to p. However, fish larvae may not assimilate lipid as well as protein, and lipid assimilation may actually decline with increasing consumption (see Discussion of Chapter 5). If my version of the egestion function is correct, potential variation in waste losses is much greater than was previously thought. Furthermore, waste losses follow the same positive trend with consumption as suggested for the activity multiplier. Thus, variation in activity costs with respect to food consumption or food abundance may be partly explained by poor estimation of waste losses. Other components or parameters in the bioenergetics model which are currently treated as constants may also be quite variable. Specific dynamic action (S in Table 7.1), for example, is usually modelled as a constant proportion of digestible energy but probably declines with increasing consumption (Beamish and MacMahon 1988). I feel there is sufficient potential for both measurement error in energy budget components such as consumption, and for misallocation of other components such as waste losses, to conclude that interpretations of variation in the single component measured by difference should be viewed with caution. Activity may indeed be a highly variable component of the energy budget but other expenditures may also vary markedly.

Bioenergetics models in their current state suffer from a lack of species-specific and life-stage-specific studies on the various energy budget components. Parameter values taken from the literature may not apply to the species or life stage under study. Reviews of particular energy budget components, such as metabolism (e.g. Giguère et al. 1988), illustrate the potential interspecific variation in parameter estimates for larval fish. Parameters for temperature functions used in the model, particularly CQ and CTO (Bartell et al. 1986), should

also be examined. A further problem is the choice of energy densities (Hewett et al. 1991). In this study I used a walleye:zooplankton energy density ratio of ~ 1.7. Previous models for larval percids used ratios of 1 (Post 1990; Fox 1991) and 1.3 (Madon and Culver 1993). Obviously, the choice of caloric densities can have a great effect on model predictions. However, wet weight caloric densities used in bioenergetics models are frequently estimated from the literature. Because most published energy densities of fish and invertebrates are based on dry weight (reviewed by Cummins and Wuycheck (1971)), assumptions about moisture contents can have a big influence on caloric densities used in bioenergetics models. Furthermore, because preserved fish larvae and zooplankton may lose considerable dry weight while changing little in wet weight (Appendices B and C), moisture contents estimated from preserved samples may be biased high. I feel confident that my choice of energy densities for walleye larvae and zooplankton were reasonable estimates of the mean energy densities during the study period. However, both energy density and moisture content can vary substantially. In salmonids, lipid:protein ratios, and thus energy densities, increase with consumption (Brett et al. 1969). Moisture contents of fish generally decrease with consumption or fish condition (Brett et al. 1969; Salam and Davies 1994). Furthermore, both can vary seasonally. Thus, a constant energy density or moisture content for walleye larvae and their prey under all food regimes and over all dates seems unlikely. Because energy densities are a central part of the bioenergetics approach, future modelling exercises should put more effort into direct determinations of the energy contents of fish, food, and faeces whenever possible.

The results of this research have several implications for understanding the consumption and growth of postlarval walleye. Food consumption in short-term, laboratory studies is generally higher than daily consumption in the field. Consumption in natural environments may be limited as much by digestion as the ability to find and capture prey. The functional response of walleye to their zooplankton prey is evident in the field but the range of prey abundances over which consumption increases appears to be quite narrow. The greatest effect of zooplankton abundance on walleye consumption probably occurs at mean densities < 0.05 mg·L^{-1} (~ 37 cyclopoid copepods·L^{-1}). The strong relationship between growth and temperature and the weak relationship between growth and food abundance provides further

evidence that mean zooplankton abundances in the range observed in the ponds are generally not limiting to walleye larvae. The relationship between growth and consumption in the field qualitatively resembles the pattern predicted from laboratory studies. But, growth in this study was never < 0.05 $\%\cdot d^{-1}$, and appeared to be limited by consumption only at the lowest range of observations. In addition, this study indicated that maximum growth rates in the field are much higher than my laboratory-derived estimates (Chapter 6). Finally, my method and possibly other currently-used methods of estimating consumption of fish larvae in the field appears to be inadequate. Current methods should be validated and more reliable methods investigated. In the future, bioenergetics models may provide a means to reliably estimate consumption in the field. However, in their present form, bioenergetics models still appear to be a rather crude tool for analyzing larval fish energetics.

Chapter 8:
General Discussion

Limits to Feeding and Growth: Prey Abundance and Distribution

Walleye inhabit a wide range of lakes differing greatly in productivity (Rawson 1960). Thus, different populations are likely to encounter quite different mean densities of zooplankton during the postlarval period. Unfortunately, few studies have sampled freshwater zooplankton communities with respect to their suitability for larval fish. Most surveys tend to sample the entire water column and concentrate their sampling during the summer months, whereas most larval fish utilize only the epilimnion and many, including walleye, enter the pelagic community in the spring. These shortcomings must be kept in mind when examining the results of zooplankton surveys.

I have summarized published estimates of the mean zooplankton abundances during the walleye postlarval period for several walleye lakes in Manitoba, northwestern Ontario, and the northern United States (Table 8.1). Estimates range from 3.1 prey·L^{-1} for oligotrophic Sydney Lake to > 50 prey·L^{-1} for most sampling dates on eutrophic Dauphin Lake. The differences in abundances between lakes in this summary may be a bit exaggerated. Sampling generally included the entire water column, and thus, abundance estimates from deeper, oligotrophic lakes would be expected to be lower simply because a larger proportion of the volume sampled represents hypolimnion. I suspect that a comparison amongst these lakes based on epilimnetic samples would still show lower zooplankton abundances in the oligotrophic lakes, but the differences among lakes would be less marked.

Invertebrate prey frequently exhibit patchy distributions both spatially and temporally. For zooplankton, spatial distributions may vary both vertically and horizontally. Plankton patchiness is evident at small scales (cm to m) as well as much larger scales both in the ocean (Steele 1978; Owen 1989) and in lakes (reviewed by Malone and McQueen (1983)). In the ocean, the patchiness of zooplankton distributions at fine scales generally increases at lower wind speeds, during daylight, and under more oligotrophic conditions (Owen 1989). From their review, Malone and McQueen (1983) defined four basic types of zooplankton patches in lakes; i) large scale (> 1 km diameter), ii) small scale (10-1000 m diameter), caused

Table 8.1. Estimated mean abundances of crustacean zooplankton (excluding nauplii) in various North American lakes with self-sustaining walleye populations. Data were selected from the sampling dates nearest to the walleye postlarval period. Estimates were corrected for gear bias and sampling efficiency wherever possible.

Source	Location	Lake	Date	Abundance (prey·L^{-1})
Friesen and Mathias (1990)	S Manitoba	Dauphin	26-28 May 1982	51
			10-11 June 1982	67
			25-26 May 1983	12
			20-23 June 1983	71
			2-3 May 1984	51
			3-6 June 1984	80
Patalas and Salki (1992)	Manitoba	Winnipeg (N basin)	4-12 June 1969	9.6
		Winnipeg (S basin)	4-12 June 1969	32
Salki (1992)	NW Ontario	Trout	14 June 1989	6.7
		Orange	14 June 1989	9.9
		Linge	14 June 1989	37
		Musclow	14 June 1989	5.3
		Sydney	14 June 1989	3.1
Selgeby (1974)	North and South Dakota	Oahe	May 1969	13
			June 1969	62

by wind-induced water movement, iii) Langmuir circulation aggregations, and iv) swarms (0-10 m diameter), potentially caused by biotic factors. These patches tend to vary in their species composition, and their persistence, as well as in the conditions under which they form (reviewed by Malone and McQueen (1983)). Swarms represent the extreme case where prey densities within a patch may be several orders of magnitude higher than the mean density in the surrounding water body. Distribution patterns may vary with lake size. Patalas and Salki (1993) observed greater abundances of crustacean zooplankton in offshore regions of smaller lakes, but in inshore regions of larger lakes. Patchiness in zooplankton distribution may be even stronger on the vertical axis of lakes (Pinel-Alloul and Pont 1991). Zooplankton will undergo active diel vertical migration to avoid predators (reviewed by Lampert (1989); Bollens et al. 1992; Dini and Carpenter 1992). In lakes, zooplankton abundances may be affected by inflowing rivers. Patalas and Salki (1992) noted relatively high zooplankton densities in Lake Winnipeg near the inflows of the Saskatchewan and Red Rivers which drain sedimentary regions, and relatively low zooplankton abundances near the inflow of the Winnipeg River which drains nutrient-poor soils of the Canadian Shield. Freshwater zooplankton populations also show distinct temporal distributions. Abundances are generally lower in early spring and increase from spring through early summer (Friesen and Mathias 1990; Mazumder et al. 1992; Patalas and Salki 1992).

The results of my research indicate that walleye postlarvae would probably require mean prey abundances > $30 \cdot L^{-1}$ to avoid starvation (Chapter 6) but may require prey abundances up to $100 \cdot L^{-1}$ to maximize growth (Appendix A). However, the mean prey abundances necessary to feed and grow at maximal rates and to meet maintenance food requirements decline with walleye size (Chapters 3, 6, and 7). Reported mean abundances in lakes (Table 8.1) are frequently < $30 \cdot L^{-1}$ and all are below the $100 \cdot L^{-1}$ level. Thus, if walleye had to feed at these prey densities, starvation may occur and growth would almost certainly be sub-optimal. For a given mean prey abundance, low levels of aggregation would allow walleye to feed at higher rates (see Discussion of Chapter 3). Thus, in environments with low mean zooplankton abundance the feeding success of walleye larvae may depend on the heterogeneity of their prey's distribution. This is thought to be the case for the larvae of some marine fishes as well (Lasker 1981; Peterman and Bradford 1987). Diel vertical migration of

zooplankton may further reduce zooplankton availability to walleye larvae unless the larvae follow the zooplankton migration pattern. Little is known of the ability of fish larvae to associate with zooplankton patches in nature. However, the presence of self-sustaining walleye populations in many oligotrophic lakes (Rawson 1960; Ryder 1972) suggests that at least a small proportion of walleye larvae come to be passively or actively associated with high density patches, or that they are able to feed successfully at very low zooplankton densities. Observed patterns of temporal variation in zooplankton abundance may also pose a problem for walleye larvae. As they are one of the earliest species to enter the spring ichthyoplankton of many lakes, walleye larvae may experience lower or more variable zooplankton abundances than other freshwater species. However, this effect may be offset for riverine-spawned larvae if their spawning river contains water which is warm and/or nutrient-rich relative to the receiving lake. In such cases, the walleye larvae would enter one of the more productive zones of the lake.

Limits to Feeding and Growth: Prey Size, Species, and Biochemical Composition

Size and species distributions in the zooplankton communities of lakes can vary greatly. Lakes with abundant planktivores generally have pelagic communities dominated by smaller zooplankton species (Lynch 1979; Johannsson and O'Gorman 1991). The size distribution of zooplankton may also differ between the littoral and the pelagic zones within lakes depending on relative predation pressures in the two environments (Gliwicz and Rykowska 1992). The scarcity of large-bodied zooplankton could result in reduced growth and survival of YOY fishes which require progressively larger prey during development (Crowder et al. 1987). Zooplankton species also differ greatly in their susceptibility to capture (see Discussion of Chapters 3 and 4) and this can affect their value as a food source to fish larvae.

Throughout this research I have assessed food limitation based on total biomass or energy of zooplankton consumed with little regard to the biochemical composition of the prey. This has been a common approach in feeding studies of fish. For example, most foraging models utilize net energy intake per unit of time as a measure of feeding success and assume that no essential nutrients are limiting. Generally, carnivorous fishes are assumed to be energy-limited whereas herbivorous fishes are assumed to be nutrient-limited (Townsend and Winfield 1985). However, recent research suggests that reduced growth and starvation

mortality of larvae, even among carnivores, may result from the deficiency of one or more dietary nutrients. The susceptibility of a fish species to nutrient limitation will depend on its own dietary requirements, the availability of the nutrient in the prey population, and the ability of the species to assimilate the nutrient.

For larval fish, essential fatty acids (EFA) are considered the major limiting nutrient in a zooplankton diet (Watanabe et al. 1983). The specific fatty acids which are essential appear to vary among fish species. In general, freshwater species require linolenic acid (18:3ω3) and to a lesser extent linoleic acid (18:2ω6) whereas marine species require highly unsaturated fatty acids (HUFA), such as 20:5ω3 and 22:6ω3 (reviewed by Cowey and Sargent (1979), Watanabe et al. (1983), and Kanazawa (1985)). Awaiss et al. (1992) observed that perch larvae grew faster on diets rich in linoleic acid than on diets with lower linoleic acid contents. Similarly, the growth and survival of marine fish larvae improves with increasing dietary levels of HUFA (Davis and Olla 1992; Koven et al. 1992; Tuncer and Harrell 1992; Mourente et al. 1993). Deficiencies of EFAs appear to affect the size and proper functioning of the swim bladder (Katavić 1986; Davis and Olla 1992) and may affect vision (Navarro et al. 1993). Davis and Olla (1992) noted that walleye pollock, *Theragra chalcogramma*, larvae fed on lipid-deficient prey sank head-first and had to swim almost continuously to maintain their vertical position in the water column. Abnormal swimming behaviour and poor survival was also observed in herring larvae with low body levels of HUFA (Navarro and Sargent 1992). Condition of marine fish larvae in the field has been related to their lipid content and the lipid content of their prey (Håkanson 1989). In comparison to fatty acids, much less research has been conducted on other essential nutrients in larval fish diets. Some studies have pointed to amino acids as limiting nutrients. Dabrowski and Rusiecki (1983) suggested that the sulphur amino acid contents of most freshwater zooplankton (except rotifers) do not meet the requirements of fish. Research on marine fish larvae has indicated that free amino acids (FAA) may be necessary energy sources for first-feeding larvae and that the suitability of a prey type may depend on its FAA content (Fyhn 1989). There is also some evidence that young fish select prey based on their FAA content (Holm and Walther 1988).

Research on the biochemical composition of zooplankton has primarily examined proximate composition (i.e. total protein, lipid, and carbohydrate) rather than the availability of

essential nutrients for larval fish. The lipid content of zooplankton varies between species and varies with respect to the environment or diet of a given species. Energy density of freshwater zooplankton generally declines during the spring season (Schindler et al. 1971; Snow 1972; Appendix A of this study) suggesting that the lipid content of zooplankton also declines over this period. However, direct measurement of the lipid fraction has indicated that this pattern may vary between lakes (Wainman et al. 1993). Copepods generally have higher lipid contents (Yurkowski and Tabachek 1979) and higher energy densities (Schindler et al. 1971; Appendix A of this study) than cladocerans. Lipid contents of marine copepods vary with respect to feeding conditions which in turn can vary with patterns in water circulation (Willason et al. 1986; Attwood and Peterson 1989). Freshwater zooplankton may have lower lipid contents because of selective predation (Arts and Sprules 1988) or as a response to the chemical stimuli of predators (Dodson 1989). Thus, zooplankton may have a lower lipid content in lakes with abundant planktivores. Protein contents of zooplankton vary much more widely than lipid contents but do not appear to differ with respect to zooplankton species (Yurkowski and Tabachek 1979; Simpson et al. 1982). The essential amino acid (EAA) index of freshwater zooplankton is very high (based on salmonid requirements) suggesting that they are an adequate source of these nutrients (Yurkowski and Tabachek 1979). Not all ingested nutrients may be from prey. Some marine fish larvae can absorb dissolved amino acids by drinking water (Korsgaard 1991). The relative importance of this form of nutrition is unknown.

Prey quality could be a major limiting factor to walleye feeding and growth. Walleye select larger prey than other species of fish larvae and select progressively larger prey as they grow (Chapters 3 and 4). Small zooplankton, as is typically found in lakes with large planktivores, appears to be positively selected only during the earliest stages of exogenous feeding. Very small zooplankton, such as rotifers or copepod nauplii, are rarely eaten by walleye larvae even when they are very abundant (Mathias and Li 1982; Appendix A this study). Walleye may be forced to commence feeding on benthos or fish at an earlier age in lakes where large zooplankton are rare. There is some evidence that growth of postlarval walleye varies with prey size (Fox 1989). Even as postlarvae, walleye exhibit faster growth on fish diets than zooplankton diets (Hokanson and Lien 1986), possibly because invertebrates

contain a larger amount of indigestible material. Thus, a rapid transition from zooplanktivory to piscivory would probably be advantageous to walleye growth and survival.

It is impossible to determine from the results of my research and those of previous studies whether or not postlarval walleye growth and survival in natural environments are limited by the nutrient composition of their food. The nutrient requirements of walleye larvae have not yet been examined and little is known of the essential nutrient composition of their various prey species. Because the larval diet differs greatly from the adult diet, the larval period may indeed represent a period of nutrient limitation and walleye which progress to the adult diet of fish early in life may have a distinct advantage. The possibility seems intriguing. If such nutrient constraints exist, it would be interesting to examine how they relate to the walleye's prey selection patterns.

Limits to Feeding and Growth: Environmental Factors

Temperature is probably the most important environmental factor affecting walleye feeding and growth. Some studies on planktivorous YOY fish have suggested that temperature is more important than food availability. Mooij et al. (1994) found that growth rates of YOY fishes over 13 yr in a shallow eutrophic lake was primarily related to temperature, not food abundance, in most species examined. This is probably true for many eutrophic systems. In the Methley culture ponds, walleye growth rate was much more strongly related to water temperature than zooplankton abundance (Chapter 7). Because gut evacuation rate increases with temperature (Chapter 5), food consumption rate can also increase with temperature. Temperature also increases the walleye's rate of prey capture independently of gut evacuation (Chapter 4). This may be an important factor in environments with low prey abundances.

Because fish larvae are primarily visual feeders, feeding success can vary with light intensity (reviewed by Blaxter (1986)). Some species of fish larvae prefer lower illumination than others (Huse 1994). Combined effects of temperature and light can influence daily feeding periodicity. In walleye larvae in culture ponds, I observed increasing gut fullness during daylight hours suggesting that consumption rate increased throughout the day, peaking near dusk (Chapter 7). Similarly, feeding intensity of cod and haddock, *Melanogrammus aeglefinus*, larvae has been observed to peak near the end of daylight hours (Kane 1984).

Walleye larvae < 30 mm are positively phototactic and prefer high illumination (Bulkowski and Meade 1983). However, in my laboratory experiments walleye larvae feed very well at relatively low light intensities (Chapters 3 and 4). I doubt that light is a limiting factor under most circumstances. An exception may be if larvae are forced to feed at greater depth to avoid predation. Piscivory in late juvenile and older walleye seems to improve under more turbid conditions (Vandenbyllaardt et al. 1991). It is unknown how turbidity affects feeding in zooplanktivorous larval walleye.

A number of biotic factors may also influence walleye feeding and growth. Body size is one of the most important (Werner and Gilliam 1984; Miller et al. 1988; Pepin 1991). Prey capture success and maximum ingestible prey size both increase with body size. Apart from temperature and past feeding success, the major determinant of larval size is egg quality (Kamler 1992). Walleye size at hatch has been related to yolk and oil volume of eggs (Moodie et al. 1989). Feeding and growth may also be limited by the ability of the larvae to digest, assimilate, and evacuate their food. These factors are also influenced by body size and temperature (Kamler 1992). Digestion may be a particular problem for zooplanktivorous walleye as they appear to assimilate invertebrates less efficiently than fish (Kelso 1972). Results from this study indicate that assimilation efficiency of walleye larvae on a zooplankton diet is lower than was previously assumed (Chapter 5). Finally, predator abundance can be an important determinant of feeding success. Walleye larvae may cease feeding or feed in regions of sub-optimal prey abundance to avoid attacks by predators (reviewed by Lima and Dill (1990)). For example, Williams and Brown (1991) observed a much reduced feeding rate in larval lumpfish, *Cyclopterus lumpus*, in the presence of a predator compared to in the absence of a predator. Because the major predators of fish larvae are probably planktivorous fish, avoiding predators may require avoiding the areas which are richest in zooplankton.

Postlarval Mortality: Starvation

Starvation may be a major source of mortality for many species of fish larvae (Hunter 1981). The effects of food deprivation may be irreversible long before a larva dies, and thus the length of time that a larva can withstand food-deprivation and survive (termed point of no return) is usually much shorter than the length of time required for a larva to die of starvation. In the laboratory, food-deprived anchovy larvae of 9 mm showed 50% survival over 4-5 d and

1% survival after 7 d at 16.2 °C (Booman et al. 1991). However, histological examination of these fish indicated that liver vacuoles (presumably containing glycogen) disappeared after 1 d of starvation and liver tissue began to deteriorate after 4 d of starvation. These sub-lethal effects may be important in future survival.

The results of my research indicate that walleye postlarvae may be less susceptible to starvation mortality than was previously thought. Maintenance rations for walleye larvae estimated in my research (Chapters 6 and 7) are much lower than those estimated for smaller larvae of marine species. For example, Peterson and Ausubel (1984) estimated that Atlantic mackerel, *Scomber scombrus*, larvae required a maintenance ration of 25-75%·d^{-1}. Walleye larvae also appear to have lower maintenance rations than other freshwater species (see Discussion of Chapter 6). Censusing of culture ponds did not reveal any period of high mortality at the onset of feeding as suggested by the critical period hypothesis (Chapter 2). However, walleye mortalities during short periods of food deprivation appear to be related to the rate of weight loss (Chapter 6). Thus, short periods of weight loss caused by low prey abundances could have an adverse effect on walleye larvae. Such situations may arise in very oligotrophic lakes. Studies which have linked larval fish survival to prey abundance in freshwater have generally examined oligotrophic systems. Freeberg et al. (1990) linked growth and survival of lake whitefish larvae to zooplankton abundance in Lake Michigan where zooplankton densities ranged from 0.91 to 5.0 prey·L^{-1}.

Postlarval Mortality: Predation

Though both starvation and predation are considered major causes of larval mortality (Hunter 1981), a disproportionate amount of the research on larval mortality has examined the former mechanism. More recently, the role of predation in larval fish mortality has received greater attention (reviewed by Bailey and Houde (1989)). As with the majority of larval fish studies, much of the research examining predation mortality has focussed on marine species. Fish larvae are susceptible to both invertebrate and vertebrate (mostly fish) predators but the former are capable of preying upon a much narrower size range of fish larvae. Marine fish larvae are exposed to a diverse group of invertebrate predators including cnidarians, ctenophores, copepods, amphipods, chaetognaths, and euphausiids (Bailey and Houde 1989), whereas the major invertebrate predators of fish larvae in freshwater pelagic ecosystems are

probably cyclopoid copepods (Hartig et al. 1982; Hartig and Jude 1984; Labay and Brandt 1994). Smaller larvae (3-8 mm) are most susceptible to cyclopoid predation (Hartig et al. 1982). High densities (> 500·L^{-1}) of cyclopoid copepods will also attack and kill larger larvae such as walleye (8-10 mm) under laboratory conditions (Hokanson and Lien 1986; pers. obs. this study). In natural environments, such dense aggregations of copepods are probably localized and walleye larvae would be able to avoid them. Thus, I feel that cyclopoid copepod predation would be a minor source of larval walleye mortality in natural lakes and rivers. A number of benthic invertebrates could act as predators of walleye larvae, particularly during the earliest larval stages when larvae are negatively buoyant and spend a great deal of time on the bottom (Chapter 2). In the culture ponds examined in this study, beetle larvae (Coleoptera: Dytiscidae) were the most abundant benthic invertebrates capable of consuming fish larvae. The impact of predation by benthic invertebrates on the culture pond walleye larvae is unknown and I could find no previous studies which examined this potential source of mortality in larval fish.

Juvenile and adult fish are probably the most important predators of fish larvae. As prey, fish larvae are essentially large plankton and are probably a preferred prey item of many planktivores. They have a body shape which allows easy ingestion by gape-limited predators, are soft-bodied and easier to digest than either fish eggs or crustacean zooplankton, and often contain rich sources of proteins and lipids in the form of yolk-sacs and oil globules. In freshwater ecosystems, the major predators of pelagic fish larvae would probably be obligate planktivores such as cisco, *Coregonus artedii*, emerald shiners, *Notropis atherinoides*, alewives, *Alosa pseudoharengus*, and smelt, *Osmerus mordax*, and facultative planktivores such as yellow perch and various species of minnow (Cyprinidae) and sunfish (Centrarchidae). Benthic fishes of the littoral zone may also be important predators if larval fish drift inshore.

The vulnerability of fish larvae to predation is a function of their encounter rate with predators and their susceptibility to capture once encountered (Bailey and Houde 1989). Encounter rates increase with predator abundance, the degree of overlap between predator and larval fish distributions, and the ability of the predator to detect larval fish. Susceptibility to capture depends on the reaction time of the larva, and the burst swimming speeds of both the predator and the larva. The percentage of fish larvae which respond to attacks increases

with larval length or age (Folkvord and Hunter 1986; reviewed by Bailey and Houde (1989); Margulies 1990; Johnson et al. 1993) and larval condition. For a given body length, well-fed larvae are stronger swimmers (Laurence 1972) and are more likely to respond to and escape predators than poorly-fed larvae (Booman et al. 1991). The speed of the escape response also increases with larval size (Williams and Brown 1991). Generally, the susceptibility of a larva to capture declines as the ratio of larva size to predator size increases (Pepin et al. 1992). As larvae grow they become easier to detect (encounter rate increases) but more difficult to capture (susceptibility decreases) (Folkvord and Hunter 1986). Thus, larval vulnerability may not decline monotonically with increasing larval size but may be maximum at some intermediate size (Monteleone and Houde 1992). The vulnerability of fish larvae to predation may also depend on the availability of alternate prey such as zooplankton, benthos, or the larvae of other fish species. Predation pressure on fish larvae generally declines with increasing abundances of alternate prey (Pepin 1987; Kean-Howie et al. 1988; Luecke et al. 1990; Margulies 1990), particularly if the alternate prey are as large or larger than the fish larvae (Gotceitas and Brown 1993).

Fish larvae may employ a variety of mechanisms to reduce their vulnerability to predation (reviewed by Fuiman and Magurran (1994)). To avoid encounters with predators larvae may possess physical characteristics or behaviours which make them difficult to detect, or they may actively separate themselves spatially from their predators. Larvae may also be separated from predators by the timing and location of their parents' spawning. Cessation of movement in the presence of a predator seems to be a larval behaviour for avoiding detection (Williams and Brown 1991). Presumably, larvae avoid capture once detected by trying to swim out of the predator's path.

Because vulnerability to predation declines with decreasing light levels (Monteleone and Houde 1992), migrating downward in the water column during the day may reduce predation risk. Fish larvae exhibit diel vertical migrations in both directions (reviewed by Neilson and Perry (1990)). Some studies have observed fish larvae at greater depth during the day than the night (e.g. Kendall et al. 1994), whereas others have observed the opposite trend (e.g. Brodeur and Rugen 1993). Moving shallow during the night and deeper during the day (Type I migration) appears to be more common than the reverse pattern (Type II migration), and

where the latter is observed it can usually be related to similar movements by the fish's prey (Neilson and Perry 1990). The vertical position held by fish larvae at any one time probably represents a compromise between the risk of predation, food availability, and preferred physical conditions.

Predation can have dramatic effects on larval fish populations. Crowder (1980) attributed the collapse of several native Lake Michigan fishes to the predation by exotic planktivores (smelt and alewife) on the pelagic eggs and larvae of the native species. Tonn et al. (1992) demonstrated the strong effect of perch predation on the recruitment of crucian carp, *Carassius carassius*, in an experimentally-divided lake. The longer a cohort of fish larvae remains in a size range vulnerable to predators, the greater the predation effect. Thus, rapid growth can reduce the rate of predation mortality during the larval period (Folkvord and Hunter 1986; Bailey and Houde 1989; Luecke et al. 1990; Margulies 1990; Rice et al. 1993). Food limitation may have its greatest effect on mortality indirectly through predation. Low food abundances can lead to poor growth and a longer period of vulnerability to predators.

With the exception of studies on cannibalism (e.g. Loadman et al. 1986), few studies have examined the interaction between walleye larvae and their predators. The relationship between the vulnerability of walleye larvae to predation and walleye size are unknown. Similarly, the behaviours employed by walleye larvae to avoid detection and to avoid capture once detected have not been examined. For pelagic larvae, such as walleye, vertical migration would seem to be the only mechanism available for avoiding encounters with predators. Walleye postlarvae in Oneida Lake appear to occupy the upper 3 m of water both day and night (Houde and Forney 1970). In this study, catch-per-unit-effort (CPUE) of walleye larvae in the water column of extensive culture ponds was much higher at night than during the day (Chapter 2). This may indicate that walleye larvae reside near bottom (unsampled region) during the day and/or that walleye larvae can avoid sampling gear during the day. Lake ichthyoplankton surveys have also noted that CPUE of larval fish in the water column is much higher at night than during the day (Houde and Forney 1970; Cole and MacMillan 1984). If walleye larvae do undergo diel vertical migration, less-biased sampling procedures will have to be employed to detect it. By spawning in rivers, walleye may reduce some predation pressure on the emergent prolarvae. However, predators from the receiving lake may

congregate near river mouths to feed on the concentrations of drifting larvae. The relative effect of this predator aggregation may be reduced if larval fish abundances are sufficiently high to swamp the functional and numerical responses of the predator population. Walleye larvae may be particularly vulnerable to predators during this period because they are one of the earliest species to enter the spring ichthyoplankton (M.N. Gaboury, Manitoba Natural Resources, Winnipeg, unpubl. data), and are thus initially unable to benefit from the dilution effect of other fish larvae which act as alternate prey.

Conclusions and future directions

My results suggest that the effects of food limitation during the walleye postlarval period may be less direct than has been previously suggested. Strong evidence of starvation mortality during the postlarval period was generally lacking in this study. However, I was unable to simulate low food abundances (< 30 prey·L^{-1}) in field situations. Postlarval walleye may experience a critical period of starvation mortality in some oligotrophic systems, but probably not in more productive systems. Though preferred sizes of prey may be lacking, walleye larvae appear capable of consuming a wide range of prey items in order to meet their maintenance requirements. The greatest effect of food limitation on postlarval survival may be indirect. Over the range of prey abundances experienced by walleye larvae in all lakes, conditions of positive but sub-optimal growth are probably more common than conditions of negative growth. Thus, the greatest effect of food limitation on survival may be to prolong the period when walleye are most susceptible to predation. The significance of food limitation during the postlarval period to future year-class strength is difficult to judge from this study. I suspect that postlarval feeding, growth, and survival, in general, would be most important to future year-class strength under conditions where larval hatch was low and density dependent effects were minimal. However, this remains to be tested by future studies.

I see my research as a starting point for numerous future studies. The interaction between walleye larvae and their predators deserves much attention, both at the individual and population level. Feeding studies should concentrate more on feeding and growth at low prey abundances (< 30 prey·L^{-1}). Most studies, including my own, have examined larval feeding ecology based on means rather than variances of prey abundance. An examination of how walleye larvae relate to patchy zooplankton distributions could provide an indication of how

they feed under conditions of low prey abundance. Studies requiring manipulation of prey abundance would be technically difficult but could be very revealing. The role of gut evacuation in limiting daily food consumption should also be examined. Specifically, it would be interesting to determine at what prey abundance daily food consumption is limited by digestion not prey abundance. Feeding studies should also develop more reliable methods of estimating daily food consumption in the field. This would vastly improve bioenergetics modelling. In general, the modelling approach requires many additional refinements. Moving from modelling population means to size distributions with individual-based models (e.g. Rice et al. 1993) may prove more useful for examining population-level effects of food limitation and predation. Finally, the relative importance of food abundance during the larval period could also be examined indirectly through life history studies. Because larval feeding success and survival depends on size at hatch (Miller et al. 1988), and size at hatch depends on egg size (Kamler 1992), walleye females may produce fewer, larger eggs in environments with low abundances of larval prey (Lack 1954). Thus, if postlarval feeding and survival is a strong determinant of walleye reproductive success, one would expect a negative correlation between egg size and the abundance of the postlarval food source. Examining egg size variation would make an interesting inter-population study for walleye because of their wide distribution.

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Appendix A:

Walleye culture ponds at Methley Beach, Manitoba: Management, sampling methods, environmental conditions, zooplankton dynamics, and postlarval walleye diet and growth

Introduction

Larval walleye are often one of the least abundant species found in the spring ichthyoplankton of lakes with self-sustaining walleye populations (Faber 1967; Leslie and Timmins 1992). This low abundance coupled with the spatial and temporal heterogeneity of natural lakes makes field research on larval walleye quite difficult. For this reason, I chose to conduct my field research on larval walleye by sampling extensive culture ponds. These are ponds used to rear walleye from the prolarval to the juvenile stage for the purpose of stocking lakes, rivers, and reservoirs. Though not identical to natural conditions, the culture pond environment reflects natural conditions much better than laboratory aquaria. In addition, culture ponds can be experimentally manipulated much more easily than natural systems. Interspecific predator and competitor abundances can be controlled, and food densities can be altered through fertilization.

The purpose of this appendix is to describe the research conducted on the extensive culture pond system at Methley Beach. Though the content is largely methodological, I have included some results which were not presented in previous chapters in order to better describe the environmental conditions of the culture ponds. I have focussed on information gathered during the walleye postlarval period. Data collected during the juvenile period including juvenile harvest and production figures are provided in Johnston et al. (1992).

Materials and Methods

Study Site

All field research on postlarval walleye was conducted at the Dauphin Lake Walleye Rehabilitation and Research Station at Methley Beach on the south-east shore of Dauphin Lake, Manitoba. The facility had four 1-ha rectangular earthen ponds for walleye culture. Each pond had a maximum capacity of 15 000 m^3 and ranged in depth from 1 m at the shallow end to 2 m at the deep end. The ponds were filled with water pumped from Dauphin

Lake and were drained by gravity through an underground pipe system which connected the deep end of each pond with Dauphin Lake. Water pumped into the ponds was filtered through 500 µm Nitex® mesh to prevent unwanted fish introductions. The ponds were drained during the late summers of 1985, 1987, 1988, and 1989 to harvest the fish and were refilled during the following autumn or winter of each year. Valves at the outlet of each pond allowed them to be drained separately. The underground pipe system fed into an open concrete catch kettle situated between the ponds and the lake. By screening off the kettle outflow, walleye accumulated in the kettle during draining. This allowed the fish to be sampled and enumerated prior to transport and stocking.

Pond Treatments

The ponds were used for walleye culture during the spring and summer of 1985, 1987, 1988, and 1989. The ponds were left filled in 1986 but were not stocked. Following ice-off in late April the ponds were fertilized by towing dehydrated, powdered alfalfa in a mesh bag behind an outboard motor boat. By weight, the alfalfa powder had a C: N: K: P ratio of 403.2: 12.5: 10.4: 1. The amounts of alfalfa added and the rate of addition differed among the four years (Table A.1). Equal amounts were added to each pond in 1985 and 1987. In 1988, the fertilization rate was varied in an attempt to introduce a gradient of productivity among the four ponds and in 1989 the ponds were not fertilized. Alfalfa was added in a single treatment after ice-off in 1987 but in four equal doses at one week intervals following ice-off in 1985 and 1988. In addition, 46.0 kg of ammonium chloride (12.0 kg N) and 3.7 kg of phosphoric acid (1.0 kg P) were added to each of ponds 1 and 3 on 13 May 1987.

Zooplankton from Dauphin Lake were introduced incidentally to the ponds in all years at the time of refilling. The zooplankton of Dauphin Lake consisted primarily of small-bodied plankters with low numbers of Cladocera relative to Copepoda (Friesen and Mathias 1990). *Daphnia pulex* and some other zooplankton species were collected from local farm dugouts and introduced to pond 1 on 15 May 1985 and pond 4 on 17 May 1985 to increase the abundance of large-bodied plankters in the pond communities.

Walleye were stocked within 1 to 3 d of hatching. The number of fish introduced was estimated volumetrically. Stocking densities differed among years (Table A.2) but were always less than 10 larvae·m^{-3}. In addition to walleye, an assortment of other species including

Table A.1. Alfalfa meal additions and total nutrient additions for each of the Methley Beach walleye culture ponds in 1985, 1987, 1988, and 1989. All added nutrients came from the alfalfa meal except in 1987 when inorganic fertilizer was also applied to ponds 1 and 3 (see text).

Year	Pond(s)	Alfalfa additions ($kg \cdot ha^{-1}$)	Total nutrient additions ($kg \cdot ha^{-1}$)	
			N	P
1985	All	750	22.5	1.8
1987	1,3	750	34.5	2.8
	2,4	750	22.5	1.8
1988	1,3	750	22.5	1.8
	2	0	0.0	0.0
	4	1500	45.0	3.6
1989	All	0	0.0	0.0

Table A.2. Origin of stock, stocking density and stocking date for prolarval walleye introduced into the Methley Beach walleye culture ponds in 1985, 1987, 1988, and 1989.

Year	Pond(s)	Stock	Stocking Density ($fish \cdot ha^{-1}$)	Stocking Date
1985	All	Crean Lake, Saskatchewan	74 000	22 May
1987	All	Crean Lake, Saskatchewan	84 000	25 May
1988	All	Lake Manitoba (Swan Creek)	40 000	13 May
		Crean Lake, Saskatchewan	40 000	20 May
1989	1	Lake Manitoba (Swan Creek)	60 000[1]	23 May
	2,4	Lake Winnipegosis (Duck Bay)	70 000	17 May
	3	Falcon Lake, Manitoba	88 000	1 June

[1] probably an overestimate; some larvae lost during stocking

fathead minnow, brook stickleback, Iowa darter, and emerald shiner, were added to the ponds as juvenile walleye forage in July, 1985. Incomplete drainage of the ponds in 1985 allowed forage fish to survive until refilling and many were still present in the ponds at the beginning of the 1987 field season. Large numbers of them were removed by trap-netting but they were not completely eliminated. Some juvenile walleye from 1985 also survived until 1987 in pond 1 (4 fish) and pond 2 (30 fish) and were present throughout the culture period. Thus the pond fish assemblages were quite diverse in 1987. Walleye was the only fish species in the ponds during the postlarval period (May-June) for 1985, 1988, and 1989.

Pond Sampling and Monitoring

Pond temperature was recorded at a depth of 75 cm throughout the postlarval period using a 7-day continuous-recording Weksler® temperature recorder (Model #06MNL). Secchi depth readings were taken at the deep end of each pond at weekly intervals in all years except 1989. Water samples were collected for phytoplankton and chemical analyses on several dates in 1985, 1987, and 1988.

Quantitative zooplankton samples were taken at approximately weekly intervals throughout the walleye postlarval period in all years. Additional samples were collected before and after the postlarval period in 1985 and 1987. All collected organisms were killed by immersion in 95% ethanol to prevent distortion of body shape then preserved in 3-5% formalin buffered with 2% saturated sodium borate solution by volume (de Bernardi 1984). Sampling was conducted by Department of Fisheries and Oceans' (DFO) personnel in 1985 and 1987 and by myself in 1988 and 1989. The sampling procedures changed over the study period.

In 1985 an integrated tube sampler was used. Collections were made by vertically lowering a clear acrylic tube (internal diameter 76 mm) to the pond bottom, capping the top end, raising the bottom end close to the water surface and capping the bottom end. The zooplankton were concentrated by pouring the tube of water through a 73 µm mesh net. Two samples, each consisting of four tubes, were taken for each pond on each sample date. Samples were taken at the deep end of the ponds early in the season and at mid-pond during the remainder of the year.

In an attempt to increase the zooplankton sample sizes from those collected in 1985, horizontal tows were conducted in 1987. Each tow consisted of hauling a 25 cm diameter, 73

µm mesh net horizontally from the deep end to the shallow end of the pond. To integrate the sample over depth the net was hauled for 22.5 m at each of the following depths; 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25 m. By late spring it was suspected that increasing amounts of filamentous algae were clogging the nets and reducing filtering efficiency. For all subsequent tows a flowmeter was mounted inside the mouth of the net to estimate volume filtered. In later calculations it was assumed that filtering efficiency was 100% for the first two sampling dates (23 April and 13-14 May) when filamentous algae was sparse. The efficiency for other tows taken prior to the use of the flowmeter were corrected by using flow readings from the next nearest sampling dates. Two replicate tows were taken for each sample date by pond combination.

The sampling procedure used in 1988 and 1989 was chosen as a compromise between techniques used in 1985 and 1987. Vertical hauls were taken with paired Wisconsin nets (25 cm diameter; 73 µm mesh). Each sample consisted of three hauls taken at three equally-spaced points along a transect parallel to the longitudinal axis of the ponds. Three samples corresponding to three transects were taken at each sample date by pond combination. Evans and Sell (1985) found that the filtering efficiency of a 73 µm-mesh net increased with decreasing haul depth and that efficiency was near 80% for a 6 m deep haul. Because of the shallow haul depth in this study (1-2 m), it was assumed that net clogging was negligible and that filtering efficiency was 100%.

In addition to the quantitative samples, several qualitative samples of zooplankton were collected in 1988. These zooplankton were sieved into S, M, and L fractions, killed by immersion in 95% ethanol, then frozen in ~15 mL of pond water at -18 °C. These samples were later used to determine dry weight-length relationships and energy densities.

Walleye were sampled primarily during the postlarval period and occasionally during the later juvenile period. In this study I defined the postlarval period as the life stage where zooplankton was the primary food source. This corresponded approximately to walleye in the 8-25 mm size range. Fish which had not begun exogenous feeding were classified as prolarvae and fish larger than 25 mm were classified as juveniles. Sampling was conducted by DFO personnel in 1985, by myself and DFO personnel in 1987, and by myself in 1988 and 1989. The first sample date in each year was set to roughly coincide with the onset of first

feeding and subsequent samples were taken at 4- to 10-d intervals in correspondence with the zooplankton sampling schedule. Postlarval fish were collected by trawling paired bongo samplers mounted on the front of an outboard motor boat. The bongo samplers were 315 µm mesh conical nets attached to aluminum mouth-reducing cones and were modified from the river drift samplers of Burton and Flannagan (1976). Late postlarval and juvenile fish were collected from shore using a 10 mm square mesh beach seine. Quantitative sampling to assess survival was conducted at night between 23:00 and 04:00 (Chapter 2). Qualitative sampling to examine gut contents was conducted between 09:00 and 20:00 h (Chapter 3). Captured fish were killed in a solution of MS-222 (ethyl *m*-aminobenzoate methanesulfonate) and either preserved in 3-5% buffered formalin (day samples) or frozen in 15 mL of pond water (night samples). The fish sampling program removed only 2-3% of the number stocked in any one year and was therefore not considered to be a significant source of mortality.

Analyses

Quantitative zooplankton samples were dyed with Rose Bengal prior to examination. Analyses were carried out on whole samples from 1985, 1988, and 1989 and on subsamples, representing 2.25% of the original sample, from the 1987 horizontal tows. In 1985 and 1987 the samples were sorted by sieving through Nitex® sieves. The procedure involved passing the zooplankton through progressively smaller mesh sizes and yielded five size fractions hereafter referred to as XL (extra large, >1050 µm), L (large, 505-1050 µm), M (medium, 315-505 µm), S (small, 202-315 µm) and XS (extra small, 73-202 µm). XL, L, M and S fractions were counted under a dissecting microscope at 10x power whereas the XS fraction was counted under a compound microscope at 40-100x power. When numbers in a given fraction were high the plankters were diluted in 40 mL of water and two 1-mL subsamples were removed, with replacement, for counting. In 1988 and 1989 entire unfractionated samples were each diluted in 2500 mL of water and ten 5.35-mL subsamples were taken, with replacement, using a glass tube. Taxonomic identifications were made using Pennak (1978) and Edmondson (1959). I revised my identifications of several species following consultation with zooplankton researchers (B.J. Hann, University of Manitoba, and A. Salki, Freshwater Institute, pers. comm.). Copepod nauplii and rotifers were enumerated, except in 1989, but not identified beyond this taxonomic level.

Using an ocular micrometer and a dissecting microscope at 40x power, length and width measurements were taken on a subsample of 200 plankters, excluding copepod nauplii and rotifers, for each pond and sample date in all years except 1985. The number of organisms measured for each taxon was determined by its relative abundance in the sample. Zooplankter length was measured from the anteriormost point of the body to the base of the caudal spine in Cladocera and to the base of the caudal setae in Copepoda (Lawrence et al. 1987). Zooplankter width was measured as the second longest body dimension. This corresponded to body width in Copepoda and to dorso-ventral depth in Cladocera. I assumed that walleye feeding was more limited by zooplankton width than length and thus estimated plankton size distributions based on width. Length-width relationships were determined to estimate width for all plankters on which it was not measured. The edible portion of the zooplankton community was defined differently in 1985 than in subsequent years. For 1985, the edible portion was defined as the sum of S, M, and L fractions. In all other years the edible portion was defined as all particles, except copepod nauplii and rotifers, with widths less than or equal to the maximum edible particle width (MAX, mm) defined as

[A.1] $$MAX = -1.570 + 0.239 \cdot FL$$

where FL is mean walleye fork length (mm). This relationship was developed from gut content data from field samples (Fig. A.5). If mean FL was > 12 mm, all plankters, except nauplii and rotifers, were considered edible. Copepod nauplii and rotifers were considered as inedible for all sizes of walleye as they were rarely utilized as food in this study or in previous studies (Mathias and Li 1982).

Log_e(dry weight) vs. log_e(length) relationships were constructed for the various crustacean taxa from the Methley ponds following the guidelines of McCauley (1984). Frozen zooplankton samples were thawed at room temperature. For each taxon, individual plankters were measured under a dissecting scope with an ocular micrometer and were sorted into 0.1 or 0.2 mm length categories. For each category, groups of 10-30 individuals were placed on pre-weighed aluminum foil trays (~1.3 mg), oven-dried for 8 hr at 60 °C, moved to a desiccator for 15-30 minutes and then weighed to 0.1 µg on a Cahn Electrobalance®. I converted these preserved dry weights to fresh dry weights using the fresh:frozen dry weight ratios of Appendix

C before calculating the dry weight-length regressions. When estimating the dry weight of plankters from these regressions I used the correction factor of Bird and Prairie (1985) to correct for bias due to transformation. For the less common zooplankton taxa, dry weight was estimated using the regression of the taxon which most closely resembled its body shape.

Some dry weight-length relationships were constructed using organisms which were not collected from the Methley ponds. For *Ceriodaphnia sp.* and ostracods, length-weight regressions were built using individuals sampled from a water retention pond along Waverley Boulevard in Winnipeg. For chironomid larvae and pupae, I used individuals taken from walleye gut samples from the Methley ponds. Only undigested organisms from the most anterior part of the walleye digestive tracts were used. However, because these organisms were stored in formalin, they were corrected for dry weight loss using the conversion formula for postlarval walleye of Appendix B. For the remaining invertebrate taxa, dry weight formulae were taken from the literature (Mathias 1971; Smock 1980; Eaton 1983; Lawrence et al. 1987). I modified the formulae of Mathias (1971) and Eaton (1983) to account for dry weight losses due to preservation. Dry weights of ingested larval fish were estimated from the weight-length relationship of larval walleye raised in the laboratory (Chapter 3). The dry weight formulae for all walleye food organisms are summarized in Table A.3.

Zooplankton collected for calorific determinations were thawed and oven-dried on glass slides at 60 °C for 24 h. Samples were then homogenized by pulverizing with a mortar and pestle and pressed into pellets of ~ 8-12 mg. The pellets were placed on pre-weighed platinum trays, dried again for a minimum of 8 h, desiccated for 1 h, weighed to the nearest 0.01 mg on a Cahn Electrobalance®, then burned in a Phillipson microbomb calorimeter (Gentry Instruments, Inc., Aiken, S.C.) following standard calorimetric techniques (Prus 1975). Platinum trays were reweighed after bombing to determine the total weight of unburned residue. Energy density was calculated on both a total dry weight and a residue-free dry weight basis. Calorific values were corrected for changes due to freezing using the fresh:frozen energy density ratios of Appendix C.

Walleye collected during day sampling were analyzed for diet composition. Each fish was measured to the nearest 0.1 mm using an ocular micrometer and dissecting scope for the smaller larvae and a scientific ruler mounted under a dissecting scope for the larger larvae

Table A.3. Fitted parameters (a,b), correction factors (CF), and regression statistics for $\log_e$(dry weight) vs. $\log_e$(length) relationships used to estimate biomass of various organisms consumed by postlarval walleye. Dry weight (DWT) in μg is estimated from length (L) in mm by the formula $DWT = a \cdot L^b \cdot CF$ with the exception of *Hyallela azteca* where L is replaced by head length or 0.10·L. The correction factor was estimated using the formula of Bird and Prairie (1985) and was assumed to equal 1 when it was not calculable. Dry weight data used to build relationships in this study were corrected for losses due to preservation as outlined in Appendix C. Relationships taken from other studies were corrected for expected weight losses due to preservation where necessary (see text).

Taxon	a	b	CF	F	(df_{error})	R^2	Collection	Source
Crustacean Zooplankton								
Bosmina sp.	12.63	2.70	1.016	56.91	(6)	0.90	Methley ponds	this study
Ceriodaphnia sp.	10.85	2.57	1.017	85.57	(9)	0.90	Waverley pond	this study
Diacyclops sp.	4.27	3.70	1.062	171.43	(21)	0.89	Methley ponds	this study
Daphnia sp.	4.38	3.63	1.016	820.67	(26)	0.97	Methley ponds	this study
Diaptomus sp.	3.47	3.36	1.068	78.71	(13)	0.86	Methley ponds	this study
Copepod nauplii	3.42	2.25	1.005	-	(58)	0.98	Lake 223, Ontario	Lawrence et al. (1987)
Other Invertebrates								
Hyallela azteca	393.69	3.27	-	-	-	-	Marion Lake, B.C.	Mathias (1971)
Ostracods	27.78	2.80	1.023	196.00	(7)	0.97	Waverley pond	this study
Chironomid larvae	0.95	2.81	1.123	59.91	(13)	0.82	Methley ponds	this study
Chironomid pupae	3.49	2.78	1.163	44.85	(29)	0.61	Methley ponds	this study
Chaoborus sp.	0.93	2.43	-	-	-	0.89	Lake Norman, N.C.	Eaton (1983)
Sigara sp.	38.01	2.53	-	-	(51)	0.64	various rivers, N.C.	Smock (1980)
Caenis sp.	6.90	2.61	-	-	(12)	0.69	various rivers, N.C.	Smock (1980)
Larval Fish	0.25	3.25	1.011	599.08	(310)	0.66	Methley ponds	this study

(generally >15 mm). Walleye length was measured as total length until a definite fork appeared in the tail and as fork length thereafter. Contents from the entire digestive tract were removed, identified, and counted. Food particles were measured using a dissecting scope and ocular micrometer. All food organisms, up to a maximum of 10 individuals per taxon, were measured from each of the walleye guts. Crustacean zooplankton lengths and widths were measured as described above. For other invertebrates and larval fish length was always measured as total length along the longest body axis excluding setae and appendages. The dry weight composition of the walleye diet was determined by converting the lengths of ingested food items to dry weight using the formulae of Table A.3.

Diet breadth was examined using two indices. The Levins standardized index of niche breadth (Levins 1968; Krebs 1989), B_A, was calculated by the formula

[A.2] $$B_A = ([1 / \Sigma p_j^2] - 1) / (n - 1)$$

where p_j is the fraction of items in the diet that are of food category j, and n is the number of food categories. Hurlbert's standardized index of niche breadth (Hurlbert 1978; Krebs 1989), B_A', was calculated by the formula

[A.3] $$B_A' = ([1 / \Sigma(p_j^2/a_j)] - a_{min}) / (1 - a_{min})$$

where p_j is as defined for the Levins index, a_j is the proportion of all available resources consisting of resource j, and a_{min} is the minimum observed a_j. Because the abundance of benthic prey in the ponds was never assessed the latter index could only be used to estimate the breadth of the zooplankton portion of the diet. I separated food items into 13 equally-spaced categories based on $\log_e$(prey biomass) and p_j values were estimated from the numbers of prey in each category. Means and variances of the diet breadth indices were calculated by jackknifing using the FORTRAN subroutine of Matloff (1980). I examined the relationships between diet breadth and other pond variables using multiple regression techniques (Freund and Littell 1981). Means were weighted by the inverses of their standard errors.

In order to account for temperature differences among the four years I examined growth as a function of cumulative degree days above 10°C (CDD) rather than as a function of time.

CDD was initialized to 0 at the day of hatching and was calculated from the temperature records at increments of 0.25 d thereafter. Walleye growth was analyzed only during the postlarval period. I used growth data up to and including 200 CDD which roughly corresponded to a mean FL of 25 mm.

Results and Discussion

Physical and Chemical Conditions and Primary Production

The physical and chemical conditions of the Methley Beach culture ponds changed considerably over the study period but were considered suitable for walleye culture. Water temperatures during the spring were generally much higher in 1987 and 1988 than in 1985 and 1989 (Fig. A.1). The late spring and early summer temperatures were close to the 22°C optimum for juvenile walleye growth as determined by Smith and Koenst (1975). However, temperatures during the postlarval period were generally below this optimum, particularly in 1985 and 1989. Temperatures never reached the upper lethal range of 27.0-31.6 °C (Smith and Koenst 1975). Ammonia concentrations in some ponds rose to 800 $\mu g \cdot L^{-1}$ or more on at least two occasions but there was no evidence of any adverse effects to the fish (Johnston et al. 1992). Chemical analyses in 1988 revealed that chlorophyll and particulate P, N, and C were all relatively high in May following pond fertilization but declined by early June and remained at low levels for the remainder of the culture period (Johnston et al. 1992). Chlorophyll and particulate nutrient concentrations were consistently lower in pond 2 (unfertilized) and higher in pond 4 (high fertilization treatment) in 1988. Thus the chemical effects of fertilization on the ponds were evident but temporary and the productivity of the ponds appears to have been low.

Secchi depth tended to increase, peak, and then decline slightly over the spring and early summer (Fig. A.2). The increase phase of this trend was much more gradual in 1985 than in 1987 and 1988. Readings for ponds 3 and 4 were generally shallower than those for the other two ponds in all years. During May 1988, Secchi depth was consistently shallower in the heavily fertilized pond, pond 4 (Fig. A.2). However, by early June pond 4 Secchi depths were similar to those of the other ponds. Because of the inverse relationship between secchi depth and chlorophyll concentrations, secchi depth has often been used as an indicator of primary

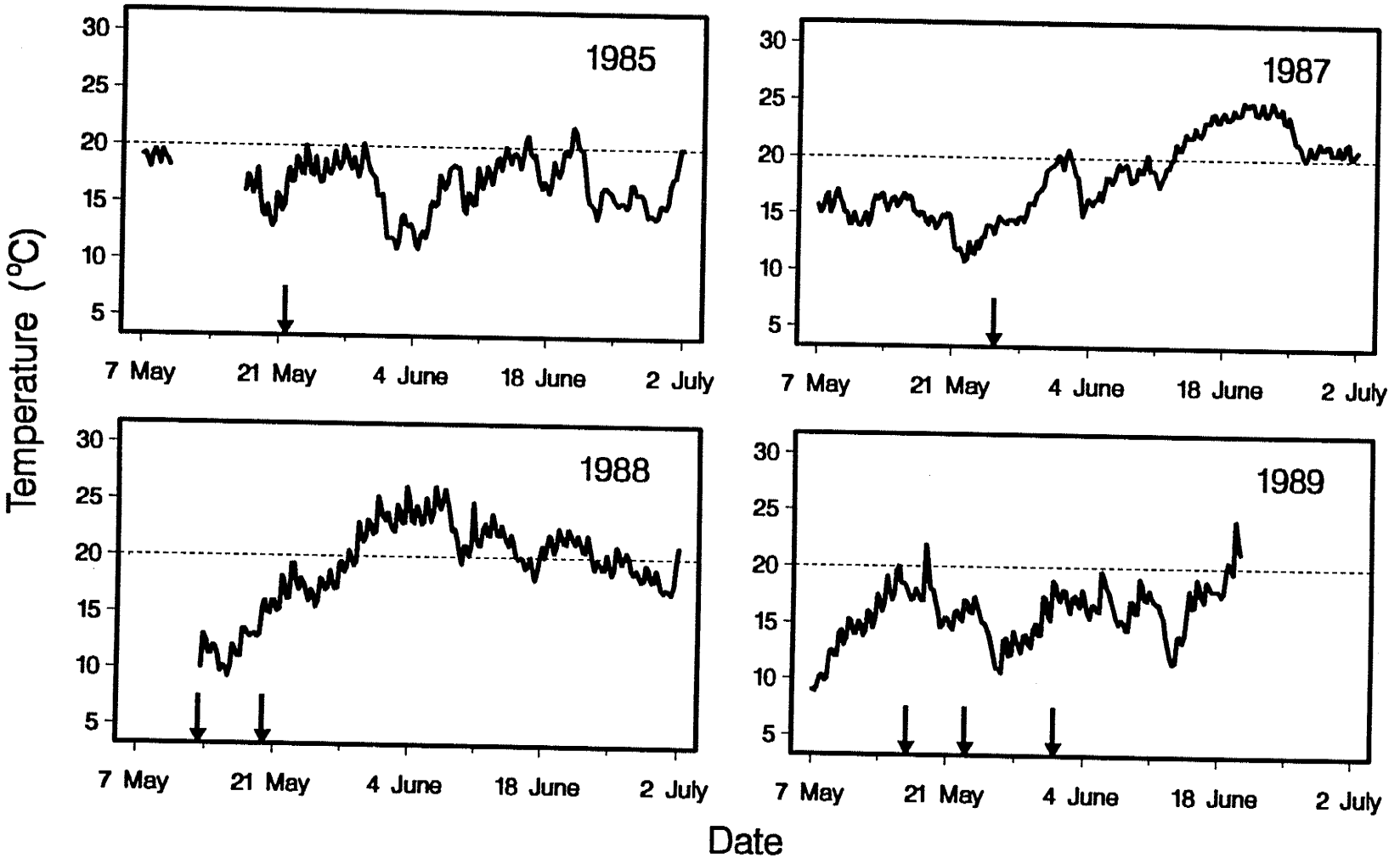

Figure A.1. Water temperature of the Methley Beach walleye culture ponds during spring of 1985, 1987, 1988, and 1989. Reference line indicates 20 °C. Arrows indicate walleye stocking dates as listed in Table A.2.

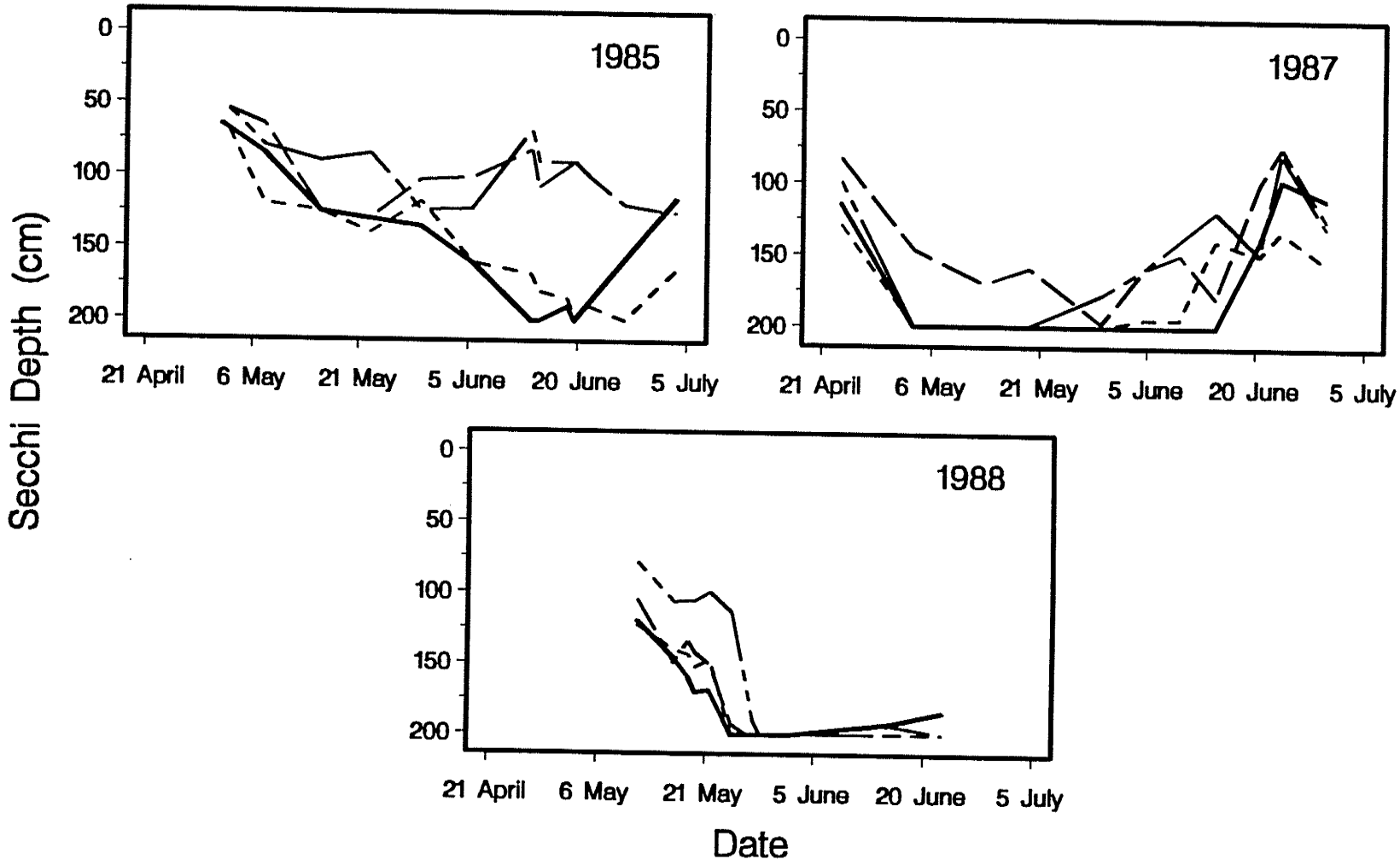

Figure A.2. Secchi depth readings of the Methley Beach walleye culture ponds (———— = pond 1, - - - - - - - - - = pond 2, —— —— = pond 3, —— - - —— = pond 4) during 1985, 1987, and 1988. Readings of 200 cm indicate that the disc was seen resting on bottom and that the true reading was actually greater.

production (Almazan and Boyd 1978). A significant negative correlation was also observed between chlorophyll concentration and secchi depth in the Methley Beach ponds in 1988 (Johnston et al. 1992). Boyd (1982) suggested that a Secchi visibility of 40 to 80 cm was desirable for pond culture of warmwater species. The relatively high readings obtained in the Methley Beach ponds, particularly during 1987 and 1988, suggest that the abundance of phytoplankton and other organic particles may have been low. In lakes, the biomass of zooplankton is positively related to the biomass of phytoplankton and zooplankton production is strongly related to phytoplankton production (McCauley and Kalff 1981). Thus the standing stock and production of zooplankton in the ponds may have been limited by primary production and ultimately by nutrient availability. Fertilization appears to have stimulated productivity early in the season but this effect diminished quickly.

Filamentous algae became very abundant in all ponds in 1987, forming extensive mats over the sediments. Only small clumps of filamentous algae were seen in other years. Submergent macrophyte growth was sparse in both 1985 and 1987, but well established in the deeper ends of all ponds in 1988 and 1989. It is difficult to say whether the increase in macrophyte growth caused phytoplankton abundance to decline through nutrient competition or simply that low phytoplankton abundance allowed greater light penetration and greater macrophyte growth. Dense stands of submerged macrophytes can cause diel oxygen shifts as great as 8 $mg \cdot L^{-1}$ (Carpenter and Lodge 1986). However, there was no evidence of low oxygen concentrations in the culture ponds. Macrophyte growth may have enhanced the production of benthic organisms during the latter years of this study.

Secondary Production

A total of five species of Copepoda and eleven species of Cladocera were identified from the zooplankton communities of the culture ponds (Table A.4). Seven of these species were not found in Dauphin Lake (Friesen and Mathias 1990) and were established either by the zooplankton introductions or by natural dispersion. The dominant crustaceans in order of decreasing abundance were *Diacyclops bicuspidatus thomasi*, *Daphnia pulex*, and *Bosmina longirostris*. Some other species were abundant in certain ponds at certain times. Among the copepods, *D. b. thomasi* was the only abundant cyclopoid. The only calanoid, *D. siciloides*, rarely exceeded densities of $15 \cdot L^{-1}$ and was always at densities below $3 \cdot L^{-1}$ in 1988 and 1989

Table A.4. Crustacean zooplankton species identified from the Methley Beach walleye culture ponds. Species not found in the Dauphin Lake crustacean community are indicated by ** (modified from Friesen and Mathias (1990)).

COPEPODA
Diacyclops bicuspidatus thomasi Forbes
Acanthocyclops vernalis Fischer
Cyclops navus Herrick **
Diaptomus siciloides Lilljeborg
Eucyclops agilis (Koch)
CLADOCERA
Alona quadrangularis (Müller) **
Bosmina longirostris (Müller)
Ceriodaphnia quadrangula (Müller)
Chydorus sphaericus (Müller) **
Daphnia pulex Leydig **
Daphnia retrocurva Forbes
Diaphanosoma leuchtenbergianum Fischer
Leptodora kindtii (Focke)
Macrothrix rosea (Jurine) **
Pleuroxus denticulatus Birge **
Simocephalus vetulus Schødler **

(Table A.5). Following the introduction of *D. pulex* in 1985, the cladoceran communities changed greatly. Initially, the Cladocera were dominated by small-bodied species, particularly *B. longirostris*, and the only common large-bodied species was *Daphnia retrocurva* (M.K. Friesen, Freshwater Institute, Winnipeg, pers. comm.). This was similar to the Dauphin Lake cladoceran community (Friesen and Mathias 1990). One month later, large Cladocera, predominantly *D. pulex*, outnumbered small Cladocera in pond 1 but small Cladocera continued to dominate in the other ponds (Table A.5). *D. pulex* gradually increased in abundance over 1985 and became the dominant large cladoceran in ponds 1, 2, and 4 by 1987 and in pond 3 by 1988. *D. retrocurva* was rarely captured after 1985. With the exception of pond 3 in 1987, *B. longirostris* was generally a minor component of the zooplankton communities after 1985. *Chydorus sphaericus* was also of minor importance after 1985. *Simocephalus vetulus* was not captured in 1985 but was abundant in pond 3 in 1987 forming up to 27% of the adult crustacean community. *S. vetulus* declined thereafter as *D. pulex* increased in abundance. *Leptodora kindtii* was captured only occasionally in fish sampling gear. Another large plankter, *Chaoborus sp.* (Diptera), was rare in the ponds during 1985 and 1987 but became fairly common in the latter two years of this study.

There was a general trend of declining crustacean zooplankton abundances from 1985 to 1989 (Table A.5). Copepod densities, excluding nauplii, were highest in 1985 reaching peaks above $200 \cdot L^{-1}$ in all ponds (Table A.5). Similar densities were seen in ponds 1 and 3 in 1987 but not in ponds 2 and 4, and not in any ponds in subsequent years. This may be partly due to the narrower range of sample dates in later years. However, sample dates in 1988 and 1989 corresponded to the dates of peak abundance in 1985. The highest densities of Cladocera were also seen in 1985 reaching over $400 \cdot L^{-1}$ in pond 3 and over $1000 \cdot L^{-1}$ in pond 4 (Table A.5). Cladoceran densities exceeded $50 \cdot L^{-1}$ on only one occasion after 1987. Peak densities of Cladocera generally occurred later in the year than the peak densities of Copepoda.

The structure of zooplankton communities depends not only on the trophic status of the system but also on the type and abundance of competitors and predators. In this study the zooplankton community was also influenced by the successful introduction of large-bodied Cladocera. In general, large Cladocera will outcompete suspension-feeding rotifers (Gilbert

Table A.5. Mean densities (particles·L^{-1}) of major zooplankton taxa in the Methley Beach walleye culture ponds during 1985, 1987, 1988, and 1989. *Daphnia sp.*, *Simocephalus sp.*, *Ceriodaphnia sp.*, and *Leptodora sp.* were categorized as large Cladocera whereas all other Cladocera listed in Table A.3 were categorized as small. Note that in 1985 Cladocera were separated into large and small size categories for only two sample dates. Dates of walleye first feeding in 1985 and 1987 were 25 May and 29 May respectively. First feeding corresponded with the first zooplankton sample date for each pond in 1988 and 1989.

Year	Pond	Date	Large Cladocera	Small Cladocera	Cyclopoid copepods[1]	Calanoid copepods[1]	Nauplii	Rotifers	Total Crustacea[1]
1985	1	1 May	0.08		111.89	7.01	169.05	123.62	118.97
		8 May	0.07		22.78	1.43	184.50	69.77	24.27
		15 May	0.18	0.09	140.54	2.37	162.02	9.80	143.20
		22 May	0.78		152.72	2.06	59.33	0.78	155.56
		29 May	6.48		192.04	4.43	13.30	2.42	202.96
		5 June	44.23		359.67	25.53	3.98	0.92	429.43
		13 June	84.87	52.02	165.83	16.36	11.76	2.61	319.09
		27 June	44.22		32.60	11.97	6.53	0.65	88.79
		4 July	22.42		90.65	9.85	15.93	3.37	122.92
1985	2	2 May	0.49		174.61	9.34	151.52	24.68	184.44
		8 May	0.20		99.13	5.45	198.34	55.35	104.77
		15 May	0.15	0.08	48.75	1.23	125.12	35.75	50.21
		22 May	2.74		284.28	3.61	47.33	3.92	290.63
		29 May	26.97		231.08	11.17	25.99	0.44	269.22
		5 June	92.84		215.34	11.02	6.80	9.05	319.20
		13 June	13.63	137.80	28.73	11.18	42.15	3.59	191.33
		26 June	196.34		60.34	10.68	29.73	3.92	267.36
		4 July	108.51		73.41	5.96	21.56	10.46	187.89
1985	3	27 April	0.12		1.68	0.19	0.46	0.00	1.98
		3 May	0.35		83.66	1.89	144.69	143.71	85.90
		8 May	0.07		71.92	3.73	134.29	54.75	75.72
		15 May	0.02	0.01	54.53	1.17	137.75	10.95	55.73
		22 May	1.62		176.91	3.81	65.58	2.88	182.33
		29 May	2.66		232.17	7.07	34.46	0.68	241.89
		5 June	24.43		172.34	10.83	58.79	1.35	208.59
		13 June	4.84	156.44	178.35	10.25	21.49	0.63	349.88
		26 June	484.34		23.76	5.09	17.31	1.31	513.18
		4 July	427.12		67.05	6.84	23.52	13.72	501.00

Table A.5. Continued

Year	Pond	Date	Large Cladocera	Small Cladocera	Cyclopoid copepods[1]	Calanoid copepods[1]	Nauplii	Rotifers	Total Crustacea[1]
1985	4	2 May	0.05		144.38	5.17	89.71	32.98	149.60
		8 May	0.00		103.85	2.72	145.30	51.32	106.58
		15 May	0.03	0.00	187.94	2.30	273.30	16.14	190.27
		22 May	0.07		257.41	3.03	93.84	2.35	260.51
		29 May	0.61		66.19	2.66	200.11	0.68	69.46
		5 June	9.04		203.74	5.19	24.03	0.32	217.98
		13 June	0.46	45.88	152.54	6.21	24.86	1.40	205.10
		26 June	733.19		93.63	7.93	51.04	1.69	834.75
		3 July	1087.65		77.68	6.86	65.35	6.86	1172.18
1987	1	23 April	1.09	0.00	4.38	0.04	2.90	0.90	5.50
		14 May	16.16	0.06	5.11	0.07	25.00	1.10	21.40
		29 May	31.79	0.00	66.10	0.61	23.90	9.20	98.50
		3 June	15.02	0.00	60.57	0.41	49.10	50.40	76.00
		8 June	106.45	0.00	80.77	2.18	63.50	1.50	189.40
		13 June	45.69	0.10	27.26	0.76	29.00	2.10	73.80
		23 June	0.00	4.10	195.11	5.60	24.00	164.40	204.80
1987	2	23 April	5.30	0.00	5.62	0.18	5.20	8.00	11.10
		14 May	7.19	0.02	7.38	0.01	35.90	1.70	14.60
		29 May	24.95	0.17	18.38	0.00	119.80	61.60	43.50
		4 June	23.20	0.00	15.90	0.40	80.30	133.90	39.50
		9 June	11.22	0.00	1.67	0.71	86.40	74.70	13.60
		14 June	0.00	0.00	1.57	0.03	8.70	927.10	1.60
		23 June	0.00	6.09	0.08	0.23	0.70	123.30	6.40
1987	3	23 April	0.05	0.01	5.50	0.04	6.80	13.40	5.60
		13 May	0.03	2.75	23.04	0.78	81.00	5.70	26.60
		29 May	11.30	77.50	295.50	3.90	811.90	205.20	388.20
		4 June	31.89	100.07	179.64	4.70	868.30	94.10	316.30
		8 June	28.99	64.77	122.44	3.80	180.70	42.70	220.00
		14 June	56.80	82.60	61.80	3.20	212.90	45.70	204.40
		23 June	1.60	138.60	28.50	18.70	331.80	32.50	187.40
1987	4	23 April	1.84	0.47	4.04	1.15	2.90	13.30	7.50
		14 May	3.77	0.00	13.48	0.74	103.00	1.10	18.00

Table A.5. Continued

Year	Pond	Date	Large Cladocera	Small Cladocera	Cyclopoid copepods[1]	Calanoid copepods[1]	Nauplii	Rotifers	Total Crustacea[1]
1987	4	29 May	22.09	0.41	43.88	0.62	146.30	2.90	67.00
		4 June	12.49	0.22	31.55	1.44	94.20	3.10	45.70
		8 June	64.44	0.49	18.52	1.65	84.50	3.60	85.10
		14 June	34.55	1.00	9.25	1.90	68.10	0.30	46.70
		23 June	0.00	10.68	25.60	1.62	85.00	12.40	37.90
1988	1	21 May	9.90	0.00	65.79	0.28	74.10	13.40	75.97
		26 May	24.85	0.14	24.42	0.28	28.40	9.60	49.70
		6 June	17.22	0.67	18.08	0.56	228.00	1.80	36.53
		21 June	20.96	1.75	30.87	2.72	27.20	86.70	56.30
1988	2	21 May	8.52	0.95	69.63	0.36	63.20	2.90	79.47
		26 May	14.58	3.23	44.97	0.25	30.70	0.60	63.03
		5 June	11.60	1.91	9.62	0.22	40.20	1.70	23.35
		21 June	0.86	10.90	14.60	1.34	74.10	19.60	27.70
1988	3	21 May	0.32	0.11	85.73	0.11	75.60	16.60	86.27
		26 May	0.51	0.62	66.51	0.18	48.90	3.40	67.83
		4 June	64.25	2.37	74.07	0.36	61.90	1.30	141.05
		21 June	17.64	1.38	18.30	1.68	60.50	0.70	39.00
1988	4	22 May	5.19	1.39	152.44	0.07	76.40	20.20	159.10
		26 May	10.67	3.62	135.07	0.21	25.20	2.00	149.57
		5 June	28.04	2.67	26.25	0.33	49.90	0.30	57.28
		22 June	9.72	0.17	15.76	0.45	74.90	0.90	26.10
1989	1	31 May	21.62	0.04	40.77	0.07	-	-	62.50
		7 June	23.69	0.04	32.12	0.08	-	-	55.93
		10 June	13.60	0.12	15.74	0.04	-	-	29.50
		15 June	7.69	0.08	20.35	0.16	-	-	28.28
1989	2	22 May	12.66	0.96	52.91	0.17	-	-	66.69
		26 May	23.21	1.42	77.60	0.00	-	-	102.22
		3 June	10.33	2.02	31.86	0.13	-	-	44.34
		7 June	11.34	1.82	20.47	0.04	-	-	33.67
1989	3	4 June	23.41	0.04	38.43	0.09	-	-	61.98
		8 June	15.07	0.05	20.72	0.09	-	-	35.93

Table A.5. Continued

Year	Pond	Date	Large Cladocera	Small Cladocera	Cyclopoid copepods[1]	Calanoid copepods[1]	Nauplii	Rotifers	Total Crustacea[1]
1989	3	13 June	7.64	0.05	7.64	0.23	-	-	15.55
		19 June	0.96	0.05	21.36	0.62	-	-	22.99
1989	4	21 May	21.22	0.11	62.86	0.23	-	-	84.42
		26 May	22.11	0.15	35.81	0.19	-	-	58.27
		2 June	11.38	0.12	39.14	0.04	-	-	50.67
		7 June	3.88	0.20	20.87	0.00	-	-	24.95

[1] excluding nauplii

1985) and small Cladocera such as *Bosmina sp.* (Lynch 1979), and will depress calanoid but not cyclopoid copepod abundances (Soto and Hurlbert 1991). The decline in abundance of small Cladocera, and to a lesser extent calanoids, was evident in the Methley ponds after *D. pulex* inoculations. Fish predation tends to drastically reduce the abundance of large Cladocera (Lynch 1979; Soto and Hurlbert 1991), but causes only moderate and negligible reductions in calanoids and cyclopoids, respectively (Soto and Hurlbert 1991). Consequently, zooplankton communities are typically dominated by rotifers, cyclopoid copepods, and small-bodied Cladocera in lakes with abundant vertebrate zooplanktivores such as Lake Ontario (Johannson and O'Gorman 1991) and Dauphin Lake (Friesen and Mathias 1990). Such plankton communities were seen in all the Methley ponds prior to *D. pulex* introductions in 1985, and in ponds 2 and 3 in 1987. In 1987, pond 3 had the largest forage fish population of any pond prior to fish removals, and pond 2 had the highest abundance of larval fish following the successful spawning of Iowa darters and brook sticklebacks. In these two cases, predation pressure on the zooplankton was most intense and probably caused the shift to small-bodied plankters in the communities. After 1987 however, the zooplankton communities never showed as distinct a shift towards small-bodied plankters as a result of fish predation. Walleye feeding alone was not sufficient to induce a major community shift during the postlarval periods of 1988 and 1989. However, such a change could have occurred later in the summer as walleye grew and consumed larger sizes and quantities of zooplankton.

Compared to the method used to estimate edible densities from 1987 to 1989, I believe that the 1985 method may have given slightly conservative estimates of edible density. Despite this, estimates from 1985 were generally much higher than those for subsequent years. The edible density available at first feeding in 1985 was at or above $100 \cdot L^{-1}$ (Fig. A.3). In subsequent years the first feeding edible densities were below the $100 \cdot L^{-1}$ level with the exception of pond 3 in 1987 and pond 4 (high fertilization treatment) in 1988. In general, edible density declined over the postlarval period in all years except 1985. The most severe depletion of zooplankton occurred in pond 2 in 1987 when edible density declined to almost $0 \cdot L^{-1}$ by mid-June. This decline was most likely the result of feeding by large numbers of brook stickleback and Iowa darter larvae. The mean edible density, calculated over the first four sample dates of the postlarval period, was correlated with the total P input ($r = 0.56$, $n =$

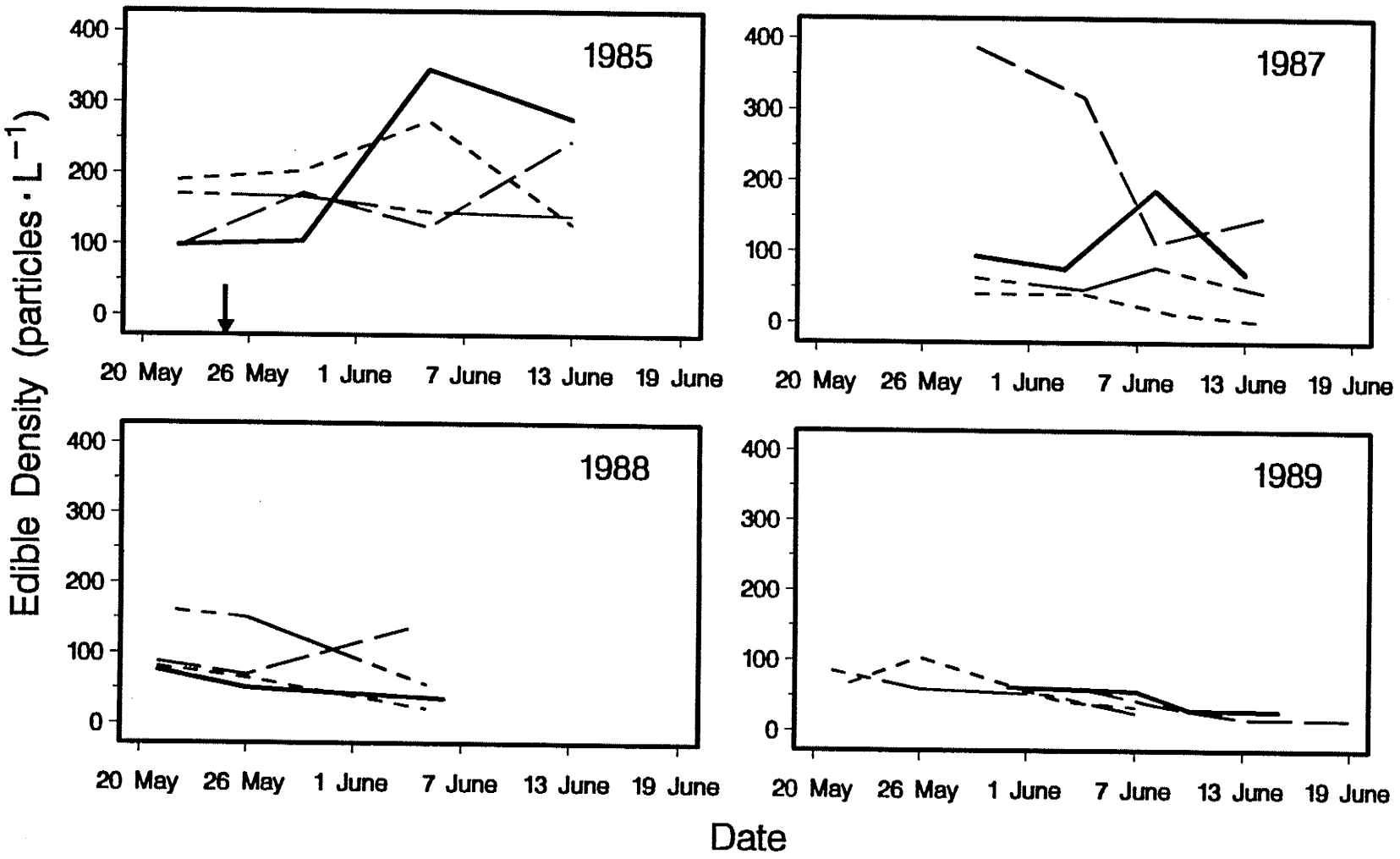

Figure A.3. Edible zooplankton densities in the Methley Beach walleye culture ponds (———— = pond 1, - - - - - - - - - = pond 2, —— —— = pond 3, —— - - —— = pond 4) during the first month of walleye feeding in 1985, 1987, 1988, and 1989. Arrow indicates approximate date of first feeding in 1985. Date of first feeding for other years corresponds to the first sample date in each pond.

16, P = 0.024).

Edible biomass was much higher in 1987 than in either 1988 or 1989 (Fig. A.4). Zooplankton size distributions and hence biomasses were not estimated in 1985. However, for the remaining three years, a significant correlation existed between total P input and the mean edible biomass as calculated over the first four sample dates of the postlarval period (r = 0.72, n = 12, P = 0.008). In 1987, pond 3 had edible biomasses similar to those in ponds 1 and 4 (Fig. A.4), even though the zooplankton composition of these ponds differed greatly (Table A.5).

Significant correlations between both the edible zooplankton density and biomass during the postlarval period and total P inputs in this study suggest that zooplankton abundance was enhanced by fertilization. McIntire and Bond (1962) observed higher abundances of both zooplankton and benthos in fertilized ponds. Hall et al. (1970) noted that zooplankton production in experimental ponds increased with nutrient additions but that community composition was not greatly affected.

Energy densities of pond zooplankton sampled in 1988 are summarized in Table A.6. Replicate subsamples were combusted for only two samples but observed subsample variation was low (cv <10%). A gradual decline in caloric content was evident over the period of 14 May to 7 June. I analyzed this relationship using ANCOVA with day of the year as a continuous variable and zooplankton size (S or L) as a class variable. There was no significant interaction between zooplankton size and day of the year, and the trend of declining energy density ($kJ \cdot RFDWT^{-1}$) with day of the year was significant (ANCOVA, F = 9.46, df = 1, 5, P = 0.028). This pattern was consistent with longer-term seasonal changes observed in other populations (Schindler et al. 1971; Wissing and Hasler 1971; Snow 1972). The S fraction of the pond zooplankton, primarily cyclopoid copepods, had a higher energy density than the L fraction, primarily daphnids, when compared on a total dry weight basis. This trend was not evident when compared on a residue-free dry weight basis because the L fraction had a much higher proportion of unburned residue than the S fraction. Because some elements may be lost during combustion the residue portion provides only a rough estimate of the ash content of a sample (Paine 1971). However, the residue data suggest that the cladocerans contained abnormally high levels of ash. Reported values of ash contents have been in the

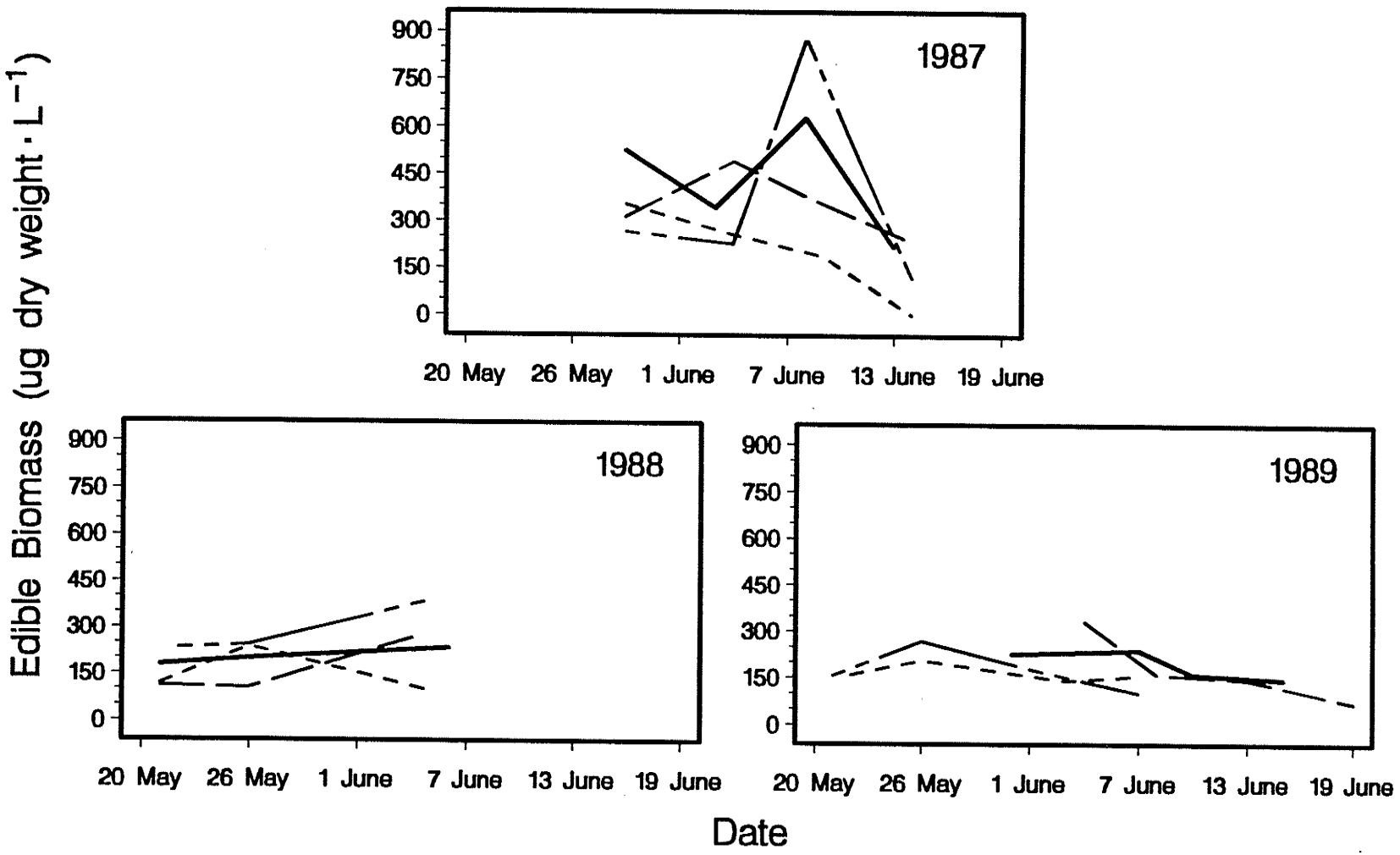

Figure A.4. Edible zooplankton biomasses in the Methley Beach walleye culture ponds (———— = pond 1, - - - - - - - - - = pond 2, ——— ——— = pond 3, ——— - - ——— = pond 4) during the first month of walleye feeding in 1987, 1988, and 1989. Date of first feeding corresponds to the first sample date in each pond for all years.

Table A.6. Energy densities of crustacean zooplankton collected from the Methley Beach walleye culture ponds on several dates in 1988 as determined by bomb calorimetry. Collected zooplankton were sieved into small (S), medium (M), and large (L) size fractions prior to freezing and bombing (see text). Zooplankton composition was defined by dry weight biomass of copepods (Cope) and cladocerans (Clad). Energy density values were calculated based on total dry weight (DWT) and residue-free dry weight (RFDWT) where the weight of residue was the dry weight of sample material remaining after combustion. Energy densities were corrected for preservation effects. All data represent single determinations with the exception of the S fraction from 29 May and the L fraction from 7 June where data represent the means of two determinations.

		Biomass (%)			Energy Density	
Date	Size	Cope	Clad	Residue (%)	kJ·g DWT^{-1}	kJ·g RFDWT^{-1}
14 May	S	100	0	9.35	23.31	26.28
21 May	L	5	95	20.64	22.34	28.76
22 May	S	98	2	4.95	23.67	25.45
	M	73	27	7.06	23.23	25.54
	L	2	98	11.35	20.55	23.69
29 May	S	96	4	3.24	23.11	24.40
	L	0	100	30.87	16.78	24.80
7 June	S	99	1	3.96	20.38	21.69
	M	60	40	13.24	19.65	23.14
	L	0	100	36.37	12.06	19.37

range of 2.3-4.6% for copepods and 4.0-12.0% for daphnids (reviewed by Cummins and Wuycheck (1971)) although some studies have recorded daphnid ash contents of 15-20% (Baudouin and Ravera 1972). Some of the excess ash may have been from gut contents (e.g. diatom frustules), as the zooplankton were sampled during daylight hours when they would have been actively grazing and their guts were not allowed to clear prior to killing and preservation. Reported caloric contents of copepods are typically higher than those of cladocerans. In their review of the literature Cummins and Wuycheck (1971) reported mean caloric values of 24.22, 24.02, and 21.04 kJ·g dry weight^{-1} for Cyclopidae, Diaptomidae, and Daphniidae, respectively.

Benthos was not quantitatively sampled during any of the years of this study. However, some trends were observed from qualitative samples. Chironomids, primarily Chironomiinae, appeared to be the most common benthic invertebrates in all years and reached very high densities in 1988. Amphipods were relatively abundant in 1985 and 1987 but were rare in the latter two years. Mayfly larvae (Ephemeroptera), primarily Caenidae, were present but relatively uncommon in all years.

Postlarval Walleye Diet and Growth

The composition of the walleye diet is summarized in Table A.7. Generally, Cyclopoid copepods and large cladocerans, primarily *Daphnia sp.*, formed the largest components of the diet in all years. In first-feeding larvae, cyclopoid copepods were usually the largest percentage of gut content biomass. Cyclopoid copepods tended to decrease in importance and large cladocerans tended to increase in importance as the larvae grew. This pattern has been observed in other extensive culture studies (Fox 1989). One notable exception was pond 3 in 1987 where cyclopoids continued to make up > 75% of the diet even when the mean larval length was > 20 mm (Table A.7). Calanoid copepods and small cladocerans were of minor importance to the diet with some exceptions. When zooplankton populations declined drastically in pond 2 during June 1987 (Table A.5), the walleye diet shifted to include greater proportions of calanoids and small cladocerans. By 13 June 1987, calanoids and small cladocerans made up 18.9% and 8.9% of the biomass consumed by pond 2 walleye, respectively. Calanoids never formed >3% of the diet on any other occasion. Small cladocerans were occasionally an important dietary component for smaller larvae but were only

Table A.7. Dietary compositions of larval walleye sampled from the Methley Beach walleye culture ponds at various dates in 1987, 1988, and 1989. Compositions were calculated based on dry weight biomass for each fish then averaged across fish within a sample. Diet categories were defined as: Cyc = cyclopoid copepods, Cal = calanoid copepods, L Clad = large cladocerans (*Daphnia sp.*, *Ceriodaphnia sp.*, *Simocephalus sp.*), S Clad = small cladocerans (*Bosmina sp.*, *Chydorus sp.*), Benthos = all aquatic insects and ostracods.

Year	Pond	Date	n	FL (mm)	Diet Composition (%)					
					Cyc	Cal	L Clad	S Clad	Benthos	Fish
1987	1	29 May	22	10.5	41.2	0.0	48.1	7.4	3.2	0.0
		3 June	20	13.7	63.1	0.0	26.7	0.0	6.9	3.3
		8 June	26	18.7	23.3	0.0	73.4	0.0	3.3	0.0
		13 June	20	22.2	66.8	0.2	23.1	0.7	2.7	6.6
	2	30 May	15	10.6	64.6	0.0	34.8	0.6	0.0	0.0
		3 June	21	13.7	8.6	0.0	87.1	0.0	0.6	3.8
		8 June	23	18.6	0.0	0.2	98.4	0.0	0.0	1.4
		13 June	38	20.5	23.0	18.9	9.4	8.9	26.6	13.1
	3	30 May	20	10.4	76.3	0.0	2.4	9.8	9.7	1.8
		3 June	31	13.2	84.7	2.1	6.1	3.3	1.6	2.2
		8 June	22	16.4	80.9	0.0	11.8	1.7	1.9	3.7
		13 June	20	21.4	84.4	2.0	8.9	0.2	4.1	0.4
	4	30 May	25	10.2	71.4	0.0	25.5	0.2	3.0	0.0
		3 June	20	12.5	34.8	0.0	64.4	0.0	0.8	0.0
		9 June	20	16.9	5.5	0.0	93.9	0.0	0.7	0.0
		14 June	28	21.8	14.7	1.0	79.9	0.0	1.0	3.4

Table A.7. Continued

Year	Pond	Date	n	FL (mm)	Diet Composition (%)					
					Cyc	Cal	L Clad	S Clad	Benthos	Fish
1988	1	21 May	20	10.3	34.8	0.0	65.2	0.0	0.0	0.0
		26 May	25	13.2	35.3	0.4	64.3	0.0	0.0	0.0
		4 June	31	23.4	1.6	0.0	54.4	0.0	44.0	0.0
	2	21 May	20	10.3	79.3	0.6	13.9	5.2	1.0	0.0
		25 May	19	12.6	33.6	0.7	60.7	5.0	0.0	0.0
		4 June	20	21.6	0.1	0.0	61.1	0.0	38.8	0.0
	3	21 May	20	10.2	62.6	1.0	24.4	11.9	0.0	0.0
		26 May	26	14.0	29.5	1.2	66.5	1.4	1.3	0.0
		4 June	20	26.1	1.0	0.0	37.8	0.0	61.2	0.0
	4	21 May	21	10.3	85.5	0.0	11.6	1.3	1.5	0.0
		25 May	20	13.0	28.9	0.0	48.2	5.1	17.7	0.0
		4 June	34	24.1	0.0	0.0	38.1	0.0	61.9	0.0
1989	1	31 May	20	10.3	63.3	0.0	9.2	3.9	23.6	0.0
		5 June	20	12.1	20.9	0.0	76.3	0.0	2.8	0.0
		9 June	25	14.2	2.6	0.0	95.1	0.0	2.3	0.0
		16 June	25	18.4	7.5	0.0	77.2	0.0	15.2	0.0

Table A.7. Continued

Year	Pond	Date	n	FL (mm)	Diet Composition (%)					
					Cyc	Cal	L Clad	S Clad	Benthos	Fish
1989	2	22 May	20	10.5	33.0	0.0	38.7	23.2	5.0	0.0
		26 May	22	11.8	17.8	0.0	75.7	4.2	2.2	0.0
		3 June	20	15.3	1.6	0.0	89.5	0.1	8.8	0.0
		7 June	20	16.6	0.0	0.0	97.5	0.0	2.5	0.0
	3	4 June	21	10.1	68.4	0.0	2.8	0.0	28.8	0.0
		8 June	20	11.2	25.5	0.3	73.2	0.0	1.0	0.0
		13 June	20	13.1	0.1	0.0	99.9	0.0	0.0	0.0
		19 June	29	17.4	20.5	0.0	43.7	0.0	35.8	0.0
	4	22 May	20	10.7	33.4	0.0	45.4	0.7	20.5	0.0
		26 May	20	11.7	13.2	0.0	78.5	1.4	6.9	0.0
		2 June	20	14.1	8.0	0.0	87.7	0.0	4.3	0.0
		7 June	20	17.2	0.5	0.0	90.6	0.0	8.9	0.0

important to older larvae when other food sources were scarce (e.g. 13 June 1987, pond 2). Benthic prey were consumed by all sizes of postlarval walleye (Table A.7). Chironomid larvae were the dominant benthic prey of first-feeding walleye whereas larger chironomid pupae were most important to the larger walleye, particularly during early June 1988. Larval fish, other than conspecifics, were only present during 1987 and were most abundant in ponds 1 and 2. Though consumed by all walleye size-classes, fish formed the largest proportion of the diet in the largest walleye. When zooplankton abundances were lowest and larval fish densities highest (13 June 1987, pond 2) larval fish made up 13.1% of the walleye diet (Table A.7).

First-feeding walleye consumed a very narrow range of prey sizes but this range increased as the larvae grew then narrowed again over the 24-28 mm size range (Fig. A.5). Minimum prey sizes remained fairly stable from first-feeding to the 24 mm stage but then increased slightly over the 24-28 mm size range (Fig. A.5). Maximum prey lengths and weights increased steadily from first-feeding to the 24 mm stage then declined slightly over the 24-28 mm size range (Fig. A.5 a,c). By contrast, maximum prey widths increased fairly rapidly from first-feeding to the 12 mm stage but then increased only slightly over the 12-28 mm size range (Fig. A.5 b). This pattern may indicate that larval walleye are primarily limited by prey width not prey length or simply that few preferred prey items with greater widths existed. Larger larval walleye were evidently capable of consuming very wide prey items (~2.5 mm) such as corixids but rarely did so (Fig. A.5 b).

Patterns in diet breadth differed between the indices of Levins and Hurlbert (Fig. A.6). Diet breadth as measured by the Levins index, B_A, showed no significant relationship with walleye mean length, zooplankton numerical density, or zooplankton biomass density (ANOVA, $F < 1$, $df = 1,40$, $P \geq 0.29$). The regression of the Hurlbert index, B_A', against $\log_e$(mean length) indicated a significant negative relationship (ANOVA, $F = 87.4$, $df = 1,40$, $P < 0.001$) but the addition of prey abundance variables to this model did not result in further significant reductions in variation. The addition of $\log_e$(zooplankton biomass density) accounted for the most additional variation (ANOVA, $F = 1.41$, $df = 1,40$, $P = 0.24$). When all prey items were considered and the Levins index was used diet breadth remained fairly stable with respect to fish size. When only zooplankton prey were considered and the Hurlbert index was used diet breadth narrowed with respect to fish size. This reflected the gradual elimination of smaller

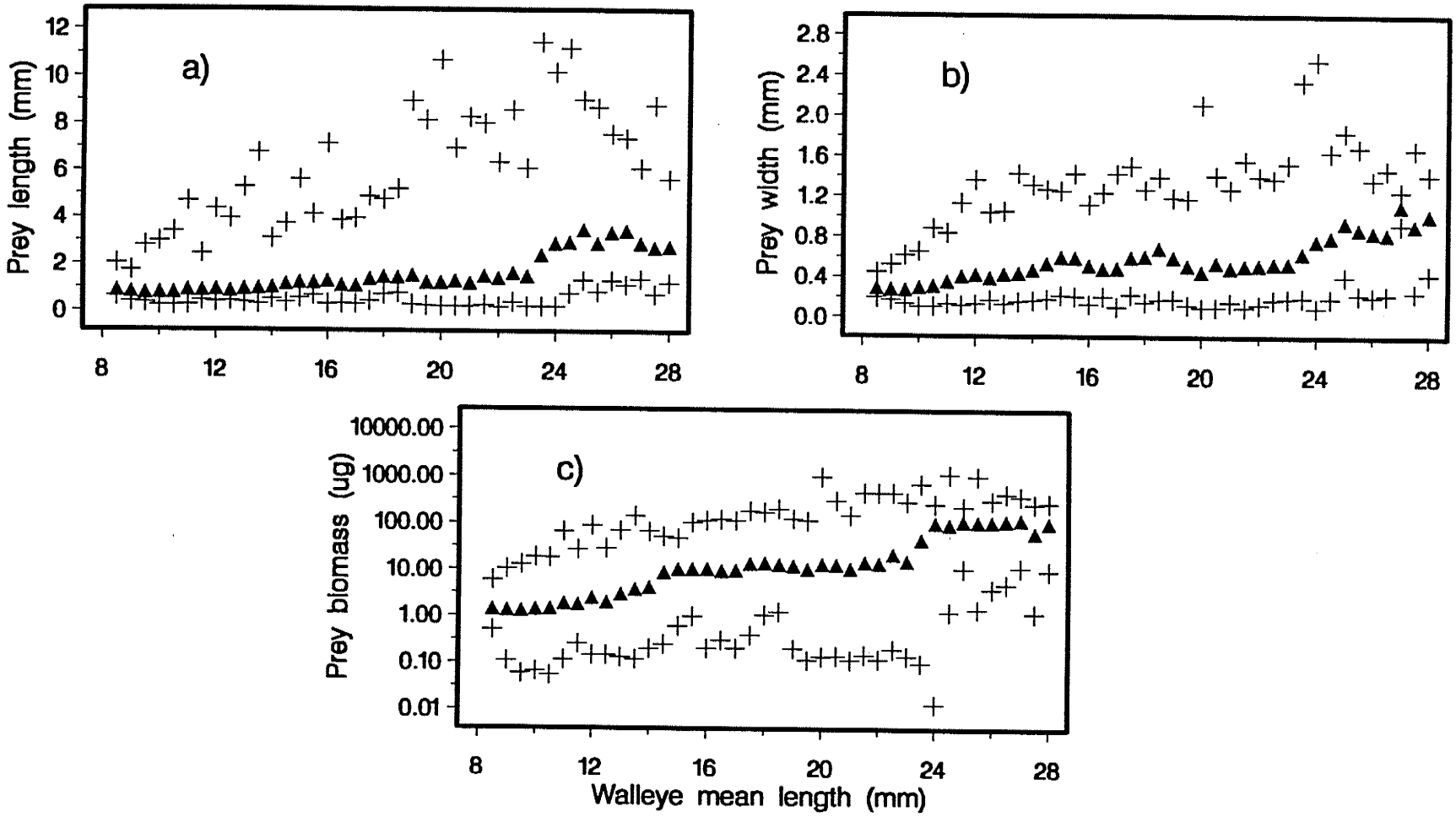

Figure A.5. Scatter plots of mean (▲), and minimum and maximum (+) ingested prey sizes vs. walleye mean length for walleye sampled from the Methley Beach walleye culture ponds. Prey size was measured as a) total length, b) body width, and c) dry weight biomass. Note logarithmic scale for c).

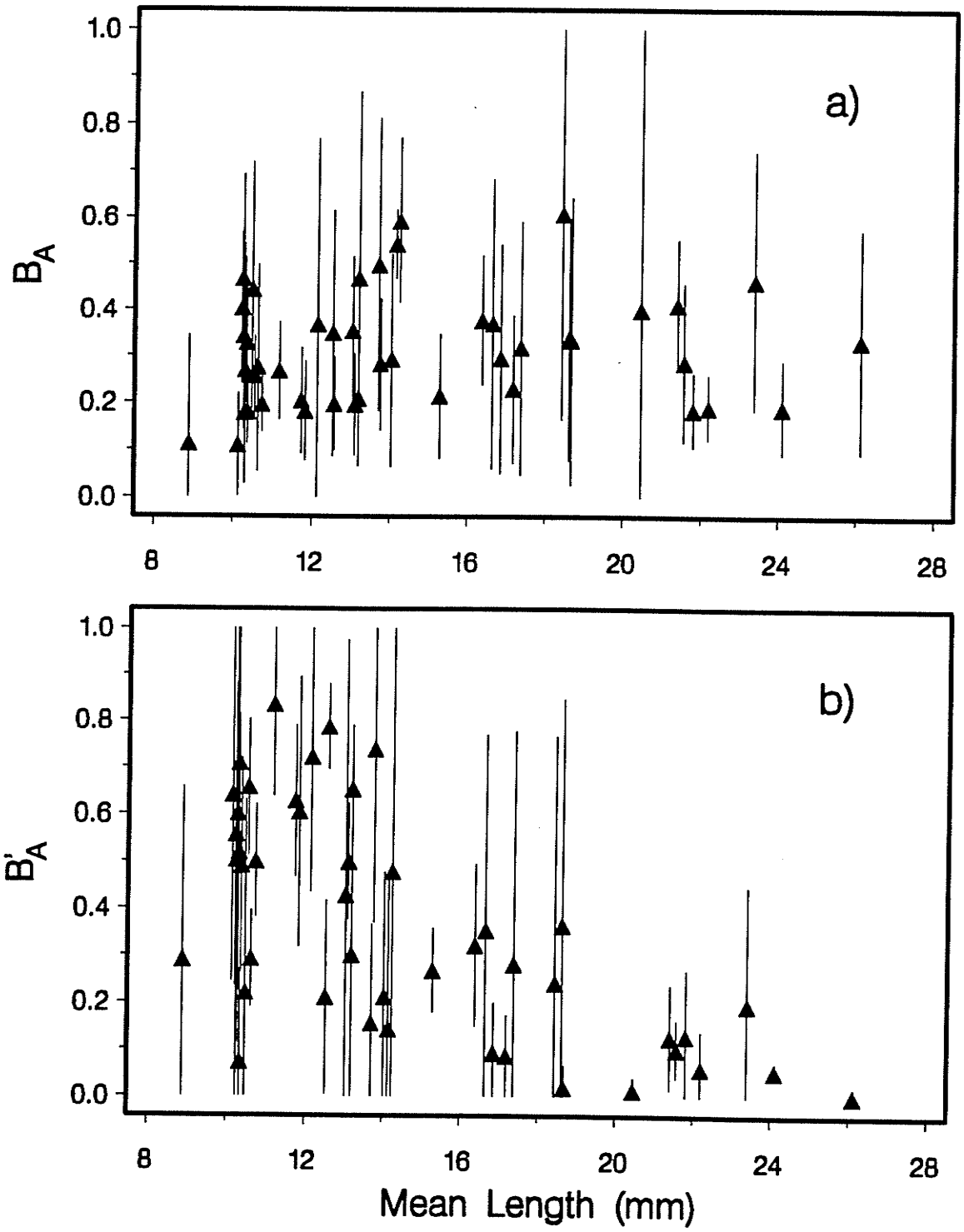

Figure A.6. Scatter plots of diet breadth vs. walleye mean length for walleye sampled from the Methley Beach walleye culture ponds. Diet breadth was calculated using a) Levins' standardized index of niche breadth, and b) Hurlbert's standardized index of niche breadth. Levins' index was calculated using all dietary items whereas Hurlbert's index was calculated for only the zooplankton portion of the diet. See text for formulae. Symbols represent jackknifed means ± 1 SE.

zooplankton size-classes from the diet as the larvae grew. Based on optimal foraging theory, it would be expected that diet breadth would decline with increasing prey abundance because less profitable prey items would be eliminated from the diet at high prey densities (Emlen 1966; MacArthur and Pianka 1966). This was apparently not the case over the range of prey densities observed in the Methley ponds. Perhaps such a pattern would be evident over a wider range of prey densities.

It is unlikely that the species composition of the pond zooplankton was ever a limiting factor to walleye feeding. Numerous studies have indicated that both cycopoid copepods and *Daphnia sp.* are readily consumed by larval walleye (e.g. Houde 1967; Spykerman 1974; Bulkley et al. 1976; Fox 1989). However, the density of edible particles may have limited feeding, particularly during 1988 and 1989. In laboratory studies, postlarval walleye feed at maximal rates in zooplankton densities at or above $100 \cdot L^{-1}$ (Mathias and Li 1982; Chapter 3). More recent studies suggest that even higher densities may be required to allow maximum consumption (see Chapter 3). Mean zooplankton densities were generally below $100 \cdot L^{-1}$ after 1985, but the heterogeneous distribution of zooplankton would undoubtedly result in local patches of much higher density. The feeding success of walleye under conditions of low mean zooplankton density probably depended on their ability to associate with high density patches. The dominance of larger-bodied zooplankton in the Methley ponds relative to natural lakes may have been advantageous for the larval walleye, as they tend to feed on larger plankters more than other species of larval fish (Bulkley et al. 1976; Keast 1980). Several studies have noted larval walleye feeding on small prey items such as diatoms, rotifers, or copepod nauplii (Smith and Moyle 1943; Hohn 1966; Paulus 1969), but it appears that such prey are not preferred (Mathias and Li 1982).

The abundance of alternative food sources such as benthos and fish may have affected walleye feeding success and the importance of these foods probably increases with larval development. Mathias and Li (1982) noted that juvenile (31-45 mm) walleye were able to feed at faster rates on *Chaoborus* larvae or sticklebacks than *Daphnia* or *Gammarus* (Amphipoda). When abundant, benthos may form 50% or more of the diet of larval walleye even at first-feeding (Fox 1989). In natural lakes, predation upon larval fish has been observed among all sizes of postlarval walleye but fish is usually only an important dietary component among

larger postlarvae (Houde 1967; Bulkley et al. 1976). Lab studies however have demonstrated that larval walleye growth is significantly higher on a diet of larval fish than on a diet of wild zooplankton (Hokanson and Lien 1986). This suggests that the relative scarcity of larval fish in the diet of young postlarval walleye may be more dependent on the walleye's ability to find and capture larval fish than on their actual preference for larval fish relative to zooplankton. The results of this and previous studies confirm that the postlarval walleye diet is very flexible and can conform to variations in the relative abundance of different prey items.

A simple linear model of mean FL versus cumulative degree days above 10 °C (CDD) described postlarval growth well and scatter plots indicated that a more complex model was not necessary. Postlarval growth showed marked year to year differences (Table A.8). The fastest growth rates (b_1 values from Table A.8) in descending order were in 1985, 1988, 1987, and 1989. The most distinct difference in growth rates between years was between 1989 and the other years. The ponds received no fertilization in 1989. Similarly, the unfertilized pond in 1988, pond 2, had the lowest postlarval growth rate in that year. Plots of postlarval growth rate versus mean edible zooplankton density over the postlarval period, mean edible zooplankton biomass over the postlarval period, and total P inputs all indicated curvilinear responses (Fig. A.7). The data suggest that postlarval growth rate increased asymptotically with increases in these variables. Growth rate reached a maximum at approximately 100 edible zooplankters$\cdot L^{-1}$ (Fig. A.7a), 200 µg edible zooplankton$\cdot L^{-1}$ (Fig. A.7b), and 2 kg P$\cdot ha^{-1}\cdot yr^{-1}$ (Fig. A.7c). When the independent variables of these relationships were transformed by $\log_e(x + 1)$, I found strong correlations between growth rate and mean zooplankton density ($r = 0.69$, $n = 16$, $P = 0.0034$), and total P inputs ($r = 0.77$, $n = 16$, $P = 0.0005$), but not mean zooplankton biomass ($r = 0.44$, $n = 12$, $P = 0.16$).

Growth during the postlarval period showed positive relationships with both zooplankton density and fertilization rates. Postlarval growth rate was maximized at zooplankton densities of roughly 100$\cdot L^{-1}$ which corresponds to the density at which maximum feeding rate was attained in laboratory studies (Mathias and Li 1982). Ball and Brown (1987) also noted high postlarval walleye growth rates associated with high zooplankton densities. Fox et al. (1989) noted much higher walleye growth rates in heavily and regularly fertilized ponds. However, rapid growth during the postlarval period did not ensure a large mean size at harvest. Mean

Table A.8. Fitted parameters and statistics resulting from the regression of mean walleye fork length (FL, mm) against cumulative degree days > 10°C (CDD) for postlarval walleye sampled from the Methley Beach walleye culture ponds in 1985, 1987, 1988, and 1989. The parameters b_0 and b_1 represent the intercept and slope of the regression respectively. Only samples collected within the first 200 CDD were used in this analysis. Walleye mean lengths ranged from 10 to 20 mm, and water temperatures ranged from 12 to 24 °C during the 200 CDD growth periods.

Year	Pond	b_0	b_1	F	(df_{error})	P>F	R^2
1985	1	6.70	0.108	54.4	(2)	0.0179	0.965
	2	6.89	0.098	51.5	(2)	0.0189	0.963
	3	7.33	0.097	459.8	(2)	0.0022	0.996
	4	7.25	0.093	260.2	(2)	0.0038	0.992
1987	1	8.08	0.093	251.1	(3)	0.0005	0.998
	2	8.31	0.083	58.5	(3)	0.0046	0.951
	3	7.71	0.085	3269.4	(3)	<0.0001	0.999
	4	7.60	0.082	1732.3	(3)	<0.0001	0.998
1988[1]	1	8.46	0.091	575.9	(2)	0.0017	0.997
	2	8.67	0.079	1861.0	(2)	0.0005	0.999
	3	8.46	0.104	827.4	(3)	<0.0001	0.996
	4	8.52	0.093	1075.3	(2)	0.0009	0.998
1989	1	8.19	0.071	84.5	(3)	0.0027	0.966
	2	8.41	0.068	542.3	(3)	0.0002	0.995
	3	8.23	0.071	101.7	(3)	0.0021	0.971
	4	8.01	0.072	195.5	(4)	0.0002	0.980

[1] Results are for Swan Creek stock only

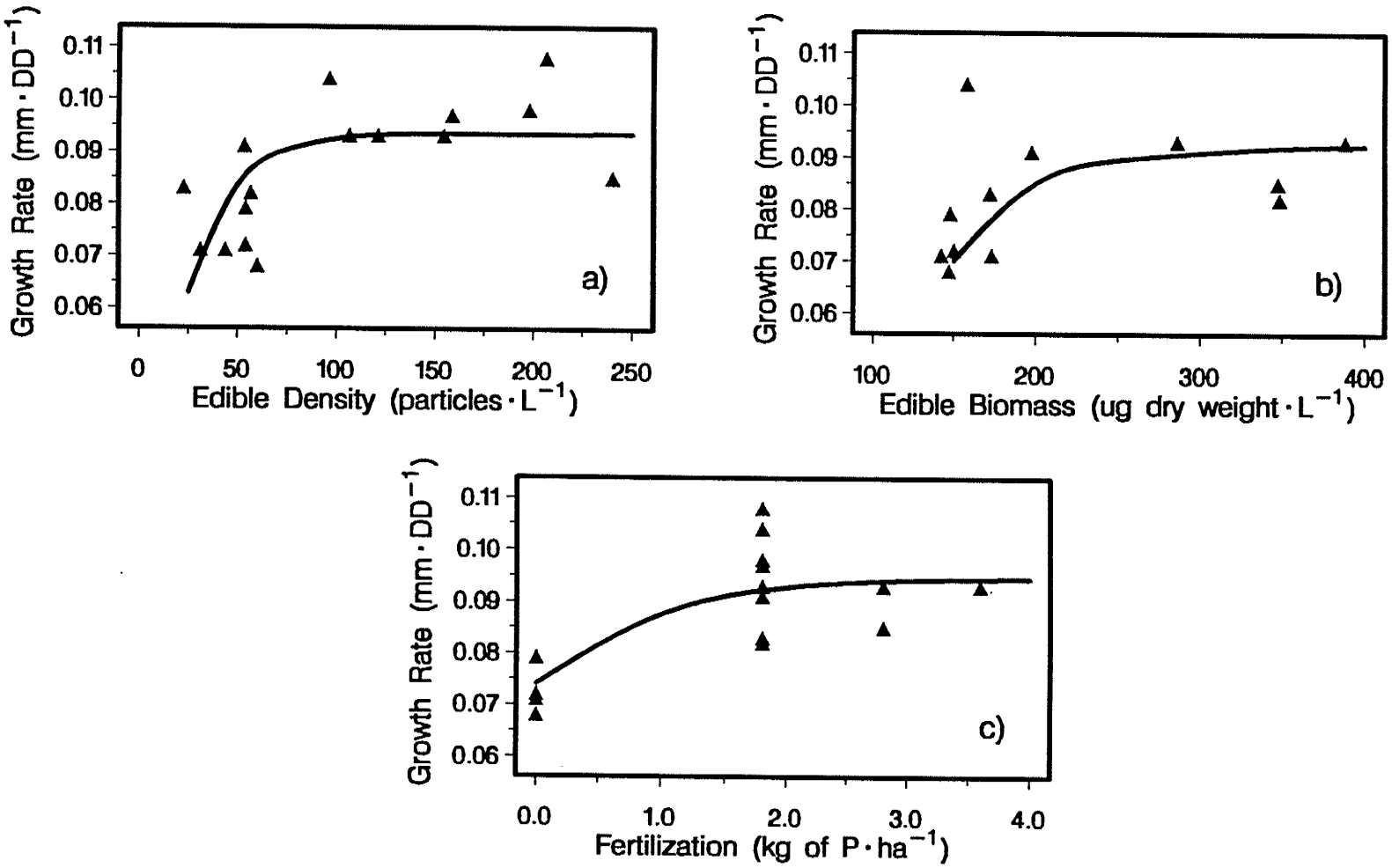

Figure A.7. Scatter plots of walleye postlarval growth rate (mm per degree day >10 °C) vs. a) mean edible density of zooplankton over the postlarval period, b) mean edible biomass of zooplankton over the postlarval period, and c) total annual P inputs. Curves were eye-fitted. Data were collected from the Methley Beach walleye culture ponds from 1985, 1987, 1988, and 1989 for plots a) and c), and from 1987, 1988, and 1989 for plot b).

FL at harvest was not significantly correlated with either postlarval growth rate or the rate of fertilization (Johnston et al. 1992). Evidently, conditions during the juvenile period obscured the effects of postlarval growth. It is likely that culture ponds, such as those used in this study, can support sufficient secondary production to support large numbers of walleye larvae. However, as the larvae grow into juveniles their consumption quickly exceeds the ponds' productive capacities. Under such conditions, most of the population exhibits slow growth from lack of invertebrate food while others become cannibalistic and exhibit rapid growth. Initially, this situation leads to a bimodal size distribution in the walleye population. However, if cannibalism begins early in the culture period, only large individuals may remain at harvest. Thus, size at harvest is probably most strongly affected by the timing of the onset of juvenile cannibalism. Juvenile cannibalism was observed in all years except 1988.

Appendix B:

Preservation effects upon larval walleye

Introduction

Accurate estimates of body length and weight are essential in ecological studies of fishes but time constraints during sampling often necessitate preserving the fish and taking these measurements at a later date. The standard technique for preservation of larval fish is storage in a formalin solution (Ahlstrom 1976; Smith and Richardson 1977; Snyder 1983). However, there are several disadvantages to the use of formalin.

Preservation in formalin has been shown to alter fish length and wet weight (e.g. Parker 1963; Stobo 1972), and dry weight (Hay 1984). Formalin may also cause loss of lipids (Morris 1972; Jones 1976) and decalcification of bone (Taylor 1977). It would therefore not be an appropriate preservative for bioenergetic studies (Dowgiallo 1975) or for studies examining otolith microstructure (Radtke and Waiwood 1980). In addition, formalin fumes are irritating and may pose a human health risk (Anon. 1989).

An alternative to chemical preservation is to freeze fish samples. The effects of freezing on length and wet weight have been examined in juvenile and adult fish (Engel 1974; Treasurer 1990) but rarely in larval fish (Fowler and Smith 1983), and no studies have examined the effects of freezing on fish dry weight. The objective of this study was to examine the relationships between fresh and preserved measurements of body length and dry weight in larval walleye subjected to freezing and to compare the results with changes observed in fish preserved in the more commonly used fixative, formalin. In addition, I wanted to examine the effect of freezing on the energy density of larval walleye.

Materials and Methods

Newly-hatched walleye larvae were obtained from the Manitoba Department of Natural Resources, raised in 120-L aquaria at 20 °C, and fed a diet of wild zooplankton. Fish were sampled on six dates from 15 May to 6 June 1988. For the three week sampling period, walleye fresh length ranged from 8.9 to 21 mm and fresh dry weight ranged from 0.51 to 12 mg.

On each sample date, fish were anaesthetized in a weak solution of MS-222 (ethyl *m*-aminobenzoate methanesulfonate). Measurements were taken to the nearest 0.07 mm using an ocular micrometer and dissecting scope for the first four sample dates and estimated to the nearest 0.1 mm using a scientific ruler mounted under a dissecting scope for the remaining sample dates. Length was measured as total length until a definite fork appeared in the tail and as fork length thereafter. Measured fish were sacrificed in a strong solution of MS-222 and immediately transferred to one of three treatments. Fish of the first group were placed on pre-weighed aluminum trays, oven-dried for 24 h at 60°C, moved to a desiccator for one half hour, then weighed to the nearest 0.1 mg. Fish of the second group were placed in vials containing 8 mL of 5% formalin buffered with 2% (by volume) saturated sodium borate solution, the standard fixative for larval fish collections (Smith and Richardson 1977; Snyder 1983). The pH of the buffered formalin was 8.6 but declined to 7.4 when fish were added. Fish of the third group were placed in plastic bags with ~15 mL of pond water (pH 8.7-8.8) and placed in a freezer at -18 °C. After 180 d in storage, the formalin-preserved fish were rinsed in distilled water, re-measured for length, then oven-dried and weighed. After 1075 d in storage, the frozen fish were thawed at room temperature, re-measured for length, then oven-dried and weighed.

To assess measurement error, 5 repeated measures of length and dry weight were taken on 5 groups of 5 small larvae (mean length 9.7 mm) and on 5 individual larger larvae (mean length 14.9 mm). Measurement error was low relative to the treatment effects. Coefficients of variation for length measurements were 0.25% for 9.7 mm larvae measured with the ocular micrometer and 0.56% for 14.9 mm larvae measured with the scientific ruler. Coefficients of variation for dry weight measurements were 1.76% for 0.43 mg larvae and 1.26% for 2.77 mg larvae.

Groups of fish were used for weighing the smaller larvae because of their low weight relative to the sensitivity of the balance. Thus, for the first four sample dates the data consisted of ten means of five fish each for each of the three treatments. On the last two sample dates the data were derived from ≥ 20 individual fish for each of the three treatments. Fresh length was regressed against preserved length using standard regression techniques (Draper and Smith 1981). As fresh weight and preserved weight were estimated from different

fish, the analysis for weight loss was slightly different. Data were categorized by rounding fresh length to the nearest 0.25 mm for both the fresh and preserved data sets. Mean weights were calculated for each fresh length category and fresh and preserved data sets were match-merged by length category. Mean fresh dry weight was then regressed against mean preserved dry weight for corresponding length categories. The percentage change in length or weight for a given size of larva was calculated as 100 x (Fresh size - Preserved size)/(Fresh size), where preserved size was estimated from the empirical relationships.

Differences in length reduction and dry weight loss between the two preservation techniques were tested by ANCOVA using SAS® software (Freund and Littell 1981). Fresh length was used as the covariate. Natural logarithms of both fresh length and preserved weight were taken prior to testing for differences in weight loss.

Experiments to examine the effects of freezing on energy density were performed separately in June 1993. Larval walleye were obtained and raised as described above. At various stages of larval development, random samples of larvae were collected, killed in MS-222, and divided into two groups of 20-30 larvae each. The first group were immediately oven-dried at 60 °C for 24 h. The second group were frozen in ~15 mL of pond water at -18 °C for 2 d, then thawed and oven-dried at 60 °C for 24 h. Each group of larvae was then homogenized by pulverizing with a mortar and pestle and pressed into pellets of ~ 8-12 mg. The pellets were placed on pre-weighed platinum trays, dried again for a minimum of 8 h, desiccated for 1 h, weighed to the nearest 0.01 mg on a Cahn Electrobalance®, then burned in a Phillipson microbomb calorimeter (Gentry Instruments, Inc., Aiken, S.C.) following standard calorimetry techniques (Prus 1975). Platinum trays were reweighed after bombing to determine the total weight of unburned residue. Energy density was calculated on both a total dry weight and a residue-free dry weight basis.

Results

Relationships between fresh and preserved measurements are illustrated in Figs. B.1 and B.2. Based on the length relationships of Fig. B.1, the predicted mean length reduction for frozen walleye ranged from 4.3% for 9 mm fish to 2.3% for 21 mm fish. At similar sizes, larvae preserved in formalin experienced mean length reductions of 2.3% and 1.8%, respectively. Dry weight loss was much more pronounced. Based on the weight relationships

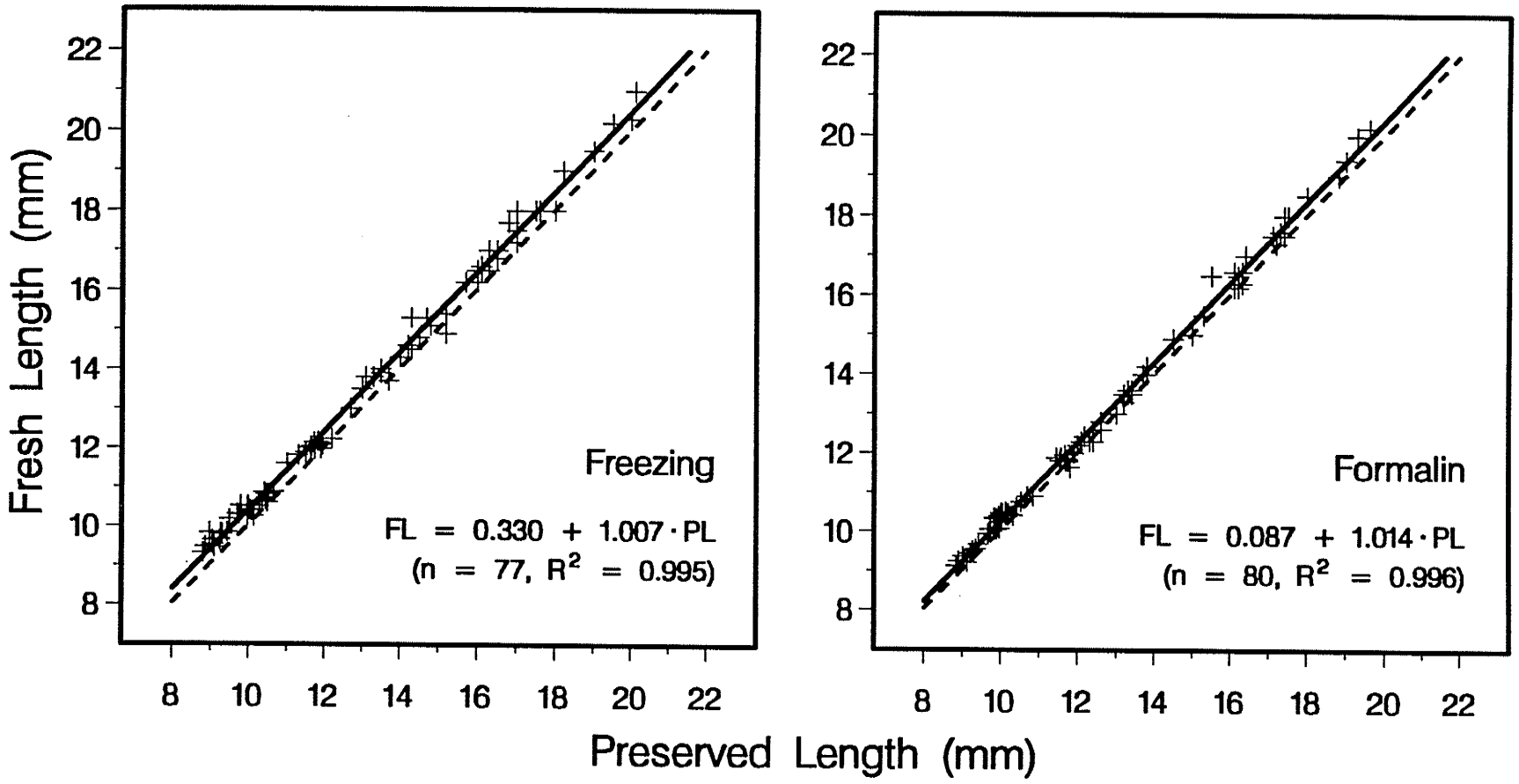

Figure B.1. Scatter plots of fresh length versus preserved length for frozen and formalin-preserved larval walleye. Symbols represent individual data values. Solid line represents the observed relationship. Dashed line represents 1:1 agreement between fresh and preserved measurements.

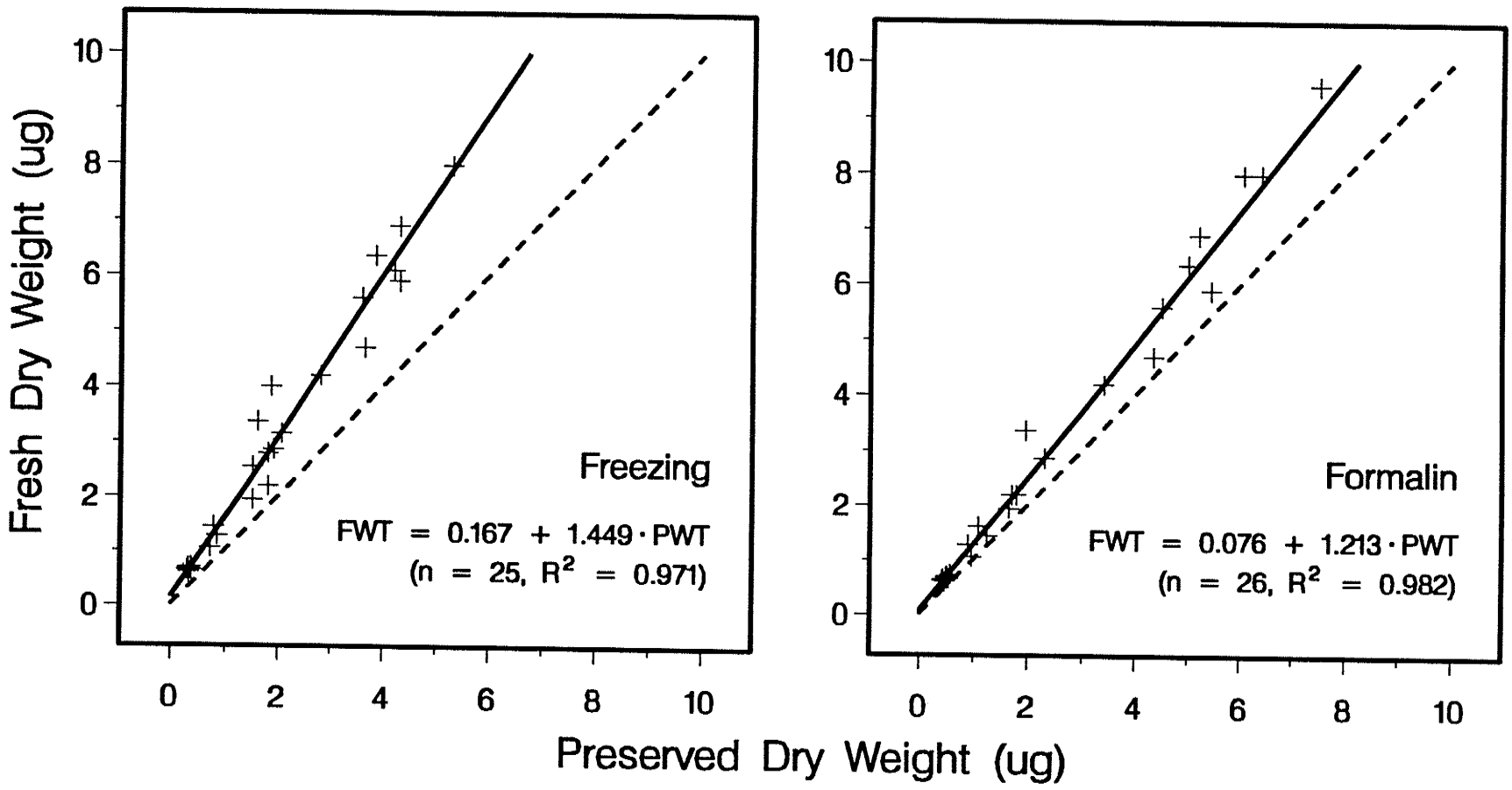

Figure B.2. Scatter plots of fresh dry weight versus preserved dry weight for frozen and formalin-preserved larval walleye. Symbols represent means. Solid line represents the observed relationship. Dashed line represents 1:1 agreement between fresh and preserved measurements.

of Fig. B.2, the predicted mean dry weight loss for frozen walleye ranged from 54.0% for 0.5 mg fish to 32.1% for 10 mg fish. By comparison, similar sized walleye subjected to formalin preservation would be expected to lose 30.1% and 18.2% of dry weight, respectively.

Slopes of the preserved length vs fresh length relationships were not significantly different between freezing and formalin treatments (ANCOVA, $F = 0.22$, $df = 1,153$, $P = 0.64$) whereas the intercepts of these relationships were significantly different ($F = 21.0$, $df = 1,154$, $P < 0.001$). Thus, frozen larvae showed a significantly greater length reduction than formalin treated larvae. Slopes of the $\log_e$(preserved weight) vs $\log_e$(fresh length) relationships were significantly different between freezing and formalin treatments ($F = 10.42$, $df = 1,153$, $P < 0.01$) and further analyses had to be conducted on restricted data sets. When ANCOVA was performed on larvae of 8-10 mm fresh length, the slopes of the $\log_e$(preserved weight) vs $\log_e$(fresh length) relationships were not significantly different between treatments ($F = 0.97$, $df = 1,17$, $P = 0.34$) but the intercepts were significantly different ($F = 39.9$, $df = 1,17$, $P < 0.001$). The same analysis for larvae of 16-18 mm fresh length also found an insignificant difference in the slopes ($F = 0.50$, $df = 1,26$, $P = 0.49$) and a significant difference in the intercepts ($F = 50.1$, $df = 1,26$, $P < 0.001$). Thus, frozen larvae lost significantly more dry weight than formalin treated larvae. However, the difference in dry weight loss between freezing and formalin treatments was significantly dependent on larval length.

Bomb calorimetry was performed on only a small number of fish samples. Generally, calorimetry was performed on single subsamples from each of the fresh and frozen fish samples at each of five developmental stages from 9.0 to 18.8 mm fresh length. However, subsample variation appeared to be low. Replicate determinations of energy density made at the 14.7 mm developmental stage (n=3 and n=2 for fresh and frozen treatments, respectively) indicated that coefficients of variation were < 3%. Energy densities were consistently higher in frozen samples than in fresh samples (Table B.1). Paired-observations *t*-tests (Steel and Torrie 1980) demonstrated significant differences between energy densities of fresh and frozen walleye samples, both when calculated on a total dry weight basis ($|t| = 4.55$, $df = 4$, $P = 0.010$) and a residue-free dry weight basis ($|t| = 2.86$, $df = 4$, $P = 0.046$). Scatter plots did not indicate any clear relationship between the fresh:frozen energy density ratio and mean larval length. Mean values of the fresh:frozen energy density ratio were 0.939 and 0.960 for

Table B.1. Energy densities of fresh and frozen postlarval walleye at several developmental stages (FL = mean fork length). Values were calculated based on total dry weight (DWT) and residue-free dry weight (RFDWT) where the weight of residue was the dry weight of sample material remaining after combustion in a bomb calorimeter. All data represent single determinations on subsamples taken from homogenates of 20-30 larvae with the exception of the 14.7 mm stage where data represent means of three determinations for the fresh fish and two determinations for the frozen fish.

			Energy Density	
FL (mm)	Treatment	Residue (%)	kJ·g DWT^{-1}	kJ·g RFDWT^{-1}
9.0	Fresh	7.29	25.90	27.94
	Frozen	4.90	26.70	28.24
11.2	Fresh	9.51	21.04	23.25
	Frozen	6.42	23.47	25.08
14.7	Fresh	9.72	22.38	24.78
	Frozen	7.08	23.09	24.85
15.4	Fresh	9.63	21.10	23.35
	Frozen	9.09	22.67	24.94
18.8	Fresh	11.58	21.22	24.00
	Frozen	9.29	22.86	25.20

dry weight and residue-free dry weight determinations, respectively. These values represent energy density increases of 6.5% and 4.2%, respectively. Unburned residue as a fraction of total dry weight increased with mean larval length (Table B.1) and was significantly higher in fresh than frozen samples (paired-observations *t*-test, $|t| = 5.04$, df = 4, P = 0.007).

Discussion

Comparisons between the freezing and formalin treatments in this study were considered justifiable despite differences in storage time. As most of the cellular changes which accompany freezing are associated with the freezing and thawing processes (Farrant 1980), it is unlikely that length and dry weight losses in the larvae were dependent on the duration of storage. In addition, numerous studies have shown that formalin-induced changes in fish occur primarily within the first few days or weeks of preservation (e.g. Heming and Preston 1981; Fowler and Smith 1983; Tucker and Chester 1984). Thus, I assumed that further reductions in length and dry weight would be insignificant beyond six months of formalin preservation.

In this study, freezing resulted in greater shrinkage than formalin preservation, but both treatments caused < 5% reduction in larval walleye length. With a few exceptions, reported mean length reductions for fish preserved in a similar manner have been ≤ 5% (Table B.2). Most of these studies have also noted that the percent reduction in length declines with increasing fish length. Weight changes resulting from preservation have been more variable (Table B.2). Freezing has been shown to result in a wet weight loss of < 3% in juvenile (Engel 1974) and adult (Treasurer 1990) fish. Reported changes in wet weight for fish preserved in formalin range from reductions of 4% (Lockwood and Daly 1975) to increases of 9% (Heming and Preston 1981). Few studies have examined dry weight loss; however, my results indicated that such losses in preserved larval walleye can be severe, particularly for frozen fish. Dry weight losses in formalin-preserved walleye were slightly higher than those reported by Hay (1984) for formalin-preserved Pacific herring, *Clupea harengus pallasi*. Preservation in ethanol may cause greater dry weight loss than formalin. Bailey (1982) estimated that 0.083 mg dry weight Pacific hake, *Merluccius productus*, lost 57.8% and 24.1% of their dry weight when stored in 80% ethanol and 3% formalin, respectively.

Table B.2. Summary of reported length and weight changes in frozen and formalin-preserved fish (na = not available). Weight changes refer to wet weight except for the results of Hay (1984) and the results of this study. All formalin mixtures were prepared with fresh water except for Fowler and Smith (1983).

Source	Species	Fresh size	Methods	% Change	
				Length	Weight
Billy (1982)	*Sarotherodon mossambicus*	18-85 mm	5 d in 10% formalin + 65 d in 37.5% isopropyl alcohol	<1	+4
Engel (1974)	*Perca flavescens*	127-171 mm, 24 g (wet)	72 h frozen at -10 to -15.5 °C	<1	-1.7
	Coregonus artedii	176-267 mm, 70 g (wet)	as above	-2.1	-2.0
Fowler and Smith (1983)	*Merluccius bilinearis*	3-15 mm	337 d in 4% neutral formalin-seawater	-4.3	na
			338 d frozen at -15 °C	-1.4	na
Glenn and Mathias (1987)	*Stizostedion vitreum*	9-33 mm	3 d in 5% formalin	-2 to -7	na
			quick frozen in -70 °C ethanol then stored 3 d in freezer	-2 to -15	na
Hay (1982)	*Clupea harengus pallasi*	10-12 mm	6 mo in 5% formalin	-1	na
Hay (1984)	*Clupea harengus pallasi*	0.106 mg (dry)	10 d in 4% formalin	na	-22.2
		0.211 mg (dry)	as above	na	-15.5
Heming and Preston (1981)	*Oncorhynchus tshawytscha*	26-40 mm, 410-544 mg (wet)	50 d in 5% formalin	-5.3	+2 to +9

Table B.2. Continued

Source	Species	Fresh size	Methods	% Change	
				Length	Weight
Jennings (1991)	*Dicentrarchus labrax*	5-70 mm	200 d in 4% formaldehyde	-5	na
Kruse and Dalley (1990)	*Mallotus villosus*	5-23 mm	168 d in 5% formalin	-5.2	na
Lockwood and Daly (1975)	*Pleuronectes platessa*	20-75 mm	1 yr in formalin	<1	-4
Parker (1963)	*Oncorhynchus keta, O. gorbuscha*	34-37 mm, 200-285 mg (wet)	218 d in 3.8% formaldehyde	-4.3	+5
Stobo (1972)	*Perca flavescens*	68-99 mm	250 d in 10% formalin	-1.4	+7.5
Treasurer (1990)	*Perca fluviatilis*	110-340 mm	70-98 d frozen at -25 °C	-1.7	-2.7
	Esox lucius	190-830 mm	as above	-5.4	-2.6
Tucker and Chester (1984)	*Paralichthys lethostigma*	9-13 mm	1 yr in 4% formalin	-2.5	na
This study	*Stizostedion vitreum*	9-21 mm, 0.5-10 mg (dry)	1075 d frozen at -18 °C	-3.3	-32 to -54
			180 d in 5% formalin	-2.1	-18 to -30

As with length reduction, dry weight loss declined with increasing fish size. Hay (1984) suggested that weight loss in formalin-preserved herring larvae was a function of the surface area:volume ratio and thus weight loss was proportionately less in larger fish. It is possible that this may be true for both freezing and formalin preservation, and that dry weight loss in juvenile and adult fish may be much less than the relationships of this study would predict. Weight loss may be greater in larval walleye because they lack scales. Stobo (1972) hypothesized that heavy scalation in spiny-rayed fishes slowed the osmotic processes involved with formalin preservation. Walleye typically begin to develop scales at a total length of 30 mm (Glenn and Mathias 1985).

The increase in the energy density of walleye which accompanied freezing suggests that disproportionately more low energy matter, such as ash or protein, is lost during freezing. In addition, the lower percentage of unburned residue in frozen fish samples suggests that ash losses may be disproportionately high. Because the chemical composition of fish larvae changes during development, I would expect that the relative increase in energy density which accompanies preservation would also vary with larval development. No distinct pattern emerged between percent change in energy density and larval size in this study. However, a trend may be evident over a wider developmental range. I was unable to find any published studies which examined the effects of preservation on the energy density of fish. However, increases in energy density following preservation has been reported for marine copepods preserved in either formalin or ethanol (Giguère et al. 1989).

Freezing appears to be a suitable preservation method where growth is estimated from length measurements. Length shrinkage was slight and comparable to that seen in formalin-preserved fish. However, freezing caused much greater dry weight loss than formalin, particularly in smaller larvae. Where estimates of larval dry weight are required, such as in energetic studies, suitable correction factors should be applied or other preservation techniques utilized.

Appendix C:

Preservation effects upon crustacean zooplankton

Introduction

Zooplankton samples are usually stored in chemical preservatives between the time of collection and analysis in the laboratory (de Bernardi 1984). Though length reduction due to preservation appears to be minor (Kuhlmann et al. 1982; Kulka and Corey 1982), dry weight loss can be severe (Omori 1978; Williams and Robins 1982; Boettger and Schnack 1986; Giguère et al. 1989). Freezing of zooplankton samples has been considered as an alternative but dry weight losses resulting from this technique also appear to be quite high (Williams and Robins 1982). Correcting for such preservation changes is essential if ecological data are to be properly interpreted.

The purpose of this study was to examine the effects of preservation on freshwater crustacean zooplankton. Specifically, I wanted to examine how the technique of killing in ethanol and freezing in pond water affected the dry weight and energy density of zooplankton.

Materials and Methods

This research was conducted at the Freshwater Institute and the University of Manitoba, Winnipeg in June 1993. Zooplankton were obtained primarily from two locations: the La Salle River, 10 km south of Winnipeg, and a water retention pond on Dalhousie Drive in Winnipeg. A single collection was made at another water retention pond along Waverley Boulevard in Winnipeg. The zooplankton of the LaSalle River and Waverley pond were primarily *Ceriodaphnia sp.* and *Bosmina sp.* with some *Daphnia sp.*, whereas the Dalhousie pond community was primarily composed of cyclopoid and calanoid copepods with some *Daphnia sp.* Zooplankton were collected with a Wisconsin net (25 cm diameter, 73 µm mesh) thrown from shore. Collected organisms were immediately brought back to the laboratory and passed through a series of Nitex® sieves to separate them into three size fractions hereafter referred to as L (large, 509-1050 µm), M (medium, 300-509 µm), and S (small, 183-300 µm). Not all fractions were present in every collection.

The effects of freezing on dry weight were examined as follows. The zooplankton size fractions were each randomly divided into two aliquots. Organisms from the first aliquot were

held live in a petri dish over ice. For each fraction, four random subsamples were drawn with an eye-dropper and placed on pre-weighed aluminum trays (~1.3 mg). Excess water was removed by capillary action by touching a piece of absorbent paper to the edge of the pans. This caused the organisms to adhere to the pan and facilitated counting. The number of organisms per pan was counted under a dissecting scope and all foreign material removed with dissecting needles. The number of organisms per pan ranged from 10-20 for the L fraction to 50-200 for the S fraction. Samples were oven-dried at 60 °C for 8 h, placed in a desiccator for 1 h, and weighed to the nearest 1 µg on a Perkin-Elmer AD-6 Autobalance. Mean individual weight was calculated as the total dry weight on the four pans divided by the total number of organisms. Plankters from the second aliquot were killed by immersion in 95% ethanol and frozen in ~15 mL of pond water at -18 °C. After 2 d, the second aliquot was thawed at room temperature and four random subsamples were placed on pans and dried and weighed as above. Plots of mean fresh dry weight vs. mean preserved dry weight were constructed separately for copepods and cladocerans. Mean fresh dry weight was regressed against mean preserved dry weight following standard regression procedures (GLM procedure, SAS Institute Inc. 1985).

The effects of freezing on caloric content were examined as follows. Zooplankton size fractions were each randomly divided into two aliquots. Zooplankton of the first group were immediately oven-dried on glass slides at 60 °C for 24 h. Zooplankton of the second group were killed by immersion in 95% ethanol and frozen in ~15 mL of pond water at -18 °C for 2 d then thawed and oven-dried on glass slides at 60 °C for 24 h. Each aliquot was then homogenized by pulverizing with a mortar and pestle and pressed into pellets of ~ 8-12 mg. The pellets were placed on pre-weighed platinum trays, dried again for a minimum of 8 h, desiccated for 1 h, weighed to the nearest 0.01 mg on a Cahn Electrobalance®, then finally burned in a Phillipson microbomb calorimeter (Gentry Instruments, Inc., Aiken, S.C.) following standard calorimetric techniques (Prus 1975). Platinum trays were reweighed after bombing to determine the total weight of unburned residue. Energy density was calculated on both a total dry weight and a residue-free dry weight basis. Differences between fresh and frozen treatments were analyzed using paired-observations *t*-tests (Steel and Torrie 1980).

Results

Plots of fresh vs. preserved mean weights along with regression equations are illustrated in Fig. C.1. Variation about the relationship was greater for Cladocera than for Copepoda. The regression slope was significantly greater than 1 for both the copepods ($|t|$ = 4.95, df = 10, P = 0.0006) and cladocerans ($|t|$ = 3.866, df = 23, P = 0.0008). However, the intercepts were not significantly different from 0 for either regression ($|t| < 0.30$, $P > 0.75$). This suggests that the process of killing in ethanol and freezing in water caused significant reduction in dry weight of crustacean zooplankton but that the reduction was not significantly dependent on zooplankton size. The mean ratios of fresh:frozen dry weight were 1.231 and 1.206 for Copepoda and Cladocera, respectively. These corresponded to dry weight losses of 18.8% and 17.1% for Copepoda and Cladocera, respectively.

Bomb calorimetry was performed on relatively few zooplankton samples. Generally, determinations were made on single subsamples from each of the corresponding fresh and frozen treatments. Replicate determinations of energy density were made for only a single size fraction on one sample date (28 June, L). The coefficient of variation in this case was 10.7%. Energy densities were consistently higher in frozen samples than in fresh samples (Table C.1). Paired-observations *t*-tests demonstrated a significant difference between energy densities of fresh and frozen zooplankton when calculated on a total dry weight basis ($|t|$ = 4.53, df = 4, P = 0.011) and a near-significant difference when calculated on a residue-free dry weight basis ($|t|$ = 2.71, df = 4, P = 0.053). Scatter plots did not indicate any clear relationship between the fresh:frozen energy density ratio and mean zooplankton dry weight. Mean values of the fresh:frozen energy density ratio were 0.915 and 0.935 for dry weight and residue-free dry weight determinations, respectively. These represented increases in energy density of 9.3% and 7.0%, respectively. Too few samples were analyzed to determine if the change in energy density differed between cladocerans and copepods. All samples were primarily (> 90%) copepods with the exception of the 13 June collection (Table C.1) which was exclusively cladocerans. The percentage of unburned residue was markedly higher in the 13 June sample relative to the other samples (Table C.1). Unburned residue as a fraction of total dry weight was not significantly different between fresh and frozen treatments (paired-observations *t*-test, $|t|$ = 0.49, df = 4, P = 0.65).

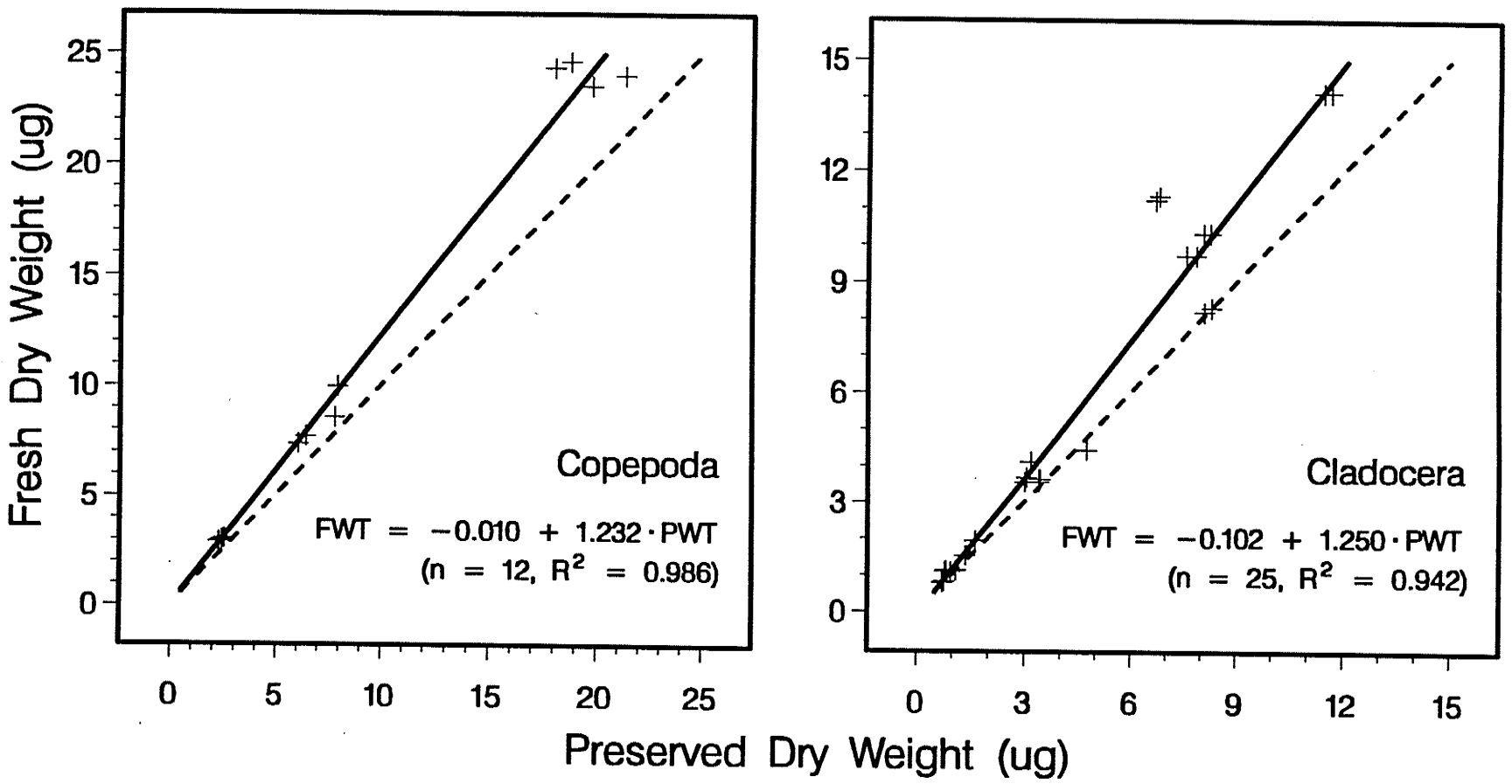

Figure C.1. Scatter plots of fresh dry weight versus preserved dry weight for frozen Copepoda and Cladocera. Symbols represent weighted means. Solid line represents the observed relationship. Dashed line represents 1:1 agreement between fresh and preserved measurements.

Table C.1. Energy densities of fresh and frozen crustacean zooplankton collected on several dates in 1993. Zooplankton were divided into small (S), medium (M), and large (L) size classes (see text). Samples were composed primarily (> 90%) of cyclopoid and calanoid copepods with the exception of the 13 June sample which was composed of *Bosmina sp.* and *Ceriodaphnia sp.* Energy density values were calculated based on total dry weight (DWT) and residue-free dry weight (RFDWT) where the weight of residue was the dry weight of sample material remaining after combustion in a bomb calorimeter. All data represent single determinations with the exception of the frozen treatment of the L fraction sampled on 28 June where data represent means of two determinations.

				Energy Density	
Date	Size	Treatment	Residue (%)	kJ·g DWT $^{-1}$	kJ·g RFDWT $^{-1}$
10 June	S	Fresh	6.49	23.59	25.23
		Frozen	2.54	25.51	26.17
13 June	S	Fresh	16.55	20.65	24.74
		Frozen	17.50	21.34	25.87
28 June	S	Fresh	6.72	21.57	23.13
		Frozen	9.64	23.61	26.12
28 June	M	Fresh	5.47	23.44	24.79
		Frozen	4.42	27.07	28.32
28 June	L	Fresh	5.23	22.36	23.59
		Frozen	3.49	24.79	23.75

Discussion

As with larval fish preservation, I assumed that the freezer storage time for the samples was not an important variable because most of the changes occurring in the preserved tissue are associated with the freezing and thawing processes (Farrant 1980). However, storage time may be an important variable in determining the weight loss of zooplankton stored in chemical preservatives. Omori (1978) noted an exponential decline in the organic weight of formalin-preserved zooplankton over time.

Weight losses of preserved zooplankton observed in this study were generally lower than those reported in previous studies (reviewed by Giguère et al. (1989)). This may be due to the choice of fluid used to preserve the plankters. In all cases I used water from the environment in which the organisms were collected. Osmotic losses would therefore be expected to be low. Omori (1978) demonstrated that marine zooplankton rinsed in distilled water lost significantly more weight than zooplankton rinsed in seawater. Williams and Robins (1982) observed a 57% loss of dry weight in marine copepods which were rinsed in de-ionised water then frozen. The observed weight loss of frozen zooplankton in this study was also less than that observed in frozen larval walleye (Appendix B). This may be due to differences in body structure. The hard exoskeletons of zooplankton may resist damage from freezing better than the soft external tissues of larval fish.

Some alternative preservation methods have been proposed to prevent dry weight losses of zooplankton and other invertebrates. Freezing in weak solutions of aldehydes appears to overcome the problem of weight loss (Salonen and Sarvala 1985; Kimmerer and McKinnon 1986). Freezing followed by lyophilization was recommended by Giese (1967) as a suitable means of preserving invertebrates for biochemical analyses. In addition, this technique has been shown to prevent the loss of radio-tracer elements in zooplankton (Berberovic and Pinto-Coelho 1989). Presumably, dry weight losses from lyophilized material would also be minimal.

Changes observed in the energy density of zooplankton as a result of freezing were similar to those observed in larval walleye (Appendix B). Giguère et al. (1989) noted energy density increases of 13-27% in chemically-preserved relative to fresh marine copepods. Similarly, Schindler et al. (1971) found that energy densities of the freshwater calanoid *Diaptomus oregonensis* increased by 15.6% and 22.9% when subjected to formalin

preservation and freezing, respectively. However, collection dates differed for fresh and preserved specimens in the latter study and therefore some of the energy density difference may be due to seasonal variation.

The higher energy densities observed in frozen zooplankton indicates that the dry matter lost during freezing is energetically less dense than what remains. It may be that certain body constituents are more easily lost than others during freezing and thawing. Giguère et al. (1989) noted that chemically-preserved copepods lost a larger percentage of inorganic matter than organic matter. Because the chemical composition of zooplankton can vary widely among different taxonomic groups (reviewed by Vijverberg and Frank (1976)), as well as seasonally within taxa (Schindler et al. 1971; Snow 1972), the dry weight loss and change in energy density may also vary taxonomically and seasonally.

This study has demonstrated that the preservation technique of killing in ethanol and freezing in pond water can result in significant changes in both dry weight and energy density of zooplankton. Though the observed weight losses were on average lower than those previously reported they were nonetheless substantial and failure to account for such changes could introduce significant errors into the interpretation of ecological studies.

Appendix D:
Laboratory feeding experiments conducted in 1987

Introduction

In Chapter 3, I presented a series of laboratory feeding experiments conducted in 1988 that examined the functional response and prey selection of walleye larvae. I initially conducted feeding experiments in 1987 following a slightly different methodology. For various reasons, I felt that the methods used and the results obtained from the 1987 feeding experiments were unsatisfactory, and thus, I repeated the feeding experiments in 1988. However, some of the results from the 1987 experiments were interesting and I felt that they should be presented. This appendix describes the feeding experiments of 1987.

Materials and Methods

This research was carried out at the Department of Fisheries and Oceans' Dauphin Lake Walleye Rehabilitation and Research Station at Methley Beach (Dauphin Lake), Manitoba in May and June of 1987. Hatchery-raised walleye larvae from two stocks (Table D.1) were brought into the laboratory within 1-3 d after hatching and raised in 120-L glass stock aquaria at 20 °C. The sides of the stock aquaria were covered with translucent green plastic sheeting. Wild zooplankton swept from the field station culture ponds were provided at least 1 d prior to the onset of feeding and maintained at densities > $200 \cdot L^{-1}$ thereafter. Experimental units were 13-L rectangular glass aquaria covered in green plastic sheeting and filled with 4 L of 20 °C (range 19-21 °C), filtered (through 45 μm Nitex® mesh) pond water. Light levels over the aquaria ranged from 3 to 5 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Laboratory experiments were conducted on six dates corresponding to walleye mean lengths of 9.44 to 11.5 mm (Table D.1). From 12 to 20 aquaria were used in each experimental run and 1-3 runs were performed in a given day (Table D.1). Within a run, each aquarium was randomly assigned a different zooplankton treatment and the runs represented replicates blocked for the time of day. Prior to each run, larvae were removed from the stock aquaria and placed in the pre-experimental chamber. The pre-experimental chamber was a 35-L aquarium filled with 20 L of filtered pond water containing newly-hatched brine shrimp (*Artemia sp.*) at a density of > $500 \cdot L^{-1}$. Larvae were allowed to feed for ~ 1.5 h before being

Table D.1. Summary of laboratory feeding experiments conducted in 1987. Walleye larvae were obtained from Crean Lake, Saskatchewan, and from the Swan Creek stock of Lake Manitoba. Each experimental run involved 20 experimental aquaria (see text). Experimental runs were replicated (blocked) 1-3 times on each experimental date. Feeding time represents a mean calculated for all experimental aquaria. Dry weights for individual larvae were estimated from length-dry weight relationships (not presented).

Date	Walleye stock	Walleye size ($\bar{X}$ ± 1 SD)		Replicates	Larvae·aquarium^{-1}	Feeding time (min)
		Length (mm)	Dry weight (mg)			
16 May	Lake Manitoba	9.88 ± 0.25	0.58 ± 0.05	1	10	165
18 May	Lake Manitoba	10.3 ± 0.40	0.70 ± 0.10	1	10	97
19 May	Lake Manitoba	10.9 ± 0.58	0.87 ± 0.18	1	10	93
28 May	Crean Lake	9.44 ± 0.23	0.50 ± 0.05	3	10	90
30 May	Crean Lake	9.71 ± 0.29	0.55 ± 0.08	3	10	95
2 June	Crean Lake	11.5 ± 0.76	1.17 ± 0.33	3	10	92

moved to the experimental aquaria. The purpose of this approach was to allow the larvae to feed continuously, and to allow them to ingest a readily identifiable food which could be used as a marker to later define which ingested zooplankton were consumed in the experimental aquaria.

Zooplankton treatments were added to the experimental aquaria while the larvae were feeding in the pre-experimental aquarium. Zooplankton were swept from the Methley culture ponds and passed through a series of Nitex® sieves to separate them into three size fractions hereafter referred to as XS (extra-small, 73 - 202 μm), S (small, 202 - 315 μm), M (medium, 315 - 509 μm), L (large, 509 - 1050 μm), and XL (extra-large, > 1050 μm). The S, M, and L fractions were used in all experiments, whereas the XS fraction was used on three experimental dates (18 May, 28 May, 30 May) and the XL fraction on only one experimental date (19 May). Samples of each fraction were preserved in 5% buffered formalin for each experimental run to determine size and species composition. Most aquaria (12 on 2 June, 16 on all other dates) were randomly assigned one of the straight size fractions (XS, S, M, L, and XL) at one of four abundances (20, 50, 100, or 200 prey·L^{-1}). Four other aquaria were given an assemblage of 4 (3 on 2 June) of the straight size fractions in equal proportions at one of four abundances (80, 100, 200, or 400 prey·L^{-1} on most dates, except 60, 75, 150, and 300·L^{-1} on 2 June). I commenced experiments by removing the larvae from the pre-experimental chamber and placing 10 larvae in each experimental aquarium. I terminated experiments by removing the fish from the experimental aquaria, sacrificing them in MS-222 and preserving them in 5% buffered formalin. Total experimental feeding time was generally 1.5 h with the exception of 16 May when larvae were allowed to feed for nearly 3 h (Table D.1).

Preserved larvae were measured to the nearest 0.1 mm using an ocular micrometer. Length was measured as total length. Preserved lengths were converted to fresh lengths using the formula of Appendix B. Ingested brine shrimp nauplii were counted. Ingested zooplankton were divided into two groups; those anterior to brine shrimp in the gut, and those posterior to brine shrimp or occurring alone in the gut. Zooplankton anterior to brine shrimp were identified to genus, enumerated and measured to the nearest 0.02 mm with an ocular micrometer as described in Chapter 3. Brine shrimp dry weight was estimated as a constant 1.62 μg·$nauplius^{-1}$. This figure was obtained by drying and weighing samples of recently-

hatched brine shrimp using methods as described for weighing zooplankton in Appendix A. Zooplankter dry weights were estimated using length-dry weight formulae developed for zooplankton of the Methley culture ponds (Appendix A). Zooplankton consumption rate was calculated for each fish as the dry weight (µg) of zooplankton anterior to the brine shrimp in the gut divided by the foraging time in the experimental aquarium (h). Similarly, I estimated the brine shrimp consumption rate for each fish as the dry weight of all consumed brine shrimp divided by the foraging time in the pre-experimental chamber (1.5 h).

Results and Discussion

These experiments required that the walleye larvae consume brine shrimp in the pre-experimental chamber prior to consuming zooplankton in the experimental aquaria. This would allow me to determine which zooplankton in the fish guts were consumed during the experiment and which were consumed in the stock tank prior to the experiment. However, very few larvae actually consumed both brine shrimp and zooplankton. Many of the Lake Manitoba larvae did not feed, and a large percentage of both stocks of larvae which did feed consumed brine shrimp or zooplankton but not both (Fig. D.1). Only for the largest larvae of the Crean stock (2 June experiments, mean length 11.5 mm) did > 20 % of the larvae consume both brine shrimp and zooplankton. The large number of non-feeders in the early trials may have simply resulted from starting the experiments at an early developmental stage. The patterns observed among those larvae which did feed are more difficult to explain.

Evidently, first-feeding walleye larvae are able to consume brine shrimp at higher rates than wild zooplankton. Mean brine shrimp consumption rates for the Crean Lake larvae (excluding non-feeders) were 10.9, 21.1, and 36.2 $\mu g \cdot h^{-1}$ for the three experimental dates corresponding to walleye mean lengths of 9.44, 9.71, and 11.5 mm, respectively. Because of the high prey densities of the pre-experimental chamber (> $500 \cdot L^{-1}$), I considered these estimates to be near the maximum consumption rates, C_{max}, attainable for walleye larvae of these sizes consuming brine shrimp. However, these estimates may be slightly conservative as the larvae were sampled ~ 1.5 h after feeding on brine shrimp had ceased. In comparison to C_{max} estimates for walleye larvae feeding on zooplankton (Chapter 3, Fig. 3.2), the C_{max} estimates for larvae feeding on brine shrimp were higher for first-feeding larvae but not for the largest (11.5 mm) larvae. The slow-moving, highly visible brine shrimp may be a much easier

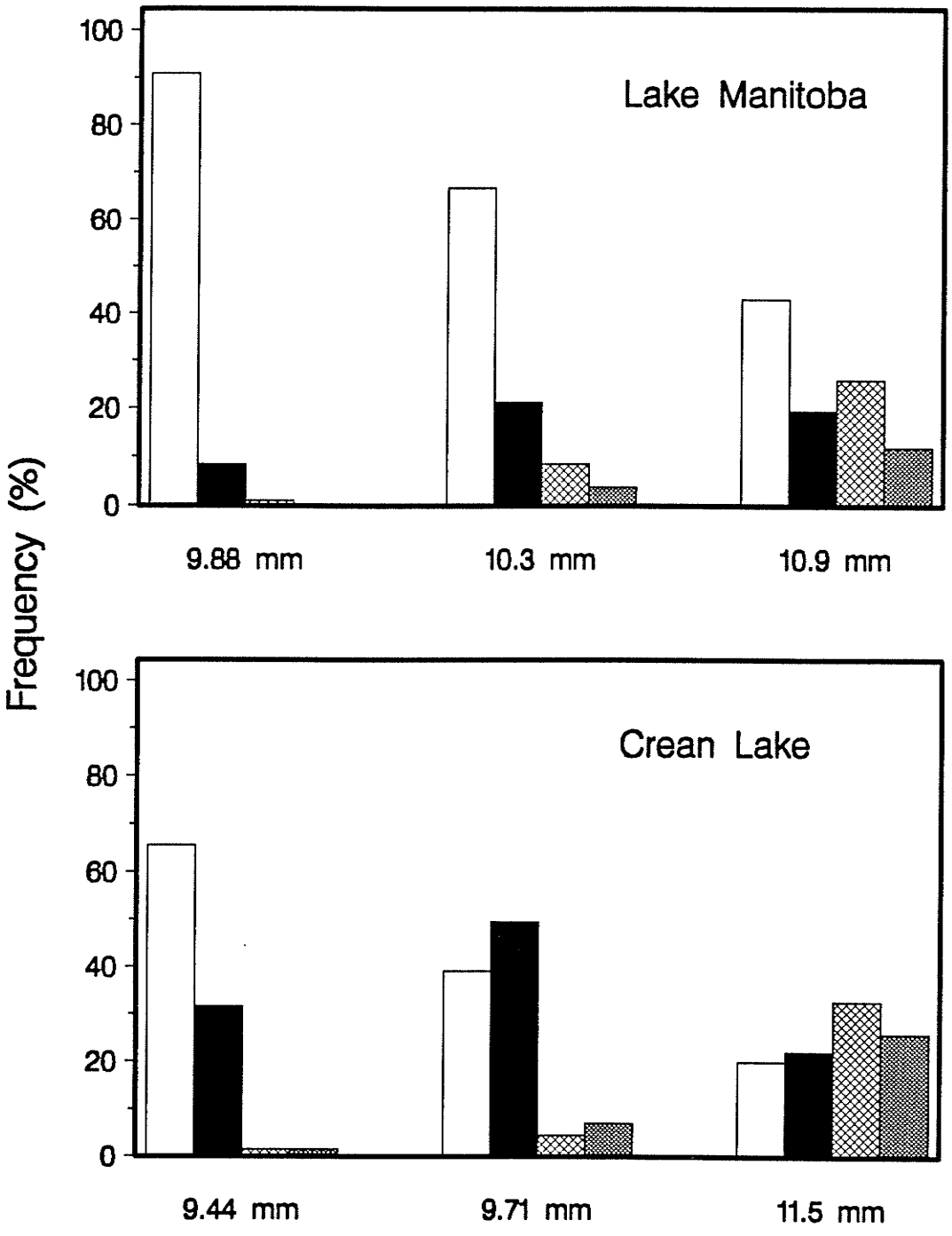

Figure D.1. Percentages of all walleye larvae guts from the 1987 feeding experiments which contained no food (□), brine shrimp only (■), zooplankton only (▨), or brine shrimp and zooplankton (▩). The six histograms correspond to the three experiments conducted using walleye of Lake Manitoba stock (top) and the three experiments conducted using Crean Lake stock (bottom) (see Table D.1). Numbers below histograms indicate the mean lengths of larvae used in each experiment.

prey to locate and capture compared to wild zooplankton. However, the relative advantages of brine shrimp to wild zooplankton as prey probably diminishes as the walleye grow. The ability of walleye larvae to consume brine shrimp at higher rates than they can consume wild zooplankton may be the primary reason why walleye larvae have been observed to exhibit higher growth on the former diet (Hokanson and Lien 1986).

I examined the functional response for only the S, M, and L zooplankton treatments of the 2 June experiments. Only walleye which consumed experimental zooplankton after consuming brine shrimp were used in this analysis. Zooplankton consumption rate increased with increasing zooplankton abundance up to 100 prey·L^{-1} but then declined over the prey abundance range of 100-200 prey·L^{-1} (Fig. D.2). Maximum observed consumption rates were lower than C_{max} estimates from the functional response models of Chapter 3 for similar-sized larvae (Fig. 3.2). The larvae may have fed at lower rates simply because their guts were nearly full. Handling times per unit of prey generally increase with predator satiation (Werner 1974; Confer and O'Bryan 1989), and thus, consumption rate would be expected to decline with increasing gut fullness. The pre-experimental treatment may have also affected the feeding behaviour of the walleye in the experimental aquaria. Switching prey types may require a period of adjustment in which the predator learns to recognize the new organism as prey and/or develops specific tactics to capture the new prey. This may partly explain why so many walleye in these experiments consumed brine shrimp or zooplankton but not both. To my knowledge, the short-term effects of switching prey communities on walleye foraging has not been examined. However, gut analysis of larvae from culture ponds indicated that the walleye diet can be quite varied (Appendix A), and thus, switching to different prey types does not appear to be a problem over longer periods of time.

The decline phase of the walleye functional response in these experiments was most pronounced for the large prey treatment and least pronounced for the small prey treatment (Fig. D.2). The effect of declining consumption rate with increasing prey abundance has been observed previously in goldfish, *Carassius auratus*, feeding on *Daphnia sp.* (Welty 1934) and in first-feeding walleye larvae foraging on large zooplankton (primarily *Daphnia sp.*, J.A. Mathias, unpubl. data). This effect may be the result of confusion from seeing many prey items at once (Marcotte and Browman 1986). Welty (1934) noted that goldfish had difficulty

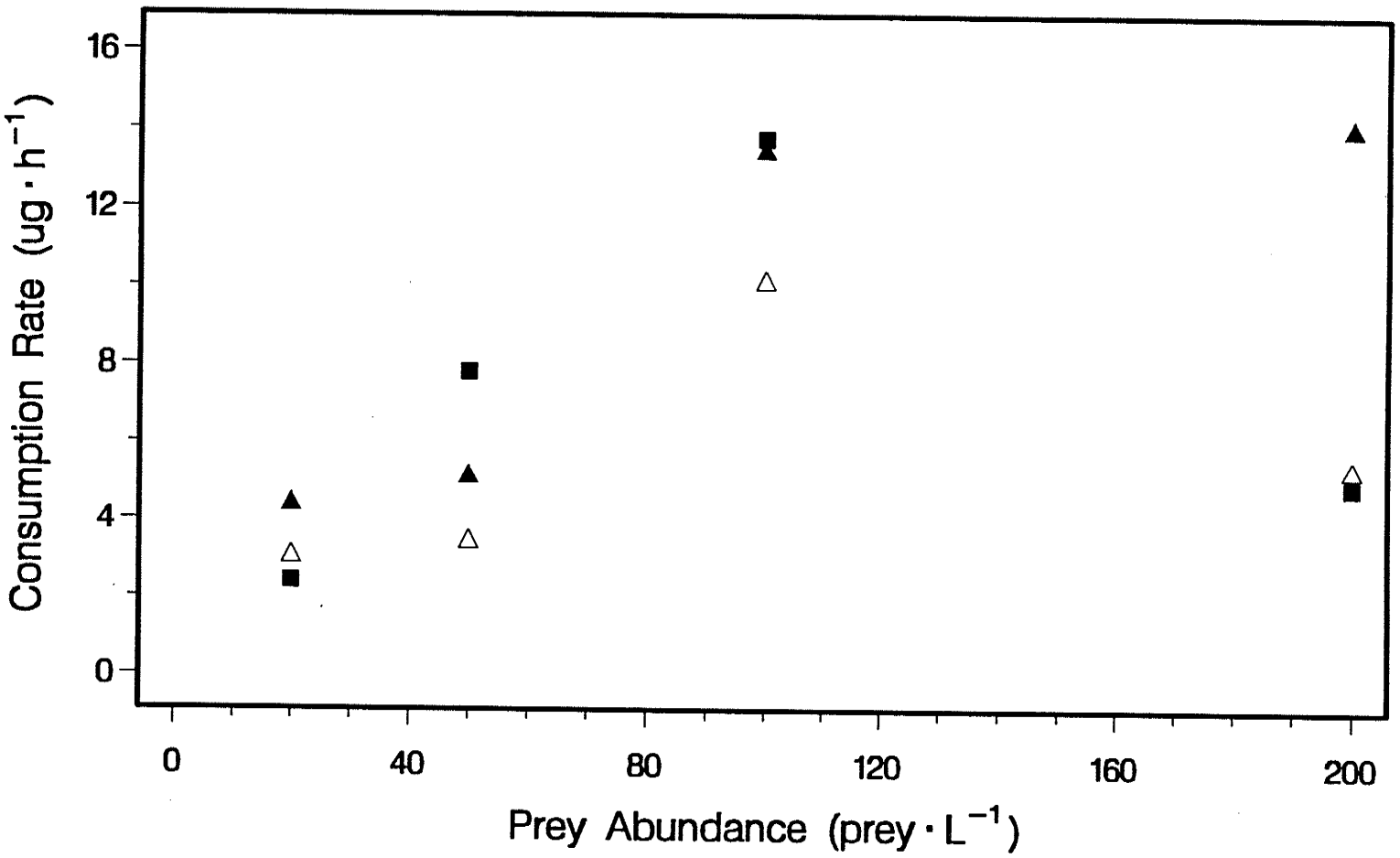

Figure D.2. Functional response of Crean Lake walleye larvae (mean length 11.5 mm) feeding on small (202-315 µm body width, ▲), medium (315-509 µm, △), and large (509-1050 µm, ■) wild crustacean zooplankton at 20 °C in the laboratory on 2 June 1987. Walleye larvae were allowed to feed at near maximal rates on brine shrimp (*Artemia sp.*) prior to these experiments. Symbols represent means of consumption estimates from three aquaria.

striking and capturing a single prey item when many others were visible. Larger prey items are more visible than small prey items, and thus, for a given numerical prey density, a larval walleye will see more large prey items simultaneously than small prey items. Thus, the trend of declining consumption rate with increasing prey abundance should be evident at lower prey abundances for large prey than for small prey. This is consistent with the pattern observed in these experiments.

Appendix E:

Assaying the chitin content of crustacean zooplankton

In Chapter 5, I estimated the assimilation efficiency of walleye larvae feeding on zooplankton using a methodology requiring only qualitative samples of food and faeces. The procedure required quantitative estimates of both the nutrient and an indigestible marker substance in both the food and faeces. Initially, I chose to use chitin as the indigestible marker substance. This appendix describes my rationale for using chitin as an internal marker, and my attempts to estimate chitin concentrations in zooplankton and walleye faeces.

The rationale for using chitin as a dietary marker is relatively simple. First, it occurs naturally in the zooplankton food of walleye larvae. Chitin forms ~ 6-8% of the dry weight of freshwater zooplankton (Yurkowski and Tabachek 1979). Second, though many species of fish possess chitinolytic enzymes in their digestive tracts (Lindsay 1984; Seiderer et al. 1987) the digestibility of chitin appears to be low (Buddington 1980; Lindsay et al. 1984). The apparent purpose of these enzymes is to break the structure of invertebrate exoskeletons to allow food passage through the gut rather than to reduce the chitin to units small enough for assimilation (Lindsay 1984). Thus, I assumed that the chitin of freshwater zooplankton was partly digestible but could not be assimilated by walleye larvae.

In reviewing the literature, I was only able to find two studies which used dietary chitin as an internal marker to estimate assimilation efficiency. Buddington (1980) used chitin, cellulose, and chromic oxide as markers to estimate the assimilation efficiency of rainbow trout fed a formulated diet. Rösch (1987) used chitin as a dietary marker to estimate the assimilation of *Daphnia pulicaria* by juvenile vendace. The method used by Buddington requires much larger samples of food and faeces than can be feasibly obtained from walleye larvae. Rösch's procedure is applicable to smaller samples but the fluorometric chitin assay which he used was not published (Doctoral thesis, in German). I chose to utilize another fluorometric assay for quantifying small amounts of chitin (10-50 µg) as described by Montgomery et al. (1990).

I followed the assay procedure outlined by Montgomery et al. (1990) with only a few modifications. A known dry weight of the chitin standard or biological sample (zooplankton or

fish faeces, see Chapter 5 for collection procedures) was added to 3 mL of phosphate buffer (10 mM, pH 7.4-7.6) along with a known quantity (20 µg) of a fluorescent-marked sugar-binding protein (lectin). The standard was chitin purified from crab shell (Sigma Chemical Co., St. Louis, MO) and this was maintained as a suspension in the phosphate buffer so that it could be added volumetrically with an Eppendorf pipette. The lectin used was succinylated fluorescein isocyathionate (FITC)-labelled wheat germ agglutinin (WGA) (Vector Laboratories, Inc., Burlingame, CA; EY Laboratories, San Mateo, CA). This lectin has been shown to bind strongly to series of 3 consecutive N-acetylglucosamine (NAG) residues (the subunits of the chitin polymer) (see references in Montgomery et al. 1990). The lectin was maintained in a solution of the buffer (1 $mg \cdot mL^{-1}$) and delivered volumetrically with an Eppendorf pipette. The buffer with sample and FITC-WGA was then incubated on a shaker table (30 min, 20 °C, 130 rpm) and filtered (0.2-µm Nuclepore polycarbonate filters) with suction using a Millipore filtration system. The filter was rinsed twice with 1 mL of fresh buffer. The filtrate was then measured for unbound FITC-WGA using a fluorometer (Perkin-Elmer LS-50 Luminescence Spectrometer) at excitation and emission wavelengths of 495 nm and 519 nm, respectively. Because the assay measures unbound FITC-WGA, the intensity of fluorescence in the filtrate should be negatively related to the quantity of chitin in the sample.

I was unable to duplicate the results of Montgomery et al. (1990). The FITC-WGA marker did not appear to bind well to the chitin particles. Intensities of fluorescence in the filtrate were roughly the same for control samples (no chitin added) and samples which had excess chitin (> 1 mg). Use of a tris buffer (10 mM; pH 7.4) rather than a phosphate buffer in the assay did not change the outcome significantly. Similarly, the FITC-WGA produced by Vector Laboratories showed only slightly better binding properties than the FITC-WGA produced by EY Laboratories. Following a suggestion by Dr. J. Whitehead (Vector Laboratories), I used FITC-labelled potato lectin rather than FITC-WGA in the assay. However, this lectin showed practically no binding whatsoever. I contacted Dr. Montgomery for advice on his methodology, but, numerous consultations with him did not lead to a solution of the problem. In reviewing the citation indices, I could not find any published studies which had used the assay of Montgomery et al. (1990). I spoke with chemists at both Vector Laboratories and EY Laboratories about the binding properties of their lectins. Both laboratories agreed to repeat

the same assay using their respective products and both found very poor binding. In one of these cases, the observed affinity of FITC-WGA for the chitin standard was three orders of magnitude less than was reported by Montgomery et al. (1990) (Dr. J. Whitehead, Vector Laboratories, pers. comm.). At this point, I chose to abandon the assay.

Why didn't the assay work? According to Dr. Whitehead of Vector Laboratories, the binding of the lectin to the chitin is probably very dependent on the particle size of the chitin standard or biological sample. Because the chitin polymer is tightly folded on itself, the FITC-WGA probably binds only to the surface of particles, and thus increasing the exposed surface area by decreasing the particle size should improve the binding. If this is true, then a standard curve relating the quantity of chitin in a sample to the filtrate fluorescence would only be useful if the biological sample contained chitin particles equal in size to the chitin standard. Obtaining equal particle sizes would probably be very difficult. Furthermore, Dr. Montgomery indicated to me that he did not try to standardize the mean and range of particle sizes between the chitin standard and biological samples used in his published assay. Dr. Whitehead also suggested that treating the chitin with acid prior to using it in the assay could open up the polymer structure and allow the lectin better access to binding sites. Chitin is commonly treated in this manner when being prepared as a substrate for enzyme assays (e.g. Seiderer et al. 1987). It is not clear whether this would eliminate the particle size problem or not. Finally, I spoke with Dr. Roger Laine, a biochemist who studies chitin at Louisiana State University. He agreed with Dr. Whitehead that the fluorometric assay as described would be very dependent on the particle sizes used in the experiment. In his opinion, the FITC-labelled lectins are most appropriate for localizing chitin in histological work and would not provide a reliable means of quantifying chitin. He suggested that the most reliable method of quantifying chitin would be to measure the quantity of NAG using an amino acid analyzer. The sample would first be digested in NaOH to remove all matter other than chitin, then subjected to strong hydrolysis (conc. HCl, 100 °C) to break the polymer into its NAG subunits. Dr. Laine felt that this approach would be applicable to small sample sizes.

Though I did not pursue chitin analysis beyond the original fluorometric assay in this study, I feel that it should be examined further in the future. Because chitin is so widespread in the invertebrates commonly consumed by young fish, and because it appears to be

relatively indigestible to most fish, it seems to be the ideal internal marker for estimating assimilation efficiency. All that is required is a means to quantify it in small samples.