EFFECT OF PREY CONCENTRATION AND LIGHT ON THE FORAGING BEHAVIOUR, GROWTH AND SURVIVAL OF ATLANTIC COD LARVAE (Gadus morhua) UNDER LABORATORY CONDITIONS

VELMURUGU PUVANENDRAN


CENTRE FOR NFLD. STUDIES
MEMORIAL UNIVERSITY
OF NEWFOUNDLAND

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

UMI®
800-521-0600

Memorial
University of Newfoundland

This is to authorize the Dean of Graduate Studies to deposit two copies of my thesis/report entitled

Effect of prey concentration and light on the foraging behaviour, growth and survival of Atlantic cod larvae (Gadus morhua) under laboratory conditions.

in the University Library, on the following conditions. I understand that I may choose only ONE of the Options here listed, and may not afterwards apply for any additional restriction. I further understand that the University will not grant any restriction on the publication of thesis/report abstracts.

(After reading the explanatory notes at the foot of this form, delete TWO of (a), (b) and (c), whichever are inapplicable.)

The conditions of deposit are:

(a) that two copies are to be made available to users at the discretion of their custodians,

OR

(b) that access to, and quotation from, this thesis/report is to be granted only with my written permission for a period of one year from the date on which the thesis/report, after the approval of the award of a degree, is entrusted to the care of the University, namely, _____ 19 ____, after which time the two copies are to be made available to users at the discretion of their custodians,

OR

(c) that access to, and quotation from, this thesis/report is to be granted only with my written permission for a period of _____ years from the date on which the thesis/report, after approval for the award of a degree, is entrusted to the care of the University; namely, _____ 19 ____; after which time two copies are to be made available to users at the discretion of their custodians.

Date 04 January 1999

Signed [signature]

[signature]
Dean of Graduate Studies

Witnessed by [signature]

NOTES

1. *Restriction (b) will be granted on application, without reason given.*

   However, applications for restriction (c) must be accompanied with a detailed explanation, indicating why the restriction is thought to be necessary, and justifying the length of time requested. Restrictions required on the grounds that the thesis is being prepared for publication, or that patents are awaited, will not be permitted to exceed three years.

   Restriction (c) can be permitted only by a Committee entrusted by the University with the task of examining such applications, and will be granted only in exceptional circumstances.

2. *Thesis writers are reminded that, if they have been engaged in contractual research, they may have already agreed to restrict access to their thesis until the terms of the contract have been fulfilled.*

Effect of prey concentration and light on the foraging behaviour, growth and survival of Atlantic cod larvae (*Gadus morhua*) under laboratory conditions.

By

Velmurugu Puvanendran

A thesis submitted to the School of Graduate
Studies in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

Department of Biology
Memorial University of Newfoundland
1999

St.John's Newfoundland

ABSTRACT

This thesis describes experiments on the responses of Atlantic larval cod to two important ecological variables, prey concentration and light in terms of behaviour, growth and survival. The first ecological variable investigated was light intensity and its effect on the foraging behaviour, growth and survival of Atlantic cod larvae from two geographical regions in the Northwest Atlantic. Larval cod originating from different geographical locations responded differently to light intensity. Larvae originating from the Scotian Shelf (SS origin) foraged, grew and survived better in low light intensity while larvae from the Northeastern Grand Banks (NF origin) performed better in high light. This difference in response to light intensity may be explained by the different spawning seasons rather a than latitudinal difference.

The next ecological variable investigated was prey concentration. Earlier studies on larval fish indicated that growth and survival of the larvae vary with prey concentration. However, the shortcoming of most of these studies involving cod larvae was that they were short term experiments. Thus, I investigated the ontogeny of foraging behaviour of Atlantic cod larvae exposed to different prey concentrations from hatching to metamorphosis. Larvae exposed to higher prey concentration outperformed the larvae reared in lower prey concentrations in all the foraging Modal Action Patterns (MAP's) investigated in this study. But the magnitude of the foraging MAP's increased as the larvae grew regardless of prey concentration. Results also indicated development of foraging behaviour was not affected by prey concentration.

Next, I investigated the growth and survival of Atlantic cod larvae reared in a wide range of prey concentrations. My previous experiment showed that the highest prey concentration used (4000 prey L^{-1}) may not be the optimal prey concentration to rear the cod larvae in the laboratory. In this second experiment, prey concentrations of 8000 and 16000 prey L^{-1} were included. Results indicated no difference in growth when prey concentration above 4000 prey L^{-1} were used. Initially no difference was found in the survival of larval cod among the three highest prey concentrations (4000, 8000 and 16000 prey L^{-1}) but continuous use of prey concentrations above 4000 prey L^{-1} beyond 3 weeks post-hatch reduced the survival considerably. Initially, mortality rates of cod larvae were higher in prey concentrations lower than 4000 prey L^{-1}. Beyond 3 week post-hatch no significant difference was found in mortality rates among any of the treatments. Observations on foraging behaviour of larval cod indicated that larvae reared in higher prey concentrations foraged more efficiently than larvae reared in the lower prey concentrations. Observations from this study emphasize the importance of behavioural observations to explain any difference in growth variables between the treatments. Results indicated that for intensive rearing larval cod require a prey concentration of 4000 prey L^{-1} to sustain reasonable growth and survival.

I also investigated foraging, growth and survival of Atlantic cod larvae (NF origin) reared at varying light intensities and photoperiods. Behavioural observations were also carried out in an attempt to explain any differences in the performance of cod larvae under varying light intensities. Cod larvae grew and survived better in higher light intensity

(2400 lux) and 24L:0D photoperiod. The condition index (ratio of myotome height at anus to standard length) of the larvae was also better in high light intensity and 24 hr photoperiod. Examination of the foraging MAP's indicated that cod larvae reared in higher light intensity captured the prey more efficiently than larvae reared in low light.

Predator responses (functional, developmental and numerical) of larval cod to different prey concentrations were investigated in an attempt to further study some observations made in my earlier experiments. In this experiment prey consumption rates were investigated in terms of both age and size. Results indicated that the cod larvae exhibited a type II functional response where prey consumption increases with increasing prey concentration asymptotically at a decelerating rate. Developmental response of the cod larvae was closely correlated to the size. Prey consumption rates increased as the larvae grew. During the first two weeks post-hatch, larvae exposed to low prey concentrations (<1000 prey L^{-1}) did not feed enough to sustain sufficient growth and subsequently could not survive beyond three weeks.

Dedication

I dedicate this thesis to some of my family members. First of all to my parents who provided all the facilities and encouragement. Secondly to my elder brother, Dr.V.Ravindran, who encouraged me to pursue my post graduate study and pointed my way towards Joe. He was always very supportive and his encouragement enlightened me throughout my study. Thirdly to my daughter and son. They always brighten my life with their smile and laugh. That kind of cheering which I always wanted specially during my last couple of years of study.

ACKNOWLEDGMENTS

Firstly I thank to my wife, Daisy (Chandrika) for her support in all possible ways and for the encouragement throughout my program. I would not have done this without her.

I thank my supervisor, Dr. Joe Brown. He is the best supervisor for any student to have. He was very generous, supportive and most importantly understanding. I am very grateful for the financial support he provided throughout my study.

Thanks to my supervisory committee members, Drs Garth Fletcher and Derek Burton. Special thanks to Vee for his patience for editing my manuscripts and the support he offered while he was in OSC.

Thanks to Leslie who helped me to overcome the social barrier during the early stages of my study and for being my best friend. Also thank to Leslie and Danny for providing companionship to travel through the mystery (or misery !!) of larval rearing world. Many thanks to Donna for her support in live food production and for friendship.

Thanks to old lab buddies Grant, Jim and Sandy for making my early days at the lab enjoyable. I will not forget the fun we had along with Leslie, Vee and you too Trevor.

Many thanks to ever growing list of Joe's current students, Adrian (the dangerous guy), Avery (thanks for your statistical expertise), Ben, Craig, Jessica, Karen, Kelly, Louise, Marc, Martha, Russ and Scott and also to Dena and Olav. Thanks also to all of Joe's honour's and summer students for their help.

Thanks to Dr. Larry Crim's lab for providing all the help and allowed to use their

lab whenever I needed. Also my thanks to Betty for allowing me to use the electro balance. I also thank Dr.Richard Rivkin for allowing me to use his unpublished data.

Thanks to everybody at OSC, specially to Jim Devereaux, Damian, Danny, Jerry, Steve, Terry, Jim Fowler (thanks for the rides), Ross Wilson, Delores, Gen (you were always very kind), Helen, Winnie and all the custodians.

Thanks to everyone in the Biology Dept. General Office in particular Gail, Christine, Shirley and Shena. Many thanks to Ian Emerson for giving the opportunity to instruct the first year lab. Special thanks to all the lab instructors for their friendship specially to Cynthia, Darryl, Debbie, Ed, Linda, Paul and Renee and to Pat.

Thanks to my badminton buddies Benedict, Uditha, Harshana, Rupika and Ming for helping to diffuse my stress. It was a great help guys.

Importantly, I thank my brothers, sisters, brother in-laws, sister in-laws and friends for their support both morally and sometimes financially during my extended study period.

Many thanks to the Graduate School, Memorial University for the graduate fellowship they have provided to me during the first two years of my program.

CONTENTS

Tables

Tables cont..

Tables cont.,

Figures

Figures cont.,

Figures cont.,

Chapter One: General Introduction

Interest in larval fishes and factors influencing recruitment variation has increased rapidly in recent decades. The growth and survival of fish larvae has long been an area of great interest to scientists because of the inconsistent relationship between the size of the spawning populations of fish and subsequent year classes (Houde 1987). The precise determination of stock-recruitment relations early in the life history of fishes may lie in a better understanding of survival and growth in the larval stages. Studies on larval growth, survival and mortality appear to be an important aspect in determining the factors controlling the fate of fish populations.

During the early life of fish, mortality agents such as predation, disease, starvation and adverse physical factors (temperature, salinity) act on each stage. Larval mortality is a critical component in models and hypotheses that debate the ecology and evolution of differing reproductive characteristics exhibited by both marine invertebrates and vertebrates. In most of these models, predation and starvation are assumed to be a major source of larval mortality (Houde 1987). Studies on marine fish larvae suggest that both predation and starvation play important roles in the development, growth and survival of larvae from hatch to metamorphosis. Predation is common to all stages from egg to juvenile but starvation is a major factor during the exogenous feeding stage of the larval period (Houde 1987). Starved larvae are generally vulnerable to predation due to lower growth rates which extend their critical larval period and they are thus prone to size

selective predation (Heath 1992).

Most of the experimental studies carried out in recent decades on the growth and survival of larval fish primarily centre on the relationship between larvae and ecological structure (Blaxter 1980, Frank and Leggett 1986, Blaxter 1988, Blom et al. 1991, Castro and Cowen 1991, Brander and Hurley 1992, Gotceitas et al. 1996). The majority of laboratory studies on larval foraging and survival have not considered the role of predation or other factors. In nature, the combined effects of foraging conditions, predation pressure and abiotic factors affect the development and behaviour of the fish larvae and can result in an increase or decrease in survival rates. Species of commercial importance have been widely used in these studies in the laboratory and research has focused on behavioural adaptations with ecological interests in mind.

Field evidence suggests that larval fish are less susceptible to starvation (Hunter 1981, Theilacker 1986, Heath 1992). Most field studies report low prey concentrations relative to those used in laboratory studies (Pederson et al. 1989, Cowan and Houde 1990). Two possible reasons have been put forward to explain the discrepancies of the 'over estimation' of prey concentration in experimental rearing systems compared to the reported prey concentrations in nature. Firstly, Frank and Leggett (1986) and Frank (1988) suggest that this discrepancy may partly be due to inadequate sampling procedures. On a large scale, prey concentration in the ocean may be low but prey usually occur in patches and these patch concentrations are reported to be substantial enough to sustain reasonable growth and survival (Lasker 1978). Prey concentrations in the patches may

exceed or at least be on par with the prey concentrations reported from laboratory studies. The sampling procedures used in the field are inadequate to measure prey in the patches. Also, it has been pointed out that most of the early larval stages of fish prey upon small items less than 200 μm (Houde 1973) and field sampling equipment rarely retains this size range of zooplankton (Laurence 1977). Furthermore, at larger spatial scales, the sampling procedures disturb the heterogeneity of prey. These errors in estimating the prey abundance may under-estimate the effect of starvation on recruitment and other prey-predator interactions.

Secondly, in nature, larval densities are much lower than the densities of prey (Cushing 1983, Fossum and Ellertsen 1994, McLaren and Avendaño 1995) thus leading to a much higher prey-predator ratio than that used in most rearing experiments (Goshorn and Epifanio 1991, Gotceites et al. 1996). Some mesocosm studies have shown that cod larvae can be successfully reared at prey concentrations similar to those reported from nature (Kvenseth and Øiestad 1984, Blom et al. 1991, Oterrå 1993, van der Meeren and Næss 1993). Gotceitas et al. (1996) suggested that the prey:predator ratio may play a role in the growth and survival of larval fish. In their study on larval Atlantic cod, they reported that the ratio of prey and predators in the field and laboratory are similar and in some cases the ratio in the laboratory was lower than that reported from the field. Oterrå (1993) in his experiment on larval cod in large plastic enclosures found high survival during the first month of exogenous feeding but lower growth rates and increased mortality were observed following week four. The author related this to the presence of insufficient food

at the relatively high larval stocking densities. Houde (1975) showed that prey to larval ratio plays an important role in the growth and survival of sea bream (*Archosargus rhomboidalis*) larvae. Sea bream larvae reared at low prey concentration produced significant survival only at low larval densities while high prey concentrations produced better growth and survival at low and high larval densities. Results from these studies indicate that the combination of prey concentration and larval density (prey:predator ratio), could significantly influence the growth and survival of finfish larvae.

At hatch most larval fish are poorly developed. Thus, larvae are more vulnerable to mortality due to both predation and starvation than later stages (Blaxter 1988). Mortality throughout the larval stage is size specific and declines with growth and development (Folkvord and Hunter 1986.) As larvae develop there is a simultaneous emergence of associated behaviours. For instance, the development of fins and locomotor muscles and the refinement of sensory systems will influence swimming and foraging activity (Blaxter 1986; Noakes and Godin 1988). It seems reasonable that a larva's ability to locate and capture food should improve with growth, development and experience. Several studies have shown larval foraging behaviour to change with size. Browman and O'Brien (1992a) documented the ontogeny of search behaviour in white crappie larvae (*Pomoxis annularis*). In their study, fish size was found to have a significant overall effect on foraging behaviour . Similar results were reported for the golden shiner *(Notemigonus cryleucas*) (Browman and O'Brien 1992b), northern anchovy (*Engraulis mordax*) (Hunter 1972) and herring (*Clupea harengus*) (Blaxter and Staines 1971).

Growth and survival of larval fish during early development stages is largely influenced by feeding conditions (Frank and Leggett 1986, Van der Meeren and Næss 1993). The availability of suitable prey is critical at the early larval stage. Most marine fish larvae, at hatching, have limited yolk reserves and are poorly developed and need to begin feeding before all the yolk reserves become exhausted. Prey concentration, type and size are some of the important factors that influence the foraging and development of foraging behaviour of the fish larvae. Inadequate or inappropriate prey organisms in the vicinity of the fish larvae usually result in lower growth rate and condition, and consequently, high mortality. More importantly, first feeding larvae are more vulnerable to inadequate prey than later larval stages because of the transition of feeding mode, that is, from endogenous to exogenous feeding. If they do not find food before the yolk reserve becomes exhausted, they die from starvation.

Variability in both prey abundance and prey size can produce unpredictable foraging environments. When prey concentrations are low or prey are of inappropriate size, larvae may be forced to feed on energetically unfavourable prey items in order to achieve maintenance diets. As a result, larvae may be forced to search greater volumes of water and increase foraging time to obtain lower energetic gains (Lasker 1978). Growth often slows or becomes negligible under conditions like this and larvae can experience degeneration of muscles and other tissue types, thereby resulting in impaired behavioural responses. Once successful at first-feeding, a larva's susceptibility to starvation may decrease with increasing size (Jordan unpub. data), as the larva establish energy reserves

and develop an extended behavioural repertoire.

Not surprisingly, light also plays an important role in the growth and survival of larval fish (Blaxter 1975; Batty 1987). It is well known that most marine fish larvae are visual predators and require a threshold light intensity to initiate foraging. Reports indicate that the threshold light intensity for some marine fish larvae averages about 0.1 lux (Blaxter1986). However, in order to achieve a better feeding incidence the light intensity should be much higher than the threshold level and optimal intensity varies depending on the species (Blaxter1986).

At hatching in most marine larval fish, the eyes are unpigmented and become pigmented by first feeding. At first feeding, in many species, the larva has only a pure cone retina (Blaxter and Staines 1970). The rod cells appear in the retina of the eye sometime before metamorphosis and the pure cone retina becomes a duplex retina. Once the duplex retina has been established, the process of light-dark adaptation occurs. Although the pure cone retina is adequate for first feeding, given that there is appropriate light, the presence of rods in the retina is important for movement perception and visual acuity (Neave 1984). After metamorphosis, the juveniles move down in the water column where the light intensity is low (Shand 1994). Thus changes in the visual system could be associated with changes in both habitat and behaviour. Although most marine larval fish have a pure cone retina at first feeding, in contrast, some deep-sea larvae (e.g. an anguillid and a macrourid larvae) have a pure rod retina at hatching which possibly helps them to forage in very low light (Munz 1958). It would appear that the variation and change in eye pigments and

structure is related to the diversity of the environments that the larvae encounter and reflect different visual tasks that the animal has to face. It seems that the light conditions that the larvae experience may influence the timing of the development of the rod cells in the retina.

Light can also influence the behaviour of animals, through its variation in intensity, wavelength, polarization and diurnal and seasonal variation (Munz 1975; McFarland 1986). In marine invertebrates, swimming activities depend on the diurnal changes in light intensity. Most marine invertebrates show a diurnal periodicity in swimming with the peak activity occurring during night (Segal 1970). Light may also act as an orienting stimulus for marine invertebrates. The responses of the animal may be simple, consisting of random movements in which the speed of movement or the frequency of turning depends upon the light intensity (photokinesis), or directed movements in which the animal moves directly towards or directly away from the light source (phototaxis). The availability of light during the early life stages of fishes also affects the normal development of the eye. In the cichlid *Haplochromis burtoni* (Zeutzius and Rahmann 1984) and rainbow trout (*Salmo gairdneri*) (Rahmann et al. 1979), light deprivation in the early larval stage affects the normal development of the eye and reduces visual acuity. In contrast, halibut yolk-sac larvae develop abnormaly in the presence of light (Bolla and Holmefjord 1988; Skiftesvik et al. 1990). All these studies imply that unnatural light conditions may affect the normal development of the eye and consequently affect the growth and survival of larvae. In addition, development of the visual system also influences the foraging behaviour of larval

fish. Increased visual acuity produces a larger visual field in which larvae can detect more prey as well as predators. This allows larvae to feed faster and more efficiently (Noakes and Godin 1988) which in turn affects growth and survival.

Geographic variation in life history among populations of the same species has been well documented in reptiles (Ferguson and Talent 1993), fishes (Blaxter and Hempel 1963; Houde 1989; Fleming and Gross 1990; Castro and Cowen 1991; Present and Conover 1992; Mathias et al. 1993), and some invertebrates (Lonsdale and Levinton 1985; Young 1991). Studies which have examined geographic variation in life history among fish populations, have dealt mostly with salmonids and adult fish (Fleming and Gross 1990; Present and Conover 1992; Mathias et al. 1993). However, very little work has been done on geographic variation in the early life history of fishes (Blaxter and Hempel 1963; Houde 1989; Castro and Cowen 1991). It has been hypothesized and demonstrated that animal populations which are geographically separated, respond differently to particular environmental variables (Ferguson and Talent 1993, Hunt von Herbing and Boutilier 1996). These differences could be interpreted as an evolutionary response or adaptation to different environmental constraints that each population experiences in nature (Ricker 1972). Although some of these differences appear to have a genetic component, in many cases it has been difficult to establish how selective pressure has resulted in the suite of differences observed (Beacham et al. 1988).

As discussed earlier, prey availability is generally considered an important regulator of recruitment (Cushing 1972). Solomon (1949) proposed functional and

numerical responses of predators, pathogens and parasites in relation to increasing numbers or density of prey or host. The functional response describes the relationship between the concentration (or density) of the prey (or host) and the number of prey (host) items that is ingested (or infested) per unit time. Usually increases in prey numbers increase the consumption rates and result in higher reproduction rates (reproductive numerical response) or survival rates (non-reproductive numerical response) or both (Solomon 1949, Nunny 1985). Holling (1965) proposed three types of functional responses depending on whether the feeding response increases with increasing prey concentration 1) linearly to a maximum (the type I), 2) asymptotically at a decelerating rate (the type II), or 3) in a sigmoid function, the type III. Initially Holling's proposal drew the attention of many investigators to test the model, mostly on arthropod predator/parasitism systems (Holling 1966, Mori and Chant 1966, Huffaker et al 1969), but it has been extended to other systems including fish (Murdoch 1973, Hassell et al.1977, Houde and Schekter 1980).

Studies of predator-prey systems involving fish as predators have shown that consumption rate of fish could be described by either a type II or type III functional response (Houde and Schekter 1980, Miller et al. 1992, Winkler and Orellana 1992). Such differences in the type of functional response exhibited by different fish species could be due to different modes of feeding exhibited by various predator species to different prey species. Various rates of prey consumption at different prey concentrations may also result in a differential response in development and growth within a species. Studies on fish

show that first feeding larvae improve their foraging ability as they develop (Rosenthal and Hempel 1970, Houde and Schekter 1980, Miller et al. 1992). Thus, fish larvae exposed to a sub-optimal prey concentration tend to grow slowly compared to those exposed to optimal prey concentrations which leads to a size variation within a cohort. Miller et al.(1992) in their investigation on body size and functional response of three fish species demonstrated a size dependency in the functional response during development. Thus such studies on the predatory responses of larval fish should enhance our understanding of the dynamics of larval fish and its relevance to growth and survival.

My research investigates the effects of some ecological factors on the foraging behaviour, growth and survival of Atlantic cod (*Gadus morhua*) larvae. Atlantic cod is an ideal model for the study of geographic variation in their response to environmental characteristics due to their extended range from the arctic seas to temperate oceans (Scott and Scott 1988). Other studies (Cross and Payne1978, Pogson et al. 1995, Hunt von Herbing and Boutilier 1996) showed that there appear to be one or more separate stocks of Atlantic cod among and within the regions. Thus, in the second chapter of my thesis, I will investigate how different light levels affect the growth and survival of the larvae from two different cod populations. The idea of doing this occurred to me when I was attempting to develop a rearing protocol for larval cod from two populations. This chapter explains how the environmental factors could differentially influence larval behaviour, growth and survival of larvae from the two different populations.

The swimming and foraging behaviour of larval cod has been investigated by

several researchers but these studies have been done for only a particular developmental stage and carried out over a few days (Skiftesvik 1992, Munk 1995). No one has investigated the ontogeny of foraging behavior for an extended period. In chapter three I investigate the ontogeny of cod foraging behaviour from hatching to metamorphosis in relation to varying prey concentration.

Results from the experiment described in Chapter three did not provide a full picture of the effects of prey concentration on the growth and survival of larval cod as it was mainly designed to study the development of foraging behaviour. It was not clear from the results that whether 4000 prey L^{-1} was the optimal prey concentration for intensive rearing of larval cod or the growth and survival would continue to increase with further increase in prey concentration. Thus as a next step, I conducted a further experiment using prey concentrations higher than 4000 prey L^{-1} and monitored the growth and survival of the larvae from week 2 post-hatch. Although, this experiment will investigate mainly the growth and survival of larval cod, behavioural observations will be used to explain any differences in growth and/or survival between the treatments. Chapter five examines the effect of light intensity and photoperiod on the foraging, growth and survival of Atlantic cod (NF origin) larvae. Both of the above experiments (Chapter four and five) have been done with an aim to develop a rearing protocol of these fish species in intensive rearing conditions. Chapter six explains the different responses that are involved with prey concentration and larval feeding behaviour. It examines what type of functional, developmental and numerical responses larval cod exhibit to different prey

concentrations. In the final chapter, I discuss the results of all the experiments in terms of the natural ecology and aquaculture of Atlantic cod and emphasize the importance of behavioural observations in larval studies.

Chapter Two: Effect of light intensity on the foraging and growth of Atlantic cod larvae: interpopulation difference?

Introduction

Geographic variation in growth and survival among populations of the same species has been well documented in reptiles (Ferguson and Talent 1993), fishes (Blaxter and Hempel 1963, Houde 1989, Fleming and Gross 1990, Castro and Cowen 1991, Present and Conover 1992, Mathias et al. 1993), and some invertebrates (Lonsdale and Levinton 1985). Although studies have examined geographic variation in growth and survival among fish populations, most of these have dealt with salmonids and adult fish (Fleming and Gross 1990, Present and Conover 1992, Mathias et al. 1993) and only a little work has been done on geographic variation in the early life history of fishes (Blaxter and Hempel 1963, Houde 1989, Castro and Cowen 1991). It has been hypothesized and demonstrated that animal populations which are geographically separated, respond differently to particular environmental variables (Ferguson and Talent 1993). These differences could be interpreted as an evolutionary response or adaptation to different levels of environmental constraints that each population experiences in nature (Ricker 1972). Although some of these differences appear to have a heritable (i.e. genetic) component, in many cases it has been difficult to establish how selective pressure has resulted in the suite of differences observed (Beacham et al. 1988).

Atlantic cod, *Gadus morhua*, is an ideal model for the study of geographic variation in their response to environmental characteristics. Its range extends from the

arctic seas to temperate oceans and within each region there appear to be one or more separate stocks (Scott and Scott 1988). For example, Cross and Payne(1978) using electrophoretic and immunochemical characteristics, suggested the existence of genetically discrete sub-populations of Atlantic cod within restricted geographic areas off eastern North America. Recently, Pogson et al. (1995) using complementary DNA (cDNA) probes showed that populations of cod along the northeast coast of Newfoundland and along the coast of Nova Scotia are genetically discrete. Cod populations along the east coast of Canada spawn at different times. Surprisingly little work has been done on intra-population variations of Atlantic cod, despite their wide distribution. Nothing has been done to examine effects of geographic variation in the early life history of Atlantic cod until recently Hunt von Herbing and Boutilier (1996) examined the effect of temperature on the activity and metabolism of larval cod from the two populations (NF and SS origin).

Light, in particular, plays an important role in the growth and survival of larval fish (Blaxter 1975, Batty 1987). Light can influence the behaviour of fish, through its variation in intensity, wavelength and polarization and diurnal and seasonal variation (Munz 1975, McFarland 1986). The availability of light during the early life stages of fishes also affects the normal development of the eye. The response of larval fish to a particular characteristic of light is species specific. In the cichlid *Haplochromis burtoni* (Zeutzius and Rahmann 1984) and rainbow trout *Salmo gairdneri* (Rahmann et al. 1979), light deprivation in the early larval stage affects the normal development of the eye and reduces visual acuity. In contrast, halibut (*Hippoglossus hippoglossus*) yolk-sac larvae develop

abnormally in the presence of light (Bolla and Holmefjord 1988). Despite an impressive amount of research on the early life history of Atlantic cod larvae, no investigations have been done on the effects of light on growth and feeding.

Preliminary experiments on the foraging, growth and survival of cod larvae from the Scotian Shelf (SS; latitude 44°30'N) and Northeast Grand Bank (NF; 47°30'N) in the Ocean Sciences Centre (OSC), Memorial University of Newfoundland, showed that growth and survival between the two groups differed under different light intensity. I set up laboratory experiments to test the working hypothesis that light intensity would differentially affect the growth and survival of the larvae from these two geographically separate cod populations.

Materials and methods

Collection of eggs

Naturally spawned fertilized eggs were collected from Scotian Shelf (SS) broodstock maintained at Dalhousie University, Halifax, Nova Scotia and from Northeastern Grand Banks (NF) broodstock maintained at the OSC. The SS broodstock spawn naturally from November through January (Brander and Hurley 1992) while the NF broodstock spawn from April through July (Fahay 1983, Myers et al. 1993). Thus, experiments were conducted at different times of the year, but otherwise protocols were identical. SS eggs were collected in early December 1993 while NF eggs were collected in late May 1993. At the time of egg collection temperature in the brood stock tanks was

between 4-6° C for NF and 5-7°C for the SS broodstock. Similar temperatures were reported in the field during late fall for the SS (Smith 1989) and summer for NF (Myers et al. 1993). SS eggs were transported to the OSC and incubated under the same condition as NF eggs. Light intensity in the incubation room was 300-400 lux. Eggs were incubated between 5-7°C in 250L circular tanks with water flow and aeration. Dead eggs were siphoned out daily and antibiotic solution (mixture of tetracycline(100mg/L) and penicillin(60mg/L)) was sprayed on the eggs to control any bacterial and fungal infections. Incubation time for both NF eggs (13 days) and SS eggs (14 days) was similar. When 50% of the eggs had hatched, larvae and eggs were transferred to experimental tanks and this was taken as day 0 of the experiment.

Preliminary experiments.

Prior to the main experiment, a series of preliminary experiments was carried out to develop protocols for the rearing of cod larvae through metamorphosis. In one experiment, I duplicated the conditions used by Norwegian scientists (Ellertsen et. al 1980, Solberg and Tilseth 1987) including low light intensities (<100 lux). In these experiments I used a light intensity of ~10 lux/0.19 μE m^{-2} and a 16L:8D photoperiod and temperature was maintained between 7-9°C. Laboratory-reared rotifers and/or *Artemia* sp were used at four prey concentrations ranging from 500 to 4000 prey per litre. The results showed that SS larvae grew and survived better than NF larvae. Both the populations grew better in 4000 prey/L.

In a second preliminary experiment, I used 24h light (of appropriate intensity) for both NF and SS cod larvae and prey levels of 4000 prey/l. For both populations, survival was higher under the continuous light regime than under 16L:8D. Previous studies indicate that other fish species achieve a better growth and/or survival using 24 hr photoperiod (Kiyono and Hirano 1981, Duray and Kohno 1988).

Experimental set up

All experiments were carried out at the OSC in a temperature controlled room maintained at 8°C. Water temperature in the experimental tanks was measured daily in the morning. The room was subdivided into two chambers by an opaque black plastic curtain. One chamber was assigned as high light (HL) intensity treatment (12.92 μE m^{-2}/680 lux) and the other a low light (LL) intensity (0.19 μE m^{-2} /8.5 lux) treatment. These light intensities were chosen based on the results from my preliminary experiments. The experimental tanks were 30 L rectangular glass aquaria (38 cm in depth) with two tanks per treatment. Three sides of each aquarium were covered by opaque black plastic. The front was not covered to facilitate the behavioural observations. Two 90-watt incandescent bulbs, one each above each of the HL tanks, and two 7.5-watt incandescent bulbs, one each above each of the LL tanks were used. Both type of bulbs produce a smooth continuous spectrum ranging from 400-700 nm (General Electric (GE) Company, 4400 Cox Road, P.O. Box 4410, Glen Allen, VA, USA 23058-4410). All tanks were covered by a sheet of blue-green plastic to ensure an even distribution of light into the

tanks. Low light tanks were covered with two blue green plastic sheets to achieve 8.5 lux. Light intensity inside the tanks was measured using a light meter (SPER Scientific light meter 840006 for measurements in lux and Li-Cor Quantum photometer, model L1-189 for measurements in $\mu E\ m^{-2}$), held just above the water surface. All measurements were taken when the covers were on. A 24hr photoperiod was used.

Initially, tanks were filled with filtered, UV treated sea water. Larvae were transferred to the experimental tanks at 50% hatch. Larval stocking density was 40 larvae/l. For the first week, there was no exchange of water. After one week a flow of 100-200 ml/min. was started which was gradually increased to 700-800 ml/min. during the fourth week. Green algae (*Isochrysis* sp) were added to the tanks daily from day one to the end of the experiment. Cultured, HUFA-enriched (highly unsaturated fatty acid) rotifers (*Brachiomus plicatilis*) and/or *Artemia salina* were used as prey. From day 3 to day 10 post-hatch rotifers were used as prey. As the larvae grew a mixture of rotifers and *Artemia* (1:1) were used. Prey concentration was maintained at 4000 prey/L. To maintain this prey level, a 10ml water aliquot was sampled daily from each tank at different depths (just below surface, mid water column, and just above bottom). The number of prey was counted and prey levels were adjusted to 4000 prey/L, if necessary. The blue-green covers and presence of aeration through a air stone and an air lift helped to reduce the patchiness of the prey (Ellertsen et al. 1980, Gulbrandsen 1991).

Data collection

Ten larvae were sampled on day 0 and thereafter five larvae from each tank (10 per treatment) were arbitrarily chosen for morphometric measurements and dry weights at 5 day intervals over the duration of the experiment (43 days). Using a dissecting microscope, standard length (mm) and presence or absence of food in the gut in proportion to gut volume (empty, 25%, 50%, 75% and full; McLaren and Avendaño 1995), were recorded. After measurements, each larva was rinsed in fresh water and placed on a pre-weighed piece of aluminum foil and dried in an oven for 24-48 hrs at 65°C. To calculate the larval dry weight, larvae and foils were weighed to the nearest 0.0001 mg using an electronic microbalance.

Behavioural observations were recorded from day 1 to day 31 post-hatch for NF stock, and from day 1 post-hatch to day 43 post-hatch for SS stock using a Tandy 102 event recorder. I could not collect behavioural data for NF cod larvae beyond day 31 due to technical problems. Observations were conducted twice a week and all the observations were made by an observer seated in front of each tank between 10 am and 12 noon. During each observation period, a larva was observed for one minute. The occurrence (beginning and end of the event) of any of five foraging Modal Action Patterns or two activities (swim or motionless) (MAP's; Barlow 1977; Table 2.1) performed by the larva was recorded. In total, five larvae were observed in each tank (10 per treatment). In this Chapter, I combined the frequencies (MAP's/min.) of orientation, success, miss, and pass into a category termed foraging frequency.

Table 2.1: Operational definitions of Feeding Modal Action Patterns (MAP's) for larval cod.

MAP	Definition
Swim	- forward movement of larva through water column accomplished by caudal fin action.
Motionless	- larva is not swimming.
Orient	- larva stationary and fixates on a prey item.
Bite	- larva attempts to capture prey.
Success	- prey is captured.
Miss	- prey is not captured.
Pass	- larva orients on a prey item but does not bite, larva then swims in another direction.

Foraging frequency = Orient + Success + Miss + Pass.

The experiment was carried out for 43 days and terminated when the majority of the larvae were past metamorphosis. Metamorphosis was determined externally by the disappearance of the continuous fin fold and subsequent formation of discrete fins. At the end of the experiment, the numbers of surviving larvae were recorded.

Data analysis

All data were tested for normality (SAS 1988). The foraging frequency and gut fullness index data were not normal, and a non-parametric one-way ANOVA (Wilcoxon Rank Statistic) was used to determine the effect of light level ($p \leq 0.05$).

The effects of light level and age on standard length and swimming of cod larvae were analysed by two-way analysis of variance ($p \leq 0.05$).The Tukey test was used for a multiple comparison among different light treatments and locations (SS/NF) for each week.

Results

By the end of the second week, there was a significant difference in standard length (ANOVA, ($F_{[1,161]}$=29.3; $p<0.0001$) among NF cod larvae raised under high and low light intensity conditions. NF larvae reared under high light grew more from week three until the end of the experiment (Fig. 2.1a and Table 2.2). In contrast, SS cod larvae grew significantly better under low light. In fact, SS larvae reared under high light did not survive beyond the fourth week (Fig. 2.1b). Analysis of the data for the first four weeks

showed that the standard length of the SS larvae reared under low light was significantly higher ($F_{[1,92]}$=5.99; p<0.00163) than that of SS larvae reared under high light.

Overall, there was no significant difference between the standard length of SS larvae reared under low light and NF larvae reared under high light ($F_{[1,163]}$=1.27; p<0.2622). However, at hatching the SS larvae were larger in length than the NF larvae but NF larvae reared under high light exceeded the standard length of SS larvae by the end of two weeks. There was no significant difference between the growth of NF larvae under high light and SS larvae under low light at weeks 3 and 4, but NF larvae reared under high light were significantly larger than SS larvae reared under high light at weeks 3 and 4 (Table 2.2). SS larvae reared under low light were significantly larger than NF larvae reared under the same condition ($F_{[1,156]}$=87.09; p<0.0001), but there was no significant difference at weeks 3 and 4. After four weeks, SS larvae were significantly larger than the NF cod larvae (Table 2.2).

The duration of swimming of NF larvae was significantly higher ($F_{[1,185]}$=25.28; p<0.0001) under high light than low light (Fig 2.2a and Table 2.3). This higher swimming activity probably resulted in a higher encounter rate with the prey which resulted in an increased foraging frequency under high light condition. The mean foraging frequency of NF larvae was significantly higher under high light (Z=-4.27284, df= 1; p=0.0001) than for larvae under low light (Fig 2.2b). The gut fullness analysis also confirmed higher rate of successful prey encounter of NF larvae under high light than under low light. The index of gut fullness of NF cod larvae was significantly higher (Z=4.46398, df=1, p=0.0001) under

high light than low light conditions(Fig. 2.2c).

There was no significant difference in swimming duration ($F_{[1,189]}$=0.86; p=0.356) between SS larvae reared under low and high light (Fig 2.3a and Table 2.3). However, the foraging frequency of larvae under low light conditions was significantly higher than that under high light conditions (Z=-7.02919, df=1; p=0.0001) (Fig 2.3b). This was reflected in gut fullness index (Fig. 2.3c), which was significantly higher under low light (Z=-2.91237, df=1; p=0.0036) than high light conditions. At the end of the experiment the survival of NF cod larvae was higher in high light compared to low light. SS larvae did not survive in high light, but in low light survival of SS larvae was much higher than NF larvae (Fig 2.4).

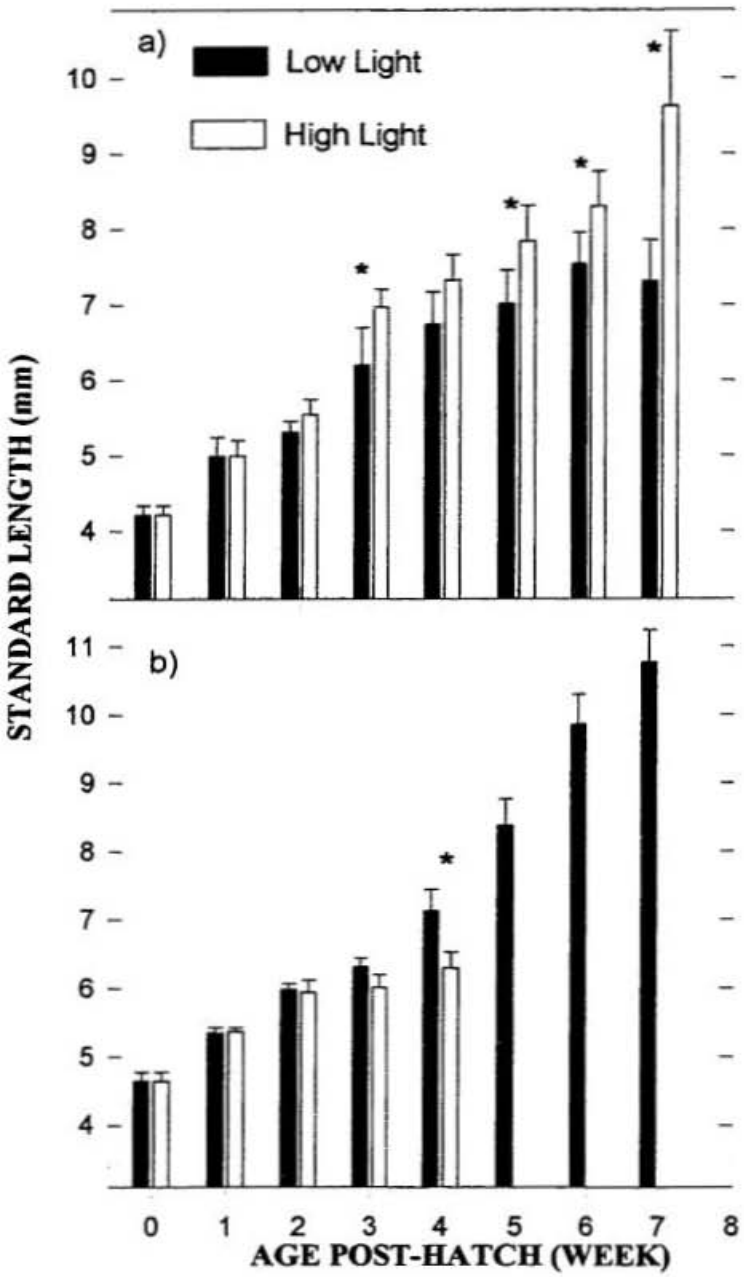

Figure 2.1. Mean standard length (mm) of ; a) Newfoundland and, b) Scotian Shelf cod larvae reared under low (8.5 lux) light and high (680 lux) light conditions over age (weeks). Values are mean ± SE. n= 10-20 larvae per week. * indicate significant difference.

Table 2.2 Results of the Tukey analysis comparing the mean standard length (mm) of cod larvae from SS and NF under two light levels (LL and HL) from week 1 to week 7 post-hatch. Values are the differences in mean standard length between two treatment comparisons.

Treatment Comparisons	Weeks post-hatch 1	2	3	4	5	6	7
NFHL and SSLL	-0.388*	-0.430*	0.268	0.194	-0.536	-0.728*	-1.141
NFHL and NFLL	0.006	0.241	0.421*	0.582	0.823*	1.443*	2.318*
NFHL and SSHL	-0.397*	-0.400*	0.574*	1.024*	----	----	----
NFLL and SSLL	-0.394*	-0.671*	-0.153	-0.388	-1.359*	-1.493*	-3.459*
NFLL and SSHL	-0.403*	-0.641*	0.153	0.441	----	----	----
SSLL and SSHL	-0.007	0.030	0.306	0.829*	----	----	----

*- indicates a significant difference between the treatments ($p<0.05$).

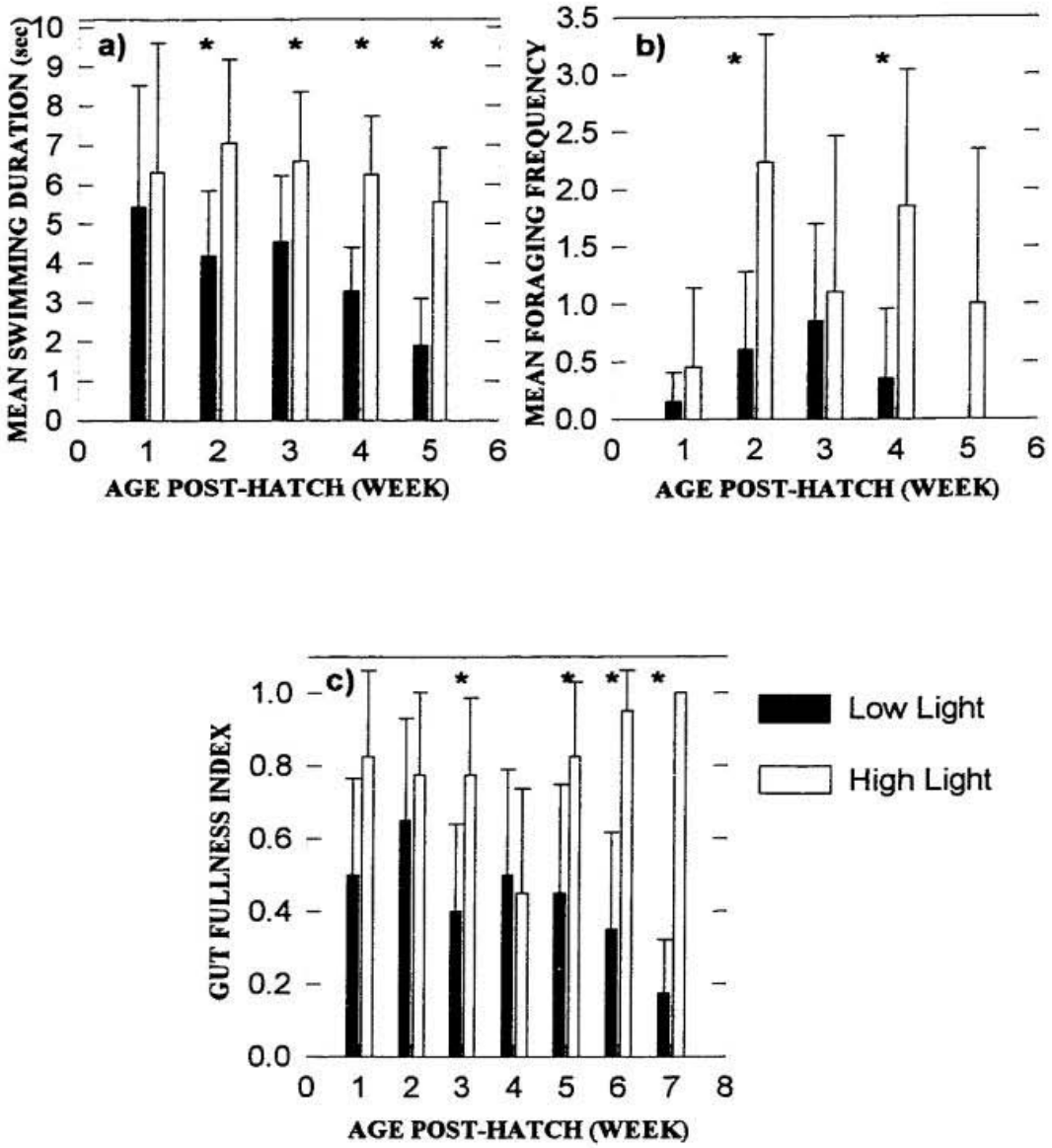

Figure 2.2. a) Mean swimming duration (sec), b) mean foraging frequency, and c) mean gut fullness index of Newfoundland cod larvae reared at low and high light conditions. Values are mean ±SE. * indicates significant difference. n=20 larvae per week.

Table 2.3 Results of the Tukey analysis comparing the mean swimming duration(sec) of cod larvae from SS and NF under two light levels (LL and HL) from week 1 to week 5 post-hatch. Values are the differences in mean standard length between two treatment comparisons. * - indicates a significant difference between the treatments ($p<0.05$).

Treatment Comparisons	Weeks post-hatch 1	2	3	4	5
NFHL and SSLL	1.515	1.153	0.550	1.025	1.510*
NFHL and NFLL	0.875	2.873*	2.055*	2.965*	3.650*
NFHL and SSHL	2.940*	1.288	1.565	1.515	----
NFLL and SSLL	0.640	-1.720	-1.505	-1.940*	-2.140*
NFLL and SSHL	2.065	-1.585	-0.490	-1.450	----
SSLL and SSHL	1.425	0.135	1.015	0.490	----

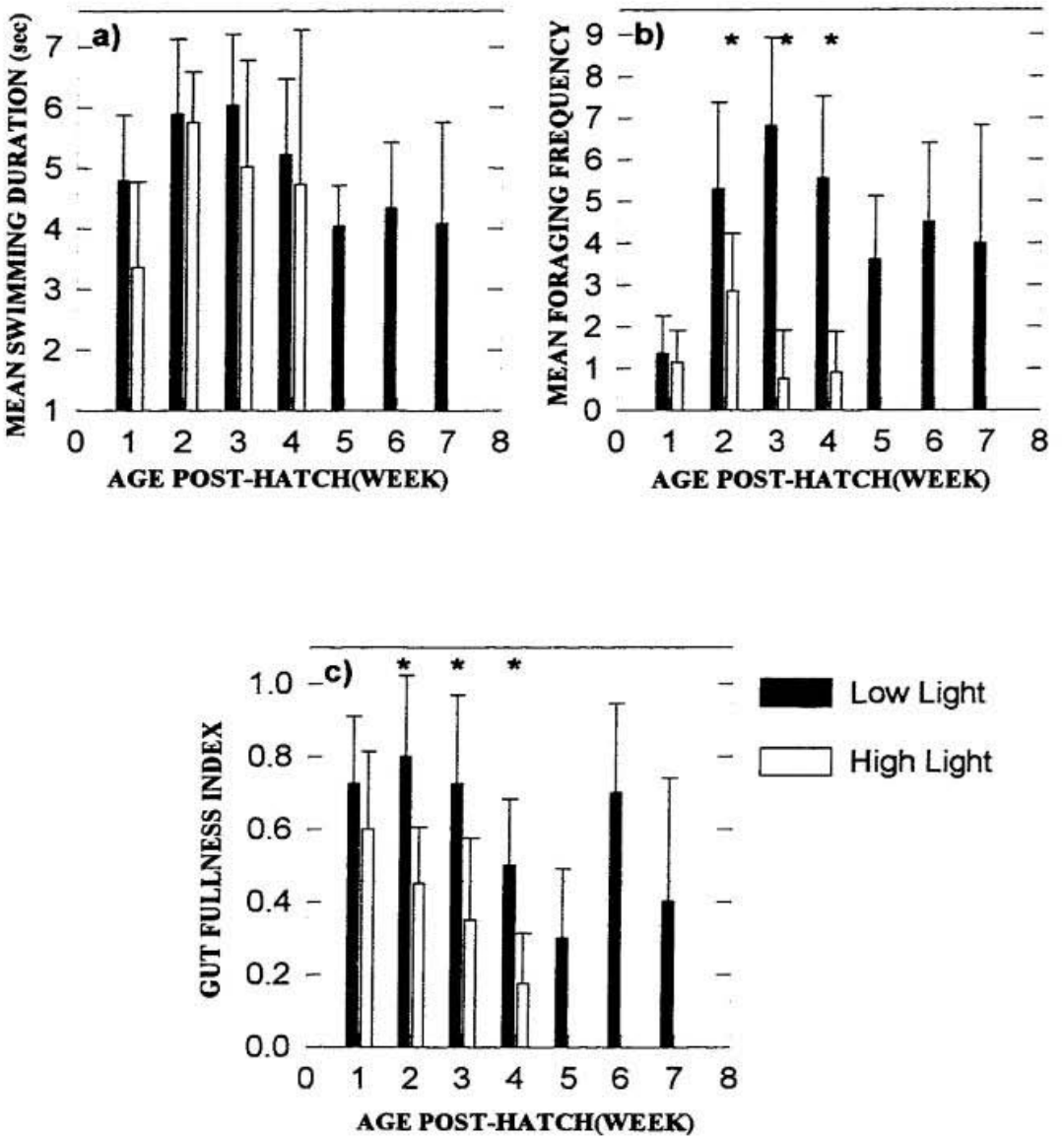

Figure 2.3 a) Mean swimming duration (sec), b) mean foraging frequency, c) mean gut fullness index of Scotian Shelf cod larvae reared at low and high light conditions. Values are mean ± SE. * indicates significant difference. n=20 larvae per week.

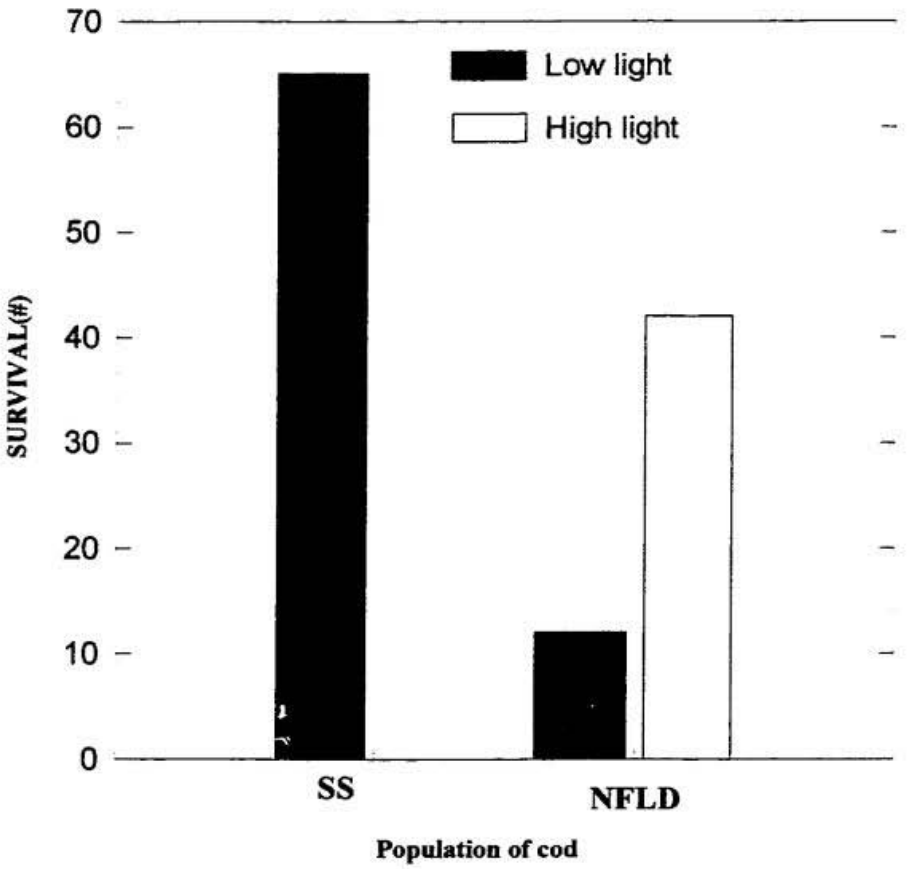

Figure 2.4. Total number of Scotian Shelf and Newfoundland cod larvae surviving at the end of the each experiment in relation to the light levels at which they were reared.

Discussion

Overall, my results showed a significant difference in the swimming, foraging, growth and survival of Atlantic cod larvae from two populations in relation to light intensity. For example, NF cod larvae foraged more, grew faster and had higher survival under high light intensity, while SS cod larvae performed better under low light conditions. Even though eggs/larvae were collected at different times of the year from natural spawning, the experimental conditions (temperature range, light intensity, photoperiod, prey type/density) were identical for both populations. Although I reported light intensities both in lux and μEm^{-2}, other studies reported either on lux or μEm^{-2}. Since these units cannot be inter-converted, I will report whatever the units those studies used to measure the light intensity.

It is apparent that light intensity affected the foraging ability of larvae differentially. The ecological reason for this result may be traced to the different spawning seasons of each population. The population of cod I studied from the Scotian Shelf typically spawn over the period of November-January (Brander and Hurley 1992) while cod on the Grand Bank typically spawn from April-July (Fahay 1983). Winter sea surface light levels at the latitude of the Scotian Shelf range from 3180-1360 lux from November to January respectively, while the spring/summer sea surface light levels at the latitude of Northeastern Grand Bank range from 13,000-20,000 lux from April to July (Nielsen 1974, Blaxter and Batty 1990). Thus larvae on the Grand Bank might possibly be expected to experience ten-fold higher light levels compared to cod larvae on the Scotian Shelf from

the fall/winter spawning. As such, the differences between larvae from these two populations noted in this study may reflect an adaptation of these larvae to local conditions.

Anderson and de Young (1995) reported that cod larvae in the offshore and inshore areas of northeastern Newfoundland occupy the top 40 m (5-35 m) of the water column during summer months. Field data from Conception Bay, Newfoundland (inshore of the Northeastern Grand Bank) showed that light intensity during the month of July, at 40 m depth, ranges between 10-30 μEm^{-2} (R.Rivkin, unpublished data).Thus in nature, cod larvae from NF experience similar light intensities to those of the high light treatment. On the other hand, cod larvae from SS occur at a depth of 20-30 m in late fall (Mckenzie 1940). O'Reilly et al. (1987) reported that the surface sun light over the SS diminishes to 1% between 20-45 m. So the larvae from SS would likely experience a light intensity of 13-31 lux in late fall which is comparable to the low light intensity (8.5 lux) used in this experiment.

Survival of NF cod larvae at the end of the experiment was higher under high light than low light. In contrast, SS larvae survived only under low light. The reason SS larvae did not forage or survive beyond four weeks in high light intensity, is difficult to explain. Swimming of SS larvae was not significantly different between high and low light intensities, but the foraging activity and the gut fullness of larvae were lower in high light. The latter result suggests that at high light intensity, SS larvae did not forage as efficiently as they did at low light intensity. The reason that SS cod larvae foraged poorly under high

light is not obvious. In nature, larvae can vertically migrate, so that if unfavourable conditions occur, they could migrate to more favourable depths in the water column (Lough and Potter 1993).

As in most marine larval fishes, cod larvae have a poorly developed visual system at hatching. The eyes of most fish larvae become more pigmented during first feeding (Blaxter 1986). Most pelagic larvae, investigated so far, have a pure cone retina at first feeding (Blaxter and Staines 1970). During metamorphosis, this pure cone retina becomes a duplex retina and the juveniles move down into the water column (Shand 1994). The changes in the visual system could be associated with changes in both habitat and feeding behaviour. In contrast, some deep-sea larvae (e.g. an anguillid and a macrourid larva) have a pure rod retina at hatching (Munz 1958). It would appear that the variation and change in eye pigments can be related to the diversity of the environments, e.g., in different light intensities at various depths, larvae encounter. It will also reflect the different visual tasks the animals have to face. Regardless, all these observations imply that the presence of rods in the retina help the larval fish to cope with a darker environment. If this is the case, then fish larvae which experience low light levels may have a higher concentration of rods in their retina to facilitate searching for prey. Based on these results, SS larvae might be expected to develop a greater concentration of rods in their retina early in the first feeding stage compared to NF larvae which begin foraging in a high light environment.

Sizes of marine fish larvae are influenced by egg size which in turn is influenced by female condition (Chambers and Waiwood 1996) and the environmental conditions

experienced during the embryonic stage (Miller et al . 1993). In my study, incubation conditions were similar for eggs from both NF and SS populations. Thus, the size difference at hatching between the larvae from two populations may be due to the differences in egg size. Unfortunately, I could not verify this as the egg sizes were not measured. Further, egg size varies substantially over years within the same populations and also across batches for same female in same season (McKenzie 1940, Miller et al. 1993). Thus, even though my results were consistent with the hypothesis, precautions should be taken when comparing with other studies.

Lagomarsino and Conover (1993) reported variation in environmentally induced sex determination process (ESD) in Atlantic silverside from different latitudes. In their studies, larvae from higher latitudes, experiencing low temperatures, produced a higher percentage of females while fish from lower latitudes, experiencing higher temperatures, produced mainly males. This incongruity was mainly attributed to differences in temperature experienced during the spawning season, and suggested that sex determination in silverside is controlled by an interaction between genetic factors, phylogenetic factors and temperature. A similar scenario may apply in the case of the SS and NF cod populations. A portion of cod in the Scotian Shelf spawn during winter, larvae experience low light levels, and based on my results, display better growth and survival at low light conditions. In contrast, NF cod spawn during summer (high light), and larvae performed better under high light conditions. As was in silverside, the underlying mechanism for the difference in response to light in larval cod may also be controlled by

genetic factors and/or light, but needs more investigation.

The other attribute of light which has been shown to have an effect on aquatic organism is the spectral quality (Munz and McFarland 1973, Hobson et al. 1981, Levine and McNichol 1982). The studies reporting these effects have been field studies dealing with adult populations and to the best of my knowledge no study has experimentally determined how spectral quality might influence the growth and survival of larval fish. Shand (1993) reported that the abrupt change in the spectral sensitivity of the goatfish (*Upeneus tragula*) eye coincided with metamorphosis and the benthic mode of life. Juvenile pollack (*Pollachius pollachius*) also showed a progressive change in the spectral absorbency during their late pelagic stage (Shand et al. 1988). In both cases, changes in the spectral sensitivity correspond to changes in life style, i.e. from pelagic to benthic life. Since I investigated only the pelagic stage up to late larvae of Atlantic cod, spectral quality may not affect feeding behaviour over the course of my experiment. Shand et al.(1988) also showed that the change in spectral sensitivity in the pollack is correlated more to age/size than to season. Thus the early life-stage larval cod that I investigated may not go through the developmental changes of the eye related to spectral sensitivity until later in larval life.

In summary, my results demonstrate that different light intensities had an influence on activity, foraging, growth and survival of two populations of Atlantic cod larvae. This result also suggests that for the successful rearing of fish larvae, we have to understand the role various environmental factors plays in influencing larval growth and survival.

Chapter Three: Effect of prey concentration on the foraging behaviour of larval Atlantic cod from hatching to metamorphosis.

Introduction

Larval fish are poorly developed at hatch. Due to this, larvae are vulnerable to both predation and starvation. Mortality during this stage is size specific and declines with growth and development (Folkvord and Hunter 1986, Blaxter 1988) which is related to the simultaneous emergence of behaviours and changes in morphology. For instance, the development of fins and locomotor muscles and the refinement of sensory systems influence swimming and foraging activity (Blaxter 1986, Noakes and Godin 1988) and are size related. It seems reasonable that a larva's ability to locate and capture food should improve with growth, development and experience. Numerous studies have shown larval foraging behaviour changes with size. Browman and O'Brien (1992a) documented the ontogeny of search behaviour in white crappie larvae (*Pomoxis annuularis*). In their study, fish size was found to have a significant overall effect on foraging behaviour . Similar results were reported for the golden shiner *(Otemigonus cryleucas*) (Browman and O'Brien 1992b), northern anchovy (*Engraulis mordax*) (Hunter 1972) and herring (*Clupea harengus*) (Blaxter and Staines 1971). In addition to increasing body size, development of the visual system also influences the foraging behaviour. Increased visual acuity produces a larger visual field in which larvae can detect more prey as well as predators, thereby allowing the larvae to feed faster and more efficiently (Noakes and Godin 1988).

Growth and survival of larval fish during the early development stages is largely influenced by feeding conditions (Frank and Leggett 1986, van der Meeren and Næss 1993). Variability in both prey abundance and prey size can produce unpredictable foraging environments during the early stages of larval ontogeny. When prey concentrations are low or prey are of inappropriate size, larvae may be forced to feed on energetically unfavourable prey items in order to achieve maintenance diets. As a result, larvae may be forced to search greater volumes of water and increase foraging time to obtain lower energetic gains. Growth is often slow or negligible under these conditions and larvae can experience degeneration of muscle and other tissue types, thereby resulting in impaired behavioural responses (Laurence 1972; Skiftesvik 1992,1994). A larva's susceptibility to starvation may decrease as larvae grow, establish energy reserves and develop an extended behavioural repertoire.

It is surprising that in spite of the tremendous amount of work which has been done on Atlantic cod (*Gadus morhua*) larvae, there is no detailed information available on the ontogeny of foraging behaviour or on the effect of prey concentration on cod larval growth and survival in intensive rearing systems. Munk (1995) observed foraging behaviour of larval cod under different prey concentrations but his experiment was carried out only for a five day period on 2-3 week old larval cod. His work addressed important issues but provided no information on the effect of prey concentration on the progressive development of foraging behaviour of larval cod over an extended period. Meanwhile, other mesocosm studies on cod larvae which examined the effect of feeding condition did

not specifically study the effect of a range of prey concentrations on larval behaviour, growth and survival (Gamble and Hoodie 1984, Blom et al. 1991, van der Meeren and Næss 1993).

The aim of this study was to describe the foraging behaviour and activity of cod larvae under laboratory conditions from hatching to metamorphosis and to determine whether changes in this behaviour are related to prey concentrations. The main focus of this paper is on the development of foraging behaviour of larval cod and how this changes with age. I also relate any difference in growth and survival of the cod larvae under different prey concentrations to variation in the foraging behaviour during the study period.

Materials and Methods.

Fertilized Atlantic cod eggs were collected from a naturally spawning captive brood stock maintained at the Ocean Sciences Centre, Logy Bay, Newfoundland. Eggs were incubated in floating rectangular baskets at 6°C until hatch. When 50% of the eggs had hatched (day 0), ten larvae were sampled from the basket for initial morphometric measurements. All experiments were carried out in a temperature-controlled room maintained at 8°C. The water temperature was maintained between 7-9°C. The experimental tanks were 30 l rectangular glass aquaria with two tanks per treatment. Three sides of each aquarium were covered by opaque black plastic. The front was not covered to facilitate behavioural observations. Overhead fluorescent light tubes were used

to provide a light intensity of 700 lux at the water surface and light was measured using a light metre (SPER Scientific light metre 840006). A 24h light photoperiod was used.

Tanks were supplied with filtered sea water. Initially the water flow rate was about 100-200 ml min^{-1}. As the larvae grew, this was gradually increased to 500-600 ml min^{-1}. Green algae (*Isochrysis* sp) were added daily to the tanks throughout the experiment. When nearly 100% of the larvae had hatched (day 1), approximately 1200 larvae (40 larvae per litre) were transferred to each of ten experimental tanks. Four prey concentrations were used, 500, 1000, 2000 and 4000 prey per litre with a control (no prey). Experimental prey concentrations were chosen on the basis of previous laboratory studies which found increased larval growth and survival through metamorphosis at high prey concentrations, whereas growth and survival were significantly reduced in larval cod reared in low prey concentration.

Laboratory-reared, enriched rotifers (*Brachionus plicatilis*) and/or *Artemia* nauplii were used as prey. From day two to day 15 post-hatch, enriched rotifers were used as prey. As the larvae grew, a mixture of rotifers and *Artemia* nauplii were used. Larvae were offered food from day two post-hatch. An up-welling aeration system (Ellertsen et al. 1980) was used to ensure a homogeneous prey distribution within the tank. To maintain a constant prey level within each experimental tank, before adding the prey, a 10 ml water aliquot was sampled from each tank at different depths (just below surface, mid water column, and just above bottom). The number of prey was counted and prey levels were adjusted twice a day, once in the morning (9.00) and again in the afternoon (15.00).

Data collection

Behavioural observations were conducted from day two until day 44 post-hatch. Observations were conducted twice a week in the morning and all observations were made by an observer seated quietly at eye level, 30 cm in front of the aquaria. Prior to an observation session, the desired quantity of prey was introduced evenly into the tanks. Observation commenced two minutes after adding the food. The focal animal technique (Altman 1974) was used and an arbitrarily selected larva was observed for a one minute interval. During each observation period, ten larvae from each tank (20 per treatment) were observed. The occurrence (both frequency and duration) of any of six Modal Action Patterns (MAP's; Barlow 1977; Table 3.1) performed by the larva was recorded using an event recorder (Tandy 102) and behavioural software (Observer; Noldus Information technology, Wageningen, Netherlands).

Five larvae from each tank (ten per treatment) were arbitrarily chosen for length determinations once a week over the duration of the experiment (from day six to day 46). Using a dissecting microscope equipped with a micrometer, standard length (mm) and the presence or absence of food in the gut in proportion to gut volume (gut fullness) were recorded. Gut fullness index was visually estimated into five categories; empty, 25% full, 50% full, 75% full or full (McLaren & Avendaño 1995).

The experiment was carried out for 46 days post-hatch. The experiment was terminated when the majority (80%) of the larvae in the high food treatment had metamorphosed which was determined externally by the disappearance of the continuous

fin fold and subsequent formation of discrete fins. At the end of the experiment, the number of surviving larvae in each treatment was recorded.

Data analysis

Prior to analysis, data were tested for the assumptions required to perform analysis of variance. Normality was tested by using Kolomogorov-Smirnov statistic (SAS 1988) and plots of residuals and predicted values were examined. The behavioural data were not normally distributed and available transformation methods could not normalize the data. However, according to central limit theorem, normality can be relaxed in cases where sample size is large (in my case n=840, Johnson and Wichern 1992). The effects of prey concentration and age (days) on swimming, all foraging data, standard length and gut fullness index were analysed by two-way ANOVA (SAS 1988; $p \leq 0.05$). When significant results were obtained, Tukey's studentized (HST) test was used to determine which means differed.

Results

Results from ANOVA showed significant interactions between prey concentrations and larval age for all behavioural MAP's, except for pass (Table 3.2). These results indicate these MAP's varied with larval age and the influence on these MAP's was different depending on which prey concentrations the larvae were exposed to.

The swimming of the larvae was typically characterized by short, intermittent

bursts produced by the action of the caudal area of the body, followed by periods where larvae remained motionless. Initially (day two) most of the larvae occupied the water surface and were motionless except for frequent short swimming bouts, but by day five larvae had moved down in the water column. Prey concentration had a significant effect on swimming duration of larval cod (Table 3.2). Differences in swimming duration among treatments became apparent from day five post-hatch. Larvae reared in lower prey concentrations swam significantly more than larvae reared in higher prey concentrations. From day nine onwards, larvae in the control and 500 prey L^{-1} treatment swam significantly less than the larvae in other treatments (Fig. 3.1).

The foraging behaviour of larval cod consisted of four MAP's; orient, capture, miss and pass (Table 3.1). The frequency of the foraging MAP's were significantly different (Table 3.2) among the treatments. Larvae reared in 4000 prey L^{-1} oriented and captured significantly more prey than the larvae reared in lower prey concentrations (Fig. 3.2a, 3.2b). Larvae reared in 4000 prey L^{-1} oriented more towards prey than larvae from the other treatments until day 37 (Table 3.3). Except in the later part of the experiment (day 30), larvae reared in 4000 prey L^{-1} foraged significantly more than the larvae in all other treatments (Table 3.4). Initially, larvae from all treatments had a high frequency of miss which started to decline from day 12 onwards in higher prey concentrations (Fig.3.3a). There was no significant difference in miss frequency between the treatments, except for 1000 prey L^{-1} (Table 3.5). Initially, frequency of pass increased to day 12 and leveled off to day 27 and then increase up to day 37. Generally, the incidence of pass was

more frequent in larvae reared at 4000 prey L^{-1} than larvae from lower prey concentrations (Fig. 3.3b).

The frequency of the capture and miss MAP's were pooled together to create the variable "attack" which was used to calculate capture success.

Capture success= (capture frequency / attack frequency)*100

Larvae reared in 2000 and 4000 prey L^{-1} showed a steady increase in capture success from day 2 onwards and reached 100% on day 27 and 23 respectively (Fig. 3.4a). The mean time spent per orient increased initially for all treatments and declined to its minimum between 27 and 30 days post-hatch (Fig.3.4b). When searching for prey, larval cod would swim a short distance and became stationary (pause) scanning (determined from the movement of the head region and eye of larvae) for prey. Not all pauses resulted in successfully locating the prey. When a prey was not located, the larvae continued swimming. However, the ratios between the frequency of orient and pause & frequency of attack and pause increased as the larvae grew (Fig. 3.5a & 3.5b). The ratios between orient and pause & attack and pause were higher for larvae reared in 4000 prey L^{-1} throughout the study. Once prey was detected, a larva would orient and fixate on the prey. After fixating on the prey, the larva either attempted to capture it or would move towards the prey but not try to capture it then swim in another direction (pass). Not all orients resulted in an attack or a pass. Sometimes a larva would orient towards the prey but would uncoil the body and continue to swim without moving towards the prey.

The gut fullness index was significantly different among treatments (ANOVA,

df=4; f=37.36; p=0.0001). Larvae reared in 2000 and 4000 prey L^{-1} had a higher gut fullness index compared to larvae from lower prey concentrations (Fig. 3.6a). Initially there was no significant difference in gut fullness between larvae reared in 2000 and 4000 prey L^{-1}, but from day 20 onwards (except for day 27) larvae from 4000 prey L^{-1} had a significantly higher gut fullness index. However, on day 46 gut fullness index of larvae from the 4000 prey L^{-1} treatment was lower than that was recorded in any other sampling day. Standard length of the cod larvae was significantly influenced by prey concentration (ANOVA, df=4, f=121.49, p>0.0001). Larvae reared at low prey concentrations (0-2000 prey L^{-1}) were significantly smaller than the larvae reared at 4000 prey L^{-1} (Fig. 3.6b).

Table 3.1. Operational definitions of Feeding Modal Action Patterns (MAP's) for larval cod.

MAP	Definition
Swim	- forward movement of larva through water column accomplished by movements of caudal area of body.
Pause	- larva motionless (similar to 'non-swimming' of Munk 1995).
Orient	- larva motionless and fixates (determined by larva's eye movement) on a prey item (similar to 'approach and attack position' of Munk 1995).
Capture	- larva bites and ingests prey. Larva moves towards prey by a posterior drive of the tail (similar to 'attack' of Munk 1995).
Miss	- larva fails to capture prey after a bite.
Pass	- larva orients and fixates on a prey item and moves toward the prey but does not bite, larva then swims in another direction.

Attack = Capture frequency + Miss frequency

Table 3.2 Results of a two-way ANOVA (age and prey concentration) on the swimming and foraging MAP's of larval cod at different prey concentrations. Significance at 0.05 level.

MAP	source	df	F-value	Pr. > F
Swim	Model	41	13.30	0.0001
	Error	798		
	Age	12	17.10	0.0001
	Concentration	4	21.53	0.0001
	Age × Concentration	25	7.54	0.0001
Orient	Model	41	15.51	0.0001
	Error	798		
	Age	12	19.33	0.0001
	Concentration	4	26.43	0.0001
	Age × Concentration	25	3.52	0.0001
Capture	Model	41	11.59	0.0001
	Error	798		
	Age	12	11.72	0.0001
	Concentration	4	28.98	0.0001
	Age × Concentration	25	2.37	0.0002
Miss	Model	41	4.75	0.0001
	Error	798		
	Age	12	8.98	0.0001
	Concentration	4	7.19	0.0001
	Age × Concentration	25	2.21	0.0006
Pass	Model	41	5.17	0.0001
	Error	798		
	Age	12	8.45	0.0001
	Concentration	4	5.51	0.0002
	Age × Concentration	25	0.77	0.7768

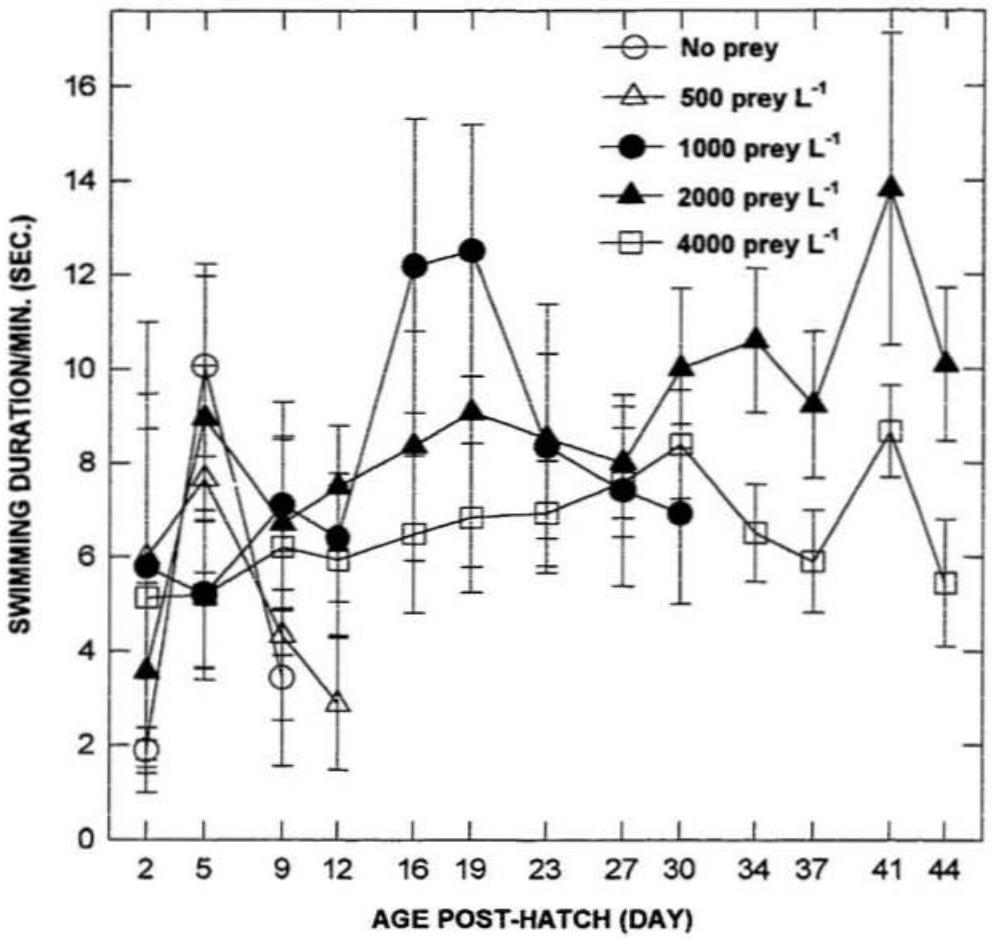

Fig 3.1. Mean swimming duration of Atlantic cod larvae reared in different prey concentrations. Values are mean ± se. n=20 larvae per sample per treatment.

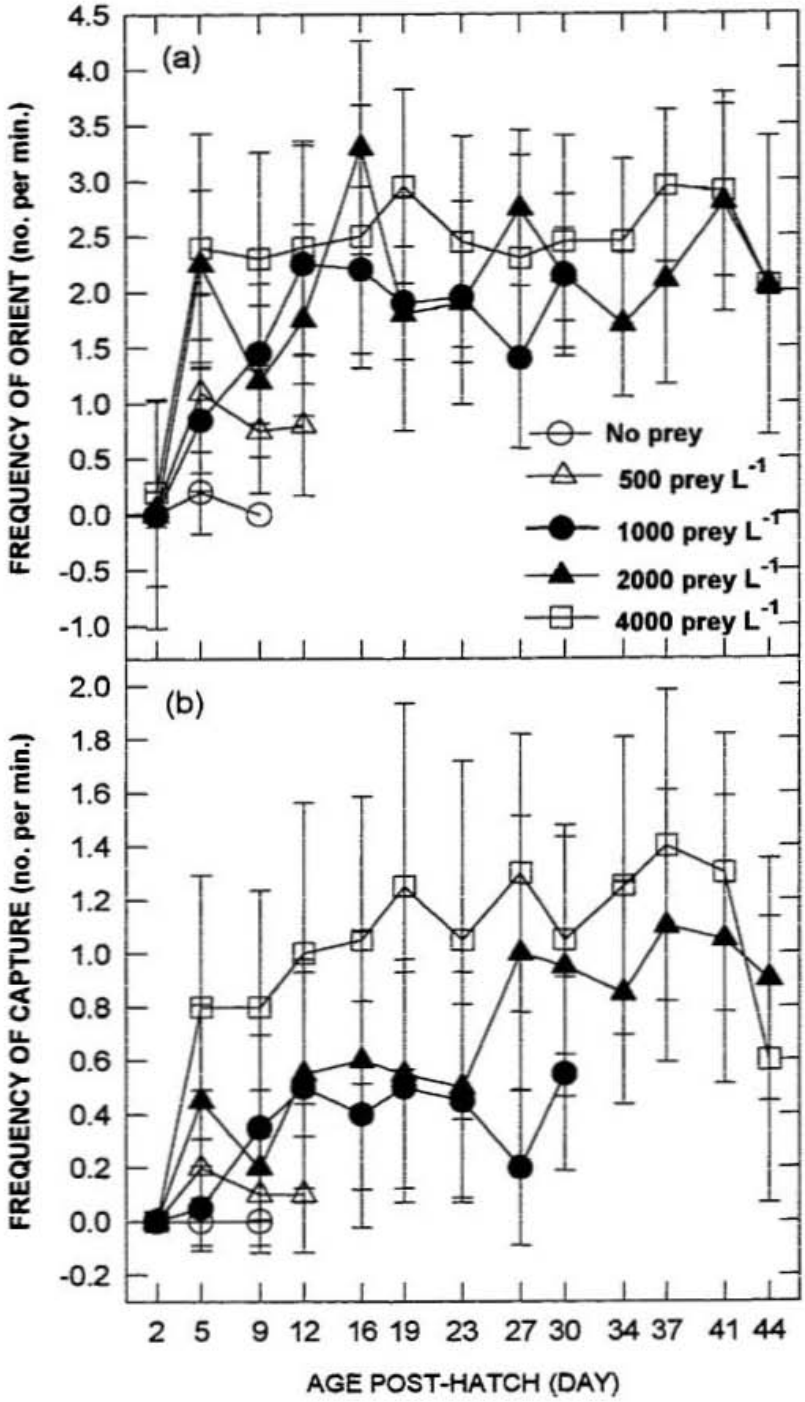

Fig 3.2. The mean frequency of foraging MAP's exhibited by Atlantic cod larvae reared in different prey concentrations. a) orient b) capture. Values are mean ± se. N=20 larvae per sample per treatment.

Table 3.3 Results of least mean square comparisons examining the differences between means of orientation frequency of larval cod under different prey concentrations. Values are the differences in mean orientation frequencies between two treatment comparisons. * indicates significance at 0.05 level.

	Treatment comparisons									
Age(d)	0-500	0-1000	0-2000	0-4000	500-1000	500-2000	500-4000	1000-2000	1000-4000	2000-4000
2	-0.05	0	0	-0.20	0.05	0.05	-0.15	0	-0.20	-0.20
5	-0.90	-0.65	-2.05*	-2.20*	0.25	-1.15*	-1.30	-1.40*	-1.55*	-0.15
9	-0.75	-1.45*	-1.20*	-2.30*	-0.70	-0.45	-1.55*	0.25	-0.85	-1.10*
12	---	---	---	---	-1.45*	-0.95	-1.60*	0.50	-0.15	-0.65
16	---	---	---	---	---	---	---	-1.10*	-0.40	0.80
19	---	---	---	---	---	---	---	-0.10	-1.15*	-1.05*
23	---	---	---	---	---	---	---	-0.05	-0.55	-0.50
27	---	---	---	---	---	---	---	-1.35*	-0.90	0.45
30	---	---	---	---	---	---	---	0	-0.30	-0.30
34	---	---	---	---	---	---	---	---	---	-0.75
37	---	---	---	---	---	---	---	---	---	-0.85*
41	---	---	---	---	---	---	---	---	---	-0.10
44	---	---	---	---	---	---	---	---	---	0

Table 3.4 Results of least mean square comparisons examining the differences between means of capture frequencies of larval cod under different prey concentrations. Values are the differences in mean capture frequencies between two treatment comparisons.
* - indicates significance at 0.05 level.

	Treatment comparisons					
Age(d)	500-1000	500-2000	500-4000	1000-2000	1000-4000	2000-4000
5	0.15	-0.25	-0.60*	-0.40*	-0.75*	-0.35
9	-0.10	-0.25	-0.70*	0.15	-0.45*	-0.60*
12	-0.40	-0.45	-0.90*	-0.05	-0.50	-0.45
16	---	---	---	-0.20	-0.65*	-0.45
19	---	---	---	-0.05	-0.75*	-0.70*
23	---	---	---	-0.05	-0.60*	-0.55*
27	---	---	---	-0.80*	-1.10*	-0.30
30	---	---	---	-0.40	-0.50*	-0.10
34	---	---	---	---	---	-0.40
37	---	---	---	---	---	-0.30
41	---	---	---	---	---	-0.25
44	---	---	---	---	---	0.30

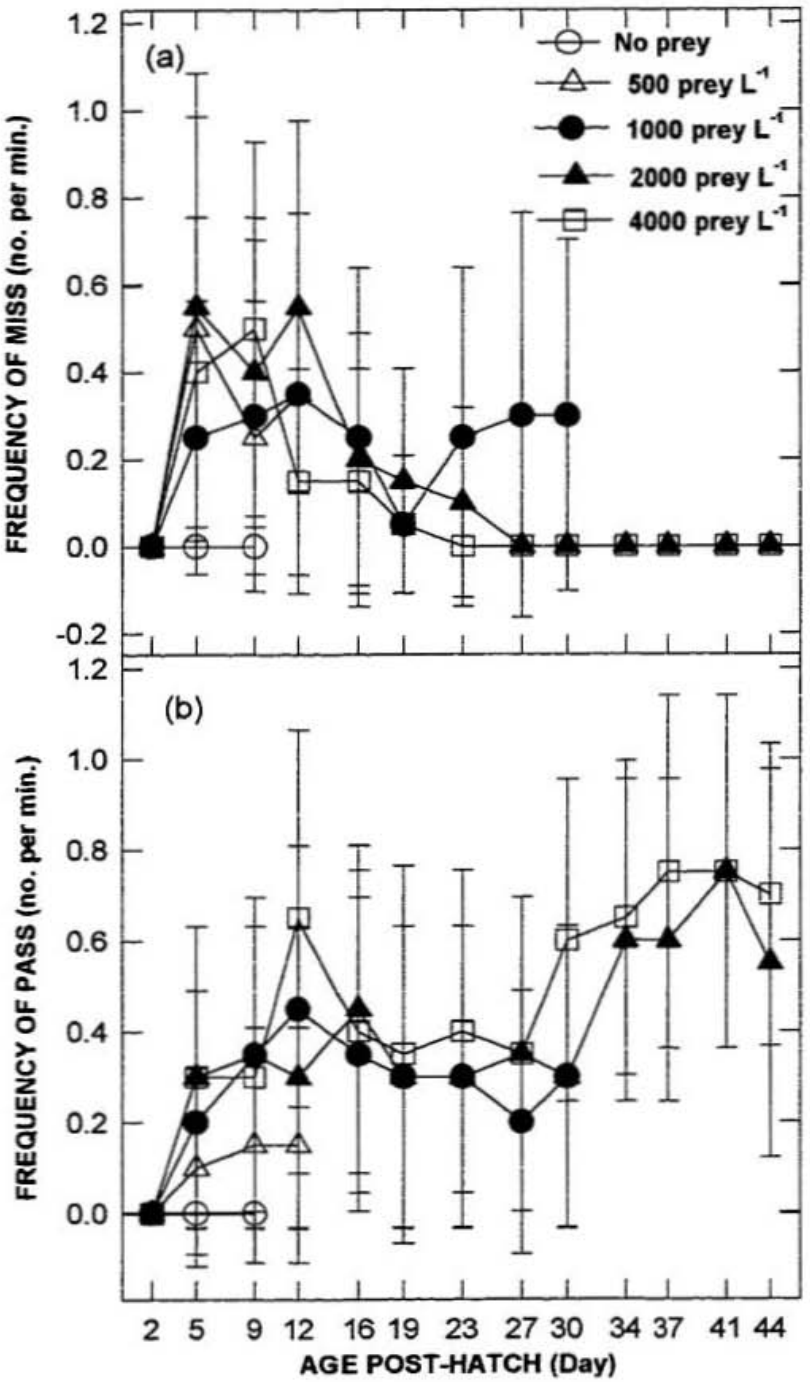

Fig 3.3. The frequency of foraging MAP's exhibited by Atlantic cod larvae reared in different prey concentrations. a) miss, and b) pass. Values are mean ± se. N=20 larvae per sample per treatment.

Table 3.5 Results of least mean square comparisons examining the differences between means of miss frequencies of larval cod under different prey concentrations. Values are the differences in mean miss frequencies between two treatment comparisons. * indicates significance at 0.05 level.

	Treatment comparisons					
Age(d)	500-1000	500-2000	500-4000	1000-2000	1000-4000	2000-4000
5	0.25	-0.05	0.10	-0.30	-0.15	0.15
9	-0.05	-0.15	-0.25	-0.10	-0.20	-0.10
12	0	-0.20	0.20	-0.20	0.20	0.40
16	---	---	---	0.05	0.10	0.05
19	---	---	---	-0.10	0	0.10
23	---	---	---	0.15	0.25	0.10
27	---	---	---	0.30*	0.30*	0
30	---	---	---	0.30*	0.30*	0

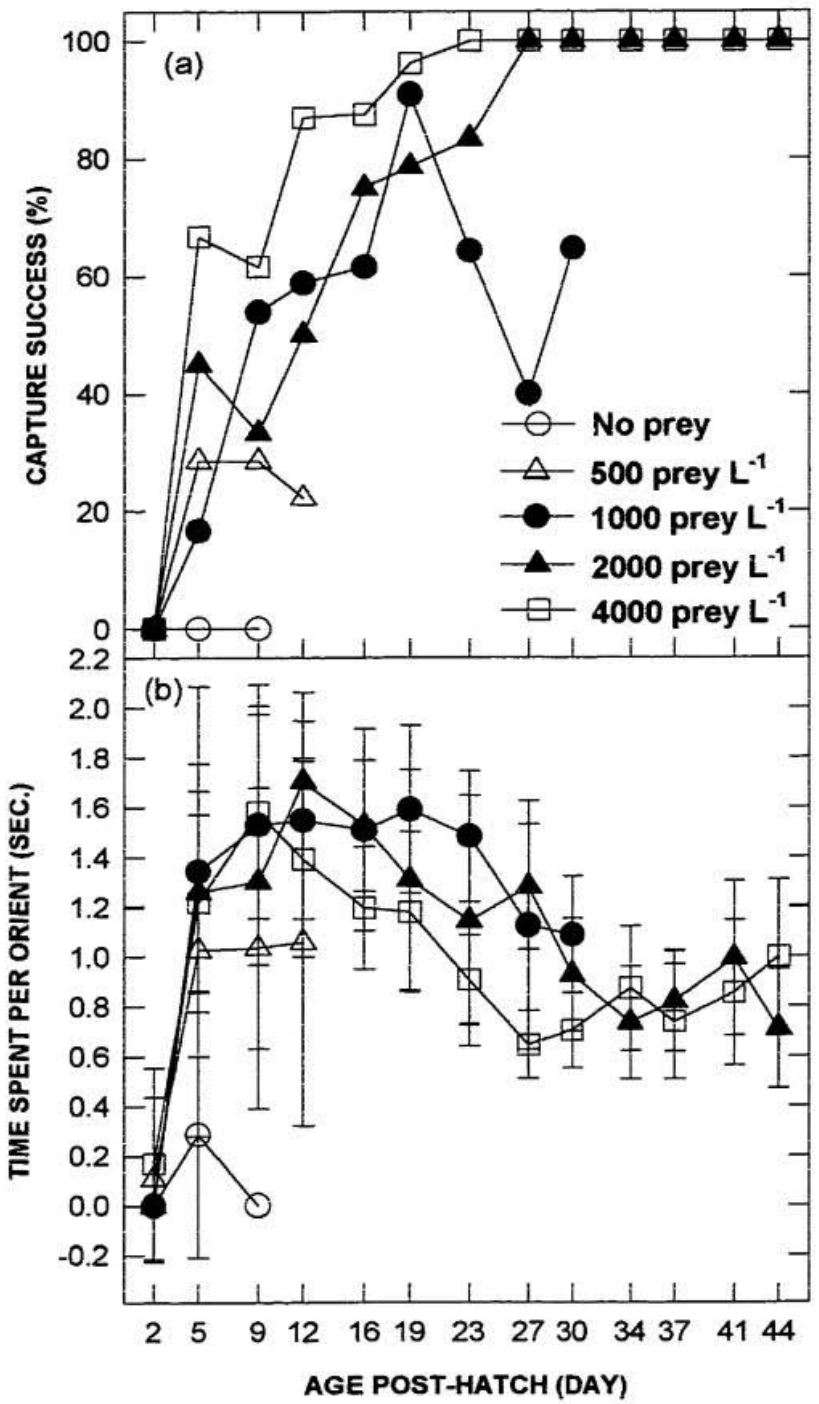

Fig 3.4. a) Capture success b) mean time spent per orient of Atlantic cod larvae reared in different prey concentrations. Values are mean ± se. N=20 larvae per sample per treatment.

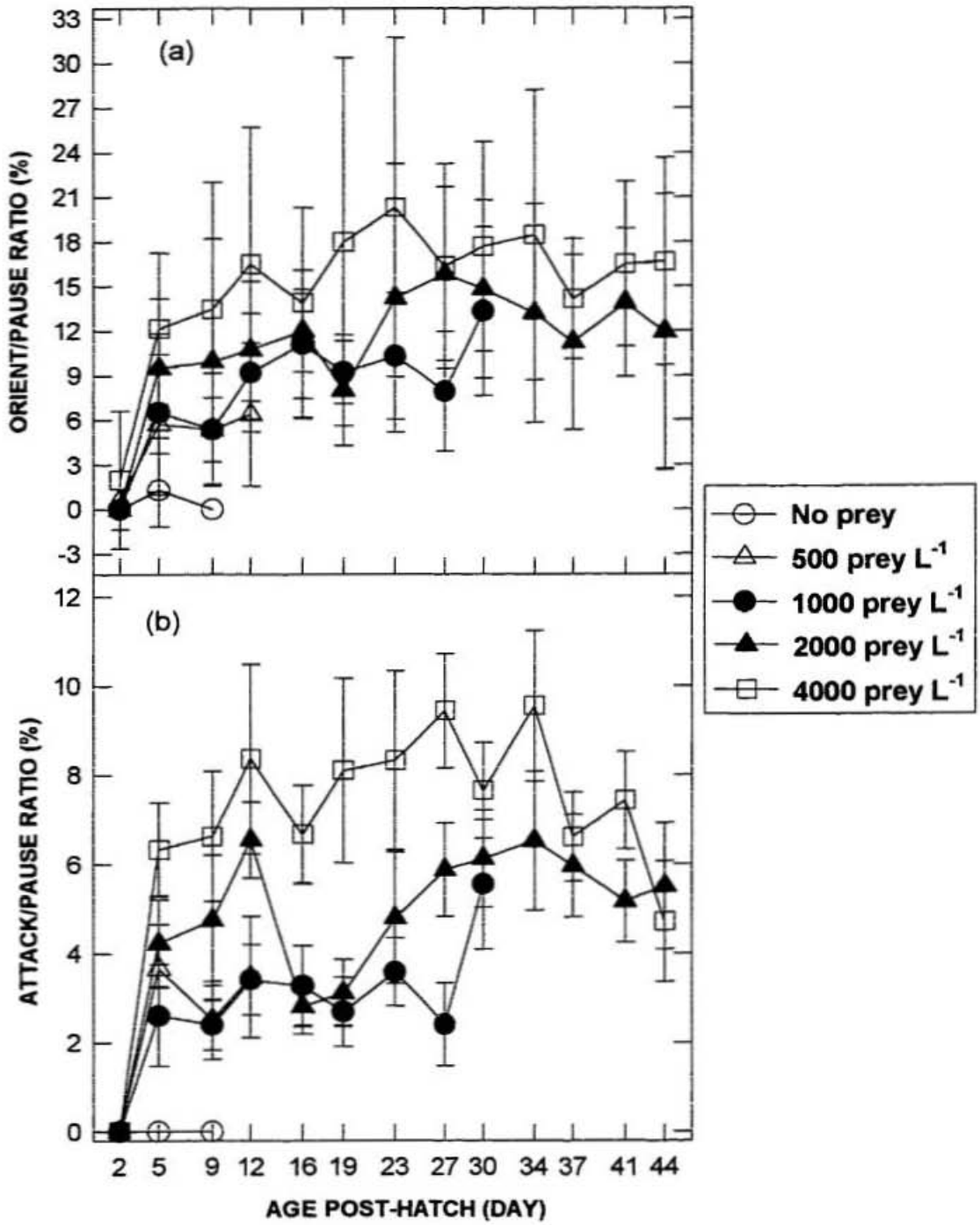

Fig 3.5. Ratio between number of a) orients and pauses and, b) attacks and pauses of Atlantic cod larvae reared in different prey concentrations. Values are mean ± se. N=20 larvae per sample per treatment.

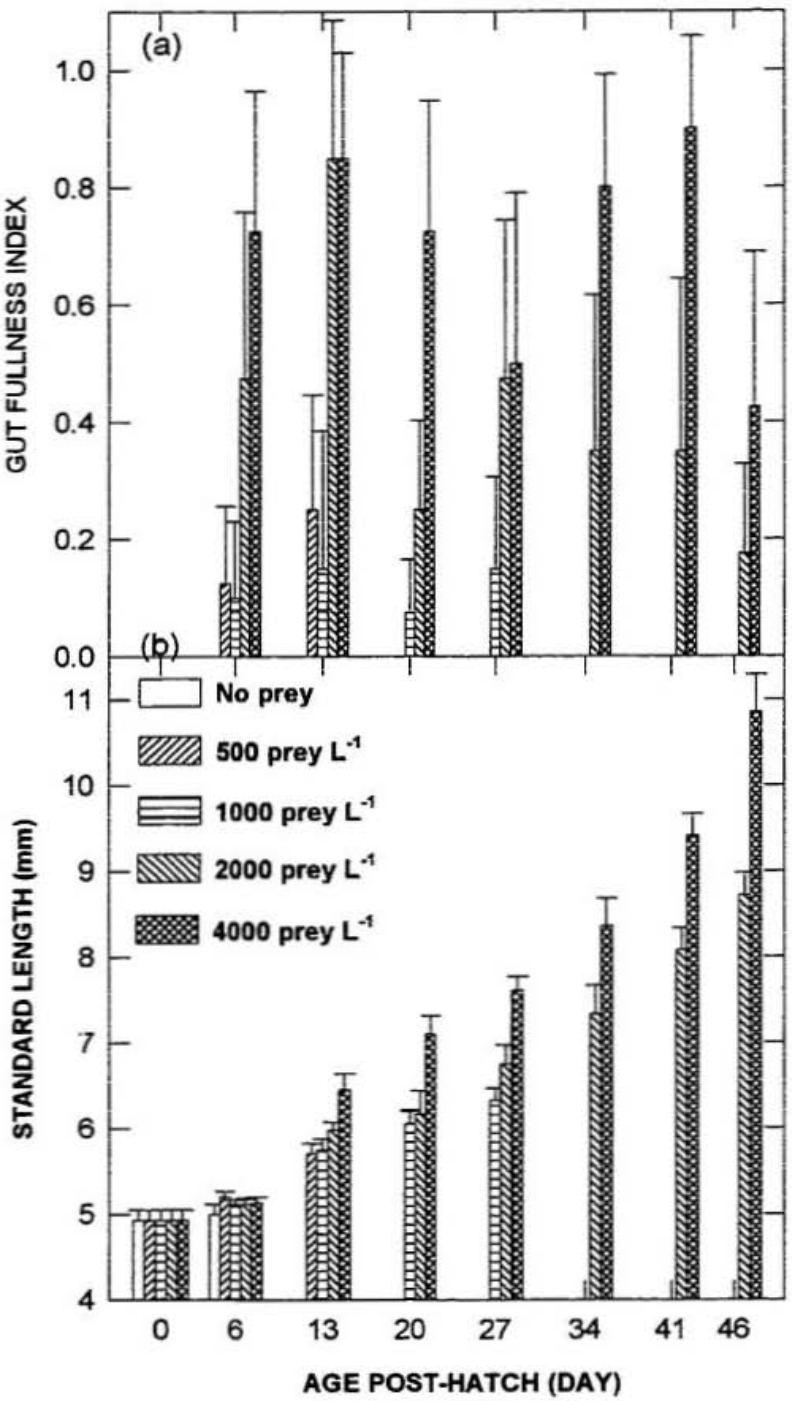

Fig 3.6. a) Mean gut fullness index and, b) standard length (mm) of Atlantic cod larvae reared in different prey concentrations. Values are mean ± se. N = 10 larvae per sample per treatment.

Discussion

Previous research examining larval cod behaviour has typically been of short duration (Ellersten et al. 1980, Skiftesvik and Huse 1987, Skiftesvik 1992, 1994, MacKenzie and Kiørboe 1995, Munk 1995). Also in these studies and others, (Gamble and Houde 1984, Blom et al. 1991, van der Meeren and Næss 1993) the prey concentration was much lower than that used in my experiments. However, the prey to larval ratios of 50-200 in those mesocosm experiments were very much comparable to the ratio of 12.5-133.3 in my experiments (see also Gotceites et al.1996).

Foraging of larval cod consisted of four feeding MAP's: orient, capture, miss and pass. Munk (1995) also observed similar behaviours in his work but used slightly different terminologies (Table 1). The feeding MAP's occurred between intermittent swimming bouts. This type of foraging behaviour, where larvae travel short distances, stop and move again if prey is not detected, has been termed a saltatory search strategy. Browman and O'Brien (1992a, 1992b) documented similar search strategies in golden shiner (1992a) and white crappie (1992b) larvae. In my study, larval cod, regardless of the prey concentration, showed an increase in attack/pause and orientation/pause ratios over time. The ratio was higher in 4000 prey L^{-1} than the lower prey concentrations throughout the study period. Browman and O'Brien (1992b) also reported that the ratio of attack/pause increased as the crappie larvae grew. The increase in the ratio as the larvae grow could be associated with the morphological changes and experience in locating and attacking the prey. Similar observations of changes in foraging behaviours with age and/or size have

been reported in other fish species (Gallis 1990, Croy and Hughes 1991) and these changes were attributed to experience (Gallis 1990). Pass behaviour increased as the larvae grew and the reason for this was not clear. I suspect that the larvae may "choose" more profitable prey among the available prey.

The frequency of foraging in larval cod varied with larval growth and prey availability. Larvae reared at high prey concentrations foraged more, grew faster and survived longer than larvae reared at lower prey concentrations. Overall, larval survival was found to be related to foraging environment, i.e. prey concentration. Other laboratory studies examining the effects of prey concentration on larval fish have also reported increased foraging rates, growth and survival at higher prey concentrations. (Wyatt 1972, Laurence 1974, Houde 1977, Munk and Kiørboe 1985).

It has been observed that if larval fish do not successfully initiate and maintain feeding before a 'critical point' after yolk absorption, then swimming and foraging will be reduced and mass mortalities will result (Blaxter 1988). This critical point is termed the point of no return (PNR) or the time of irreversible starvation (Blaxter 1988). The time to reach this point is temperature and species dependent. For larval cod, Ellertsen et al. (1980) determined this to be 10-12 days post-hatch at 6°C and Laurence (1978) reported it as 10 days at 7°C. They also reported that at this point cod larvae show a marked decrease in foraging activity and increased buoyancy. In my studies, larvae which were reared at 8°C and subjected to low (no prey and 500 prey L^{-1}) prey concentrations, showed a decrease in swimming and foraging by day 9 and total mortality occurred in

these low concentrations by day 11 and 15 respectively. These results suggest that a prey concentration of 500 prey L^{-1} was not sufficient to prevent larval mortality from starvation in intensive rearing systems.

Yin and Blaxter's (1987) studies on larval herring, cod and flounder and Skiftesvik's (1992) studies on cod and turbot (*Scophthalmus maximus*) larvae documented similar declines in foraging intensity and activity as starved larvae reached the PNR. Even though higher activity levels should increase the likelihood of locating prey, lower activity levels associated with starvation in larval fishes may be a strategy employed to conserve energy, perhaps delaying the time to irreversible starvation. Those trends were not observed in my experiment for larval cod reared at higher prey concentrations. Under these conditions, foraging and swimming activity increased at week two coinciding with successful transition from endogenous to exogenous feeding. Increased activity associated with foraging behaviour would maximize a larva's probability of locating prey items. Successful capture of prey by larvae in high food treatments was reflected in their growth as well as their gut fullness.

Associated with foraging is prey capture success, commonly defined as the ratio of feeding attempts to the number of successful bites (Drost 1987). A larva's ability to feed generally involves some degree of learning. In my experiment, not all of the early feeding attacks were successful. The failure to successfully capture a prey item may be the result of larvae aiming inaccurately, a slow capture attempt, or the prey item moving out of the larva's visual field. In my study, capture success increased with larval age and was highest

in 4000 prey L^{-1}. At this higher prey concentration, encounter rates with prey items should be highest, thus providing larvae with increased foraging opportunities. As the prey concentration decreases, search volume and search time should increase which may result in increased foraging opportunities. However, this was not supported from my experiment. My results showed that the encounter rate (frequency of orient) of larvae reared in low prey concentrations was significantly lower than the encounter rate of cod larvae reared in high prey concentrations. Furthermore, the energy expenditures associated with locating food items should, therefore, be higher at lower prey concentrations.

In many species of fish larvae, capture success improves rapidly with experience and morphological development (Blaxter 1986, Drost 1987). For example, Ellertsen et al. (1980) observed that at the onset of exogenous feeding, larval cod had a feeding success of 32-62% which increased to 90% towards the end of yolk absorption (7-12 days post-hatch). They attributed this increase in capture success rates to improved manoeuverability at the time of first feeding. Similarly in my experiment, larval cod were observed to have a feeding success ranging from 16.7-66% during day five post- hatch which increased to 50-87% by day 12 post-hatch. Solberg and Tilseth (1987) observed a capture success of 22% at day seven post hatch for larval cod reared at less than 200 prey L^{-1} which was similar to my 500 prey L^{-1} treatment. But my 4000 prey L^{-1} treatment had a 66% of capture success. The low success rate of larval cod at low prey concentration indicates cod need higher prey concentrations to survive through metamorphosis. In other marine species, capture success at the onset of first feeding is much lower; 6% in herring (Rosenthal and Hempel

1970), 10% in northern anchovy (Hunter 1972) and 17% in American shad (*Alosa sapidissima*) (Ross and Backman 1992), all of which increased with growth and development.

It does not seem unreasonable that a larva's ability to locate and capture a prey increases with both morphological development and experience. Miller et al. (1992) observed dramatic improvements in foraging abilities of larval alewife (*Alosa pseudoharengus*), yellow perch (*Perca flavescens*), and bloater (*Corigonus hoyi*) as they grew. Browman and O'Brien (1992b) showed similar results in white crappie larvae as the proportion of aborted attacks decreased with fish size. In my study, cod larvae reared at 2000 and 4000 prey L^{-1} demonstrated similar improvements in foraging capabilities (i.e. attack success) with age. Increases in gut fullness and standard length reflect this foraging success.

In larval cod it appears that the development of efficient foraging behaviour is closely associated with the development of structures. Skiftesvik (1992) showed that unfed cod larvae reduce swimming after seven days post hatch whereas fed cod larvae became more active which she correlated with the better morphological development of fed cod larvae. Yin and Blaxter (1987) showed that the myotome height of starved cod larvae was reduced after day 10 post-hatch and the larvae did not grow in length. Little or no growth observed in poorly fed larvae often results in impaired development of the sensory system and the deterioration of body tissue (musculature and liver), which can hinder locomotor capabilities. Ellertsen et al. (1980) have shown that under poor feeding

conditions, the foraging behaviour of larval cod can become less efficient. Laurence (1972) in his studies on largemouth bass (*Micropterus salmoides*) larvae, showed that fed larvae were always more active than unfed larvae. He correlated this to the development of fins, muscle mass, and increased body length which improved the larva's manoeuverability, attack speed and swimming behaviour. Altogether these features would be expected to play a role in improving foraging behaviour in the larvae. Conversely, under sub-optimal foraging conditions, starvation can seriously hinder larval growth and in turn the development of associated behaviours. The small traces of prey observed in the guts of cod larvae reared in lower prey concentrations during week two of my experiment suggests a poor nutritional environment which resulted in weaker larvae that quickly approached the point of starvation. As a result of this deteriorating condition, a decline in foraging behaviour was observed.

Kjorsvik et al. (1991) studied the early development of the digestive tract in larval cod, as well as some of the consequences associated with starvation and their effect on larval morphology. They observed that starved larvae showed a gut morphology markedly different from feeding larvae. Starvation induced cellular degeneration, shrunken epithelial cells and reduced microvilli, as well as liver and pancreas degeneration. Periods of starvation were reported to cause irreversible damage to the gut, which ultimately reduced digestive and absorptive efficiencies. These results suggest that the early effects of starvation may still allow the larvae to consume prey, but digestion would be inefficient. This may explain the presence of small amounts of food in the guts of the dead larvae

reared in lower prey concentrations. In comparison, under optimal feeding conditions, Kjorsvik et al. (1991) reported an increased ability of the gut to absorb lipids and proteins. Therefore, the better growth observed in larval cod reared at higher prey concentrations (2000 and 4000 prey L^{-1}) may reflect an increased ability of the gut of these larvae to absorb such food nutrients.

It is not possible to extend the absolute changes in survival caused by increased prey concentrations to the field especially due to the absence of predation pressure on the larvae in rearing conditions. In nature, combined effects of foraging conditions, predation pressure and abiotic factors that affect the development and behaviour of the fish larvae would result in an increase or decrease in survival rates. But field evidence suggests that larval fish are not prone to starvation mortality (Theilacker 1986). Most field studies report very low prey concentrations relative to that reported in the laboratory studies. Frank & Leggett (1986) and Frank (1988) suggested that this disagreement may be partly due to inadequate sampling procedures. On a large scale, prey in the field may be low. Prey in the ocean usually occur in patches and prey concentrations in these patches are reported to be substantial in order to sustain reasonable growth and survival (Lasker 1978). Prey concentrations in these patches may exceed or at least be on par with the prey concentrations reported in laboratory studies. Lasker (1978) also reported that larval anchovy spend more time in these patches. Most sampling procedures are inadequate to measure the prey concentration in the patches. Field sampling of zooplankton usually involves a larger spatial scale and the dragging of the sampling gear through these patches

disturbs the heterogeneity of the prey. Also, it has been pointed out that most of the early larval stages of various fish species prey upon smaller prey items of less than 200 μm (Houde 1973), and the field sampling rarely retains this size range of zooplankton (Laurence 1977).

In my experiment I used rotifers and *Artemia* naupilii as prey which are not he natural prey of larval cod. In nature, larval cod primarily feed on copepod naupilli (Last 1978). Studies have shown that copepod naupilli are evasive prey (Shuvayev 1978) whereas rotifers and *Artemia* naupilli are limited escape responses (Drost 1987). Thus, it make difficult to extend the finding of my experiment to wild but still my results would contribute substantially to the understanding of foraging behaviour of larval cod.

In conclusion, it is evident that foraging behaviour of larval cod is influenced by the foraging environment. At high prey concentrations, frequencies of the foraging MAP's were higher from early in the developmental stage thus, larvae had the ability to feed more than larvae reared in lower prey concentrations. Although the frequencies of foraging MAP's were higher for larvae reared in higher prey concentrations, development of foraging behaviour was not influenced by prey concentrations. Lesser swimming activities and higher feeding incidences of the larvae reared in high prey concentration enabled them to grow and develop quickly thus shortening the critical period and increasing their overall potential for survival.

Chapter Four: Effect of prey concentration on foraging activity, growth and survival of Atlantic cod larvae reared in laboratory conditions.

Introduction

Studies on the growth and survival of fish larvae are important for both aquaculture and the larval fish ecology as larval stage is characterized by high mortality. Under natural conditions the primary sources of mortality are starvation and predation (Hunter 1972). Under larviculture conditions, problems with start-feeding are the major source of mortality. During start-feeding the concentration, type and size of prey are important factors affecting both growth and survival in larval fish (Margulies 1993). Inadequate or inappropriate prey organisms in the vicinity of the larvae usually result in lower growth rates, poor condition and consequently high mortalities (Werner and Blaxter 1980, Tsai 1991, Cushing and Horwood 1994, Welker et al. 1994).

Atlantic cod (*Gadus morhua*) is an important commercial species in the north Atlantic in both wild fisheries and aquaculture (Tilseth 1990). Despite a tremendous amount of research that has been done on cod larvae, no published data are available on the effects of different prey concentrations on larval growth and survival, especially with respect to intensive rearing systems. Munk (1995) observed larval cod foraging behaviour under different prey levels but his experiments were carried out for only five days on selected stages of larval cod. His study was not designed to provide information on the effect of prey concentration on the growth or survival of cod larvae. Other studies have examined the effect of available prey type and concentration on growth and survival of

larval cod but in these studies the prey concentration varied throughout the experiment (Kvenseth and Øiestad 1984, Blom et al. 1991, Oterrå 1993, van der Meeren and Næss 1993). Moreover, these studies did not address the effect of a range of prey concentrations on larval growth and survival.

In my earlier study on effect of prey concentrations on the foraging behaviour of Atlantic larval cod (Chapter 3), results showed that foraging, growth and survival increased with increasing prey concentrations and did not show a plateau at higher prey concentrations. This indicates that optimal prey concentration for rearing of larval cod in the laboratory may be higher than 4000 prey L^{-1}. Furthermore, in that study I did not monitor the mortalities and reported only the ultimate survival of the larval cod at the end of the experiment. Other studies indicate that mortalities are high during the first feeding larval stage (Tucker 1992).

The aim of this study was to determine the optimal prey concentration required to rear cod larvae in intensive systems. I included observations on the swimming and foraging activity of the larvae, and determined whether the differences in growth and survival of cod larvae under the different prey concentrations were related to variations in their foraging behaviour.

Materials and Methods.

Fertilized Atlantic cod eggs were collected from a naturally spawning captive broodstock maintained at the Ocean Sciences Centre, Logy Bay, Newfoundland. Eggs

were incubated at 8°C until hatch. When 50% of the eggs had hatched, 10 larvae were sampled for morphometric measurements and this was taken as day zero (week 0) of the experiment.

I used two, 30L rectangular glass aquaria per experimental treatment. All four sides of the aquaria were covered by opaque black plastic. All experimental aquaria were kept in a thermoregulated water bath maintained at 8°C. The light level at the water surface was 1200 lux (SPER Scientific light meter 840006) and a 24h period of light was used. The light intensity and photoperiod were chosen from previous experiments carried out in our lab.

Each experimental aquarium was supplied with filtered (down to 1 μm using particle filters), UV sterilized sea water at a rate of six L hr^{-1}. Green algae (*Isochrysis* sp) were added to the tanks (Tucker 1992). When nearly 100% of the larvae had hatched (day 1), 1200 larvae (40 larvae L^{-1}) were transferred to each of 14 experimental aquaria. Seven prey concentrations were chosen, 250, 500, 1000, 2000, 4000, 8000 and 16000 prey L^{-1}. Experimental prey concentrations were chosen from previous studies conducted in our laboratory (Gotceites et al. 1996, Chapter 3) and were comparable to those used in other studies where larval cod were reared under laboratory conditions (Laurence et al. 1981).

Enriched rotifers (*Brachionus plicatilis*) were used as prey from day three to day 19 post-hatch. A mixture of rotifers and *Artemia franciscana* nauplii were used as prey beyond day 20. Larvae were fed three times per day. To maintain the desired prey concentration within each experimental tank, a 10-20 ml water aliquot was sampled from

each aquarium at different depths (just below surface, mid water columns, and just above bottom) before each feeding. The number of prey items in each sample was counted and prey concentrations were adjusted as needed. Each experimental aquarium was aerated which ensured a homogeneous distribution of prey within each aquarium.

The experiment was stopped when most of the larvae (90-100%) in the high food treatment and at least some larvae from the low food treatments (20-50%) were past metamorphosis. Metamorphosis was defined in this study as when the continuous fin fold disappeared and discrete fins were formed.

Data collection

Initially, 10 larvae were sampled from the egg incubation basket on day zero (week 0) and five larvae from each tank (10 per treatment) were arbitrarily chosen for morphometric measurements every week over the duration of the experiment. Using a dissecting microscope, standard length (mm), head depth (measured posterior to the eye), eye diameter (along the body axis), and myotome height (posterior to the anus) were measured.

Condition of the larvae was calculated using a relationship between two morphometric measurements, the standard length (mm) and myotome height (mm) (Koslow et al. 1985).

$$\text{Condition index} = \text{myotome height} / \text{standard length} \qquad (1)$$

Length-specific growth rates (SGR) of larvae were determined using the following

relationship:

$$SGR = (\ln(L_t) - \ln(L_0)) / t) \times 100 \quad (2)$$

Where L_t is the mean final length (mm), L_0 is the mean initial length (mm), and t is the duration between initial and final sampling (days) (Buckley et al. 1987, Cowan and Houde 1990).

Mortalities were removed twice a day from each experimental aquarium from day 15. Instantaneous mortality rates were calculated from the following model:

$$Z = (\ln(N_t) - \ln(N_0)) / t) \times 100 \quad (3)$$

Where Z = Instantaneous mortality rate (d^{-1}), N_t is the number of larvae alive at time t, N_0 is the number of larvae alive at time 0 and t is the duration in days (Cowan and Houde 1990). At the end of the experiment, the number of surviving larvae in each treatment was recorded.

Behavioural observations were conducted twice a week from day two post-hatch until day 40 post-hatch. The focal animal technique (Altman 1974) was used to observe an arbitrarily selected larva for a one minute interval. This was done for a total of ten larvae from each tank (20 per treatment). During each observation period, I recorded the occurrence of six Modal Action Patterns - MAP's (See Table 3.1; Barlow 1977). Occurrence of any of the six MAP's was recorded using an event recorder. The frequencies of miss and capture were pooled to create the variable 'attack' which was then used to calculate the capture success and attack rate (See Chapter 3) using the following relationship:

Capture success= (frequency of capture / frequency of attack)*100 (4)

Attack rate = (frequency of attack / frequency of orientation)*100 (5)

Data analysis

Prior to analysis, normality was tested by using the Kolomogorov-Smirnov statistic (SAS 1988) and plots of residuals and predicted values were examined. The effect of prey concentration and age on standard length, condition index, survival, specific mortality rate (Z) and length-specific growth rate (SGR) were analysed by two-way analysis of variance ($p \leq 0.05$). Condition index data were log transformed (SAS 1988) to satisfy the normality requirements of ANOVA. The Tukey test or multiple t-tests were used for subsequent comparison among different prey concentrations for each week. Results from multiple t-tests for survival were compared with p-values corrected for the number of prey treatments and sampling dates (Bonferoni method, $p \leq 0.05/(6\times2) \leq 0.0042$).

The behavioural data could not be normalized by any available transformation methods. However, according to the central limit theorem, normality can be relaxed in cases where sample size is large (in my case n=1400; Johnson and Wichren 1992). The effect of prey concentration and age on swimming and all foraging variables were analysed by two-way ANOVA (SAS 1988; $p \leq 0.05$). When significant results were obtained, Tukey's studentized (HST) test was used to determine which means differed.

Results

Growth (standard length) of the cod larvae was significantly influenced by prey concentration. Effects of prey concentration and age on standard length of cod larvae were significant (Table 4.1). Larvae reared under low prey concentrations (250-2000 prey L^{-1}) were significantly smaller than the larvae reared at 4000-16000 prey L^{-1} (Fig. 4.1a). No significant difference was found between the treatments in larval standard length until week two except for 250 and 500 prey L^{-1} treatments in which larvae were significantly smaller from week one till the end of the experiment. From week two to the end of the experiment, larvae reared in 4000-16000 prey L^{-1} were significantly larger than the larvae reared in all lower prey concentrations (Table 4.2). Prey concentration and age had a significant influence on the length-specific growth rate of larval cod (Table 4.1). Except for week three, larvae reared in 4000-16000 prey L^{-1} had higher length-specific growth rates than larvae reared in other prey concentrations (Fig. 4.1b).

Prey concentration and age had a significant effect on the condition of the cod larvae (Table 4.1). Larval cod reared at 4000 prey L^{-1} were in better condition than larvae reared at all other prey concentrations Fig. 4.2a). Condition of cod larvae reared at 4000 L^{-1} increased from the start of the experiment to the end while larvae from the other prey treatments showed lower condition index than they had at hatch until week three (Fig. 4.2a). From week four onwards larvae reared in 4000 prey L^{-1} had significantly higher condition index than larvae reared in lower (1000 and 2000 prey L^{-1}) prey concentrations (Table 4.3).

Instantaneous mortality rates (Z) were significantly influenced by prey concentration and age (Table 4.1). Z was significantly higher in lower prey concentrations (500-2000 prey L^{-1}) in week two and week three compared to all three higher prey concentrations. Beyond four weeks, no significant difference was found in Z among the treatments (Table 4.4 and Fig. 4.2b). Both prey concentration and age had a significant effect on the survival of larval cod (Table 4.1). A significantly higher percentage of larvae survived when reared at 4000-16000 prey L^{-1} than when reared at 500-2000 prey L^{-1} at week two ($F=67.72$, $df=5$, $p<0.0001$) and week six ($F=42.33$, $df=4$, $p<0.0005$). Survival among higher prey concentrations (4000-16000) or among lower prey concentrations (500- 2000) were not significantly different at week two and six (Table 4.5). Although, survival among the three higher prey concentrations were similar at week two, the percentage of cod larvae surviving at week six was higher in 4000 prey L^{-1} followed by 8000 and 16000 prey L^{-1} (Fig. 4.3). Larvae reared in 250 and 500 prey L^{-1} survived only to the end of week two (11 days) and four (24 days) respectively.

Prey concentration and age had a significant influence on swimming duration , orientation, attack, and capture of larval cod (Table 4.6). In week one there was no difference in any of these behaviours among the larvae reared in different prey concentrations (Fig. 4.4a, 4.4b, 4.5a and 4.5b). From week two, larvae reared in 4000-16000 prey L^{-1} swam less than larvae reared at lower prey (500-2000) concentrations. In week two, larvae in 250 prey L^{-1} swam less than the larvae in other treatments (Fig. 4.4a). Larvae reared under 4000-8000 prey L^{-1} oriented to more prey than larvae reared in 250-

2000 prey L^{-1}. Among the low prey concentrations, larvae reared at 2000 prey L^{-1} experienced more prey encounters than larvae from other low prey concentrations (250-1000 prey L^{-1}). Differences in prey encounters between larvae reared in higher prey concentrations and 2000 prey L^{-1} disappeared from week five onwards (Fig. 4.4b).The attack rates were higher in larvae reared in 250-2000 prey L^{-1} than in the higher prey concentrations from week two to week four and were similar among all the treatments at week five and six (Fig 4.5a). Although the attack rates were higher in larvae reared at lower prey concentrations, larvae reared in higher prey concentrations showed significantly higher capture success than larvae from lower prey concentrations. From week four onwards larvae from 2000 prey L^{-1} showed a similar capture success as the larvae from higher prey treatments (Fig. 4.5b).

Table 4.1 Results of a two-way ANOVA (age and prey concentration) on standard length, SGR, condition, mortality rate (Z) and survival of larval cod at different prey concentrations. Significance at 0.05 level.

Variable	source	df	F-value	Pr. > F
Standard	Model	33	223.88	0.0001
length	Error	306		
	Age	5	1208.30	0.0001
	Concentration	6	50.64	0.0001
	Age × Concentration	22	4.49	0.0001
SGR	Model	33	6.16	0.0001
	Error	306		
	Age	5	21.88	0.0001
	Concentration	6	5.41	0.0001
	Age × Concentration	22	2.34	0.0001
Condition	Model	33	122.72	0.0001
	Error	306		
	Age	5	642.14	0.0001
	Concentration	6	29.01	0.0001
	Age × Concentration	22	2.10	0.0031
Mortality	Model	26	95.56	0.0001
rate	Error	27		
	Age	4	91.30	0.0001
	Concentration	5	327.71	0.0001
	Age × Concentration	17	18.21	0.0001
Survival	Model	10	50.54	0.0001
	Error	11		
	Age	1	97.37	0.0001
	Concentration	5	20.71	0.0008
	Age × Concentration	4	3.34	0.0508

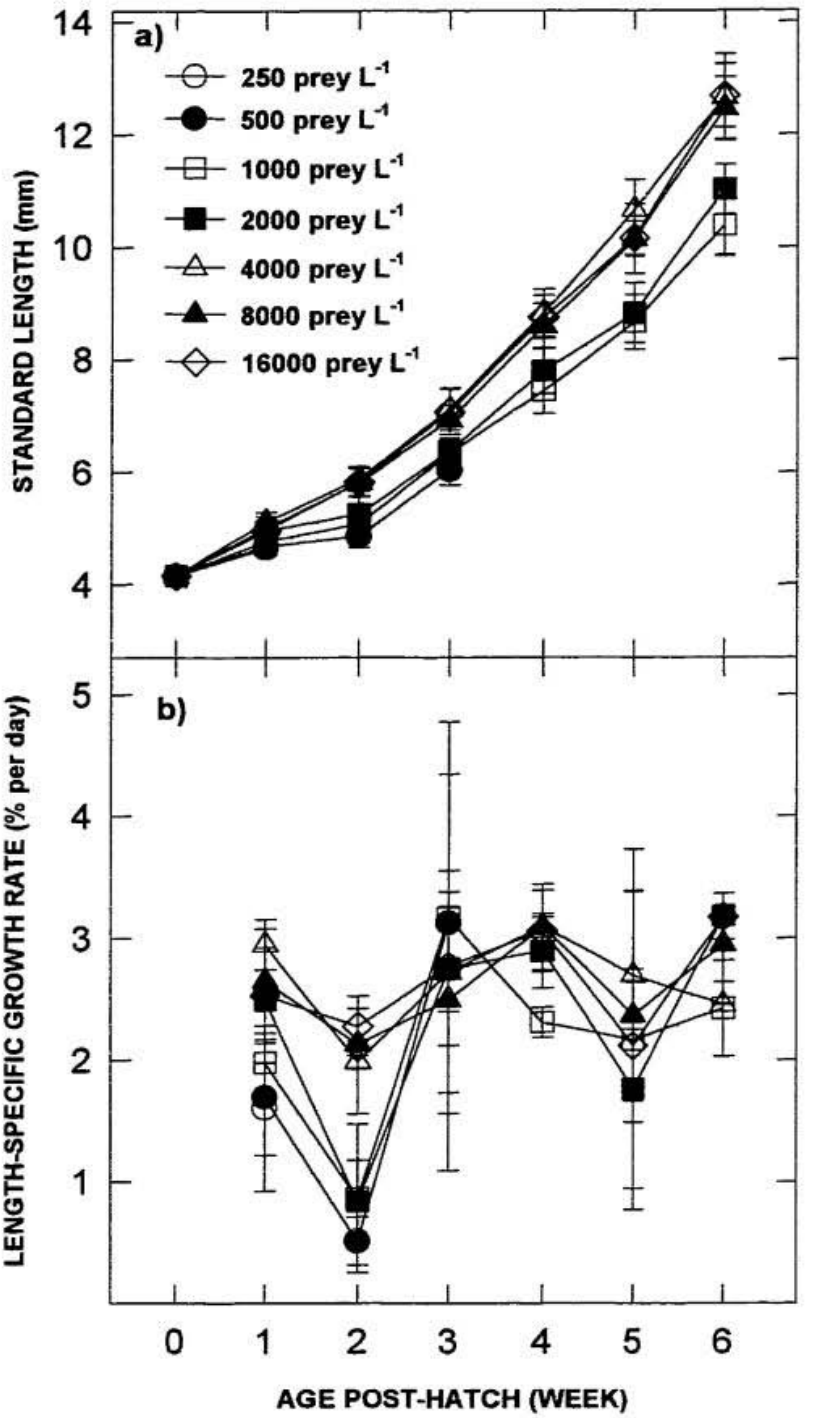

Fig 4.1. Change in mean (± se) a) standard length and, b) length-specific growth rates (SGR), of Atlantic cod larvae reared at different prey concentrations. N = 10 larvae per week.

Table 4.2 Results of the Tukey analysis comparing standard length (mm) of cod larvae reared under different prey concentrations from week one to week six post-hatch. Values are the differences in mean standard length between two treatment comparisons.
* - indicates significance at 0.05 level.

Treatment comparisons	Age (weeks) 1	2	3	4	5	6
16000-8000	-0.04	0.012	0.148	0.166	0.017	0.222
16000-4000	-0.148	0.053	-0.041	-0.071	-0.5	0.029
16000-2000	0.011	0.571*	0.7*	0.959*	1.334*	1.666*
16000-1000	0.19	0.746*	0.737*	1.312*	1.494*	2.305*
16000-500	0.283	0.97*	1.031*	----	----	----
16000-250	0.313*	----	----	----	----	----
8000-4000	-0.108	-0.065	-0.189	-0.237	-0.517	-0.193
8000-2000	0.051	0.559*	0.552*	0.793*	1.317*	1.444*
8000-1000	0.23	0.734*	0.589*	1.146*	1.477*	2.083*
8000-500	0.323*	0.958*	0.883*	----	----	----
8000-250	0.353*	----	----	----	----	----
4000-2000	0.159	0.624*	0.741*	1.03*	1.834*	1.637*
4000-1000	0.338*	0.799*	0.778*	1.383*	1.994*	2.276*
4000-500	0.431*	1.023*	1.072*	----	----	----
4000-250	0.461*	----	----	----	----	----
2000-1000	0.179	0.175	0.037	0.353	0.16	0.639
2000-500	0.272	0.399	0.331	----	----	----
2000-250	0.302	----	----	----	----	----
1000-500	0.093	0.224	0.294	----	----	----
1000-250	0.123	----	----	----	----	----
500-250	0.03	----	----	----	----	----

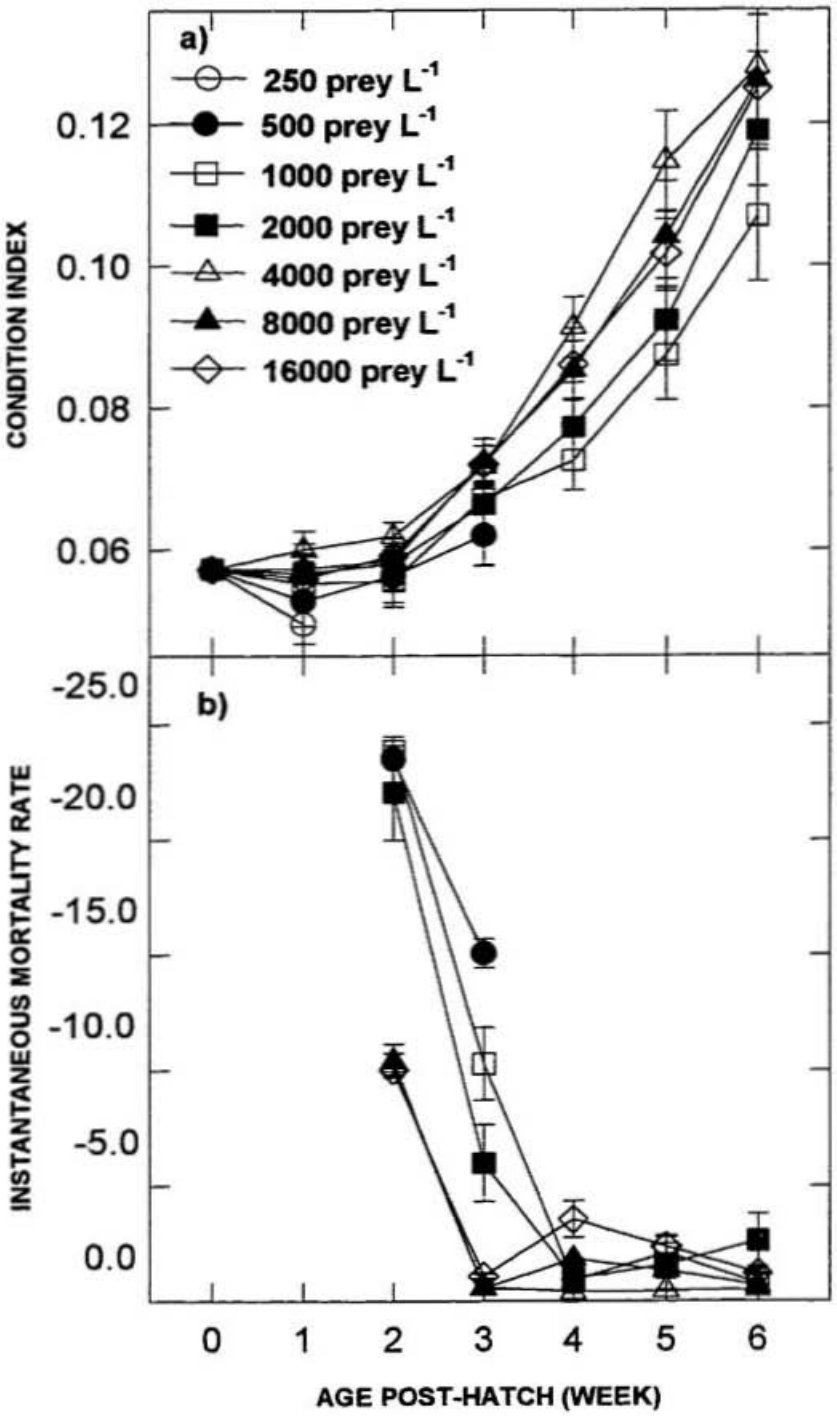

Fig 4.2. Change in mean (± se) a) condition index and b) instantaneous mortality rates (Z) of Atlantic cod larvae reared at different prey concentrations. N = 10 larvae per week.

Table 4.3 Results of the Tukey analysis comparing the condition index of cod larvae reared under different prey concentrations from week one to week six post-hatch. Values are the differences in mean condition index between two treatment comparisons.

* - indicates significance at 0.05 level.

Treatment	Age (weeks)					
comparisons	1	2	3	4	5	6
16000-8000	-0.0104	0.005	-0.0018	0.0035	-0.009	-0.0022
16000-4000	-0.031	-0.0198	0.0015	-0.0263	-0.052*	-0.0077
16000-2000	-0.0054	0.0091	0.0361	0.0479*	0.043	0.0227
16000-1000	0.0057	0.0284	0.0304	0.074*	0.0674*	0.0697*
16000-500	0.0256	0.0229	0.0667*	----	----	----
16000-250	0.0526*	----	----	----	----	----
8000-4000	-0.0206	-0.0248	0.0033	-0.0298	-0.0426	-0.0055
8000-2000	0.005	0.0041	0.0379	0.0444	0.052*	0.0249
8000-1000	0.0161	0.0234	0.0322	0.0705*	0.0764*	0.0719*
8000-500	0.036	0.0179	0.0685*	----	----	----
8000-250	0.063*	----	----	----	----	----
4000-2000	0.0256	0.0289	0.0346	0.0742*	0.0946*	0.0304
4000-1000	0.0367	0.0482*	0.0289	0.1003*	0.119*	0.0774*
4000-500	0.0566*	0.0427	0.0652*	----	----	----
4000-250	0.0836*	----	----	----	----	----
2000-1000	0.0111	0.0193	-0.0057	0.0261	0.0244	0.047
2000-500	0.031	0.0138	0.0306	----	----	----
2000-250	0.058*	----	----	----	----	----
1000-500	0.0199	-0.0055	0.0363	----	----	----
1000-250	0.0469	----	----	----	----	----
500-250	0.027	----	----	----	----	----

Table 4.4 Results of the Tukey analysis comparing the instantaneous mortality rates of larval cod reared under different prey concentrations from week two to week six post-hatch. Values are the differences in mean instantaneous mortality rates between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment	Age (weeks)				
comparisons	1	2	3	4	5
16000-8000	0.393	-0.521	-1.73	-1.05	-0.527
16000-4000	0.21	-0.504	-3.152	-1.916	-0.696
16000-2000	12.02*	4.892	-2.569	-0.791	1.401
16000-1000	13.85*	9.2*	-2.654	-0.306	-0.452
16000-500	13.495*	13.986*	----	----	----
8000-4000	-0.183	0.017	-1.422	-0.866	-0.169
8000-2000	11.627*	5.413	-0.839	0.259	1.928
8000-1000	13.455*	9.721*	-0.924	0.744	0.075
8000-500	13.102*	14.507*	----	----	----
4000-2000	11.81*	5.396	0.583	1.125	2.097
4000-1000	13.638*	9.704*	0.498	1.61	0.244
4000-500	13.285*	14.49*	----	----	----
2000-1000	1.828	4.308	-0.085	0.485	-1.853
2000-500	0.475	9.094*	----	----	----
1000-500	-0.353	4.786	----	----	----

Table 4.5 Results of least-square means analysis, t-values (p-value in parentheses) comparing the survival (%) of cod larvae from different prey treatments at week two and six. * - indicates significance at 0.0042 level.

Treatment comparisons	Age (weeks post-hatch) 2	6
16000-8000	0.64 (0.5447)	-1.77 (0.1362)
16000-4000	0.35 (0.7353)	-3.73 (0.0135)
16000-2000	10.54 (0.0001)*	5.96 (0.0019)*
16000-1000	11.19 (0.0001)*	6.44 (0.0013)*
16000-500	11.10 (0.0001)*	----
8000-4000	-0.29 (0.7833)	-1.96 (0.1073)
8000-2000	9.90 (0.0001)*	7.74 (0.0006)*
8000-1000	10.54 (0.0001)*	8.21 (0.0004)*
8000-500	10.45 (0.0001)*	----
4000-2000	10.19 (0.0001)*	9.69 (0.0002)*
4000-1000	10.83 (0.0001)*	10.18 (0.0002)*
4000-500	10.74 (0.0001)*	----
2000-1000	0.64 (0.5430)	0.48 (0.6528)
2000-500	0.55 (0.5996)	----
1000-500	-0.09 (0.9308)	----

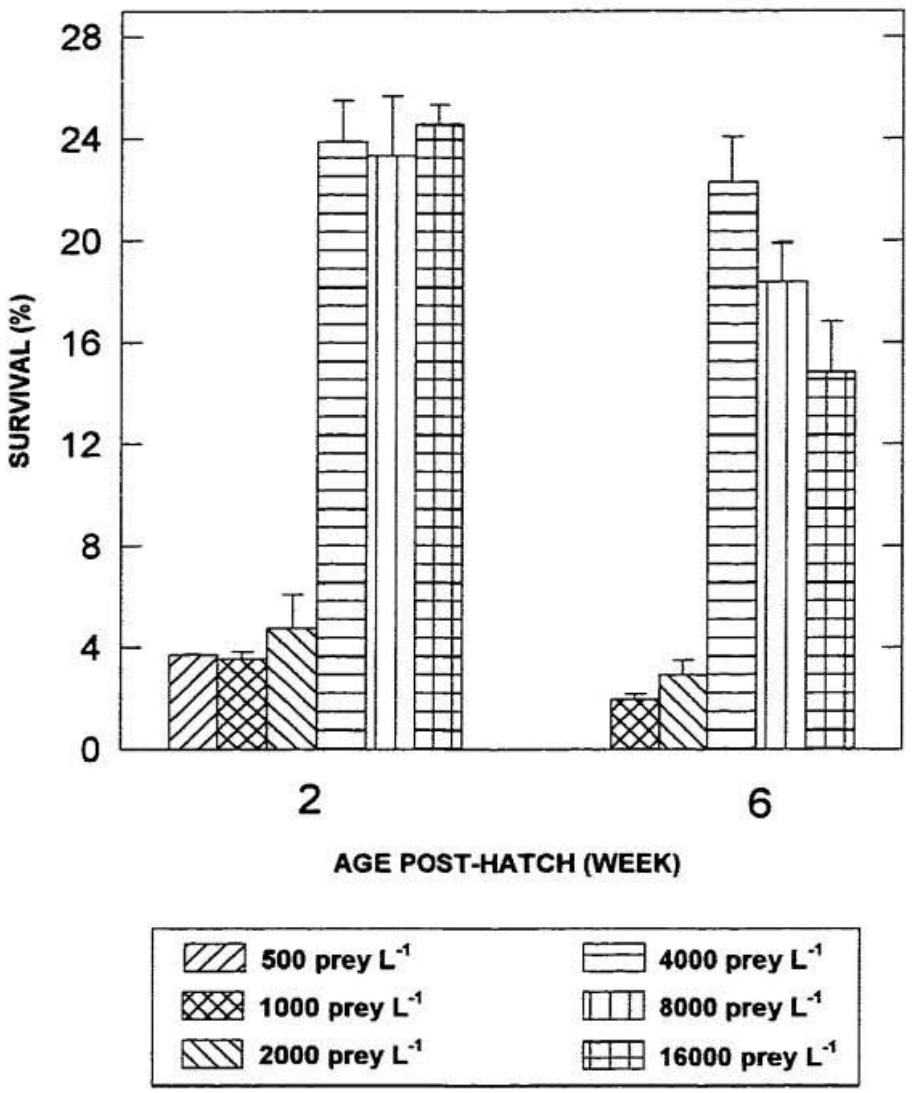

Fig 4.3: Percentage of Atlantic cod larvae surviving at week two and week six post-hatch at prey concentrations of 500-16000 prey L-1.

Table 4.6 Results of a two-way ANOVA (age and prey concentration) on swimming and foraging MAP's of larval cod at different prey concentrations. Significance at 0.05 level.

Variable	source	df	F-value	Pr. > F
Swim	Model	35	18.44	0.0001
	Error	1364		
	Age	5	79.20	0.0001
	Concentration	6	19.44	0.0001
	Age × Concentration	24	2.93	0.0001
Orient	Model	35	76.28	0.0001
	Error	1364		
	Age	5	209.0	0.0001
	Concentration	6	136.0	0.0001
	Age × Concentration	24	11.82	0.0001
Capture	Model	35	49.44	0.0001
	Error	1364		
	Age	5	175.34	0.0001
	Concentration	6	61.57	0.0001
	Age × Concentration	24	4.14	0.0001
Attack	Model	35	23.07	0.0001
	Error	1364		
	Age	5	74.2	0.0001
	Concentration	6	23.37	0.0001
	Age × Concentration	24	7.33	0.0006

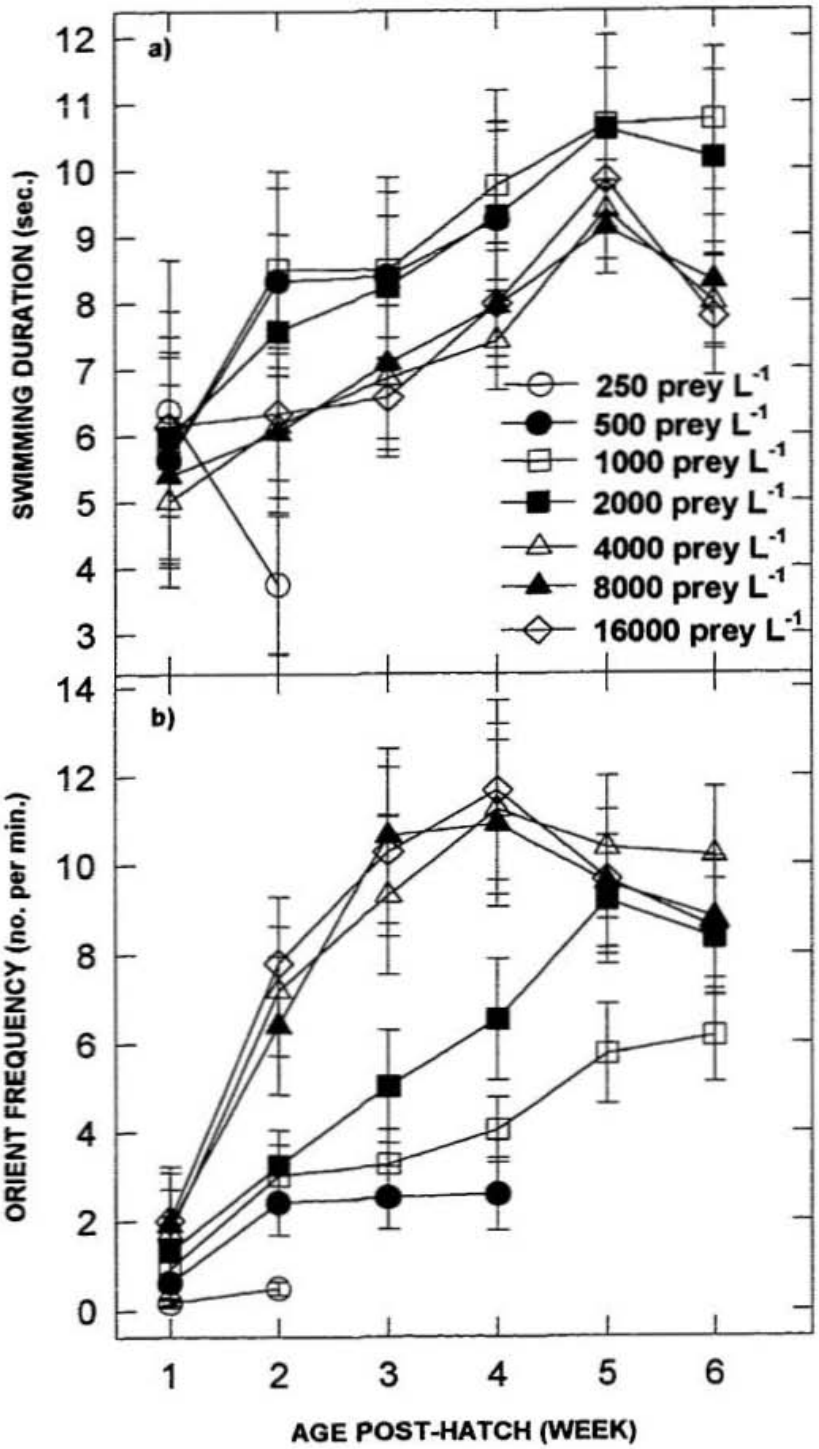

Fig 4.4. Change in mean (± se) a) swimming duration, and b) number of orient of Atlantic cod larvae reared at different prey concentrations. N =40 larvae per week.

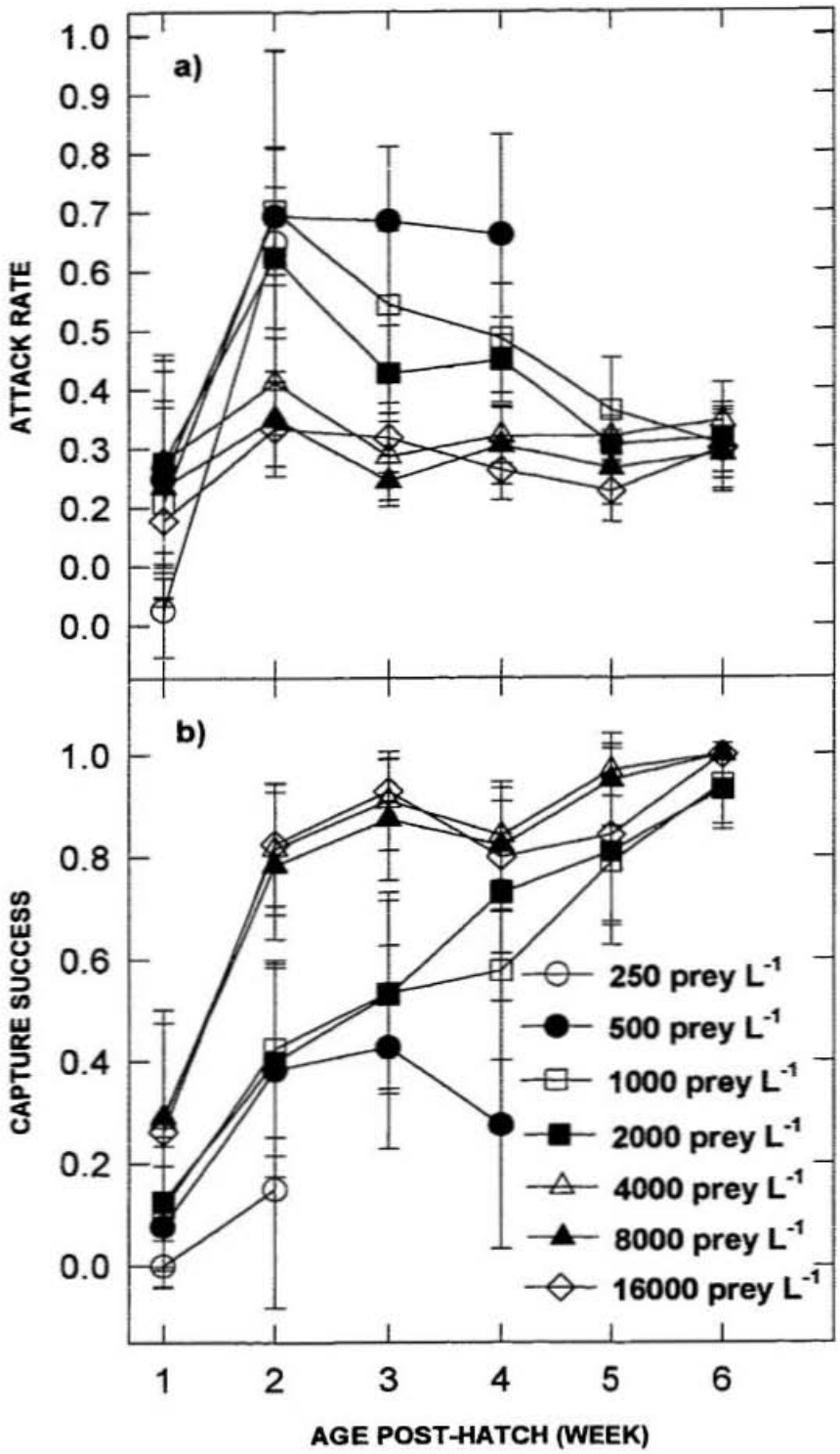

Fig 4.5. Change in mean (± se) a) attack rates, and b) capture success of Atlantic cod larvae reared at different prey concentrations. N =40 larvae per week.

Discussion

In the earlier study with larval cod (Chapter 3), over the range of the prey densities (0-4000 prey L^{-1}) used, I did not find the upper threshold of prey concentration which resulted in the best growth and survival. In that study, larval growth and survival increased with increasing prey concentration and no plateau was observed. In the present study, larval growth and survival increased from 250 to 4000 prey L^{-1} and did not significantly increase at 8000 and 16000 prey L^{-1}. Although there was no difference in growth among larvae reared at higher prey treatments, survival of the larvae was highest at 4000 prey L^{-1} at the end of the experiment. The prey concentration required for maximum growth and survival of larval cod fell within the range of prey concentrations I used. These results suggest the optimal prey concentration for rearing larval cod under intensive systems would be in the range of 4000 prey L^{-1} at a stocking density of 40 larvae L^{-1}.

Failure to survive beyond three weeks in low prey concentration at a relatively high stocking density (40 larvae L^{-1}), suggests the importance of adequate prey in the surrounding environment during first feeding. The failure to initiate sufficient feeding before the yolk is exhausted is one of the important factors causing mortalities in fish larvae (Blaxter 1986). In cod larvae, the yolk becomes exhausted between 10 -12 days post-hatch at 6°C (Ellertsen et al. 1981). In the 250 prey L^{-1} treatment, larvae survived only 11 days at 8°C suggesting that failure to initiate sufficient feeding could be a reason leading to mass mortality. Though small traces of food were observed in larval stomachs at low prey concentrations, my results suggest this was not sufficient to meet the energetic

demands of the larvae or to prevent larvae from starving. During my experiment, larvae reared in lower prey concentrations experienced high mortalities after yolk absorption. I suggest the failure to initiate sufficient first feeding may be due to insufficient prey surrounding the larvae in the 250 and 500 prey L^{-1} treatments. Buckley (1979), using biochemical indicators of growth (RNA-DNA ratios) to study the effect of prey density on growth of larval cod, found that larvae reared below 1000 prey L^{-1} showed all the symptoms of starvation while larvae reared at 1000 prey L^{-1} showed an increase in RNA and DNA content. My results also support this as larvae reared below 1000 prey L^{-1} did not survive to metamorphosis and likely died of starvation. Buckley did not include prey concentrations higher than 1000 prey L^{-1} in his study, but my results suggest the minimum threshold prey concentration for the survival of larval cod is 1000 prey L^{-1} and the optimum is 4000 prey L^{-1}.

The use of morphological indicators of condition has been criticized (Hay 1981, McGurk 1985), especially for preserved larval specimens. Preservation of larvae usually results in shrinkage and/or damage that complicates the usage of morphological condition indices. However, in my study, all measurements were made on live anaesthetized larvae. Koslow et al. (1985) used several morphological indices of wild caught cod larvae and found that the relationship between body height at the anus (myotome height) to length was the most sensitive to environmental conditions, including prey concentration. Using this relationship, my results showed that the condition of larval cod was significantly better for larvae reared at higher prey concentrations. The lower condition of larvae reared at

250-500 prey L^{-1} indicates the larvae experience a poor feeding situation and did not recover before all the larvae died of starvation. Similarly, Yin and Blaxter (1986) also found that for starving cod larvae, the body depth decreased in spite of an increase in body length.

My study showed that the growth rate of those larvae reared in lower prey concentration was much lower than the larvae reared in high prey concentrations. Similar results have been reported for other species (Houde 1978, Dowd and Houde 1980, Werner and Blaxter 1980, Welker et al. 1994). I found that larvae reared in 2000 prey L^{-1} showed lower growth rates at the start, but by week three, these larvae achieved growth rates similar to those larvae reared in 4000 prey L^{-1}. This result may be due to the fact that only a small fraction of larvae are successful in capturing a prey at first feeding in low prey concentrations and as they grow the surviving larvae may have similar success in capturing prey as the larvae in higher prey concentrations. A closer look at the foraging data confirms this as 40-50% of the larvae observed in the 2000 prey L^{-1} treatment failed to capture a prey successfully from day five to day 19 while only 0-10% of the larvae reared in 4000 prey L^{-1} failed to capture a prey successfully during the same period. Beyond day 19, larvae reared in 2000 and 4000 prey L^{-1} had a similar capture success. High prey concentrations are often required at the time of first feeding to achieve better growth and survival and late larval stages may grow better at lower prey concentration (Hunter 1972). Initially, the larvae have limited experience in accurately aiming at prey and morphological structures, such as the mouth, are not well developed (Blaxter 1986). As the larvae grow,

they get more experience in capturing prey and the associated muscle development sustains more swimming. This enables larvae to encounter and successfully capture more prey, even at low prey concentrations. Apart from this, the prey to predator ratio may also have contributed to the similar growth rates between 2000 and 4000 prey L^{-1} by week three. During the first two weeks of first feeding, larvae reared in 2000 prey L^{-1} had higher mortalities compared to larvae reared in higher prey concentrations. Due to this higher mortalities, the prey to predator ratio in 2000 prey L^{-1} had become comparable or even higher than in 4000 prey L^{-1}.

A small increase in the body size of the larvae may increase feeding performance to a large extent (Miller et al. 1992, Gill and Hart 1996). Gill and Hart (1996) in their studies with three-spined stickleback (*Gasterosteus aculeatus*) have shown that 7 and 12% increase in body size for a 40 mm fish significantly increased the number and size of the prey captured. They attributed the change in capture to an increase in jaw length (gape) and greater stomach capacity. In my study, larvae reared at 4000-16000 prey L^{-1} were 6.6-18.8% larger than the larvae reared at 1000 and 2000 prey L^{-1} at a given age. This difference in size provided an advantage for these larvae compared to larvae reared at lower prey concentrations and increased their capture success with a given prey size.

Larvae reared at low prey concentrations (500-2000 prey L^{-1}) swam more compared to larvae reared at the higher prey concentrations. This decreased swimming activity at higher prey concentrations may be due to an increased probability of encounter with the prey. Munk (1995) also found increased swimming activity with lower prey

concentrations in his experiments with cod larvae. In their experiment on the feeding behaviour and swimming of larval herring in relation to prey concentration, Munk and Kiørboe (1985) found herring larvae reared in lower prey levels displayed about a 100% higher swimming activity than larvae reared at higher prey concentrations. Higher swimming activity is energetically costly to fish larvae (Munk and Kiørboe 1986), such that larvae reared at high prey levels can invest more energy toward growth while larvae reared in low food levels may have to spend more energy in extended periods of swimming to find sufficient prey to survive. Therefore a trade-off between energy for swimming or growth, at lower prey levels could be an important factor governing growth and survival of larvae under these conditions.

It has been observed that, if larval fish do not successfully initiate and maintain feeding behaviour by a critical point in time (point of no return-PNR) after yolk absorption, then swimming, foraging and survival will be reduced (Laurence 1972, 1978, Blaxter 1980, Ellertsen et al. 1980). In my experiment, the larvae reared at 250 prey L^{-1} showed a remarkable reduction in swimming activity following the first week, suggesting that larvae reared at 250 prey L^{-1} were unable either to initiate or to maintain sufficient feeding. This reduction may be a strategy employed by fish larvae to conserve energy, perhaps delaying the time to irreversible starvation, or may be due to their weakened condition. Regardless, if they do not find enough food to sustain their normal activities and growth before they reach PNR, the larvae are subjected to starvation mortality.

All the foraging MAP's of larval cod varied with age and prey concentration. A

lower number of orientations, higher attack rates and lower capture success in larvae reared at lower prey concentrations (≤2000 prey L^{-1}) indicated that these larvae tried to attack any prey encountered. However larvae reared at higher prey concentrations (≥4000 prey L^{-1}) displayed lower attack rates but a higher capture success. This suggests that larvae reared at higher prey concentrations attack only the prey that could be successfully captured and/or are more profitable. Higher capture success at later stages in larvae reared at low prey concentrations show larvae gained more experience in aiming and capturing the prey. It could have been achieved by the larvae through better manoeuvring of prey, increased mouth gape and/or learning (Blaxter 1986, Drost 1987, Miller et al 1992) .

Mortality rates of cod larvae in my study were higher in lower prey concentrations while mortalities of larvae reared in higher prey concentrations were lower during the first three weeks. At low prey concentrations because prey had to be shared among more larvae during the first weeks of first feeding, larvae grow slowly which increased the length of the critical period. Buckley et al. (1987) studied the mortality of larvae of three marine fish species and found the mortality rates were higher at low prey concentrations and approached an asymptote as prey concentrations increased. Cushing and Horwood (1994) suggested that mortality in larval fishes through predation and other natural causes would be greater the longer it takes to achieve metamorphosis. They also showed that larvae at sub-optimal prey levels take longer to metamorphose (if at all), while larvae above the optimal prey levels pass the metamorphosis stage very early. In my study, cod larvae reared above 4000 prey L^{-1} metamorphosed at least one week earlier than larvae

reared at concentrations lower than 4000 prey L^{-1}.

Mortalities among the three high prey treatments were similar until I started to use *Artemia* nauplii as prey. Within a few days of adding *Artemia* nauplii, larvae reared at prey concentrations higher than 4000 prey L^{-1} showed increased mortalities, especially at 16000 prey L^{-1} treatments. I did not measure the nitrogen metabolites in the rearing tanks, yet several studies have found that excessive use of *Artemia* nauplii usually results in fouling stress in fish and shrimp larvae (Houde 1975, Gopalakrishnan 1976, Katavić 1986, Van der Wal and Nell 1986). Léger et al. (1986) in their review on the use of *Artemia* as a food source for larval shrimp and fish, cautioned that the excessive use of *Artemia* in rearing systems may cause fouling of the system and health hazards. Katavić (1986), noticed the involvement of *Artemia* on the mass mortality of sea bass (*Dicentrarchus labrax*) larvae following the switch from rotifers to *Artemia*.

Results of my experiment also showed that during the first 2 weeks post-hatch survival of larval cod ranged from 5-25% depends on the prey concentration. It implies that while 20% of larval mortality could be explained by prey concentration other 80% should be explained by something other than prey concentration. Similar results were reported for larval cod in mesocosm studies (Ellertsen et al. 1981, Blom et al. 1994) and in laboratory (Gotceitas et al. 1996) where larval mortalities reached 80-90% after 2 weeks post-hatch. Studies have shown that growth and survival larval fish also depends on the quality of the eggs (Chambers and Waiwood 1996). Quality of the eggs usually depends on the condition of the parents (especially female) during the spawning season

(Chambers and Waiwood 1996). Other environmental variables such as light can also affect the survival during the first feeding (Chapter 5). Results from the light intensity experiments (Chapter 5) showed that at 2400 lux light intensity about 55% of cod larvae reared (in prey concentration experiments light intensity was 1200 lux) survived after 2 weeks post-hatch. Thus it is possible that the remaining 80% of the mortality could be explained by the maternal effect on larval quality and other environmental variables such as light intensity.

The prey concentrations that have been most successful for rearing of larvae in intensive culture systems are usually greater than that found in nature (Goshorn and Epifanio 1991, Gotceites et al. 1996). In nature, larval densities are much lower than the densities of prey (Cushing 1983, Fossum and Ellertsen 1994, McLaren and Avendaño 1995) thus leading to a much higher prey-predator ratio than I used in my study. Some mesocosm studies have shown that cod larvae can be successfully reared at prey concentrations similar to those reported from nature (Kvenseth and Øiestad 1984, Blom et al. 1991, Oterrå 1993, van der Meeren and Næss 1993). Nevertheless, in those studies, the prey- predator ratio was higher than I used in my present study. Oterrå (1993) in his experiment on larval cod in large plastic enclosures found high survival during the first month but lower growth rates and increased mortality following week four. The author related this to the presence of insufficient food at relatively high larval stocking densities. Houde (1975) showed that prey to larval ratio play an important role in the growth and survival of sea bream (*Archosargus rhomboidalis*) larvae. Sea bream larvae reared at low

prey concentration produced significant survival only at low larval densities while high prey concentrations produced better growth and survival at low and high larval densities. Results from these studies and mine indicate that the combination of prey concentration and larval density could significantly influence the growth and survival of the cod larvae.

In conclusion, my results showed that the foraging, growth, condition and survival of larval cod is influenced by prey concentration. Above 1000 prey L^{-1}, larval cod feed sufficiently to sustain reasonable growth and survival. The optimal prey concentration for rearing larval cod was found to be 4000 prey L^{-1}. This optimal prey concentration provides the opportunity to forage efficiently and grow faster. This shortens the 'critical' larval period and increases their potential for survival. My results also suggest that prey concentration may be reduced for the later stages of larval cod. Economically, a reduction in the length of operation through fast growth and reduced use of live food would be beneficial for aquaculture practices.

Chapter Five: Foraging, growth and survival of Atlantic cod larvae at different light intensities and photoperiods: a laboratory evaluation.

Introduction

Studies have shown that most marine fish larvae are visual feeders (Blaxter and Staines 1970, Hunter 1981, Blaxter 1986). Blaxter and Staines (1970) reported a pure cone retina during first feeding in many fish species. Thus, first feeding larval fish require a "threshold" light intensity to initiate feeding (Blaxter 1986). For most marine fish larvae, this threshold has been suggested as 0.1 lux. Feeding incidence increases with increasing light intensities and the light intensity for efficient foraging varies with fish species (Blaxter 1986). During development, rods appear in the retina of the eye and the single cone retina gradually transforms into a duplex retina. Development of a duplex retina has been seen as an adaptation to changing habitats and light environments (Neave 1984).

Light plays an important role in the growth and survival of larval fish (Blaxter 1975, Batty 1987). Light can influence the behaviour of fish, through its variation in intensity, wavelength and polarization and diurnal and seasonal variation in photoperiod (Munz 1975, McFarland 1986). The response of larval fish to a particular characteristic of light is species specific. In the cichlid *Haplochromis burtoni* (Zeutzius and Rahmann 1984) and rainbow trout *Salmo gairdneri* (Rahmann et al. 1979), light deprivation in the early larval stage affects the normal development of the eye and reduces visual acuity. In contrast, halibut (*Hippoglossus hippoglossus*) yolk-sac larvae develop abnormally in the presence of light (Bolla and Holmefjord 1988). It has also been shown that larvae of the

same species from different populations exhibit differential responsiveness. My earlier study on larval cod and light showed a differential effect of light intensity on the growth and survival of two populations of Atlantic cod (*Gadus morhua*) larvae (Chapter 2).

Atlantic cod is an important commercial species and has been considered as a potential aquaculture species in the Atlantic region (Tilseth 1990). Its range extends from the arctic seas to temperate oceans (Scott and Scott 1988). Because of their wide geographic range, larvae from different regions may be exposed to different environmental conditions during their early life. Light conditions change with latitude in terms of intensity and photoperiod which may affect larval growth and survival (Suthers and Sundby 1996). Despite an impressive amount of research on the early life history of Atlantic cod larvae, no investigations have been done on the effects of light on growth and survival. To date studies have focused on the effect of light intensity either on the behaviour of starving Atlantic cod larvae (Skiftesvik 1994), on the growth of yolk-sac larvae (Solberg and Tilseth 1987), on the feeding incidence of the first feeding larval stage (Huse 1994) or on the differential responsiveness of larvae to varying light intensities from two populations (Chapter 3). Although these studies provided some information on the effect of light intensity on the feeding and behaviour of cod larvae, the effect of light intensity on growth and survival for an extended period has not been explored.

Studies on the effects of photoperiod on larval growth and survival of various species of fish larvae have produced mixed results. In a continuous light regime, rabbitfish (*Siganus guttatus*) larvae (Duray and Kohno 1988) and gilhead sea bream (*Sparus aurata*)

larvae (Tandler and Helps 1985) showed better growth and survival while sea bass (*Dicentrarchus labrax*) larvae (Barahona-Fernades 1979) showed a reduced growth and survival. Although some evidence exists from the field that cod larvae from higher latitudes exposed to continuous light during the summer show better feeding and growth than cod larvae from lower latitudes (Pedersen et al. 1989, Suthers and Sundby 1996), no studies has been done on the effects of photoperiod on cod larval growth and survival.

Preliminary experiments on larvae from the Northeast Grand Bank in Ocean Sciences Center (OSC), Memorial University of Newfoundland, showed that higher light intensity and extended photoperiods result in better growth and survival. I set up laboratory experiments to study the effects of light intensity and photoperiod on the foraging, growth and survival of larval cod from hatching to metamorphosis.

Material and methods

Fertilized eggs were collected from Northeastern Grand Bank broodstock maintained at the OSC. Eggs were incubated at 6-8°C in a 250 L circular tank with water flow and aeration. Light intensity in the incubation room was 300-400 lux. Dead eggs were siphoned out daily. When nearly 100% of the eggs had hatched, 1200 larvae were transferred to each of the experimental tanks and this was taken as day 0 of the experiment. Both light intensity and photoperiod experiments were conducted concurrently using larvae from the same batch.

Light intensity experiment

Experiments were carried out in a temperature-controlled room maintained at 8°C. Water temperature in the experimental tanks was measured daily in the morning. Four light intensities (300, 600, 1200 and 2400 lux) were chosen. Photoperiod was continuous. These light intensities and photoperiod were chosen based on the results from my preliminary experiments. The experimental tanks were 30 L rectangular glass aquaria (38 cm in depth) with two tanks per treatment. All sides of each aquarium were covered by opaque black plastic. Either100- or 150-watt incandescent bulbs were used to produce appropriate light intensity. Both type of bulbs produce a smooth continuous light spectrum ranging from 400-700 nm (General Electric (GE) company, 4400 Cox road, P.O. Box 4410, Glen Allen, VA, USA 23058-4410). Two 100-watt light bulbs were suspended over each of the tanks at different heights to produce 300 (60 cm from the surface water) and 600 lux (25 cm from the surface water) light intensity and two 150-watt incandescent bulbs were used to produce either 1200 lux (65 cm from the surface water) or 2400 lux (30 cm from surface water). Light intensity inside the tanks was measured in lux using a light meter (SPER Scientific light meter 840006) held just above the water surface.

Photoperiod experiment

Three photoperiods, 24L:0D (continuous light), 18L:6D (02.00-08.00 dark), and 12L:12D (20.00-08.00 dark), were chosen. Light intensity for this experiment was chosen as 1200 lux. Data for continuous light treatment were obtained from light intensity

experiments. Experiments were carried out either in a temperature-controlled room (continuous light) or in two water baths (for 18L:6D and 12L:12D), all maintained at 8°C. Otherwise, all experimental conditions and procedures were similar for both experiments.

For both experiments, each experimental tank was supplied with filtered (1 μm using particle filters), UV-sterilized sea water at a rate of six L hr^{-1}. Green algae (*Isochrysis* sp) were added to the tanks (Tucker 1992). Enriched rotifers (*Brachionus plicatilis*) were used as prey from three to 19 days post-hatch (dph). A mixture of rotifers and *Artemia franciscana* nauplii (1:1) were used as prey beyond day 20. Experimental prey concentration (4000 prey L^{-1}) was chosen from previous studies conducted in our laboratory (Gotceites et al. 1996, Chapter 3 and 4) and was comparable to those used in other studies where larval cod were reared under laboratory conditions (Laurence et al. 1981). Larvae were fed three times per day (09.30, 13.30, and 21.30). To maintain the desired prey concentration within each experimental tank, a 10-20 ml water aliquot was sampled from each aquarium at different depths (just below surface, mid water columns, and just above bottom) before each feeding. The number of prey items in each sample was counted and prey concentrations were adjusted as needed. Each experimental aquarium was aerated which ensured a homogenous distribution of prey within each aquarium.

The experiment was stopped at 42 days post hatch (dph) when most of the larvae (90-100%) in the high light intensity treatment and at least some larvae from the low light treatments (20-30%) were past metamorphosis. Metamorphosis was defined in this study as when the continuous fin fold disappeared and discrete fins were formed.

Data collection

Initially, 10 larvae were sampled from the egg incubation tank on day 0 (week 0). Subsequent samples were taken once a week (7,14,...42 dph) and five larvae from each tank (10 per treatment) were arbitrarily chosen for morphometric measurements over the duration of the experiment. Using a dissecting microscope, standard length (mm), head depth (measured posterior to the eye), eye diameter (along the body axis), and myotome height (posterior to the anus) were measured.

Condition of the larvae was calculated using a relationship between two morphometric measurements, the standard length (mm) and myotome height (mm) (Koslow et al. 1985).

$$\text{Condition index} = \text{myotome height} / \text{standard length} \qquad (1)$$

Weight-specific growth rates (SGR) of larvae were determined using the following relationship:

$$SGR = (\ln(L_t) - \ln(L_0)) / t) \times 100 \qquad (2)$$

Where L_t is the mean final dry weight (mg), L_0 is the mean initial dry weight (mg), and t is the duration between initial and final sampling (days) (Buckley et al. 1987, Cowan and Houde 1990). For both the light intensity and photoperiod experiments, SGR was calculated for the time intervals of 0 to 28 and 29 to 42 dph. Analysis of mortality data showed a decreasing trend in mortality rate as the larvae grew and significant differences in mortality rates between the treatments disappeared after 28 dph. Thus I was interested to determine if any similar trends were shown in the SGR values before and after 28 dph.

Mortalities were removed two or three times a day from each experimental tank from day 15. Instantaneous mortality rates were calculated from following model:

$$Z = (\ln(N_t) - \ln(N_0)) / t) \times 100 \quad (3)$$

Where Z = Instantaneous mortality rate (d^{-1}), N_t is the number of larvae alive at time t, N_o is the number of larvae alive at time 0 and t is the duration in days (Cowan and Houde 1990). At the end of the experiment, the number of surviving larvae in each treatment was recorded.

Behavioural observations were done only for the light intensity experiment and conducted twice a week from 2 dph until 41 dph. The focal animal technique (Altman 1974) was used to observe an arbitrarily selected larva for a one minute interval. This was done for a total of five larvae from each tank (10 per treatment). During each observation period, I recorded the occurrence of six Modal Action Patterns - MAP's; swim, motionless, orient, capture success, miss, and pass (Barlow 1977, also see Chapter 3). Occurrence of any of the six MAP's was recorded using an event recorder.

Data analysis

The effect of light intensity or photoperiod and age on standard length, condition index, survival, instantaneous mortality rate (Z) and SGR were analysed by two-way analysis of variance ($p \leq 0.05$). When data were not normal, appropriate transformations were performed to satisfy the normality requirements of ANOVA. The Tukey test was used for subsequent comparison among different prey concentrations for each week.

The effect of light intensity and age on swimming and all foraging variables were analysed by two-way ANOVA (SAS 1988; $p \leq 0.05$). None of the foraging variables were found normal and were thus rank transformed. When significant results were obtained, Tukey's studentized (HST) test was used to determine which means differed.

Results

Light intensity experiment

Effects of light intensity and age on standard length of cod larvae were significant (Table 5.1). Larvae reared in low light intensities (300 and 600 lux) were significantly smaller than the larvae reared in 1200 and 2400 lux (Fig. 5.1a). No significant difference was found between the treatments for larval standard length until day 21 between the two lower (300 and 600 lux) and the two higher (1200 and 2400 lux) light intensity treatments (Table 5.2). Except for 35 dph, no significant difference was found in larval standard length between the 1200 and 2400 light intensity treatments. Likewise, no significant difference was found in standard length of cod larvae reared in 300 and 600 lux treatments except for 7 dph. From day 21 to the end of the experiment, larvae reared in 1200 and 2400 lux light intensities were significantly larger than the larvae reared in lower light intensities (Table 5.2). Light intensity and age also had significant effects on the dry weight of larval cod (Fig. 5.2a and Table 5.1, 5.3).

A comparison of the SGR values for larval cod reared in four light intensity treatments at two intervals, 0-28 and 29-42 dph, revealed significant differences in SGR

values among the treatments at 0-28 dph interval (Fig. 5.3a and Tables 5.1, 5.4). Larvae reared at 2400 lux had significantly higher SGR values than larvae from 600 and 300 lux. Larvae reared at 1200 lux had significantly higher SGR values than larvae reared at 300 lux, but at the 29-42 dph interval, there was no significant difference was found in SGR values between any treatments.

Light intensity and age had a significant effect on the condition of the cod larvae (Table 5.1). Initially, condition of the cod larvae decreased but from 7 dph condition increased regardless of treatments (Fig. 5.4a). From 28 dph onwards larvae reared in 2400 lux had significantly higher condition index than larvae reared in other light treatments (Table 5.5). No significant difference was found between the condition of the larvae reared at 300 and 600 lux throughout the experiment.

Instantaneous mortality rates (Z) were significantly influenced by light intensity and age (Table 5.1). Z was significantly higher in lower light intensities (300 and 600 lux) at 14 dph compared to the two higher light intensities (Table 5.6 and Fig. 5.5a). Beyond 28 dph, except for the 300 lux treatment on 35 dph, no significant difference was found in Z among the treatments. Light intensity had a significant effect on the survival of larval cod (Table 5.1). A significantly higher percentage of larvae survived at 2400 lux than at 300-1200 lux throughout the experiment. Survival of larvae among lower light intensities (300 & 600 lux) were not significantly different throughout the experiment (Fig. 5.6a & Table 5.7).

Light intensity had a significant influence on swimming duration, orientation

frequency and capture success of larval cod (Table 5.8). In general, larvae reared in 1200 and 2400 lux spent more time in swimming than larvae reared in the two lower (300 & 600 lux) light intensities (Fig. 5.7). In most comparisons of swimming duration among the treatments, no significant differences were found from 2 dph to 27 dph. Thereafter, except for 34 dph, larvae reared at the two higher light intensities swam significantly more than larvae reared at 300 & 600 lux treatments (Table 5.9). Larvae reared at 2400 lux oriented to more prey than larvae from other treatments (Fig. 5.8a). Initially prey encounter was not significantly different among treatments, but from 16 dph larvae reared in 2400 lux had significantly higher prey encounters than larvae reared in 300 and 600 lux (Table 5.10).Increase in light intensity resulted in an increase in prey capture. In general, prey capture increased with age regardless of light intensity (Fig. 5.8b). Throughout the experiment, except for 6 dph and 34 dph for 600 lux, larvae reared in 2400 lux light intensity showed significantly higher prey capture than larvae from the two lower (300 & 600 lux) light intensities (Table 5.10). Although there was a significant difference in prey capture between the larvae reared in 2400 and 1200 lux light intensities, it disappeared after 34 dph. Except at 37 dph, prey capture was not significantly different among the two lowest (300 and 600 lux) light intensities throughout the experiment.

Light intensity ($F=12.81$, $df=3$, $p<0.001$) had a significant effect on the gut fullness index of larval cod. Larvae reared in 2400 lux had a significantly higher gut fullness index than larvae reared in 300 lux at 14, 21 and 28 dph (Table 5.11 and Fig. 5.9a). There was no significant difference found in gut fullness index of larval cod among the treatments

beyond 28 dph.

Photoperiod experiment

Photoperiod had a significant effect on the standard length, dry weight, SGR, condition index, instantaneous mortality rate and survival of larval Atlantic cod (Table 5.12). Throughout the experiment, larvae reared in continuous light were larger and heavier than the larvae reared in the other two photoperiods (Fig. 5.1b & 5.2b). From 7 dph, there was a significant difference on the standard length and dry weight of cod larvae reared in continuous light and in 18 and 12 hour photoperiod. Larvae reared in 18L:6D photoperiod were significantly larger than larvae reared in 12L:12D photoperiod from 7 dph (Tables 5.12 & 5.3).

Comparison of the SGR values for larval cod reared in the three treatments revealed a significant difference in SGR values during the time interval of 0 to 28 dph (Table 5.4). Larvae reared at 12 hour photoperiod had the lowest SGR values and SGR values increased with increasing photoperiod (Fig. 3b). But when the SGR values were calculated for 29 to 42 dph, no significant difference was found in SGR values among the 3 treatments (Table 5.4). Larvae reared in continuous light were in better condition than the larvae reared in the other treatments (Fig. 5.4b). Initially, there was no significant difference in the condition index between the larvae reared in continuous and 18 hour photoperiods but beyond 21 dph larvae reared under continuous light had significantly better condition (Table 5.5).

Instantaneous mortality rate (Z) showed a decreasing trend from 14 to 42 dph regardless of photoperiod except for 18 hour photoperiod. In general, larvae reared under12 hour photoperiod had the highest mortality rate followed by the 18 and 24 hour photoperiod treatments (Fig. 5.5b). When comparing the mortality rates, larvae reared in 24 hour photoperiod had significantly lower mortality rates than larvae reared in the 12 and 18 hour photoperiod until 28 dph. Beyond 28 dph there was no significant difference in the mortality rates of larval cod among the treatments (Table 5.6). The 24 hour photoperiod larvae had significantly higher survival than the 12 and 18 hour photoperiod treatments throughout the experiment while the 18 hour photoperiod larvae had significantly higher survival than the 12 hour photoperiod larvae (Table 5.7 and Fig. 5.6b). Comparison of the gut fullness index of larval cod reared in the 3 photoperiod treatments revealed no significant difference found except for 14 dph (Table 5.11 & Fig. 5.9b).

Table 5.1 Results of a two-way ANOVA (age and light intensity) on standard length, dry weight, SGR, condition, mortality rate (Z) and survival of larval cod at different light intensities. Significance at 0.05 level.

Variable	source	df	F-value	Pr. > F
Standard length	Model	23	192.02	0.0001
	Error	216		
	Age	5	825.46	0.0001
	Light	3	64.82	0.0001
	Age × Light	15	6.31	0.0001
Dry weight	Model	23	190.93	0.0001
	Error	216		
	Age	5	832.50	0.0001
	Light	3	57.07	0.0001
	Age × Light	15	3.85	0.0001
SGR	Model	7	10.07	0.0019
	Error	8		
	Age	1	53.72	0.0001
	Light	3	5.30	0.0264
	Age × Light	3	0.75	0.5511
Condition	Model	23	102.34	0.0001
	Error	216		
	Age	5	413.36	0.0001
	Light	3	56.77	0.0001
	Age × Light	15	7.79	0.0001
Mortality rate	Model	19	32.45	0.0001
	Error	20		
	Age	4	100.35	0.0001
	Light	3	49.61	0.0001
	Age × Light	12	5.52	0.0004
Survival	Model	19	36.08	0.0001
	Error	20		
	Age	4	12.85	0.0001
	Light	3	210.86	0.0001
	Age × Light	12	0.12	0.9997

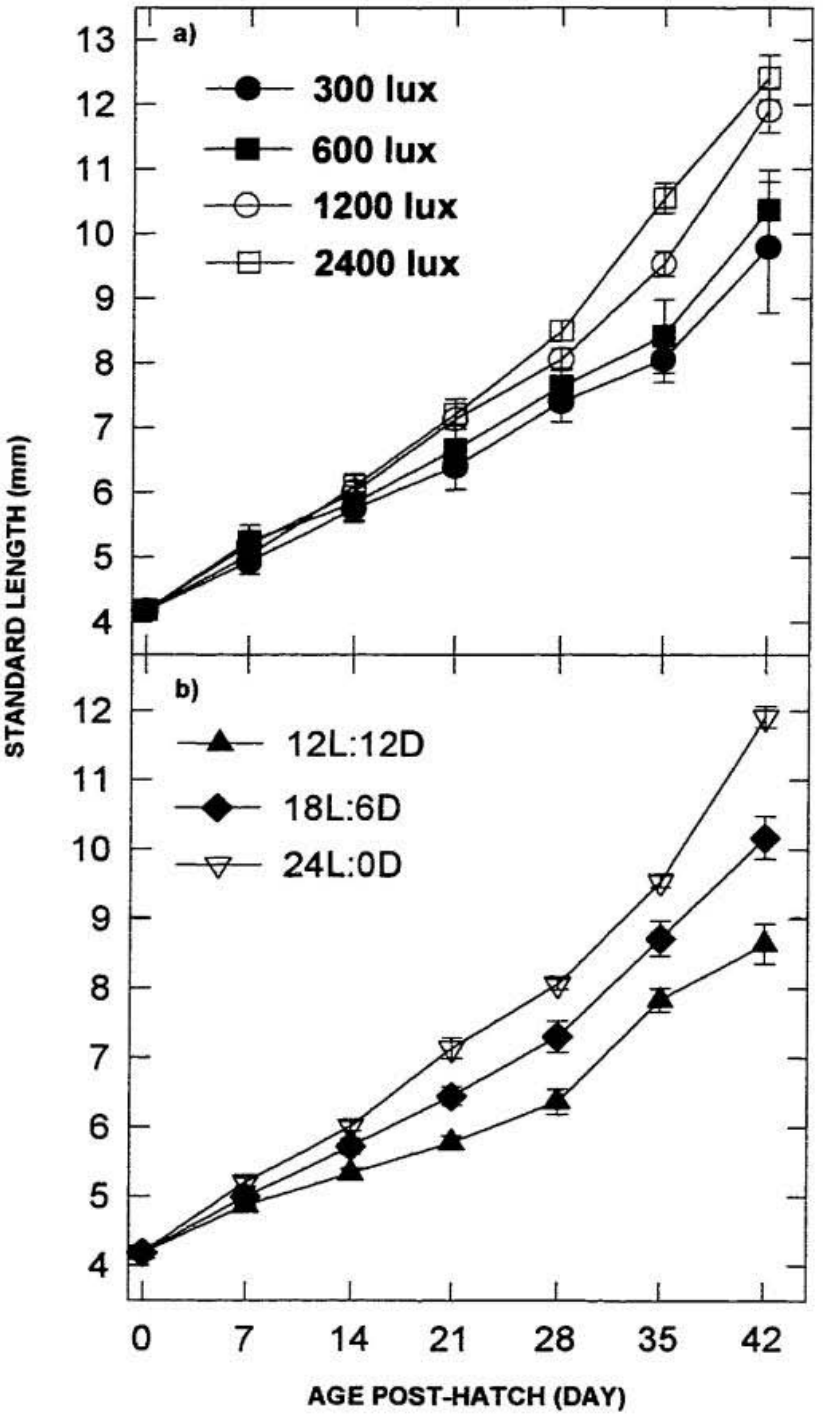

Fig. 5.1. Mean (± se) standard length of cod larvae from hatch to 42 dph reared in different a) light intensities and, b) photoperiods. N=10 larvae per sample per treatment.

Table 5.2 Results of the Tukey analysis comparison examining the mean standard length (mm) of cod larvae reared under different a) light intensities or b) photoperiods from 7 to 42 dph. Values are the differences in mean standard length between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 7	14	21	28	35	42
a) Light intensity						
2400-1200	-0.18	0.08	0.08	0.44	1.01*	0.51
2400-600	-0.22	0.25	0.55*	0.85*	2.13*	2.04*
2400-300	0.09	0.34	0.82*	1.09*	2.49*	2.62*
1200-600	-0.05	0.17	0.46	0.41*	1.12*	1.53*
1200-300	0.27	0.26	0.74*	0.66*	1.48*	2.11*
600-300	0.32*	0.09	0.27	0.25	0.36	0.58
b) Photoperiod						
24L-18L	0.21	0.29*	0.69*	0.75*	0.82*	1.74*
24L-12L	0.34*	0.69*	1.36*	1.69*	1.71*	3.26*
18L-12L	0.12	0.39*	0.67*	0.94*	0.89*	1.52*

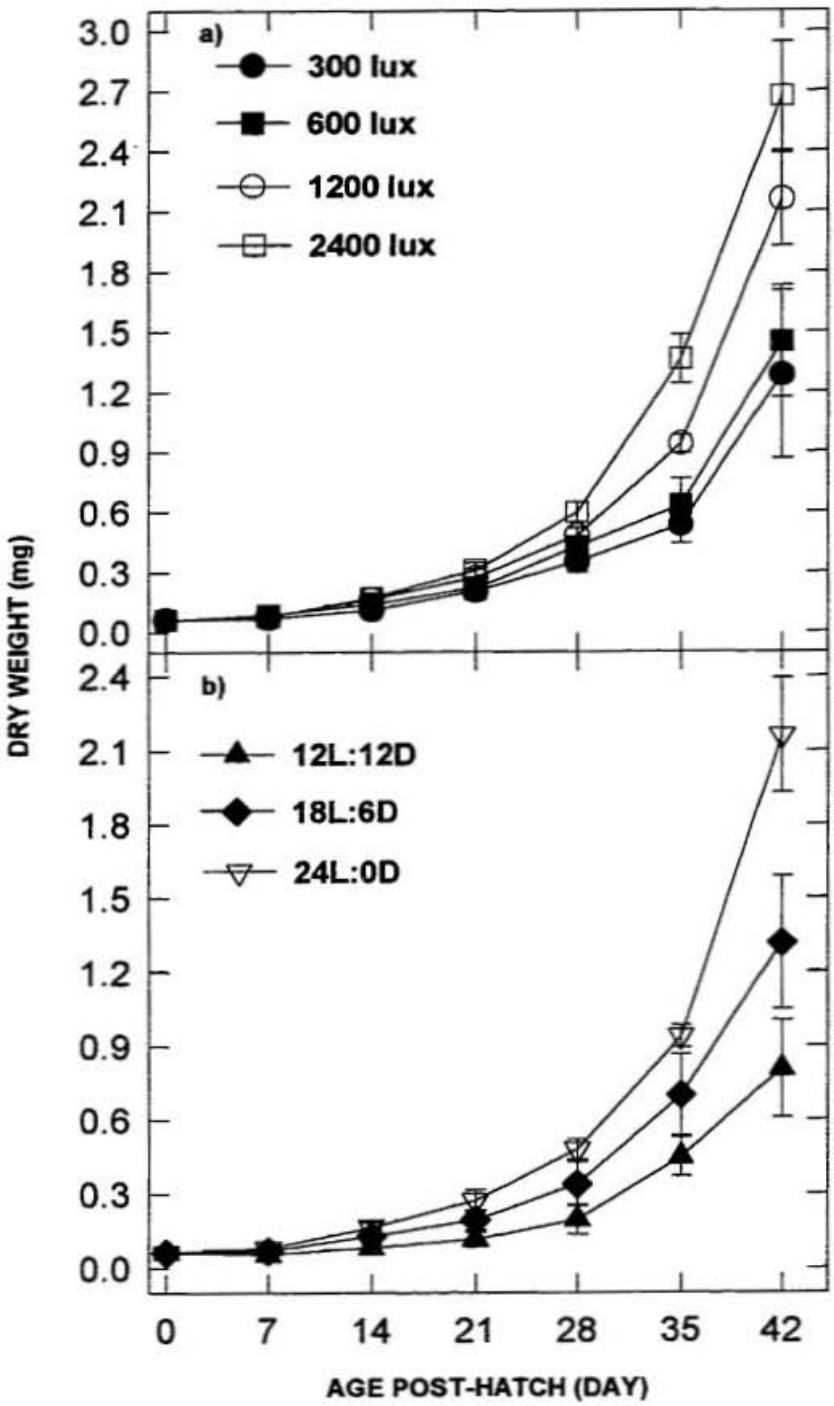

Fig. 5.2. Mean (± se) dry weight of cod larvae from hatch to 42 dph reared in different a) light intensities and, b) photoperiods. N=10 larvae per sample per treatment.

Table 5.3 Results of the Tukey analysis comparison examining the mean dry weight (mg) of cod larvae reared under different a) light intensities or b) photoperiods from 7 to 42 dph. Values are the differences in mean dry weight between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 7	14	21	28	35	42
a) Light intensity						
2400-1200	-0.002	0.008	0.035	0.117	0.425*	0.515
2400-600	-0.008	0.031	0.091*	0.171*	0.731*	1.225*
2400-300	0.008	0.062*	0.104*	0.246*	0.828*	1.390*
1200-600	-0.006	0.023	0.055	0.054	0.306*	0.710*
1200-300	0.011	0.054*	0.069*	0.129*	0.403*	0.875*
600-300	0.016	0.031	0.013	0.075	0.097	0.165
b) Photoperiod						
24L-18L	0.0094	0.0344*	0.0784*	0.1405*	0.2374*	0.8410*
24L-12L	0.0242*	0.0823*	0.1585*	0.2839*	0.4883*	1.3531*
18L-12L	0.0148	0.0480*	0.0801*	0.1433*	0.2509*	0.5122*

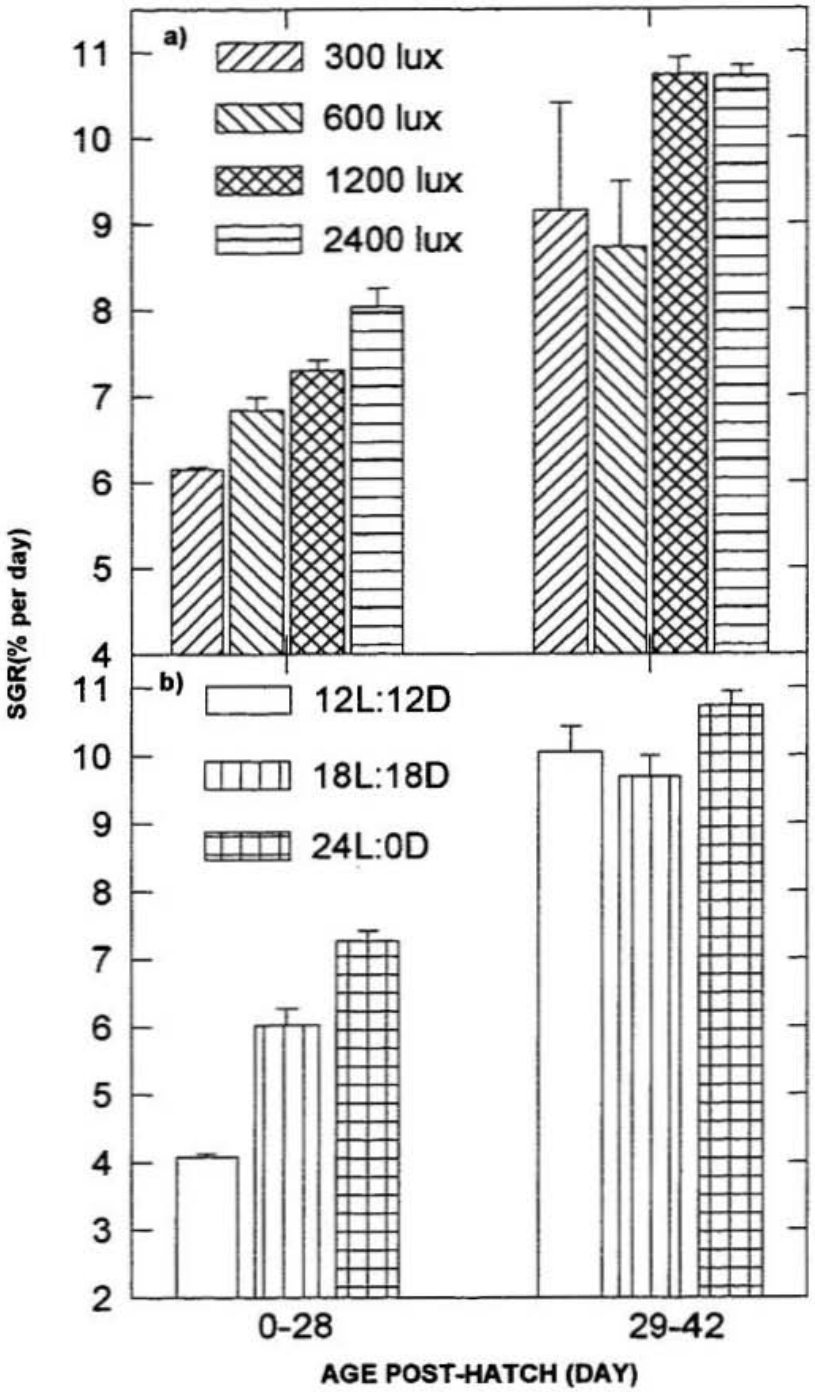

Fig. 5.3. Mean (± se) weight-specific growth rate (SGR) of cod larvae for 0-28 and 29-42 dph reared in different a) light intensities and, b) photoperiods. N=10 larvae per sample per treatment.

Table 5.4 Results of the Tukey analysis comparison examining the mean SGR (% per day) of cod larvae reared under different a) light intensities or b) photoperiods from 0 to 28 dph and 29 to 42 dph. Values are the differences in mean SGR between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 0-28	29-42
a) Light intensity		
2400-1200	0.74	-0.025
2400-600	1.201*	1.979
2400-300	1.887*	1.551
1200-600	0.461	2.004
1200-300	1.147*	1.576
600-300	0.686	-0.428
b) Photoperiod		
24L-18L	1.238*	0.685
24L-12L	3.183*	1.048
18L-12L	1.945*	0.363

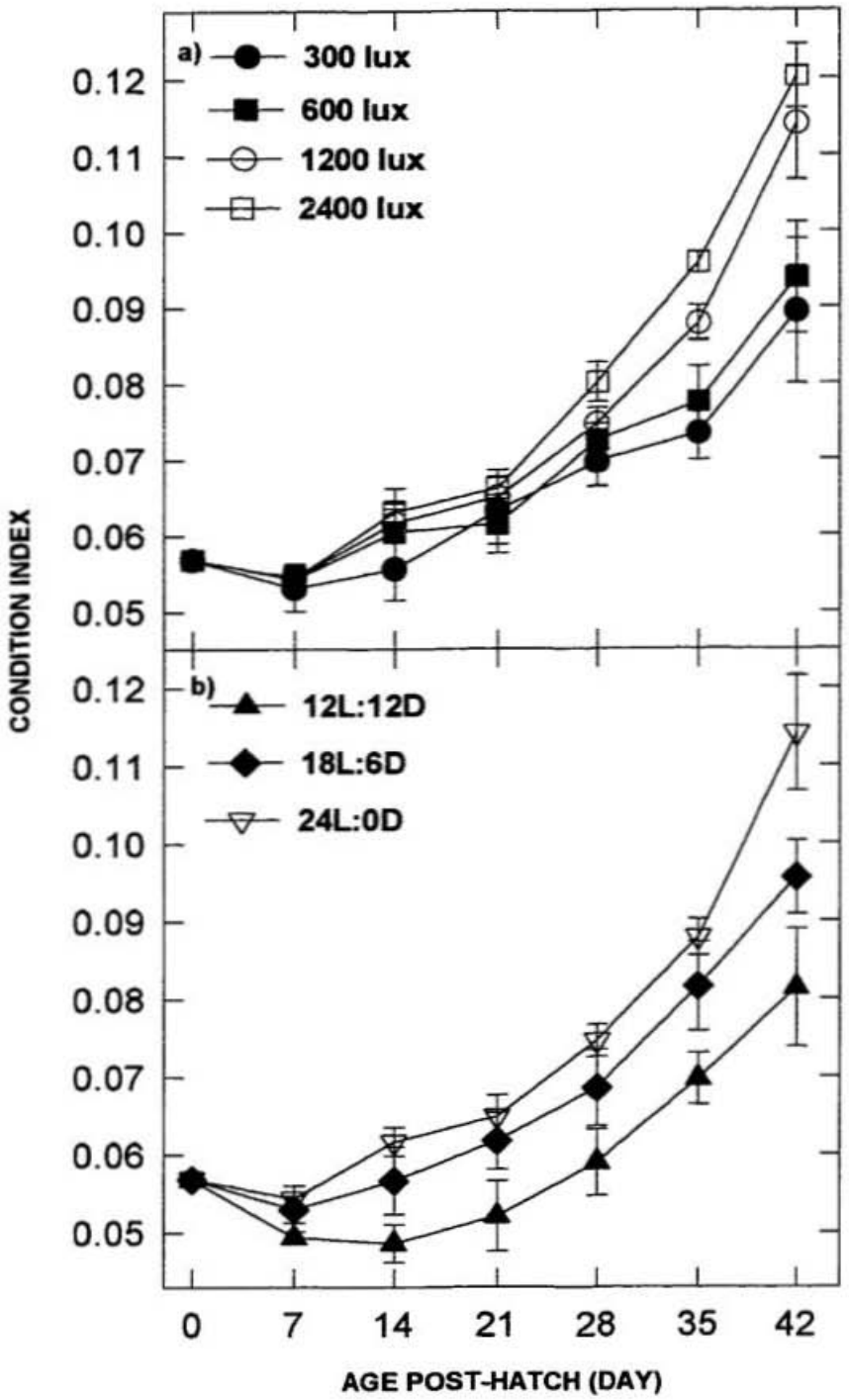

Fig. 5.4. Mean (± se) condition index of cod larvae reared in different a) light intensities and, b) photoperiods. N=10 larvae per sample per treatment.

Table 5.5 Results of the Tukey analysis comparison examining the mean condition index (ratio of myotome height and standard length) of cod larvae reared under different a) light intensities or b) photoperiods from 7 to 42 dph. Values are differences in mean condition index between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 7	14	21	28	35	42
a) Light intensity						
2400-1200	-0.0000	0.0014	0.0012	0.0054*	0.0080*	0.0062
2400-600	0.0001	0.0027	0.0047	0.0077*	0.0184*	0.0265*
2400-300	0.0013	0.0075*	0.0030	0.0105*	0.0225*	0.0309*
1200-600	0.0001	0.0013	0.0035	0.0023	0.0104*	0.0203*
1200-300	0.0014	0.0061*	0.0017	0.0051*	0.0145*	0.0247*
600-300	0.0013	0.0048	-0.0018	0.0028	0.0041	0.0044
b) Photoperiod						
24L-18L	0.0014	0.0050	0.0032	0.0061*	0.0063*	0.0185*
24L-12L	0.0050*	0.0130*	0.0129*	0.0156*	0.0182*	0.0327*
18L-12L	0.0036*	0.0081*	0.0097*	0.0095*	0.0119*	0.0142*

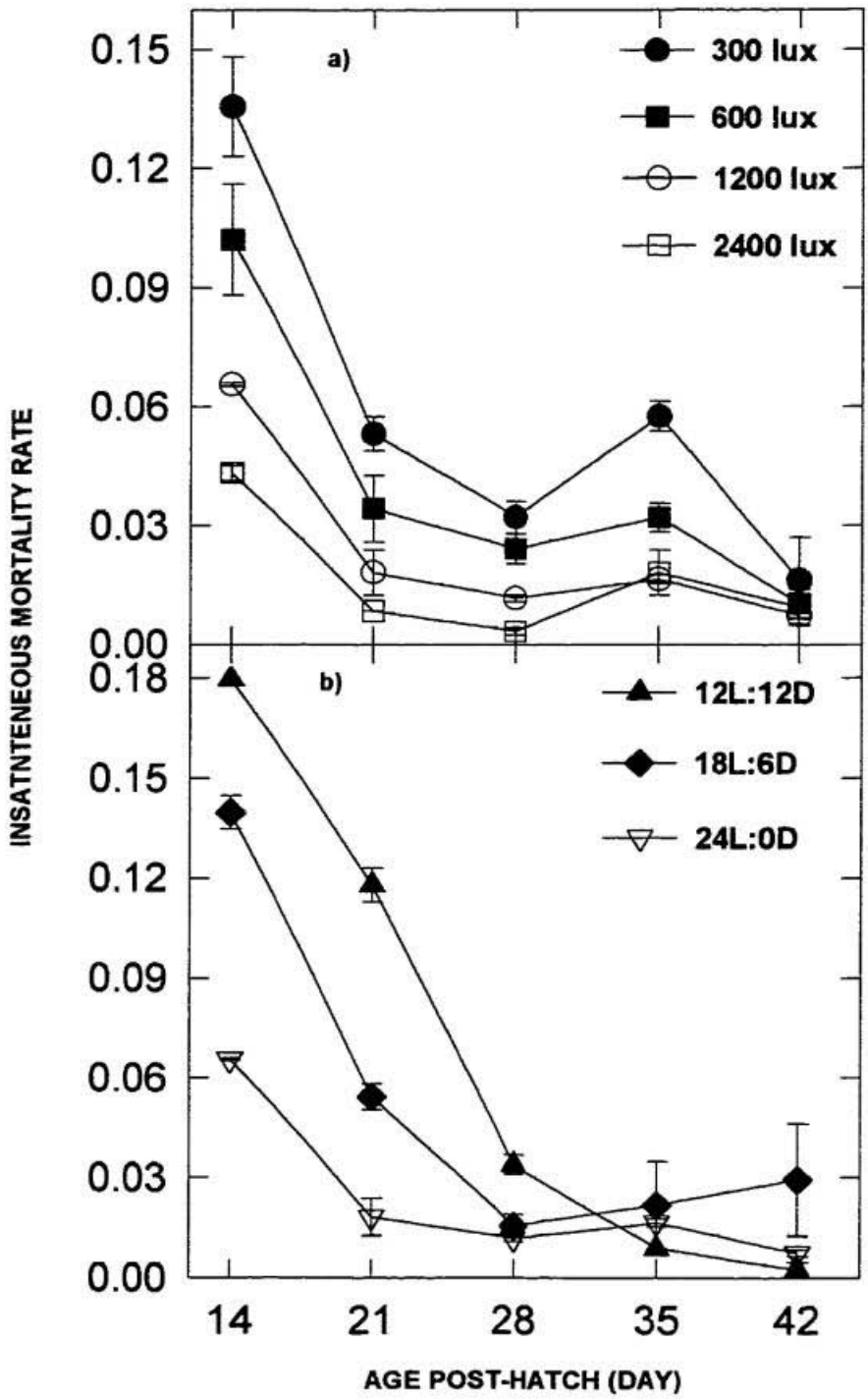

Fig. 5.5. Mean (± se) instanteneous mortality rate of cod larvae from 14 to 42 dph reared in different a) light intensities and, b) photoperiods.

Table 5.6 Results of the Tukey analysis comparison examining the mean instantaneous mortality rate (Z) of cod larvae reared in different a) light intensities or b) photoperiods from 14 to 42 dph. Values are the differences in mean Z between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 14	21	28	35	42
a) Light intensity					
2400-1200	-0.0223	-0.0097	-0.0084	0.00175	0.00195
2400-600	-0.0588*	-0.0258	-0.0209*	-0.0138	-0.00105
2400-300	-0.0923*	-0.0447*	-0.0288*	-0.0393*	-0.00685
1200-600	-0.0365	-0.0161	-0.0125	-0.01555	-0.003
1200-300	-0.07*	-0.035*	-0.0204*	-0.04105*	-0.0088
600-300	-0.0335	-0.0189	-0.0079	-0.0255*	-0.0058
b) Photoperiod					
24L-18L	-0.0741*	-0.0362*	-0.0038	-0.0053	-0.0219
24L-12L	-0.1140*	-0.0998*	-0.0222*	0.0077	0.0051
18L-12L	-0.0399*	-0.0636*	-0.0184*	0.0130	0.0271

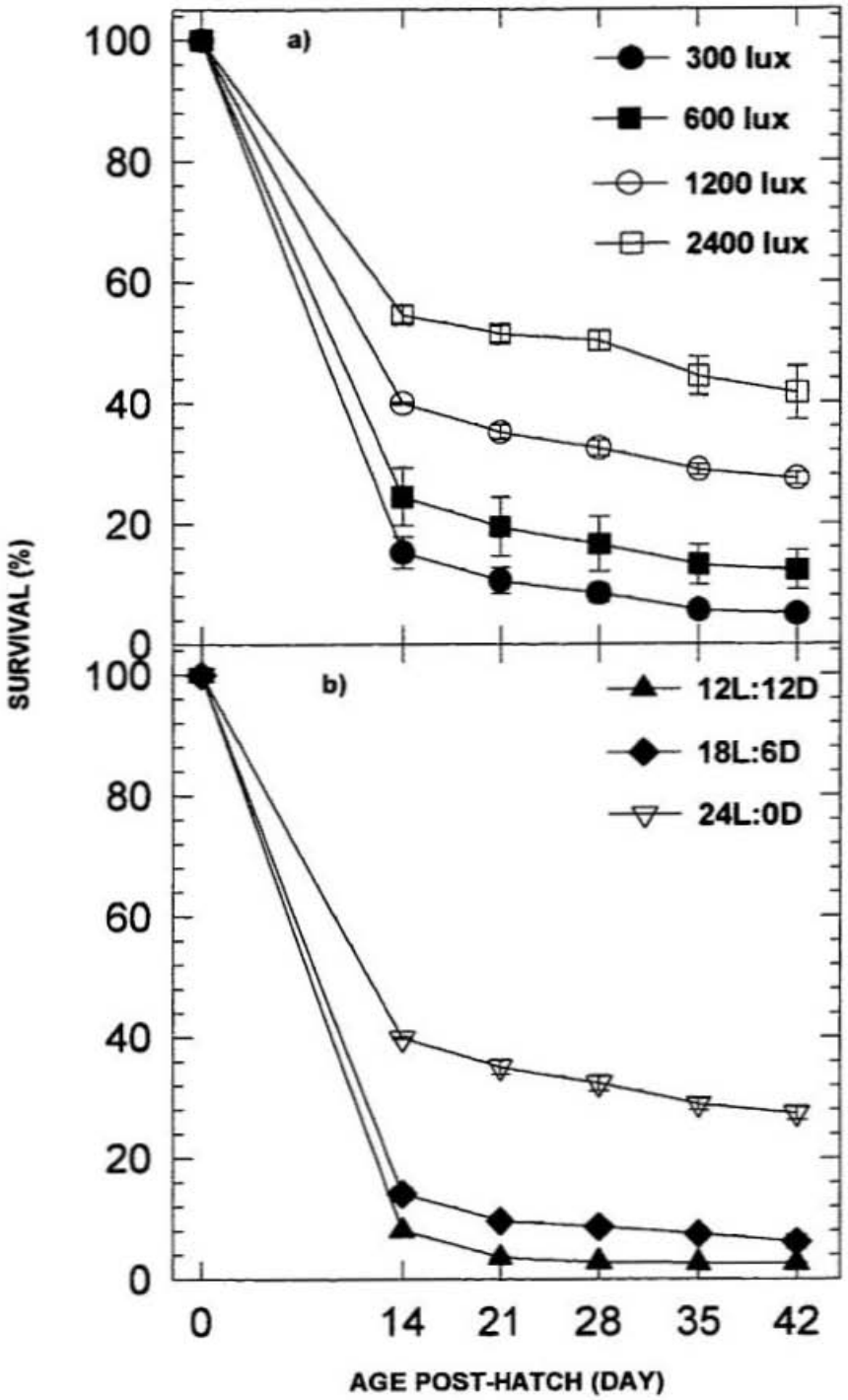

Fig. 5.6. Mean (± se) survival of cod larvae reared in different a) light intensities and, b) photoperiods.

Table 5.7. Results of the Tukey analysis comparison examining the mean survival (%) of cod larvae reared under different a) light intensities or b) photoperiods from 14 to 42 dph. Values are the differences in mean survival between two treatment comparisons.

* - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 14	21	28	35	42
a) Light intensity					
2400-1200	14.67*	16.25*	17.83*	15.46*	14.21
2400-600	30.12*	31.92*	33.67*	31.17*	29.34*
2400-300	39.34*	40.83*	41.83*	38.67*	36.63*
1200-600	15.46	15.67	15.83*	15.71*	15.13
1200-300	24.67*	24.58*	24.0*	23.21*	22.42*
600-300	9.22	8.91	8.17	7.5	7.3
b) Photoperiod					
24L-18L	25.71*	25.46*	23.71*	21.38*	21.21*
24L-12L	31.8*	31.58*	29.59*	26.21*	24.79*
18L-12L	6.09*	6.12*	5.88*	4.84*	3.58

Table 5.8 Results of a two-way ANOVA (age and light intensity) on swimming and foraging MAP's of larval cod at different prey concentrations. Significance at 0.05 level.

Variable	source	df	F-value	Pr. > F
Swim	Model	47	8.55	0.0001
	Error	432		
	Age	11	17.82	0.0001
	Light	3	33.85	0.0001
	Age × Light	33	3.16	0.0001
Orient	Model	43	9.30	0.0001
	Error	396		
	Age	10	15.76	0.0001
	Light	3	49.24	0.0001
	Age × Light	30	3.15	0.0001
Capture success	Model	43	10.45	0.0001
	Error	396		
	Age	10	29.05	0.0001
	Light	3	8.49	0.0001
	Age × Light	30	3.12	0.0001

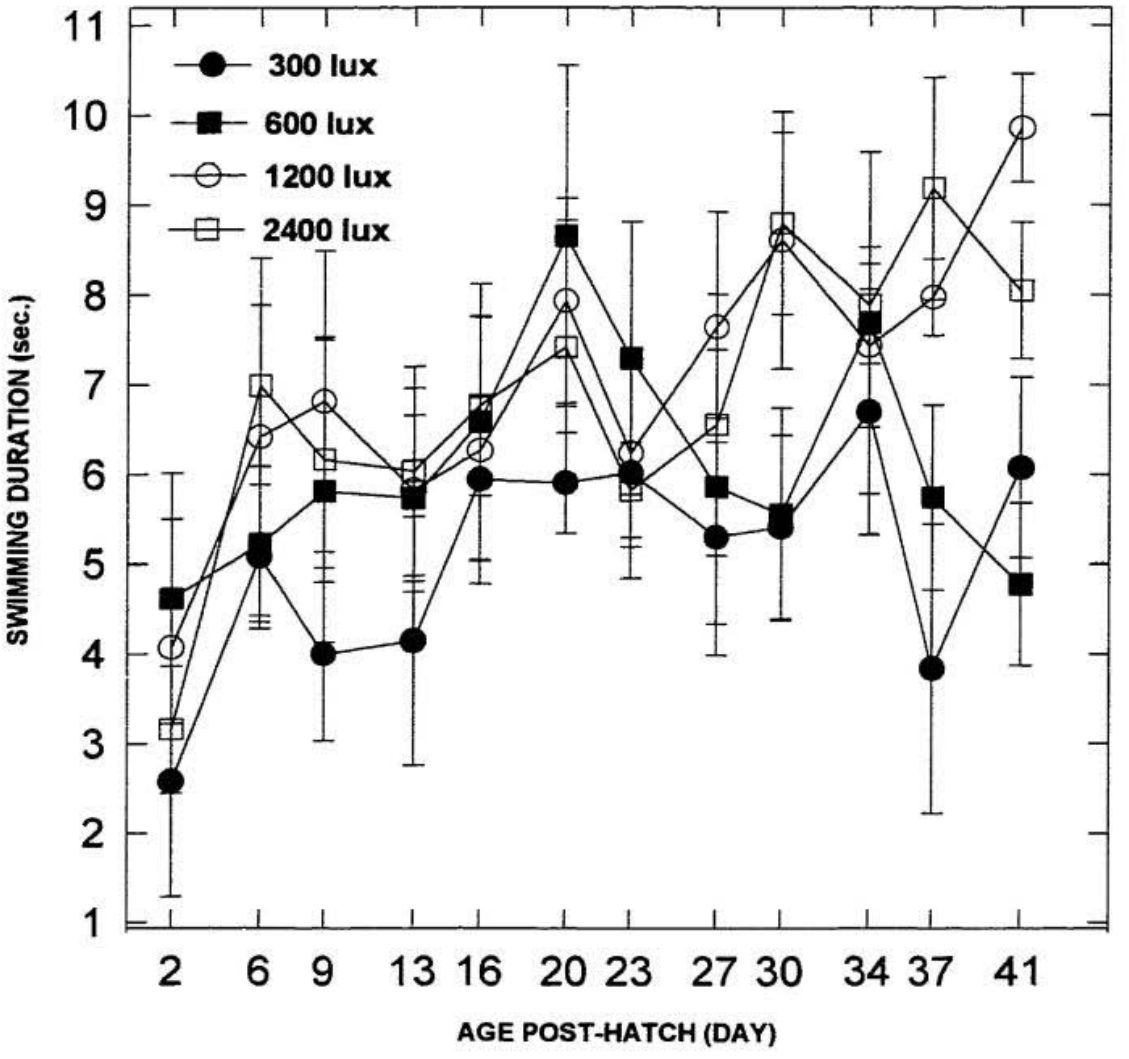

Fig. 5.7. Mean (± se) swimming duration of cod larvae from 2 to 41 dph reared in different light intensities. N=10 larvae per sample per treatment.

Table 5.9 Results of the Tukey analysis comparison examining the mean swimming duration (sec) of cod larvae reared under different light intensities from 2 to 41 dph. Values are the differences in mean swimming duration between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment	Age (dph)											
comparisons	2	6	9	13	16	20	23	27	30	34	37	41
2400-1200	-0.91	0.57	-0.65	0.21	0.50	-0.52	-0.41	-1.09	0.19	0.45	1.21	-1.81*
2400-600	-1.46	1.76	0.35	0.30	0.18	-1.24	-1.47	0.69	3.24*	0.20	3.44*	3.27*
2400-300	0.58	1.90	2.17	1.89*	0.82	1.51	-0.19	1.25	3.38*	1.19	5.35*	1.97*
1200-600	-0.55	1.19	1.0	0.09	-0.32	-0.72	-1.06	1.78	3.05*	-0.25	2.23*	5.08*
1200-300	1.49	1.33	2.82*	1.68	0.32	2.03	0.22	2.34*	3.19*	0.74	4.14*	3.78*
600-300	2.04*	0.14	1.82	1.59	0.64	2.75*	1.28	0.56	0.14	0.99	1.91*	-1.30*

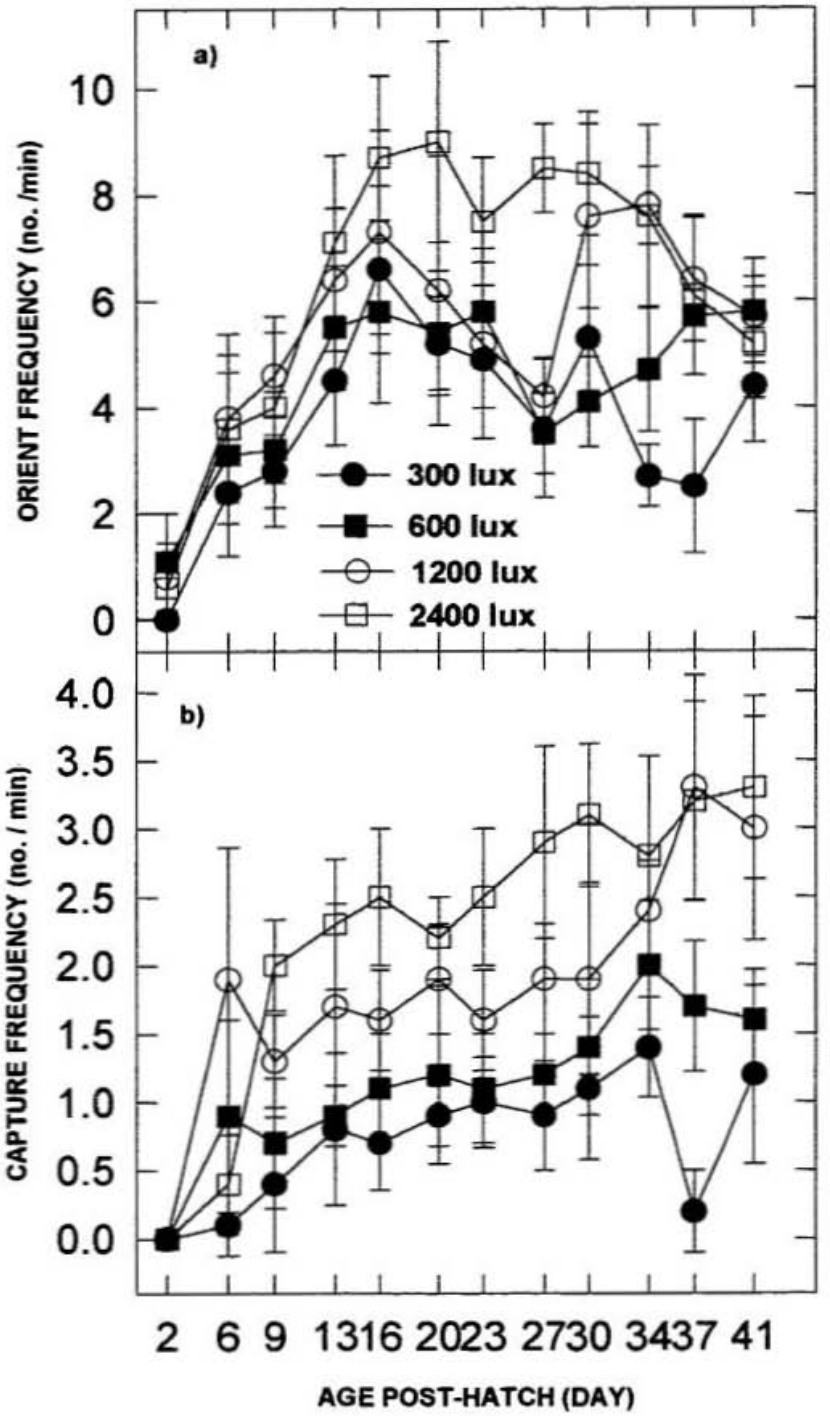

Fig. 5.8. Mean (± se) a) orient frequency and b) capture frequency of cod larvae reared in different light intensities. N=10 larvae per sample per treatment.

Table 5.10 Results of the Tukey analysis comparison examining the mean a) orientation and b) success frequencies of cod larvae reared under different light intensities from 6 to 41 dph. Values are the differences in mean orientation and success frequencies between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 6	9	13	16	20	23	27	30	34	37	41
a) Orientation frequency											
2400-1200	-0.2	-0.6	0.7	1.4	2.8	2.3*	4.3*	0.8	-0.2	-0.3	-0.5
2400-600	0.5	0.8	1.6	2.9*	3.6*	1.7	5.0*	4.3*	2.9*	0.4	-0.6
2400-300	1.2	1.2	2.6*	2.1	3.8*	2.6*	4.9*	3.1*	4.9*	3.6*	0.8
1200-600	0.7	1.4	0.9	1.5	0.8	-0.6	0.7	3.5*	3.1*	0.7	-0.1
1200-300	1.4	1.8	1.9	0.7	1.0	0.3	0.6	2.3*	5.1*	3.9*	1.3
600-300	0.7	0.4	1.0	-0.8	0.2	0.9	-0.1	-1.2	-2.0*	2.7*	1.4
b) Success frequency											
2400-1200	-1.5*	0.7	0.6	0.9*	0.3	0.9*	1.0	1.2*	0.4	-0.1	0.3
2400-600	-0.5	1.3*	1.4*	1.4*	1.0*	1.4*	1.7*	1.7*	0.8	1.5*	1.7*
2400-300	0.3	1.6*	1.5*	1.8*	1.3*	1.5*	2.0*	2.0*	1.4*	3.0*	2.1*
1200-600	1.0	0.6	0.8	0.5	0.7*	0.5	0.7*	0.5	0.4	1.6*	1.4*
1200-300	1.8*	0.9*	0.9	0.9*	1.0*	0.6*	1.0*	0.8	1.0*	3.1*	1.8*
600-300	0.8	0.3	0.1	0.4	0.3	0.1	0.3	0.3	0.6	1.5*	0.4

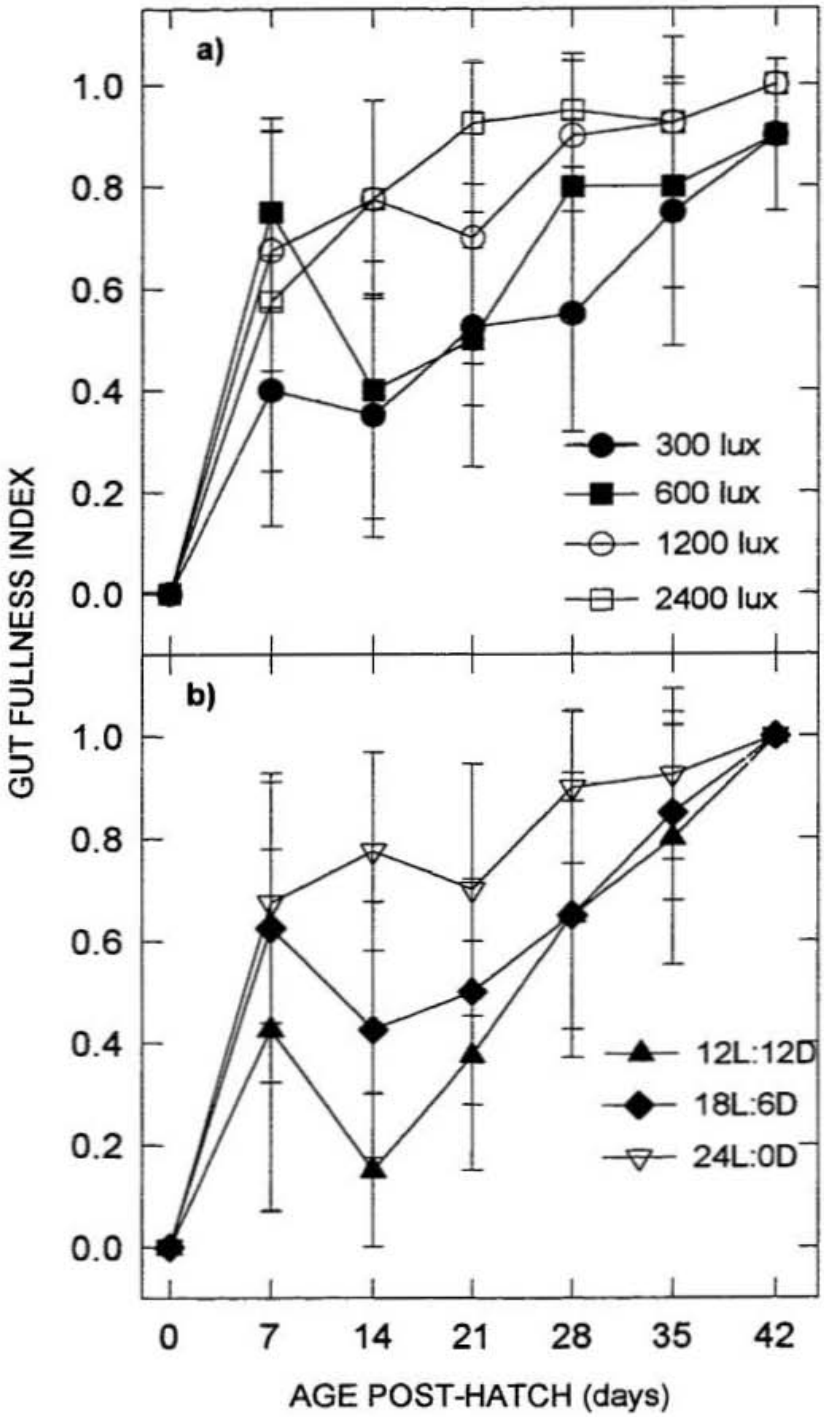

Fig. 5.9. Mean (± se) gut fullness index of cod larvae reared in different a) light intensities and, b) photoperiods. N=10 larvae per sample per treatment.

Table 5.11 Results of the Tukey analysis comparison examining the mean gut fullness index of cod larvae reared under different a) light intensities or b) photoperiods from 7 to 42 dph. Values are the differences in mean gut fullness index between two treatment comparisons. * - indicates significance at 0.05 level.

Treatment comparisons	Age (dph) 7	14	21	28	35	42
a) Light intensity						
2400-1200	-0.10	0.00	0.23	0.05	0.00	0.00
2400-600	-0.18	0.38	0.43*	0.15	0.13	0.10
2400-300	0.17	0.43*	0.40*	0.40*	0.18	0.10
1200-600	-0.07	0.38	0.20	0.10	0.13	0.10
1200-300	0.28	0.43*	0.17	0.35*	0.18	0.10
600-300	0.35	0.05	-0.03	0.25	0.05	0.00
b) Photoperiod						
24L-18L	0.05	0.35*	0.20	0.25	0.08	0.00
24L-12L	0.25	0.63*	0.33	0.25	0.13	0.00
18L-12L	0.20	0.28	0.13	0.00	0.05	0.00

Table 5.12 Results of a two-way ANOVA (age and photoperiod) on standard length, dry weight, SGR, condition, mortality rate (Z) and survival of larval cod at different light intensities. Significance at 0.05 level.

Variable	source	df	F-value	Pr. > F
Standard length	Model	17	147.40	0.0001
	Error	162		
	Age	5	429.91	0.0001
	Photoperiod	2	128.61	0.0001
	Age × Photoperiod	10	9.91	0.0001
Dry weight	Model	17	155.64	0.0001
	Error	162		
	Age	5	475.29	0.0001
	Photoperiod	2	124.45	0.0001
	Age × Photoperiod	10	2.05	0.0315
SGR	Model	5	112.30	0.0001
	Error	6		
	Age	1	468.08	0.0001
	Photoperiod	2	30.91	0.0007
	Age × Photoperiod	2	15.81	0.0041
Condition	Model	17	91.36	0.0001
	Error	162		
	Age	5	251.78	0.0001
	Photoperiod	2	113.96	0.0001
	Age × Photoperiod	10	6.64	0.0001
Mortality rate	Model	14	13.17	0.0001
	Error	15		
	Age	4	34.50	0.0001
	Photoperiod	2	5.29	0.0183
	Age × Photoperiod	8	4.47	0.0061
Survival	Model	14	337.03	0.0001
	Error	15		
	Age	4	65.47	0.0001
	Photoperiod	2	2208.35	0.0001
	Age × Photoperiod	8	4.97	0.0037

Discussion

Light intensity experiment

Previous attempts in our laboratory to raise larval cod beyond metamorphosis met with varying success (Chapter 2). Overall results suggested that survival increased when a higher light intensity was used. The mean percentage increase in survival (840%) and growth (126%) in this study at 42 dph at high (2400 lux) light intensities compared to low (300 lux)light intensities indicates that light intensity is a major factor affecting the survival and growth of larval cod.

Larval cod reared in high light intensities had better growth and survival compared to larvae reared in lower light intensities. Several studies have shown that light intensity affects the growth and survival of marine larval fish (Barahona-Fernandes 1979, Kiyono and Hirano 1981, Bolla and Holmefjord 1988,Chesney 1989). Kiyono and Hirano (1981) found that growth and survival of larval black porgy (*Mylio macrocephalus*) was increased with increasing light intensity. When reared in different light intensities, sea bass larvae (*Dicentrarchus labrax*) showed better growth and poor survival at higher light intensity but had better growth and survival at continuous lower intensity light (Barahona-Fernandes 1979). Cod larvae in my experiment performed well in higher light intensity and continuous light. Results from other studies together with mine suggest that the effects of light intensity on larval growth and survival are species specific. In some species, increased light intensity enhances the growth and/or survival, while in others it has negative effects on growth and/or survival.

SGR values for larval cod were influenced by light intensity during 0-28 dph period. Significantly higher SGR values for larvae reared in 2400 lux compared to the two low light treatments and no significantly different SGR values for 29 to 42 dph among the treatments indicate the importance of high light during the early larval stage of cod. Several studies indicated that most marine fish larvae at hatch have only a pure cone retina and rods are added to the retina as they grow (Blaxter 1975, Brancheck 1984) and the timing of the appearance of rods in retina depends on the species (Blaxter 1986). Given the fact that rods facilitate vision under dark conditions (Blaxter and Staines 1970), I speculate that larval cod may have developed rods in their retina by 28 dph thus enabling larvae at low light to feed and grow at similar rates to larvae reared in higher light intensities. Although there was a significant difference in capture success between larvae reared in lower and higher light intensities, gut fullness index was not significantly different among treatments beyond 28 dph. This suggests that although larval cod in low light intensity capture the prey less efficiently, they were able to slowly fill up their gut.

Hay (1981) and McGurk (1985) criticized the use of morphological indicators of condition for larval fish especially for preserved specimens. Preservation of larvae usually results in shrinkage and/or damage that complicates the usage of morphological condition indices. However, in my study, all measurements were made on live anaesthetized larvae. Using the relationship between length and weight was also not possible because of the allometric relationship between these two parameters during the larval stage (Cone 1989). Koslow et al. (1985) used several morphological indices of wild caught cod larvae and

found that the relationship between body height at the anus (myotome height) to length was the most sensitive to environmental conditions including prey concentration and temperature. Using this relationship, my results showed that the condition of larval cod was significantly higher for larvae reared at higher light intensities. The lower condition of larvae reared at low light intensities indicates the larvae experience a poor feeding situation.

The accessibility of zooplankton prey to visually feeding fish larvae is a function of the reaction distance to particular prey. Visual acuity and reactive distance increase with increasing light intensity (Blaxter and Staines 1970, Confer et al. 1978). The increased visual acuity and reactive distance would increase the prey encounter rate and thus enhance foraging efficiency (Millis et al 1984). Thus, reduced light intensities probably influence the relative ability to detect the prey, the reactive distance, encounter rate, and searching ability. In my experiment, larvae reared in higher light intensities had higher prey capture than larvae reared in lower light intensities. This increased foraging in higher light intensities would enable the larvae to grow faster than the larvae reared in lower light intensities.

Light intensity may have positive effects on growth and survival through a variety of mechanisms. Higher light intensity increases larval activity and swimming speed (Batty 1987), which could result in the search of greater volumes of water column Increased searching of the larvae would increase contact rate with prey. Larvae reared in higher light intensities (1200 & 2400 lux) had higher swimming duration and thus increased encounter

rates than larvae reared in lower light intensities. Food selection is also influenced by light intensity (Fossum 1983) by enhancing detectability of less conspicuous prey. My results showed that capture success of larval cod increased with increasing light intensity. Most marine fish larvae are generally considered to be visual feeders (Blaxter 1986). Various studies have shown that feeding incidence increases with increasing light intensity and vice versa (Blaxter 1986, Hunter 1981, Kiyono & Hirano 1981). Larval cod have been shown to be visual feeders and cease to feed in the dark (Ellertsen et al. 1980). Thus, reducing light intensity below an optimal level would reduce foraging rate and growth as was observed in my experiment.

Larvae reared in higher light intensities have another advantage over larvae reared in lower light intensities. Larvae reared in higher light intensities were larger than larvae reared in lower light intensity at a given age. Several studies have shown that visual acuity and reactive distance increase with increasing body size of the fish larvae (Blaxter and Staines 1971, Neave 1984). Thus being larger in body length at a given age, larvae reared in higher light intensities would have better visual acuity and increased reactive distances. Having a larger body length at a given age, in addition to higher light intensities (which would also increase the visual acuity and reactive distance) would be more advantageous for larvae reared in high light than larvae reared in low light.

Morphological constraints lessen as larval fish grow. Gill and Hart (1996) showed that in three-spined stickleback (*Gasterosteus aculeatus*) a small increase in the body length would significantly increase foraging efficiency which they attributed to the

morphological advantages such as increased gape, larger gut capacity and increased manoeuverability. In my earlier experiment (Chapter 4) with larval cod I found that larvae reared in higher prey concentrations which had a larger body length than larvae reared in lower prey concentrations (at a given age) had better feeding efficiencies. Thus larger body size at a given age of larvae reared in higher light intensities enable them to feed more efficiently, grow faster and survive better than larvae reared at lower light intensities.

My result on the effect of light intensity on the foraging of larval cod contrasts sharply with other studies. Ellertsen et al. (1980) found that larval cod fed on *Artemia* nauplii had the highest feeding incidence at 1.4 lux while when fed with dinoflagellate (*Peridinium trochoidum*) the highest feeding incidence was observed in 1000 lux. Similarly, Huse (1994) found higher feeding incidence for larval cod at 1 lux when using copepod nauplii and rotifers. All these studies were short term experiments and thus did not reveal the long term effect of light intensity on growth and foraging. Moreover, my earlier study (Chapter 2) with larval cod from two populations (Scotian Shelf and Northeast Grand Bank origin) showed that light intensity affects the foraging, growth and survival of the larvae from these two populations differently. Larvae from the Northeast Grand Bank performed better in high light levels while Scotian Shelf larvae in low light levels. So the difference between my findings and other studies involving Norwegian cod may be due to population differences. Anderson and de Young (1995) reported that cod larvae in the offshore and inshore areas of northeastern Newfoundland occupy the top 40

m (5-35 m) of the water column during summer months. Field data from Conception Bay (inshore of Northeastern Grand Bank) showed that light intensity during the month of July, at 5-40 m depth, ranges between 200-400 and10-30 μE m^{-2} (see Chapter 2).Thus in nature, cod larvae from the Northeast Grand Bank experience similar or even higher light intensities to those of my high light treatments, i.e. 1200-2400 lux.

Mortality rates of cod larvae in my study were lower in higher light intensities (1200 & 2400 lux) while mortalities of larvae reared in lower light intensities were higher from 14 dph to 35 dph. These differences in mortality rate among the treatments decreased as the larvae grew. This was not surprising since larvae reared in lower light intensities had lower success rate, and could not meet the energetic requirements to get them through the critical period stage. Thus rapid growth and lower mortality rates during the early larval stages at higher light intensities and the lower mortality rates and higher growth during late larval stages at lower light intensities indicated that 1200-2400 lux light intensity was suitable for larval growth and survival of larval cod from 1dph to 28-35 dph. Beyond this a lower light intensity would be sufficient to maintain reasonable growth and survival.

Photoperiod Experiment

Results from my experiment demonstrate that photoperiod influenced all the morphological parameters measured, the condition, SGR, mortality and survival of Atlantic larval cod. The effect of photoperiod on SGR values and instantaneous mortality

rates also varied with the age/size of the fish. Significantly lower mortality rates and higher SGR values were found under continuous light during the 0-28 dph period suggesting that larvae achieve better growth and survival during the early larval stage when reared under the longer photoperiods. The lack of significant difference in mortality rates and SGR values beyond 28 dph indicate continuous light may not be necessary during later larval stages. Similar views were expressed by other authors (Blaxter 1968, Tandler and Helps 1985).

Cod larval growth in length and weight increased with increasing photoperiod. Several other studies have also shown that extended photoperiods produced larger larvae at a given age (Kiyono and Hirano 1981, Tandler and Helps 1985, Duray and Kohno 1988, Hart et al. 1996). It seems that the relationship between photoperiod and growth is correlated to the probability of encounter between the larva and its prey. Hence, the longer the photoperiod the greater the chances of encounter, resulting in higher rates of food ingestion and growth. My results of gut fullness index could not confirm this. This may have been due to the fact that the larval samples in my study were taken hours after the early morning feeding thus all larvae would have been feeding for a period of time and would not show any difference in gut fullness index. Similarly, Kendall et al (1994) suggested that pollock larvae (*Theragra chalcogramma*) changed vertical position to extend the length of their daily feeding period. However, Barahona-Fernandes (1979) found that for larval sea bass, continuous light did not support the best growth. The higher growth was obtained at 18 hr photoperiod which resembles the natural photoperiod when

larval sea bass begin exogenous feeding. Field studies involving larval cod indicated that cod larvae feed throughout the day given the opportunity (Pedersen et al. 1989, Suthers and Sundby 1996). Pedersen et al. (1989) reported that the higher growth rate (19.5 % weight d^{-1}) of Arcto-Norwegian larval cod in early June in comparison of other months was due to the extended photoperiod which allowed the larvae to feed throughout the day. In another study, Suthers and Sundby (1996) reported that cod larvae of Arcto-Norwegian origin had a superior growth rate and were larger at a given age than cod larvae of Scotian Shelf origin. While genetic factors may contribute to these results, the authors pointed out that Arcto-Norwegian larvae have 48% more time during May-June for visual feeding than Scotian Shelf larvae. This difference was felt to play a major role in the observed differences of growth rate. Results of these field studies on larval cod support my results that larval cod reared at extended photoperiod would grow faster.

General observations of the cod larvae from the three treatments showed that the larvae from the continuous light treatment were robust and well-pigmented. Larvae reared in continuous light also showed a better condition index than larvae from shortened photoperiods throughout the experiment. These results suggest that larvae reared in continuous light did not show any stress symptoms as reported by studies on some other species (Buttle et al. 1995). My study has also demonstrated that the best survival of larval cod from hatching through metamorphosis can be obtained by using continuous light during the early larval period. Results of my study also confirm earlier claims from preliminary experiments that continuous light would enhance the survival of larval cod

(Chapter 2). Similar results have been reported for some other marine fish larvae. Duray and Kohno (1988) reported that continuous light enhanced the survival of larval rabbitfish Hart et al. (1996) found no effect of photoperiod on the survival of greenback flounder (*Rhombosolea tapirina*). In contrast, Kiyono and Hirano (1981) reported that black porgy larvae showed a higher survival at 13 hr photoperiod than extended photoperiods. Similar observations were made for sea bass larvae by Barahona-Fernandes (1979) and for sea bream (*Archosargus rhomboidalis*) by Dowd and Houde (1980). Considering these results, it seems that the effect of photoperiod on larval growth and survival is species specific.

In conclusion, my results showed that the foraging, growth, condition and survival of larval cod is influenced by light intensity and photoperiod. Although larvae survived through metamorphosis in all light intensities and photoperiods, growth and especially survival was greater for larvae reared in high intensity (2400 lux) and continuous light regime. My results also suggest that larval cod require high intensity and continuous light during the early larval stages (0- 28 dph), but this could be reduced after this period. The impact of this is to shorten the 'critical' larval period and to increase their potential for survival. From an economic perspective, a reduction in the length of the costly larval rearing period would result in cost savings.

Chapter Six : Predatory responses of larval cod: functional, developmental and numerical response.

Introduction

Increased focus on studies of mortality in the early larval stages of fish may help in understanding the stock-recruitment relationship. Small changes in mortality during this period can produce great effects on the number of recruits. The acquisition of the necessary food by fish larvae is of prime importance in survival and successful development. Without the proper quantity of food, larval growth and survival are affected. Several studies have shown that a predator's consumption increases with increasing prey concentrations which results in better growth and survival (Houde 1978, Drost 1987, Gill and Hart 1996, Chapter 4). In nature, the distribution of prey varies with scale (both in time and space), and prey are utilized differently by different species of larval fish (Houde and Schekter 1980). O'Connell and Raymond (1970) found that Anchovy (*Engraulis mordax*) larvae require higher food densities than the average densities found in the sea for better growth and survival in the laboratory while others argue that laboratory studies over-estimate the prey requirement of larval fish for enhanced growth and survival (Houde 1978).

The functional response is the change in prey consumption rate (or attack rate) that results from a change in prey concentrations and is a basic component of predator-prey relationship (Solomon 1949, Holling 1965). Holling (1965) approached the predator-prey interaction as a system of behavioural components that could be partitioned and to

which predictive mathematical modeling could be applied. Functional response of predators are characterized mainly by two parameters, the rate of attack per prey while searching and the handling time that a predator spends on average with each prey it attacks. The time spent pursuing, subduing, consuming, and resting to digest prey provides an upper limit to the number of prey that can be captured during a finite foraging period. Holling (1965) described three basic forms of functional response; type I, type II and type III. When the handling time is negligible and rate of attack per prey is constant, the response is known as type I and produces a density-independent predation rate (increases linearly to a pleateu). If the handling time is not negligible, the type of response depends on the relationship of both attack rate and handling time to prey density. A constant rate of attack per prey and constant handling time result in a type II functional response and produce an inversely density-dependent predation rate (increases to asymptote at a continuously decreasing rate). However, if the rate of attack per prey increases and/or handling time decreases with prey density, the response is known as type III and produces density-dependent predation rate (increases sigmoidally to an asymptote).

Studies have shown that predator growth and developmental rates are influenced by prey density (Gallis 1990, Gill and Hart 1996, Chapter 4). The effects of changes in these rates on the prey consumption rate of an individual have been referred to as the developmental response (Murdoch 1971). In my earlier experiments (Chapter 3 and 4), at prey concentrations that ensured survival of at least a few larvae through metamorphosis larvae showed variable growth rates. This indicated that larvae from different prey

concentrations varied in size at a given age. Thus it would be appropriate to see how the developmental responses change with larval size. Studies have shown that prey capture abilities of larval fish change as the larvae grow (Houde and Schekter 1980, Miller et al. 1992, Chapter 3 and 4). Differences in developmental rates would have an impact on the other aspects of population processes such as survivorship, fecundity and time to maturity which are usually considered part of the numerical response (Solomon 1949). Studies have also shown that different prey densities may cause differential survival of the predator (Solomon 1949, Chapter 3 and 4). The response of larval fish, in terms of survival, to different prey concentrations is termed the numerical response (non-reproductive) which describes the change in the predator density as a function of prey concentrations with time (Nunny 1985, Solomon 1949).

The objectives of this experiment were to examine the predation responses (functional, developmental and numerical) of larval cod fed at different concentrations of rotifers or *Artemia* nauplii from week 1 to 6 post-hatch. In this paper, I report the prey consumption rates of larval cod in terms of both prey concentration and age/size. I will also calculate the attack rate and handling time (components of Holling's equation) of cod larvae from direct behavioural observation and compare it with the estimated values from Holling's equation. My earlier experiments (Chapter 3 and 4) indicated that early larval stages of cod were more vulnerable to starvation mortality and grew at slower rates when reared in lower prey concentrations and that larvae reared in less than 1000 prey per litre did not survive beyond three weeks. Thus, I examine the prey consumption of larval cod

at different ages/sizes along with behavioural data and attempt to determine why larval cod did not survive beyond three weeks in low prey concentrations in my earlier experiments.

Materials and Methods

Fertilized Atlantic cod eggs were collected from a naturally spawning captive broodstock maintained at the Ocean Sciences Centre, Logy Bay, Newfoundland. Eggs were incubated in a 250 L circular fibre glass tanks at 8°C until hatch. Larvae from this cohort were used in two different experiments, one to study the effects of prey concentrations on larval growth and survival (Chapter 4) and the other for the study of functional and developmental response. This allowed me to use some data interchangeably.

Once they hatched, larvae were transferred to their respective experimental units. For the functional and developmental response study, larvae were reared in a 100L circular fiber glass tank. This tank was maintained in the same water bath as the growth and survival study thus maintaining the same rearing temperature (7-9°C) in both experiments. Larvae were stocked at 40 larvae per litre. Prey concentration was maintained at 4000 prey per litre and adjusted 3 times a day. All the rearing conditions (see material and methods in Chapter 4) were similar in both experiments or as otherwise mentioned.

For the functional and developmental response experiment, each week starting

from week 1 (day 6), larval cod were exposed to a range of seven different prey concentrations (250, 500, 1000, 2000, 4000, 8000 and 16,000 prey L^{-1}). Rotifers were used from week 1 to 3 and from week 4 to 6 *Artemia* was used as prey. Overnight, 150 larvae from the rearing tank were transferred to a 15L holding tank although only 70 larvae (10 larvae × 7 treatments) were required for the trials. These larvae were kept for12-18 hours to empty their guts. This helped to standardize the condition of the larvae used in each trial. The excess number of larvae ensured that only healthy larvae were used in the trial. Variation in size of the larvae used in the trials from each week were within 0.5 mm from week1-3 and within 1.25 mm from week 4-6. All the trials were conducted in five 2-L glass bowls maintained at 7-9°C. All the bowls were filled with 1.5L of filtered sea water.

For each prey concentration, the trial was conducted as five replicates with two larvae per replicate. Only two larvae per replicate were used to avoid observing the same larvae twice during the observations. To initiate a trial, two larvae were transferred from the holding tank to each of the five 2-L glass bowls and allowed to acclimate. The prey were added to the bowl at the experimental prey concentration and a gentle aeration kept the prey well distributed in the bowl. After 5 minutes of adding the prey, observations (Foraging MAP's; see Chapter 3 and 4 for details) on larvae were started. Each larva was observed for 1 minute and observations were made on both larvae. During this time (max. 2-3 mins. per bowl) aeration was reduced to a compromised rate to facilitate the observation and also to maintain a homogenous prey distribution. Larvae were allowed to

feed for 15 minutes (from adding the prey) and were caught by a pipette and killed with MS222. Trials for other prey concentrations were carried out in the same manner. Standard length of the larvae were measured and the prey in each larval gut were removed and counted. Prey counts and standard length measurements were completed the same day, soon after completion of the trials.

The data for numerical response were collected from the growth and survival study (Chapter 4). Numerical response data were presented only for weeks 2 and 6 because there were only minor differences found in the trend between these periods.

Data analysis

The functional response was described by fitting the data to the Holling's type II equation (Holling 1966) defined as,

$$N_{consumed} = a.\ T_t\ N / (1 + a\ T_h\ N) \qquad \text{(Eq. 6.1)}$$

where $N_{consumed}$ is the number of prey eaten, a is the attack rate or instantaneous rate of discovery (s^{-1}), T_t is the total time available for foraging (seconds), T_h is the handling time per prey and N is the prey concentration (number L^{-1}). The parameter estimates were obtained using a nonlinear regression. Data from the 5 replicates were combined to produce more accurate estimates of a and T_h. Prey saturation values, N_{max}, were calculated from T_h as

$$N_{max} = T_t / T_h \qquad \text{(Eq. 6.2)}$$

Attack rates and handling times were also calculated directly from the behavioural

data. Attack rates included the components of capture efficiencies (success, miss and pass; Spitze 1985). The handling time includes the time required for all activities associated with the capture of a prey that inhibit the capture of another prey (Holling 1966). In this view, handling time calculated from this experiment included the time required for recognition (orient), pursuit and capture. The attack rates and handling time derived from the parameter estimates and behavioural observations were then compared. Numerical response data (Survival) were fitted using least square mean non-linear regression equation.

Results

Prey consumption data when related to prey concentration fit well with Holling's type II functional response from week 1 to 6 (Fig. 6.1). Prey consumption increased relatively rapidly as prey concentrations increased from 250 to 4000 prey L^{-1} and then increased slowly asymptotically toward a plateau at higher prey concentrations. R^2 values were relatively high ranging from 0.75 to 0.9 (Table 6.1). Although the attack rates estimated from the Holling's equation were lower throughout, attack rates increased with age. Handling time estimated from the equation was very high and showed a tendency to decrease as the larvae grew. On the other hand, attack rates calculated from direct observations were higher than the values derived from the nonlinear regression. Similarly, values of handling times derived from the regression were much higher than that calculated from the direct observations.

The numbers of prey in the gut of first feeding larval cod increased as the prey concentration increased (Table 6.2). Almost 50% of the larvae in the two lowest prey concentrations (250 and 500 prey L^{-1}) did not capture a single prey during the week 1 and 2 trials. Trials in the three highest prey concentrations (4000 prey L^{-1} and above) showed that only 10% of the larvae failed to capture at least one prey on week 1 trial.

Except when larvae were ~11 mm in length, the number of prey captured by larval cod increased as the larvae grew regardless of the prey concentrations (Fig. 6.2). At around 11 mm (standard length), larvae from all treatments showed a decrease from the previous week in prey consumption rate. Although the prey consumption increased with larval size a linear relationship could not be found between prey consumption and larval size at a given prey concentration. N_{max} values were calculated from handling time values using equation 6.2 to evaluate how prey saturation values varied as the larvae grew. N_{max} values derived from direct observation and from Holling's equation showed an increase as the larvae grew. N_{max} values derived from both methods varied greatly with values from observations being much higher than from Holling's equation.

The numerical response of larval cod to a range of prey concentration followed a similar trend to the functional response (Fig. 6.3). At week 2, larval survival increased asymptotically as the prey concentration increased from 250 to 4000 prey L^{-1} and then at higher prey concentrations it tended to reach a plateau ($R^2 = 0.82$; $p<0.013$; Fig. 6.3). Although a similar trend was observed in week 6, the R^2 values were much lower

(R^2=0.58; $p<0.08$). Beyond week 3, higher mortalities were observed in prey concentrations greater than 4000 prey L^{-1} (Fig, 6.3). This resulted in a lower survival in the two highest prey concentrations compared to 4000 prey L^{-1}.

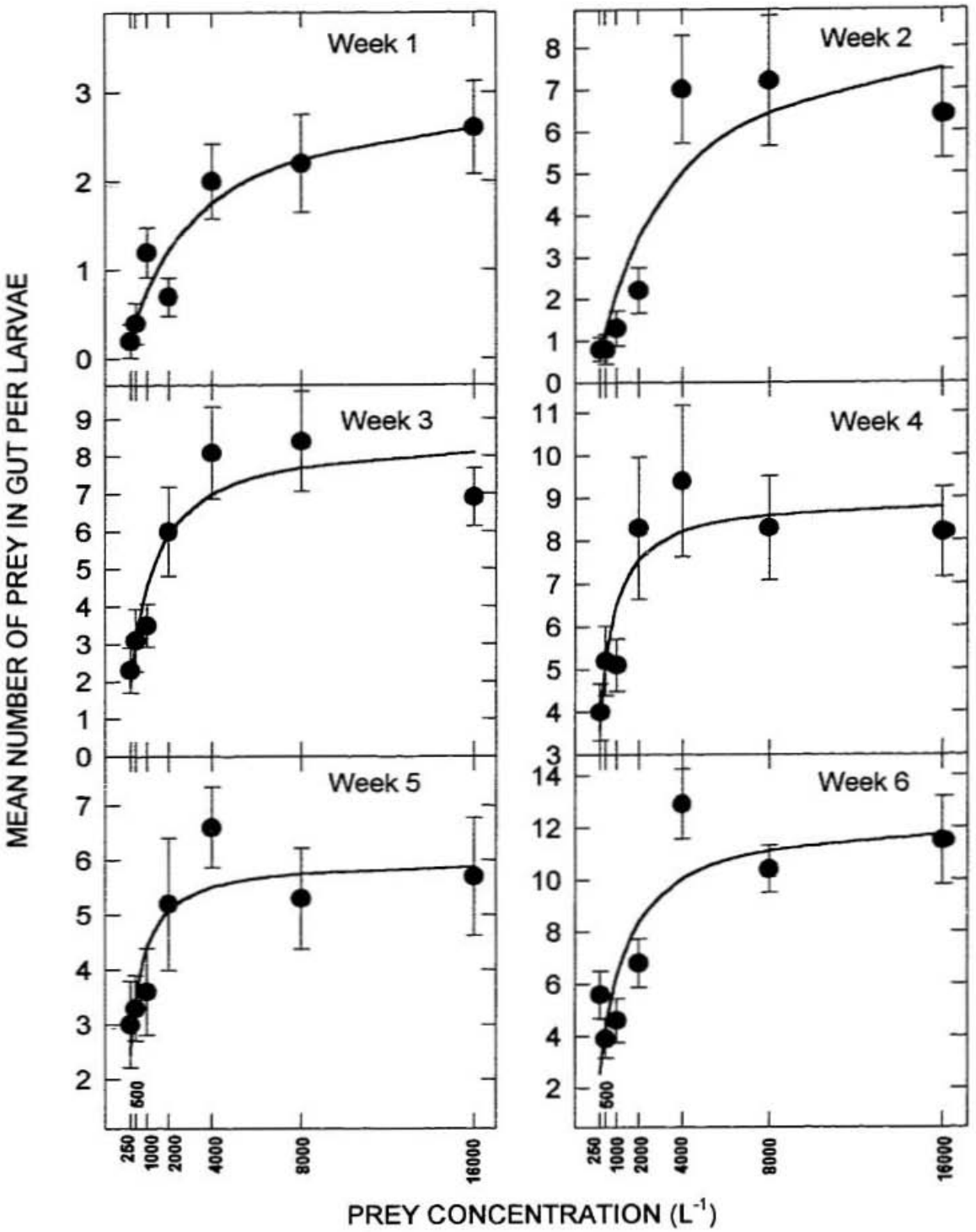

Fig. 6.1. The functional response of Atlantic cod larvae at different prey concentrations based on Holling's type II equation. Mean number of prey items eaten within 15 mins in all trials are shown.

Table 6.1. Instantaneous rate of discovery or attack rate (a), handling time (T_h), prey saturation levels (N_{max}) derived from direct behavioural observations and parameter estimates obtained from nonlinear regression, for Holling's type two functional response equation. Parameter estimates from the regression are based on the mean gut content data from the trial (n=10). Standard errors are in parenthesis.

	Behavioural Observation			Parameter Estimates			
Age (w)	a (s^{-1})	T_h (s)	N_{max} (no./60 s)	a (s^{-1})	T_h (s)	R^2	N_{max} (no./900 s)
1	1.76×10^{-2} (9.26×10^{-4})	2.39 (6.02×10^{-2})	25.1	$1.13(10^{-6})$ (3.6×10^{-7})	290.74 (46.58)	0.9	3.09
2	2.58×10^{-2} (2.06×10^{-3})	2.05 (2.75×10^{-2})	29.3	3.15×10^{-6} (1.36×10^{-6})	100.2 (22.51)	0.83	8.98
3	3.39×10^{-2} (2.65×10^{-3})	1.84 (2.42×10^{-2})	32.6	1.07×10^{-5} (3.14×10^{-5})	105.31 (9.68)	0.88	8.55
4	3.56×10^{-2} (2.22×10^{-3})	1 .4 (1.89×10^{-2})	43.0	2.64×10^{-5} (7.94×10^{-6})	99.96 (7.05)	0.83	9.0
5	3.06×10^{-2} (1.66×10^{-3})	1.14 (1.57×10^{-2})	52.7	1.85×10^{-5} (6.05×10^{-6})	149.8 (11.4)	0.8	6.01
6	4.45×10^{-2} (2.84×10^{-3})	0.98 (1.42×10^{-2})	61.4	1.43×10^{-3} (6.61×10^{-6})	72.3 (10.95)	0.75	12.45

Table 6.2. The percentage of cod larvae that had zero to 9 prey in the gut at seven prey concentrations during the 15 minutes trials.

	Number of	Prey Concentration (L-1)						
Age(w)	Prey in gut	250	500	1000	2000	4000	8000	16000
1	0	80	60	10	30	0	10	0
	1-2	20	40	90	70	80	60	60
	3-4	0	0	0	0	20	30	30
	5-6	0	0	0	0	0	0	10
	7-8	0	0	0	0	0	0	0
	≥9	0	0	0	0	0	0	0
2	0	30	40	20	0	0	0	0
	1-2	70	60	70	60	10	0	0
	3-4	0	0	10	40	0	10	20
	5-6	0	0	0	0	40	60	40
	7-8	0	0	0	0	20	0	30
	≥9	0	0	0	0	30	30	10
3	0	0	0	0	0	0	0	0
	1-2	70	60	20	10	0	0	0
	3-4	20	20	60	20	0	0	10
	5-6	10	10	20	30	30	40	30
	7-8	0	10	0	30	30	30	50
	≥9	0	0	0	10	40	30	10
4	0	0	0	0	0	0	0	0
	1-2	20	0	0	0	0	0	0
	3-4	40	40	20	20	10	10	0
	5-6	40	40	60	20	10	10	20
	7-8	0	20	20	10	30	30	30
	≥9	0	0	0	50	50	50	50
5	0	0	0	0	0	0	0	0
	1-2	40	20	20	20	0	10	10
	3-4	50	70	60	30	10	40	30
	5-6	0	10	10	10	40	20	30
	7-8	10	0	10	30	40	20	20
	≥9	0	0	0	10	10	10	10
6	0	0	0	0	0	0	0	0
	1-2	0	20	20	0	0	0	0
	3-4	30	50	30	20	0	0	0
	5-6	40	20	30	20	0	0	10
	7-8	20	10	20	30	10	20	10
	≥9	10	0	0	30	90	80	80

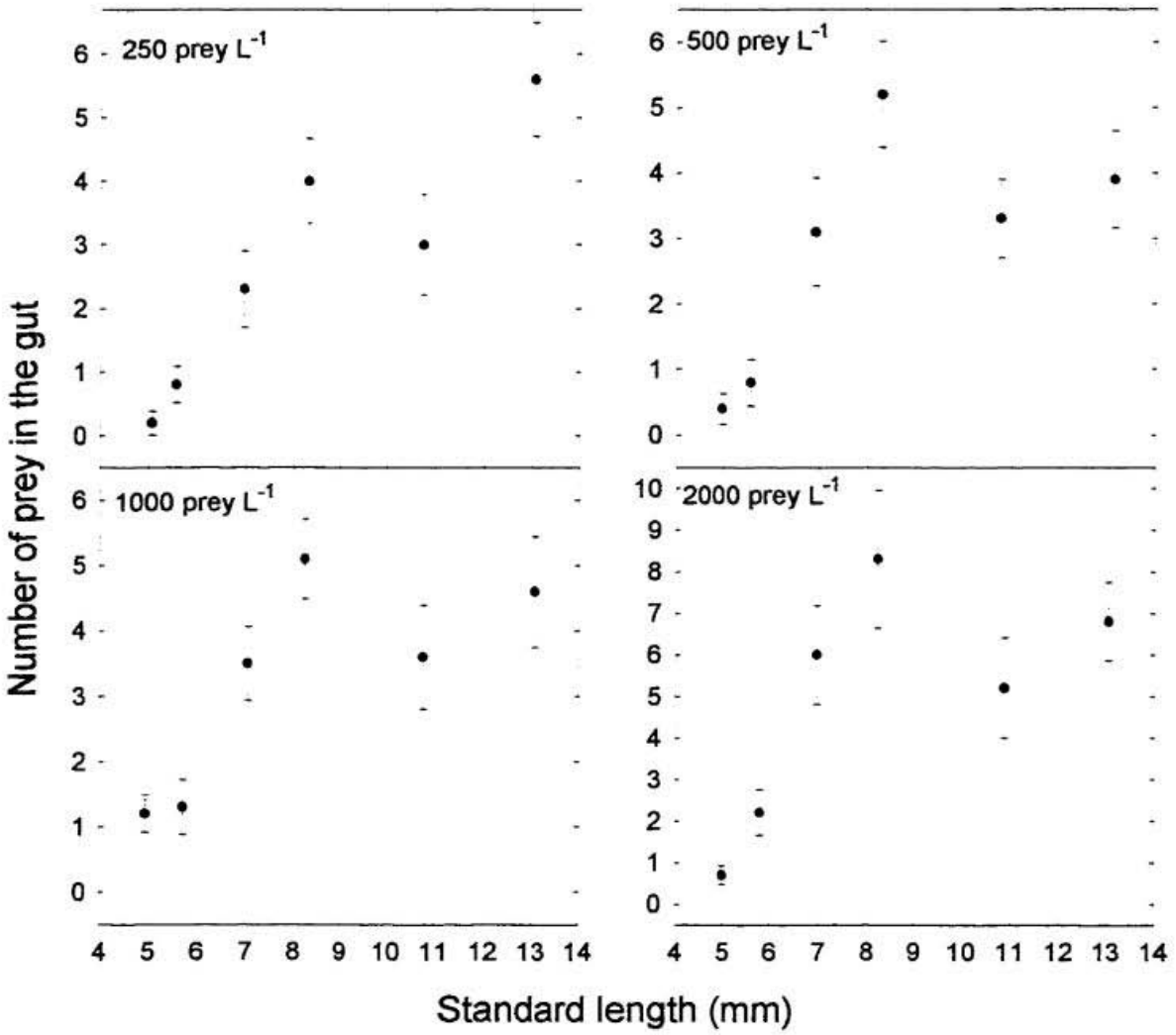

Fig. 6.2. Prey consumption rates (number per 15 mins.) of Atlantic cod larvae at various prey concentrations in relation to the standard length (mm) of the larvae.

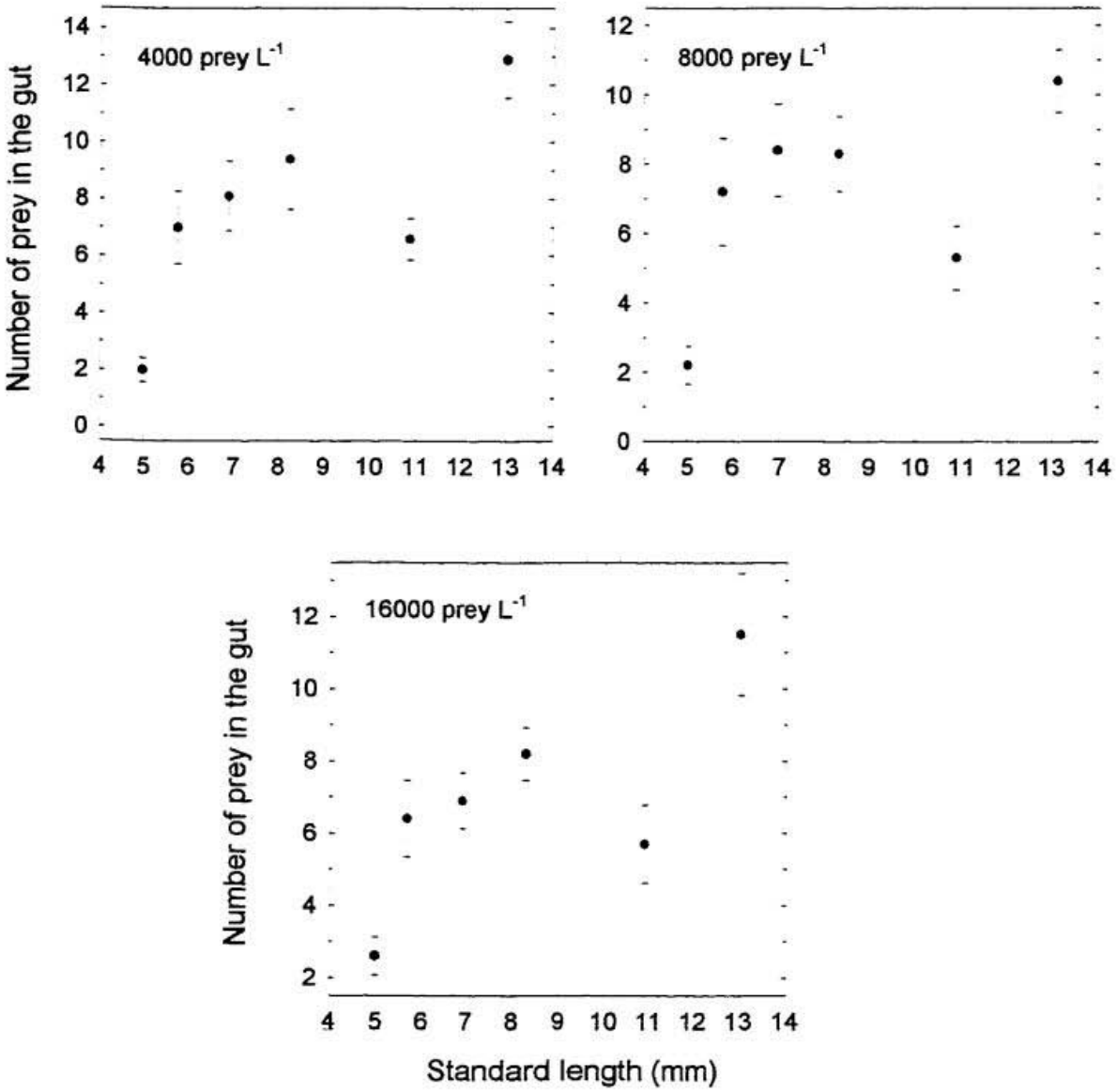

Fig. 6.2. cont. Prey consumption rates (number per 15 mins.) of Atlantic cod larvae at various prey concentrations in relation to the standard length (mm) of the larvae.

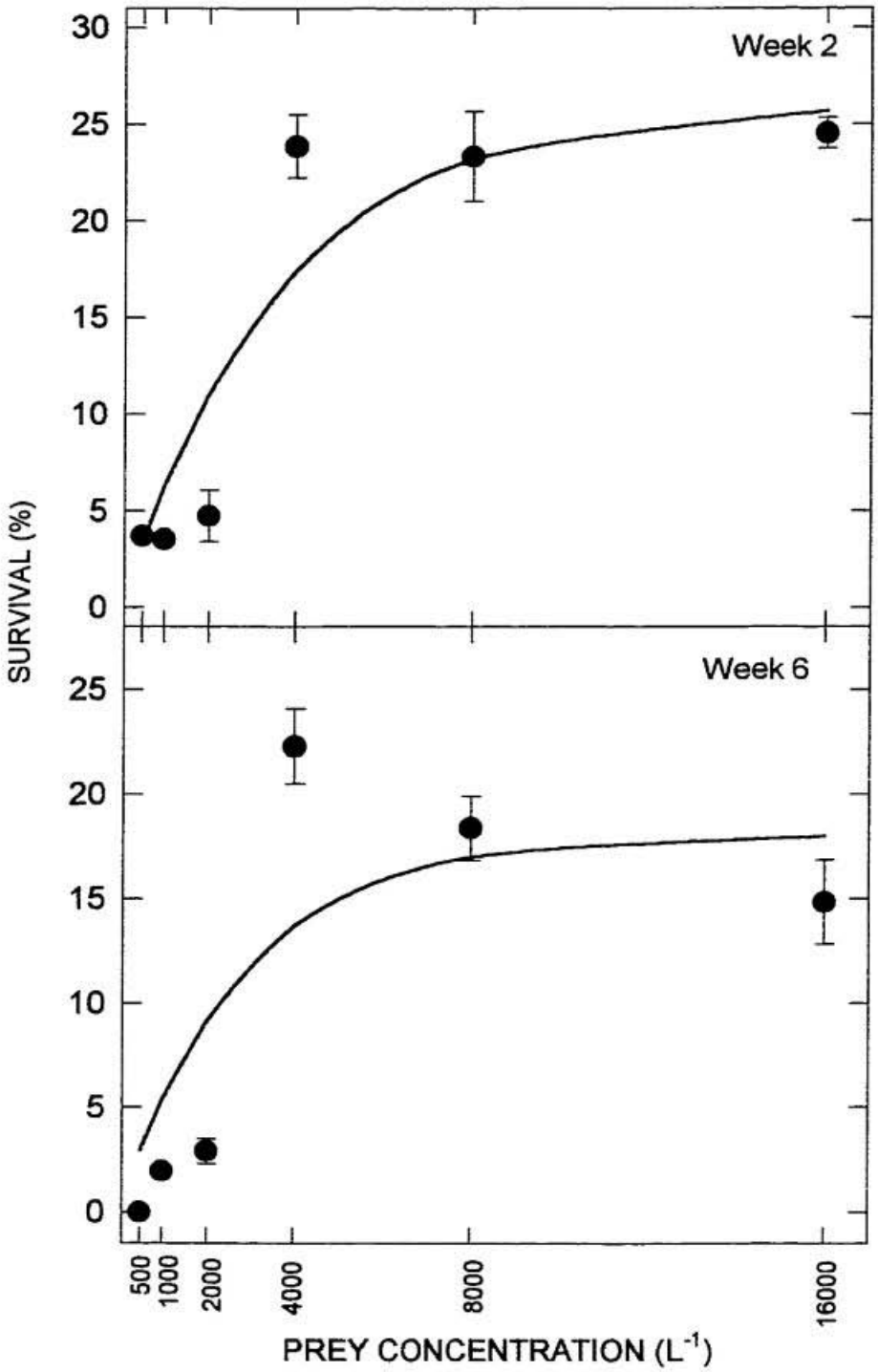

Fig. 6.3. Numerical response of Atlantic cod larvae in relation to prey concentration at week 2 and week 6.

Discussion

Over the range of prey concentrations used in this experiment (250 to 16000 prey L^{-1}), the relationship between predation rate and the prey concentrations for Atlantic cod larvae resulted in a decelerating curve; a characteristic of Holling's type II functional response. Other experiments dealing with the functional response in marine fish larvae (Houde and Schekter 1980, Miller et al. 1992) showed that the predatory response in terms of prey concentration is best described by Holling's type II equation. The feeding rates increased asymptotically at a continuously decreasing rate. Winkler and Orellana (1992) reported that the feeding response of five cyprinoid fish was best described by a sigmoid curve (type III response). They found that the instantaneous attack rates of these cyprinid fish was not independent of prey concentration as in a type II functional response and increased with an accelerating rate. In my study with larval cod, the attack rates increased with a decelerating rate which is typical for a type II functional response.

The data was only fitted with Holling's Type II response equation. Other type response equations, especially Type I, were not fitted to the data because Holling's Type I response is applicable only to filter feeders where there is no handling time involved. However, all my behavioural observations on cod (Chapter two to six) suggest that cod larvae do not filter feed. Although the handling time of larval cod is smaller (fractions of a second), still they choose and attack the prey rather a simple filter feeding.

In this experiment, values for attack rate and handling time derived from behavioural observations and Holling's type II equation did not agree. Similar

observations were made in other studies dealing with some freshwater arthropod species (Fox and Murdoch 1978, Longstaff 1980, Pastorok 1980) . Spitze (1985) pointed out that discrepancies between the values for Holling's parameter and behaviourally obtained parameters are often more a matter of semantics and experimental design than biology. Outside the Holling's equation, the terms attack rate and handling time have been used to describe various biological phenomena and the values for short-term feeding rates, detection time, pursuit time and swallowing time could be easily assessed by a trained observer. However, Holling's parameter is an instantaneous attack rate; it represents the theoretical rate of attack. It is not equivalent to any quantification of a finite feeding rate which includes pre-treatment effects and the effect of handling any prey captured during an experimental period (Spitze 1985)

On the other hand, handling time is an assessment of the relationship between present prey capture and the reduction in future foraging ability (Holling 1965). As originally defined (Holling 1965), Holling's parameter assesses the total handling time, including the effects of detection, pursuit, ingestion and digestion. Many handling time assessments from behavioural observation (this study) assess only one sub-component of the total handling time (Longstaff 1980, Pastorok 1981). In my study, detection of any 'digestive pause' which is the reduction of future strike rates, was impossible to assess and was not included in the calculation of the handling time. This may be the reason for the multi-fold increase in the handling time from Holling's equation estimate compared to the values from behavioural observation. Despite these discrepancies, estimation of these

parameters from behavioural observation could still be useful for short-term feeding trials rather than long term feeding rate predictions or a prey vulnerability analysis because the gross feeding rates are inseparable from the effect of prey handling.

The saturation prey concentration for larval cod increased as the larvae grew. Prey saturation levels increased by 303% from week 1 to 6. Houde and Schekter (1980) in their investigation on the functional response of bay anchovy, sea bream and lined sole observed increases of 229, 74 and 17% respectively, in the prey saturation values over a range of 10 to 200 μg. Miller et al. (1992) also reported similar patterns in prey saturation values for alewife (*Alosa pseudoharegus*), bloater (*Coregomus hoyi*) and yellow perch (*Perca flavenscens*).

The prey species I used in this experiment (rotifer and *Artemia)* were not the natural prey of larval cod. Although it would be difficult to relate these results to the wild conditions, their usage allows the feeding abilities to be investigated without any differential prey effects. Furthermore Holling's type II equation is valid only when a single prey is available to the predator (Abrams 1990). Thus, use of a mixture of natural prey would have complicated the interpretation of the results due to the difficulties in separating the feeding ability which depends on the differential behaviour of different prey. Using rotifers and *Artemia* as prey from week 1 to3 and from week 4 to 6 respectively, might have presented an adjusting (switching) problem (Murdoch 1969) for the larvae. But, my results did not support this. Furthermore, in the rearing tanks, the larvae had been fed with *Artemia* from day 20 (end of week 3) until the end of the experiment. Thus the

larvae used in week four were familiar with *Artemia* as prey for 7 days and the switch would not have presented any difficulties.

Results of my study showed that the prey consumption rate of larval cod increased as the larvae grew except for week 5 (~11 mm). The reason for the decrease in the prey consumption rate in week 5 was not clear. All the larvae used in week 5 were about 11-12 mm long and it is suggested that this is the size at which larval cod go through metamorphosis (Ellertsen et al. 1981, Chapter 3). In my earlier study (Chapter 3) I observed that when larval cod passed through metamorphosis, they did not fill their guts fully. Thorisson (1994) also observed an increased percentage of empty or half empty guts of larval cod during this stage. He also noticed that larval cod at this stage could be stressed easily. Thus it is possible that overnight starvation may have stressed the cod larvae during week 5, although I could not visually observe any stress symptoms. Although prey consumption rate did not increase linearly with larval length, about 65-85% of the variation could be explained by size when values from week 5 are removed from the data. My results also showed that attack rates increased while handling time decreased as the larvae grew. Changes in these parameters as the larvae grew indicate that larvae become more capable of attacking prey. Reduced handling time also allows the larvae to search and consume more prey. Several studies have shown that the predatory abilities of marine fish larvae increase with larval development (Drost 1987, Miller et al 1992, Gill and Hart 1996). These changes in foraging abilities are expressed as an increase in larval growth rates which subsequently are important determinants of larval survival (Houde

1987).

My results showed that the survival of larval cod increased as the prey concentrations increased asymptotically from 500 to 4000 prey L^{-1}. Most studies on marine larval fish dealing with prey concentration also showed similar trends (Laurence 1974, Houde 1977, 1978, Tsai 1991). Higher mortality rates in the two highest prey concentrations were not expected and I speculate that it could be due to the metabolites released by the *Artemia* (Katavić 1986) that was used as prey beyond 3 weeks. In my earlier study (Chapter 4), cod larvae reared in prey concentrations below 500 Prey L^{-1} did not survive beyond 3 weeks. Although I presented behavioural evidence that larvae reared in those lower prey concentrations did not forage compared to the larvae reared in higher prey concentrations, I could not present any direct evidence at that time. In this experiment, the detailed prey consumption data (Table 6.2) showed that the majority of the larvae fed with 250 and 500 prey L^{-1} had empty stomachs during the first week and even in the second week 30-40% larvae had empty guts. Ellertsen et al. (1981) showed that larval cod deplete their yolk-sac reserves by day 10-12 post-hatch at 6°C. Thus the cod larvae in my experiment, raised at 8°C, would have depleted the yolk reserve by day 8-9 post-hatch. Given that even those larvae that had prey in the guts by week 2 did not have more than one prey, most of these larvae would been in a weak condition after yolk depletion. Weak condition would lead to impaired behaviour which would have resulted in poor foraging thereafter.

In conclusion, larval cod prey consumption, related to prey concentration, data

fitted by Holling's type II functional response equation. Although larval cod improved their foraging abilities as they grew and developed, larval size alone described the variation well. Although estimates of Holling's parameters from behavioural observations and from the equation did not agree quantitatively, both showed similar patterns. Results from this experiment support the view that using behaviour in ecological studies should enhance our ability to understand more the dynamics of larval fish foraging and its relevance to growth and survival.

Chapter Seven : General Discussion

The experiments described in this thesis used behavioural observations on larval cod foraging to explain their growth and survival under various environmental conditions. The goal of these experiments was two-fold. One was to investigate how the larvae cope with various levels of a particular environmental variable in an ecological context. The second goal was directed to increase the growth and survival of cod larvae through metamorphosis, thus increasing the production of cod juveniles, in an aquaculture context. In each chapter, the results are discussed in the context of existing knowledge concerning larval ecology and culture. In this discussion, I will emphasize the importance of behavioural observations on larval rearing experiments and make suggestions for future experiments.

The experiment in chapter two describes the possible existence of differences in the response to varying light intensity between larval cod populations from two different geographic regions (Scotian Shelf; SS and Northeastern Grand Bank; NF). The results of this experiment showed that larval cod adapted well to the conditions prevailing at the time of first feeding. I suggest that the difference in response to the light intensity between the larvae from SS and NF was mainly due to the different spawning season rather than a pure latitudinal effect. I would have predicted that larvae from spring/summer spawning in SS would have performed better in high light conditions than in low light conditions. However, I could not carry out any experiments with larval cod from a spring/summer spawning population of SS. The question remains whether environment or genetics or

both is/are the driving force/s behind this observation. More studies could be possible involving both spawning populations of cod from SS and spring/summer spawning populations of NF at a molecular level.

The most interesting or controversial finding of the experiment in chapter two is that larval cod from Scotian Shelf (SS) did not survive beyond four weeks post-hatch at high light intensity. Behavioural observations suggested that larvae from SS reared in high light did not forage as efficiently as larvae reared in low light. If behaviourally they are not capable of capturing the prey, then the question which remains to be answered is what prevents them from doing so. Was high light intensity detrimental to the normal development of the larval eye from fall/winter spawning population of SS? I suggested in the discussion in Chapter two that SS larvae could have developed rod cells earlier than the larvae from NF thereby enabling them to feed efficiently under low light conditions. A future analysis of the histology of the eye of the cod larvae reared in low and high light could shed some light on these questions. Unfortunately the study could not be continued to answer these questions mainly due to the loss of the source of egg supply from Halifax and partly due to the restrictions imposed at that time on transferring live fish materials between different locations. Thus this area is open for future research.

The objective of the work described in Chapter three was to determine if behavioural development in cod larvae is influenced by prey concentration from hatching through metamorphosis. Earlier studies on larval cod foraging behaviour (Munk 1995, Skiftesvik 1992) failed to provide a complete picture due to the short duration of those

experiments. My results showed that foraging behaviour of larval cod varied with larval age/size and prey concentration. At high prey concentrations, frequencies of the foraging MAP's were higher from early in the developmental stage; thus these larvae had the ability to feed more than larvae reared in lower prey concentrations. This was shown by the higher encounter rates and capture success at higher prey concentrations. However, the development of foraging MAP's was not affected by the prey concentrations in all prey concentrations (500-4000 prey L^{-1}) tested. My results also showed that the time spent per orientation (one component of handling time) increased from day two in all prey concentrations but declined as the larvae grew. The timing of this decline was earlier in larvae reared in 4000 prey L^{-1}. This means larvae reared in higher prey concentration have an opportunity to spend more time searching for prey than larvae from lower prey concentrations.

In the experiment in Chapter three, I observed one foraging MAP which I termed 'pass' behaviour. During a 'pass', a larva orients and fixates on a prey item and moves toward the prey but does not bite, the larva then swims in another direction. Except for Leader (1994), this behaviour has never been reported in larval cod. Initially, larval cod had a low frequency of pass, however, from day 27 onward this increased. Generally, the incidence of pass was more frequent in larvae reared at 4000 prey L^{-1} than larvae from lower prey concentrations. The significance of this 'pass' behaviour is puzzling, but I speculate that the larval cod may 'choose' the best prey from the available prey items. In my experiment, I used two prey types beyond day 15 post-hatch. Furthermore, some prey

from the previous day also stayed in the tank (mainly in high prey concentrations), thus the larvae could encounter various prey sizes as well as types. Since I could not confirm which prey was 'passed', my suggestion is more speculative and needs further research to confirm.

Objectives of the experiments described in Chapter four were to see if 4000 prey L^{-1} was the optimal prey concentration to rear larval cod and to find out the mortality trend during the first feeding. Results of the experiment confirmed that the 4000 prey L^{-1} was the optimal prey concentration and at this prey level, growth and survival of the larval cod were at the maximum. At higher prey concentrations, larvae had two advantages compared to larvae from low prey concentrations. One was that more prey in the vicinity of the larvae enhanced foraging, and in turn larvae grew faster and by the second week larvae from the higher prey concentrations were significantly larger than larvae from lower prey concentrations. Secondly, the larger size at a given age provided an advantage in searching efficiency over the smaller larvae from lower prey concentrations. Similarly, several other studies have shown that foraging capabilities of marine fish larvae dramatically increase with the larval size (Blaxter 1986, Miller et al. 1992, Gill and Hart 1996).

Although larval growth among the three highest prey concentrations did not show any difference throughout the study period, survival was higher in 4000 prey L^{-1} compared to the 8000 and 16000 prey L^{-1} at the end of the experiment. I could not find any difference in the foraging behaviour among the larvae from these high prey concentrations,

and thus I could not explain the differences in survival behaviourally. However, other studies indicated that the excess use of *Artemia* nauplii in intensive rearing systems may cause problems as they release more metabolites and enrichments (*Artemia* nauplii were enriched with highly unsaturated fatty acids to increase their nutritional conditions) to the medium (Houde 1975, Gopalakrishnan 1976, Katavić 1986, Van der Wal and Nell 1986, Léger et al. 1986). It is speculation but cannot be ruled out.

My results also showed that initially the SGR values for larval cod were higher for the larvae reared in higher prey levels, but by week three the differences in SGR values among the larvae from high and low prey concentrations had diminished. Mortality rates were also higher in lower prey concentrations during the first two weeks post-hatch. However, from week three post-hatch no differences in mortality rates between low and high prey concentrations could be found. This raises the question whether a higher prey concentration should be maintained throughout the larval period or could it be reduced after an initial high level. Further research is needed to answer these questions arising from this experiment.

My earlier experiment (Chapter 2) on two populations of larval cod showed larval cod adapt to the prevailing natural conditions. Most Norwegian literature on larval cod and light intensity state that larvae forage well under very low light, as low as 1 lux. When the experiments described in Chapter five were carried out at light intensities as high as 2400 lux, larval cod (NF origin) grew and survived better at this higher light intensity. The highest survival (42%) and growth at 2400 lux light intensity indicate the light requirement

of larval cod cannot be generalized across the whole geographic range.

Higher growth and survival of larval cod of NF origin at higher light intensities could also be explained ecologically. Larval cod over the NF region hatch during the summer (Fahay 1983) and the light intensity during the summer in the upper oceanic layers of 5-40 m depth, ranges between 200-400 and10-30 μE m^{-2} (see Chapter 2). Anderson and de Young (1995) reported that cod larvae in the offshore and inshore areas of northeastern Newfoundland occupy the top 40 m (5-35 m). Thus, depending on the larval depth profile, it is possible that the larval cod of NF origin experience the light levels that I have used in my experiment.

The reason for the better growth and survival of larval cod reared in continuous light primarily depends on more time available for feeding. Since prey were available throughout a 24 hr period, it would result in higher feeding incidence and possibly enhance a larva's growth and survival. In nature, larval cod of NF origin generally experience 18 hour day light during summer (see Chapter 2). Several other studies have shown that marine larval fish exhibit an upward vertical migration during night (Wood 1971, Heath et al. 1988, Lough and Potter 1993). My earlier studies (Chapter 2) and experiments described in this chapter showed that although foraging abilities of larval cod is higher in high light, they still continue to feed in low light, as low as 8.5 lux. Thus, in nature, nights with moon/star light should provide enough light for the larvae to continue its feeding. My results also showed that differences in SGR values and mortality rates of larval cod from the light and photoperiod experiments decreased after week 3-4 post-hatch. This indicates

that photoperiod and light levels could be reduced to desirable levels beyond week 4.

The experiments described in Chapter six showed that larval cod exhibit a Type II functional response. My results also highlighted the discrepancies in the parameter values derived from the equation and direct behavioural observations. Behaviourally the terms attack rate and handling time have been used to describe various biological phenomena. However, in Holling's equation the parameter estimates represent a theoretical rate of attack. In a biological sense, predator's attack rate depends upon several components such as rate of prey encounter, the probability of a prey being attacked when encountered and the probability that attack will result in a capture. Any of these components could vary with prey concentration. The developmental response of the larval cod increased with larval size. Reduced handling time and improved foraging tactics of larger larvae enable them to consume more prey than smaller larvae. Studies on other marine larval fish species also showed a similar trend (Houde and Schekter 1980, Miller et al. 1992). Numerical response of larval cod showed a decelerating increase in survival with increasing prey concentration. Survival reached a maxima at 4000 prey L^{-1}.

In summary, my thesis emphasises the use of behavioural observations in larval studies. The results of the experiments described in this thesis add more depth to the existing literature of larval cod ecology and culture. In larviculture, more emphasis should be given to the consideration of prevailing natural conditions, when domesticating wild fish species, otherwise all the operations would not be operating at maximum capacity. Information from my thesis suggests that the light requirements of Atlantic larval cod

cannot be generalized across its geographical range. Thus, we should be more cautious when generalizing the ecological requirements of larval fish across a geographic region. Behavioural observations are also useful in explaining the variability in performance data (growth and survival). Results of the experiments in my thesis suggest that along with growth and survival data, behavioural observations could be a useful measure in understanding the adaptations of the larvae to a particular environment. Thus, the behaviourally based approach used in this thesis should contribute substantially to our understanding of the ecology and aquaculture of Atlantic cod larvae.

References

Abrams, P.A. 1990. The effects of adaptive behaviour on the type -2 functional response. Ecology. 71:877-885.

Altman, J., 1974. Observational study of behaviour: sampling methods. Behaviour. 91:449-459.

Anderson, J.T., and B. deYoung 1995. Application of a one-dimensional model to vertical distributions of cod eggs on the northeastern Newfoundland Shelf. Can. J. Fish. Aqua. Sci. 52:1978-1989.

Barahona-Fernades , M.H. 1979. Some effects of light intensity and photoperiod on the sea bass larvae (*Dicentrarchus labrax* (L.)) reared at the Centre Oceanologique de Bretagne. Aquaculture. 17:311-321.

Barlow, G.W. 1977. Modal action patterns. In: Sebeok TA (ed.) How Animals Communicate. Indiana University Press, Bloomington, p 98-134.

Batty, R.S. 1987. Effect of light intensity on activity and food searching of larval herring *Clupea harengus*: a laboratory study. Mar. Biol. 93:323-327.

Beacham, T.D., Withler, R.E., Murray, C.B. and L.W.Barner 1988. Variation in body size, morphology, egg size and biochemical genetics of pink salmon in British Columbia. Trans. Am. Fish. Soc. 117:109-126.

Blaxter, J.H.S. 1968. Light intensity, vision, and feeding in young plaice. J. Exp. Mar. Biol. Ecol. 2:293-307.

Blaxter, J.H.S. 1975. The role of light on the vertical migration of fish - a review. In: Evans GC, Bainbridge R, Rackham O, (eds.) Light as an ecological factor: II. Blackwell Scientific Publications, Oxford. pp. 189-210.

Blaxter, J.H.S., 1980. Vision and the feeding of fishes. In: Bardach, J.E., J.J.Magnuson, R.C.May and J.M.Reinhart (eds.) Fish behaviour and its use in capture and culture of fishes. ICLARM conference proceedings, ICLARM, Manila, Philippines. pp. 32-56.

Blaxter, J.H.S. 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. Trans. Am. Fish. Soc. 115:98-114.

Blaxter, J.H.S. 1988. Pattern and variety in development. In: Fish physiology. Vol. XIA, (Hoar, W.S. & Randall, D.J., eds.) Academic Press, London. pp. 1-58.

Blaxter, J.H.S., and R.S.Batty 1990. Herring behaviour in the light and dark. In: Herring PJ, Campbell AK, Whitfield M, Maddock L (eds.) Light and life in the sea. Cambridge University Press, Cambridge, England. p 209-220.

Blaxter, J.H.S., and G.Hempel 1963. The influence of egg size on herring larvae (*Clupea harengus* L.). J. Cons. Int. Explore. Mer. 28:211-240.

Blaxter, J.H.S., and M.Staines 1970. Pure-cone retinae and retinomotor responses in larval teleosts. J. Mar. Biol. Asso. UK. 50: 449-460.

Blaxter, J.H.S. and Staines, M.E. 1971. Food searching potential in marine fish larvae. In Forth European Marine Biology Symposium. (Crips, D.J., ed.), Cambridge University Press, Cambridge. pp. 467-485.

Blom, G., Otterå, H., Svåsand, T., Kristiansen, T.S. and B.Serigastad., 1991. The relationship between feeding conditions and production of cod fry (*Gadus morhua* L.) In a semi-enclosed marine ecosystem in western Norway, illustrated by use of a consumption model. Int. Counc. Explor. Sea (ICES) Mar. Sci. Symp., 192:176-189.

Blom, G., Svåsand, T., Jørstad, K.E., Otterå, H., Paulsen, O.I., and J.C.Holm. 1994. Comparative survival and growth of two strains of Atlantic cod larvae (*Gadus morhua*) through the early life stages in a marine pond. Can. J. Fish. Aquat. Sci., 51:1012-1023.

Bolla, S., and I. Holmefjord. 1988. Effect of temperature and light on development of Atlantic halibut larvae. Aquaculture. 74:355-358.

Brancheck, T. 1984. The development of photoreceptors in the zebrafish *Brachydanio rerio*. II. Function. J. Comp. Neuro. 224:116-122.

Brander, K., and P.C.F.Hurley 1992. Distribution of early-stage Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and witch flounder (*Glyptocephalus cynoglossus*) eggs on the Scotian Shelf; a reappraisal of evidence on the coupling of cod spawning and plankton production. Can. J. Fish. Aquat. Sci. 49: 238-251.

Browman, H.I. and W.J.O'Brien 1992a. The ontogeny of search behaviour in white

carappie (*Pomoxis annularis*). Environmental Biology Fishes. 34:1-15.

Browman, H.I. and W.J.O'Brien 1992b. Foraging and prey search behaviour of golden shiner (*Notemigonus crysoleucas*) larvae. Can. J. Fish. Aquat. Sci. 49: 813-819.

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

Buckley, L.J., Halavik, T.A., Smigielski, A.S. and G.C.Laurence 1987. Growth and survival of the larvae of three species of temperate marine fishes reared at discrete prey densities. Am. Fish. Soc. Symp. 2:82-92.

Buttle, I.G., Uglow, R.F. and I.G.Cowx. 1995. The effect of diet and photoperiod on ammonia efflux rates of the African catfish, *Clarias gariepinus* (Burchell, 1822). Aquaculture Research. 26:895-900.

Castro, L.R., and R.K.Cowen 1991. Environmental factors affecting the early life history of bay anchovy *Anchoa* mitchilli in Great South Bay, New York. Mar. Ecol. Prog. ser. 76:235-247.

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

Chesney, Jr. E.J. 1989. Estimating the food requirements of striped bass larvae *Marone saxatilis*: effects of light, turbidity and turbulence. Mar. Ecol. Prog. Ser. 53:191-200.

Checkley, D.M., 1984. Relation of growth to ingestion for larvae of Atlantic herring *Clupea harengus* and other fish. Mar. Ecol. Prog. Ser. 18:215-224.

Cone, R.S. 1989. The need to reconsider the use of condition indices in fishery science. Trans. Am. Fish. Soc. 118:510-514.

Confer, J.L., Howick, G.L., Corzette, M.H., Kramer, S.L., Fitzgibbon, S. and R.Landesberg.1978. Visual predation by planktivores. Oikos. 31:27-37.

Croy, M.I. and R.N.Hughes 1991. The role of learning and memory in the feeding behaviour of the fifteen-spined stickleback, *Spinachia spinachia*. Animal Behaviour. 41, 149-160.

Cowan, J.H.Jr and E.D.Houde 1990. Growth and survival of bay anchovy *Anchoa mitchilli* in mesocosm enclosures. Mar. Ecol. Prog. Ser., 68:47-57.

Cross, T.F. and R.H.Payne 1978. Geographic variation in cod, *Gadus morhua*, off eastern North America: a biochemical systematics approach. J. Fish. Res. Bd. Can. 35:117-123.

Cushing, D.H., 1972. The production cycle and the numbers of marine fish. Symp. Zool. Soc., London. 29:213-232.

Cushing, D.H., 1983. Are fish larvae too dilute to affect the density of their food organisms? J. Plank. Res., 5:847-854.

Cushing, D.H. and J.W.Horwood. 1994. The growth and death of fish larvae. J. Plank. Res., 16(3):291-300.

Dowd, C.E. and E.D.Houde 1980. Combined effects of prey concentration and photoperiod on survival and growth of larval sea bream, *Archosargus rhomboidalis* (Sparidae). Mar. Ecol. Prog. Ser., 3:181-185.

Drost, M.R. 1987. Relation between aiming and catch success in larval fish. Can. J. Fish. Aquat. Sci., 44:304-315.

Duray, M. and H.Kohno 1988. Effects of continuous lighting on growth and survival of first-feeding larval rabbitfish *Siganus guttatus*. Aquaculture. 72:73-79.

Ellertsen, B., Moksness, E., Solemda,l P., Strømme, T., Tilseth, S., Westgård, T., and V.Øiestad 1980. Some biological aspects of cod larvae (*Gadus morhua* L.). Fisk. DirSkr. Ser. HavUnders. 17:29-47.

Ellertsen, B., Monksness, E., Solemdal, P., Tilseth, S., Westgård, T. and V.Øiestad., 1981. Growth and survival of cod larvae in an enclosure experiment and a mathematical model. Rapp. P.-v. Réun. Cons. int. Explor. Mer., 178:45-57.

Ellertsen, B., Fossum, P., Solemdal, P., and S.Sundby. 1989. Relation between temperature and survival of eggs and first feeding larvae of northeast Arctic cod (*Gadus morhua* L.). Rapp. P.-v. Réun. Cons. int. Explor. Mer., 191:209-219.

Fahay, M.P. 1983. Guide to the early stages of marine fishes occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf. Journal of Northwest Atlantic Fishery Science. Vol. 4:1-437.

Ferguson, G.W., and L.G.Talent 1993. Life-history traits of the lizard *Sceloporus undulates* from two populations raised in a common laboratory environment. Oecologia. 93:88-94.

Fleming, I.A., and M.R.Gross 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology. 71:1- 11.

Folkvord, A. and J.R.Hunter 1986. Size specific vulnerability of Northern anchovy, *Engraulis mordax*, larvae to predation by fishes. Fish. Bull. 84(4):859-869.

Fossum, P. 1983. Digestion rate of food particles in the gut of larval herring (*Clupea harengus* L.). FiskerDir. Skr. Ser. Havunders. 17:347-357.

Fossum, P. and B.Ellertsen 1994. Gut content analysis of first-feeding cod larvae (*Gadus morhua* L.) Sampled at Lofoten, Norway, 1979-1986. ICES Mar. Sci. Symp., 198:430-437.

Fox, L.R. and W.W. Murdoch. 1978. Effects of feeding history on short-term and long-term functional response in *Notonecta hoffmanni*. J. Anim. Ecol. 47:945-959.

Frank, K.T. 1988. Independent distributions of fish larvae and their prey: natural paradox or sampling artifact. Can. J. Fish. Aquat. Sci. 40:754-762.

Frank, K.T. and W.C.Leggett 1986. Effect of prey abundance and size on the growth and survival of larval fish: an experimental study employing large volume enclosures. Mar. Ecol. Prog. Ser. 34:11-22.

Gallis, F. 1990. Ecological and morphological aspects of changes in food uptake through the ontogeny of *Haaplochromis piceatus*. In Behavioural mechanisms of food selection. (Hughes, R.N., ed.), Springer-Verlag, Berlin. pp. 281-302.

Gamble, J.C. and E.D.Houde 1984. Growth, mortality and feeding of cod (*Gadus morhua* L.) larvae in enclosed water columns and in laboratory tanks. In The propagation of cod *Gadus morhua* L.. (Dahl, E., Danielssen, D.S., Moksness, E. & Solemdal, P., eds.), Flødevigen Rapportserie 1. pp. 123-143.

Gill, A.B., and P.J.B.Hart 1996. How feeding performance and energy intake change with a small increase in the body size of the three-spined stickleback. J. Fish Biol., 48:878-890.

Gopalakrishnan, K., 1976. Larval rearing of red shrimp *Penaeus marginatus* (Creustacea).

Aquaculture. 9:145-154.

Goshorn, D.M. and C.E.Epifanio 1991. Development, survival, and growth of larval weakfish at different prey abundances. Trans. Am. Fish. Soc., 120:693-700.

Gotceitas, V., Puvanendran, V., Leader, L.L. and J.A.Brown 1996. An experimental investigation of the 'match/mismatch' hypothesis using larval Atlantic cod. Mar. Ecol. Prog. Ser. 130:29-37.

Gulbrandsen, J. 1991 Functional response of Atlantic halibut larvae related to prey density and distribution. Aquaculture. 94: 89-98.

Hart, P.R., Hutchinson, W.G. and G.J.Purser 1996. Effects of photoperiod, temperature and salinity on hatchery-reared larvae of the greenback flounder (*Rhombosolea tapirina* Günther, 1862). Aquaculture. 144:303-311.

Hassell, M.P., Lawton, J.H. and J.R.Beddington. 1977. Sigmoid functional responses by invertebrate predators and parasitoids. J. Anim. Ecol. 46:249-262.

Hay, D.E., 1981. Effects of capture and fixation on gut contents and body size of Pacific herring larvae. Rapp. P.-v. Réun. Cons. int. Explor. Mer., 178:395-400.

Heath, M.R. 1992. Field investigations of the early life stages of marine fish. Adv. Mar. Biol. 28:1-174.

Heath, M.R., Henderson, E.W. and D.L.Baird 1988. Vertical distribution of herring larvae in relation to physical mixing and illumination. Mar. Ecol. Prog. Ser. 47:211-228.

Hobson, E.S., McFarland, W.N., and J.R.Chess 1981. Crepuscular and nocturnal activities of California near shore fishes, with consideration of their scotopic visual pigments and the photic environment. Fish. Bull. 79:1-30.

Holling, C.S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Ent. Soc. Can. 45:60 pp.

Holling, C.S. 1966. The functional response of invertebrate predators to prey densitity. Mem. Ent. Soc. Can. 48:86 pp.

Houde, E.D. 1973. Some recent advances and unsolved problems in the culture of marine fish larvae. Prog. World Maricult. Soc. 3:83-112.

Houde, E.D., 1975. Effects of stocking density and food density on survival, growth and yield of laboratory-reared larvae of sea bream *Archosargus rhomboidalis* (L.) (Sparidae). J. Fish Biol., 7:115-127.

Houde, E.D. 1977. Food concentration and stocking density effects on survival and growth of laboratory-reared larvae of bay anchovy, *Anchoa mitchilli* and lined sole *Achirus lineatus*. Mar. Biol. 48, 333-341.

Houde, E.D., 1978. Critical food concentration for larvae of three species of subtropical marine fishes. Bull. Mar. Sci., 28(3):395-411.

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

Houde, E.D. 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish. Bull. 87:471-495.

Houde, E.D. and R.C.Schekter 1980. Feeding by marine fish larvae: developmental and functional responses. Env. Biol. Fish. 5(4):315-334.

Huffaker, C.B., van de Vrie, M. and J.A.McMurtry. 1969. The ecology of tetranychid mites and their natural control. A. Rev. Ent. 14:125-174.

Hunt von Herbing, H. and R.G. Boutilier. 1996. Activity and metabolism of larval Atlantic cod (*Gadus morhua*) from Scotian Shelf and Newfoundland source populations Mar. Biol. 226:607-617.

Hunter, R.J., 1972. Swimming and feeding behaviour of larval anchovy *Engraulis mordax*. Fish. Bull., 70(3):821-838.

Hunter, J.R. 1981. Feeding ecology and predation of marine fish larvae. In: R.Lasker (ed.), Marine fish larvae: morphology, ecology and relation to fisheries. Washington Sea Grant Program, Seattle, WA. p. 33-77.

Huse, I. 1994. Feeding at different illumination levels in larvae of three marine teleost species: cod, *Gadus morhua* L., plaice, *Pleuronectes platessa* L., and turbot, *Scophthalmus maximus* (L.). Aquaculture and Fisheries Management. 25:687-695.

Johnson, R.A. and D.W.Wichren 1992. Applied Multivariate Statistical Analysis. Prentice-Hall Inc. Englewood Cliffs, N.J., 594 pp.

Katavić, I., 1986. Diet involvement in mass mortality of sea bass (*Dicentrarchus labrax*) larvae. Aquaculture. 58:45-54.

Kendall Jr., A.W., Incze, L.S., Ortner, P.B., Cummings, S.R. and P.K.Brown. 1994. Factors relating to the vertical distribution of eggs and larvae of walleye pollock (*Theragra chalcogramma*) in Shelikof Strait, Gulf of Alaska. Fish. Bull. US. 92:540-554.

Kiyono, M. and R.Hirano 1981. Effects of light on the feeding and growth of black porgy *Mylio macrocephalus* (Basilewsky), post larvae and juveniles. Rapp. P-v. Réun. Cons. int. Explor. Mer. 178:334-336.

Kjorsvik, E., Van der Meeren, T., Kryvi, H., Arnfinnson, J. and P.G.Kvenseth 1991. Early development of the digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation. J. Fish Biol. 38, 1-15.

Koslow, J.A., Brault, S., Foirnier, R.O. and Hughes, P., 1985. Condition of larval cod (*Gadus morhua*) off southwest Nova Scotia in 1983 in relation to plankton abundance and temperature. Mar. Biol., 86:113-121.

Kvenseth, P.G., and V.Øiestad 1984. Large scale rearing of cod fry on the natural food production in an enclosed pond. In: E.Dahl, D.S.Daniellsen, E.Moksness, and P.Solemdal (Eds.), The propagation of cod *Gadus morhua* L., Flød. Rapp. 1, pp. 645-655.

Lagomarsino, I.V. and D.O.Conover 1993. Variation in the environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. Evolution. 47(2):487-494.

Lasker, R. 1978. The relation between oceanographic conditions and larval anchovy food in the California current: identification of factors contributing to recruitment failure. Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 173:212-230.

Last, J.M. 1978. The food of three species of gadoid larvae in the eastern English Channel and southern North Sea. Mar. Biol., 48:377-386.

Laurence, G.C., 1972. Comperative swimming abilities of fed and starved larval largemouth bass (*Micropterus salmoides*). J. Fish Biol., 4:73-78.

Laurence, G.C. 1974. Growth and survival of haddock (*Melanogrammus aeglefinus*) larvae in relation to planktonic prey concentration. J. Fish. Res. Bd. Can..

31:1415-1419.

Laurence, G.C. 1977. A bioenergetic model for analysis of feeding and survival potential of winter flounder, *pseudopleuronectes americanus*, larvae during the period from hatching to metamorphosis. Fish. Bull. 75(3):529-546.

Laurence, G.C., 1978. Comparative growth, respiration and delayed feeding abilities of larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) as influenced by temperature during laboratory studies. Mar. Biol. 50:1-7.

Laurence, G.C., Smigielski, A.S., Halavik, T.A. and B.R.Burns 1981. Implications of direct competition between larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in laboratory growth and survival studies at different food densities. Rapp.P.-v. Réun. Cons. Int. Explor. Mer. 178:304-311.

Leader, L.L. 1994. To feed to not to feed: the ontogeny of foraging and predator evidence trade-offs in larval cod (*Gadus morhua*). M.Sc. Thesis. Department of Biology, Memorial University of Newfoundland, Canada. 139 pp.

Léger, P., Bengtson, D.A., Simpson, K.L. and P.Sorgeloos 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev. 24:521-623.

Levine, J.S. and E.F.McNichol 1982. Colour vision in fishes. Sci. Amer. 246:140-149.

Longstaff, B.C. 1980. The functional response of a predatory mite and the nature of the attack rate. Aust. J. Ecol. 5:151-158.

Lonsdale, D.J. and J.S. Levinton 1985. Latitudinal differentiation in copepod growth: an adaptation to temperature. Ecology. 66:1397-1407.

Lough, R.G. and D.C.Potter 1993. Vertical distribution patterns and diel migrations of larval and juvenile haddock *Melanogrammus aeglefinus* and Atlantic cod *Gadus morhua* on Georges bank. Fish. Bull. 91:281-303.

MacKenzie, B.R. and T.Kiørboe 1995. Encounter rates and swimming behaviour of pause -travel and cruise larval fish predators in calm and turbulent laboratory environments. Limnol. Oceanogr. 40(7), 1278-1289.

Margulies, D., 1993. Assessment of the nutritional condition of larval and early juvenile tuna and Spanish mackerel (Pisces: Scombridae) in Panama Bight. Mar. Biol., 115:317-330.

Mathias, A., Chivers, D.P, and R.J.F.Smith 1993. Population differences in response of fathead minnows (*Pimephales promelas*) to visual and chemical stimuli from predators. Ethology. 93:31-40.

McFarland, W.N. 1986. Light in the sea-Correlations with Behaviours of Fishes and Invertebrates. Am. Zool. 26:389-401.

McGurk, M.D. 1985. Effects of net capture on the post preservation morphometry, dry weight, and condition factor of Pacific herring larvae. Trans. Am. Fish. Soc. 114:348-356.

McLaren, I.A. and P.Avendaño 1995. Prey field and diet of larval cod on Western Bank, Scotian Shelf. Can. J. Fish. Aquat. Sci. 52: 448-463.

McKenzie, R.A. 1940. Nova Scotian autumn cod spawning. J. Fish. Res. Bd. Can. 5(2):105-120.

Miller, T.J., Crowder, L.B., Rice, J.A. and F.P.Binkowski 1992. Body size and the ontogeny of the functional response in fishes. Can. J. Fish. Aquat. Sci. 49:805-812.

Miller, T.J., Herra, T. and W.C.Leggett 1993. An individual-based analysis of the variability of eggs and their newly hatched larvae of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. Can. J. Fish. Aquat. Sci. 52:1083-1093.

Millis, E.L., Confer, J.L. and R.C.Redady. 1984. Prey selectivity by young yellow perch: the influence of capture success, visual acuity, and prey choice. Trans. Am. Fish. Soc. 113:579-587.

Mori, H. and D.A.Chant. 1966. The influence of prey density, relative humidity, and starvation on the predacious behaviour of *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) Can. J. Zool. 44:483-491.

Munk, P., 1995. Foraging behaviour of larval cod (*Gadus morhua*) influenced by prey density and hunger. Mar. Biol. 122(2): 205-212.

Munk, P. and T.Kiørboe 1985. Feeding behaviour and swimming activity of larval herring (*Clupea harengus*) in relation to density of copepod nauplii. Mar. Ecol. Prog. Ser. 24:15-21.

Munk, P. and T.Kiørboe 1986. Feeding and growth of larval herring, *Clupea harengus*, in relation to density of copepod nauplii. Envt. Biol. Fish. 17(2):133-139.

Munz, F.W. 1958. Photosensitive pigments from the retinae of certain deep-sea fishes. J. Physiol. 140:220-225.

Munz, F.W. 1975. Behavioural studies of vision in a fish and possible relationships to the environment. In: Ali AM Vision in fishes: new approaches in research. Plenum Press, New York. pp 705-717.

Munz, F.W. and W.N.McFarland 1973. The significance of spectral position in the rhodopsins of tropical marine fishes. Vision Res. 13:1829-1874.

Murdoch, W.W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecol. Monog. 39:335-354.

Murdoch, W.W. 1971. The developmental response of predators to changes in prey density. Ecology. 52:132-137.

Murdoch, W.W. 1973. The functional response of predators. J. Appl. Ecol. 11:335-342.

Myers, R.A., Mertz, G. and C.A.Bishop 1993. Cod spawning in relation to physical an biological cycles of the northern North-west Atlantic. Fish. Oceanogr. 2:154-165.

Neave, D.A. 1984. The development of visual acuity in larval plaice (Pleuronectes platessa L.) and turbot (Scophthalmus maximus L.) J. Exp. Mar. Biol. Ecol. 78:167-175.

Nielsen, S.E. 1974. Light and primary production. pp 361-388. In: Jerlov NG, Nielsen SE (eds.) Optical aspects of oceanography. Academic Press, London. pp 361-388.

Noakes, D.L. and J-G.I.Godin 1988. Ontogeny of behaviour and concurrent development changes in sensory systems in teleost fishes. In Fish physiology, Vol. XIB. (Hoar, W.S. & Randall, D.J., eds.), Academic Press, London. pp. 345-395.

Nunny, L. 1985. The effect of long time delays in predator-prey systems. Theo. Pop. Biol. 27:202-221.

O'Connell, C.P. 1981. Development of organ systems in the northern anchovy *Engraulis mordax* and other teleosts. Am.Zool. 21:429-446.

Otterå, H., 1993. Feeding, growth and survival of Atlantic cod (*Gadus morhua*) larvae reared in replicate plastic enclosures. Can. J. Fish. Aquat. Sci. 50:913-924.

O'Reilly, J.E., Evans-Zetlin, C. and D.A.Busch 1987. Primary production. In: Backus RH

(ed.) Georges Bank. MIT press. Cambridge Mass. pp 220-233.

Pastorok, R.A. 1980. Prey vulnerability and size selection by *Chaoborus* larvae. Ecology. 62:1311-1324.

Pedersen, T., Eliassen, J.E., Eilertsen, K.S., Tande, K.S. and R.E.Olsen 1989. Feeding, growth, lipid composition, and survival of larval cod (*Gadus morhua* L.) In relation to environmental conditions in an enclosure at 70° in northern Norway. Rapp.P.-v. Réun. Cons. Int. Explor. Mer. 191:409-420.

Pogson, G.H., Mesa, K.A and R.G.Boutiler 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics, NY. 139:375-385.

Present, T.M.C. and D.O.Conover 1992. Physiological basis of latitudinal growth differences in *Menida menida*: variation in consumption or efficiency? Functional Ecol. 6:23-31.

Rahmann, H., Jeserich, G. and I.Zeutzius 1979. Ontogeny of visual acuity of rainbow trout under normal conditions and light deprivation. Behavior. 68:315-322

Ricker, W.E. 1972. Heredity and environmental factors affecting certain salmonoid populations. In: Simon RC, Larkin PA (eds.). The stock concept in Pacific salmon. University of British Columbia, Vancouver. pp 19-160.

Rosenthal, H. and G.Hempel 1970. Experimental studies in feeding requirements of herring larvae (*Clupea harengus*). In Marine food chains. (Steele, J.H.S., ed.). University of California Press, Berkeley, USA. pp. 344-364.

Ross, R.M. and T.W.H.Backman 1992. Larval American shad: Effects of age and group size on swimming and feeding behaviour. Trans. Am. Fish. Soc. 121:508-516.

SAS Institute 1988. SAS/STAT user guide, release 6.03 edn. SAS Institute, Inc., Cary, NC, USA.

Scott, W.B. and M.G.Scott 1988. Atlantic fishes of Canada. University of Toronto, Toronto.

Segal, E. 1970. Light-invertebrates. In: O.Kinne (ed.). Marine ecology, vol.1. Wiley-Interscience, London, UK. pp. 157-211.

Shand, J., Partrige, J.C., Archer, S.N., Potts, G,W, and J.N.Lythgoe 1988. Spectral absorbency changes in the violet/blue sensitive cones of juvenile pollack, *Pollachius pollachius*. J. Comp. Physiol. A. 163:699-703.

Shand, J. 1993. Changes in the spectral absorption of cone visual pigments during the settlement of the goatfish *Upeneus tragula*: the loss of red sensitivity as a benthic existence begins. J. Comp. Physiol. A. 173:115-121.

Shand, J. 1994. Changes in retinal structure during development and settlement of the goatfish *Upeneus tragula*. Brain Behav. Evol. 43(1):51-60.

Shuvayev, Y.D. 1978. Movements of some planctonic copepods. J. Hydrobiol., 14:32-36.

Skiftesvik, A.B. 1992. Changes in behaviour at onset of exogenous feeding in marine fish larvae. Can. J. Fish. Aqua. Sci. 49:1570-1572.

Skiftesvik, A.B. 1994. Impact of physical environment on the behaviour of cod larvae. ICES Mar. Sci. Symp. 198:646-653.

Skiftesvik, A.B. and I.Huse 1987. Behaviour studies of cod larvae, *Gadus morhua* L. Sarsia. 72:367-368.

Skiftesvik, A.B., Opstad, I., Bergh, Ø, Pittman, K. and L.H.Skjolddal.1990. Effects of light on the development, activity and mortality of halibut (*Hippoglossus hippoglossus* L.) yolk sac larvae. ICES C.M. 1990/f:43.

Smith, P.C. 1989. Seasonal and interannual variability of current, temperature and salinity off southwest Nova Scotia. Can. J. Fish. Aquat. Sci. 46(suppl.1):4-20.

Solberg, T.S. and S.Tilseth 1987. Variations in growth pattern among yolk-sac larvae of cod (*Gadus morhua* L.) due to differences in rearing temperature and light regime. Sarsia 72:347-349

Solomon, M.E. 1949. The natural control of animal populations. J. Anim. Ecol. 18:1-35.

Spitze, K. 1985. Functional response of an ambush predator: *Chaoborus americanus* predation on *Daphnia pulex*. Ecology. 66:938-949.

Suthers, I. and S.Sundby 1996. Role of the midnight sun: comperative growth of pelagic juvenile cod (*Gadus morhua*) from the Arcto-Norwegian and a Nova Scotian stock. ICES J. Mar. Sci. 53:827-836.

Tandler, A. and S.Helps 1985. The effects of photoperiod and water exchange rate on growth and survival of gilhead sea bream (*Sparus aurata*, Linnaeus; Sparidae) from hatching to metamorphosis in mass rearing systems. Aquaculture. 48:71-82.

Theilacker, G.H. 1986. Starvation induced mortality of young sea caught jack mackeral, *Trachurus symmetricus* determined with histological and morphological methods. Fish. Bull. 84:1-17.

Thorisson, K. 1994. Is metamorphosis a critical interval in the early life of marine fishes? Envt. Biol. Fish. 40:23-36.

Tilseth, S., 1990. New marine species for cold-water farming. Aquaculture. 85:235-245.

Tsai, C-F., 1991. Prey density requirements of the striped bass *Morone saxatilis* (Walbaum), larvae. Estuaries. 14(2):207-217.

Tucker, J.W., Jr., 1992. Feeding intensively-cultured marine fish larvae. In: G.L.Allen and W.Dall (Eds.), Proc. Aquaculture nutrition workshop, Salamander Bay. 15-17 April 1991. NSW Fisheries, Brackish water fish culture research station, Salamander Bay, Australia. pp 129-146.

van der Meeren, T. and T.Næss 1993. How does cod (*Gadus morhua*) cope with variability in feeding conditions during early larval stages? Mar. Biol. 116:637-647.

Van der Wall, E.J. and J.A.Nell 1986. Effect of food concentration on the survival and growth of Australian bass (*Macquaria novemaculeata*) larvae. Prog. Fish-Cult. 48:202-204.

Ware, D.M. 1972. Predation by rainbow trout (*Salmo gairdneri*): the influence of hunger, prey density and prey size. J. Fish. Res. Bd. Can. 29:1193-1201.

Welker, M.T., Pierce, C.L. and D.H.Wahl 1994. Growth and survival of larval fishes: roles of competition and zooplankton abundance. 1994. Trans. Am. Fish. Soc. 123:703-717.

Werner, R.G. and J.H.S.Blaxter 1980. Growth and survival of larval herring (*Clupea harengus*) in relation to prey density. Can. J.Fish. Aquat. Sci. 37:1063-1069.

Winkler, H. and C.P.Orellana 1992. Functional response of five cyprinid species to planktonic prey. Envt. Biol. Fish. 33:53-62.

Wood, R.J. 1971. Some observations on the vertical distribution of herring larvae. Rapp. P.-v. Réun. Cons. int. Explor. Mer. 160:60-64.

Wyatt, T. 1972. Some effects of food density on the growth and behaviour of plaice larvae. Mar. Biol. 14:210-216.

Yin, M.C. and J.H.S.Blaxter 1987. Feeding ability and survival during starvation of marine fish larvae reared in the laboratory. J. Exp. Mar. Biol. Ecol., 105:73-83.

Young, A.M. 1991. Temperature-salinity tolerance of two latitudinally separated populations of the long wrist hermit crab *Pagurus longicarpus* Say (Crustacea, Decapoda, Paguridae). Ophelia. 34(1):29-39.

Zeutzius, T. and H.Rahmann 1984. Influence of dark-rearing on the ontogenic development of *Arotherodon mossambicus* (Cichlidae, Teleostei): I. Effects on body weight, body growth pattern, swimming activity and visual acuity. Exp. Biol. 43:77-85.