THE EFFECTS OF PREY DENSITY AND DURATION OF PREY AVAILABILITY ON THE BEHAVIOUR, GROWTH AND SURVIVAL OF LARVAL ATLANTIC COD (Gadus morhua) AND FAT SNOOK (Centropomus parallelus)

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The effects of prey density and duration of prey availability on the behaviour, growth and survival of larval Atlantic cod (*Gadus morhua*) and fat snook (*Centropomus parallelus*)

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ABSTRACT

The main bottleneck to the mass production of juvenile seed stock for the intensive aquaculture of many marine finfish species is the high mortality associated with the larval stage. In an attempt to reduce the costs and increase larval survival for two species with aquaculture potential, experiments were performed to investigate ways of reducing the quantity of live food required.

The first experiment described in this thesis was performed in Brazil with fat snook (*Centropomus parallelus*) a species of commercial interest for which little is known about the natural history or culture techniques. Previous larviculture with this species has been done at prey densities of 30 000 prey litre. I tested a range from 30 000, to 5000 prey litre, and found no difference in survival and growth at any of these prey densities. Reducing prey densities to only 5000 prey litre will result in considerable savings in labour and cost for future culture of this species. The behaviour of the larvae is also described here for the first time. Fat snook larvae were observed to be saltatory foragers, and to employ a sigmoid (s-curve) and lunge prior to capture, until the larvae reach lengths of 3.0 mm.

The experiments with larval Atlantic cod (*Gadus morhua*) were more in depth since both the optimal prey density and foraging behaviour patterns were already known. The first experiment investigated the possibility of using a 'mismatch' (low prey density) at one of three stages (endogenous, transition from endogenous to exogenous, and exogenous) in the larval period. It was found that larval survival was maximized when a low prey density (500 prey litre was offered during the first 5 days post-hatch, and that growth and survival were maximized thereafter by offering a high prey density (4000 prey litre). The behavioural observations in this experiment did not concur with previous reports, which found that orient frequency increases with prey density. Comparisons of the methodologies between this and previous studies found that flow rates differed. Flow rates were found to modify the duration of prey availability. A second cod experiment investigated the effect of decreased duration of prey availability (controlled by flow rate) and found that survival and growth decreased with decreasing duration of prey

availability. The probable explanation for this decrease was that the larvae had less time to forage. Orient frequency of larvae that had less time to forage (high flow treatments) was 3 times higher immediately after feeding. Hourly behavioural observations demonstrated a sudden spike in orient frequency after feeding for larvae reared at high flow, which explains how larval cod are able to take advantage of prey patches that occur in their spatially and temporally variable environment.

Results of the cod experiments can be used to suggest a new larviculture protocol, which schedules low prey density during the endogenous feeding stage and then increases to high prey density thereafter. The results also demonstrate that a relationship between flow rate and duration of prey availability exists and that larval cod are sensitive to the duration of time that is available for foraging. Future larval husbandry practices should include the recording of a prey clearance curve for each tank at set flows, so that the prey dynamics of each tank can be understood. Consideration should be given to both water quality and prey clearance rates in the assignment of flow rates.

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PREFACE

Aquaculture is an extremely fast growing industry, one that has the potential to negatively impact the world's oceans and freshwater resources, just as agriculture has done in so many parts of the world over the last few centuries. Fortunately this "Blue Revolution" is occurring at a time when humankind's environmental consciousness is just beginning to blossom. There is no doubt that fish can be cultured much more efficiently than they can be harvested from the wild, and hopefully by integrating aquaculture with the high tech industry, we will be able to do so in a manner that is far more sustainable than was the "Green Revolution". It is for this reason that I decided to become involved in this field. I believe that the best way to help an industry grow in the direction that you feel it should (environmentally sustainable) is to direct it from within. The research I undertook in the preparation of this thesis was with the goal of making one little part of the industry more efficient, less expensive and less wasteful.

In memory of my late grandmother, Edythe Lytle, who taught me that all creatures on this earth must be treated equally no matter how small, or smart.

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CHAPTER 1

GENERAL INTRODUCTION

The major impediment to the large-scale production of juvenile seed stock for intensive aquaculture of many finfish species is the mass mortality associated with the larval stage (Stepien 1976; Houde and Schekter 1980; Cerqueira 1992; Puvanendran 1999). The high larval mortality of numerous commercially important marine species reflects the r-selected strategy of producing a large number of relatively small, undeveloped larvae with low individual energy investment (Hutchings 1997). At all life stages predation, starvation, disease and adverse environmental conditions threaten survival. These factors have been suggested to be the major source of mortality for the small relatively undeveloped and vulnerable larvae of many marine fish species (Ruzzante, *et al.* 1996). In the wild, the high mortality associated with the larval stage of marine fish has been attributed primarily to starvation and predation (Dekhnik, *et al.* 1970; Hunter 1972; Cushing 1976; Houde and Schekter 1978; Buckley, *et al.* 1987) that are not necessarily mutually exclusive. Research suggests that the probability of predation increases with the hunger state of larvae (Wyatt 1972; Heath 1992). Furthermore, since growth rate is directly related to the availability of food and the probability of predation decreases with size, increased food resources reduce the larval period and therefore the chances of predation mortality (Houde 1975; Duffy, *et al.* 1996). In laboratory rearing and large-scale culture conditions the effect of predation is reduced, yet mortality under these controlled conditions remains high.

The reasons for the high mortality in the laboratory in the absence of predation have been the focus for much research. Early in larval fish research, difficulties in obtaining good survival were thought to be related to a poor supply of appropriate prey items. Plankton had to be obtained from the wild (O'Connell and Raymond 1970; Houde 1975), which was unreliable and required a great deal of labour to obtain enough for large experiments, therefore keeping them small scale (Houde and Palko 1970). The high variability in zooplankton species and nutritional quality further complicated research efforts, since the composition of plankton blooms was constantly changing, making inter and intra-experiment comparisons difficult or impossible. Larval studies were greatly facilitated and intensified with the introduction of rotifers (*Brachionus* spp.) as a first feeding organism in the 1960's. This led to the first consistent success with intensive rearing of marine fish larvae: red sea bream (*Pagrus major*) reared in Japan on cultured rotifers (Lubzens, *et al.* 1989).

Rotifers are ideal as a first feeding organism for larval fish due to the availability of a variety of strains with different sizes and temperature tolerances. They are also euryhaline, and can be cultured using different food sources e.g. algae and yeast. Rotifers are thus swimming food capsules that can be enriched such that they provide the necessary nutritional requirements to the fish larvae (Lubzens, *et al.* 1989). Although their use is now ubiquitous and facilitates inter and intra-experiment comparisons, a drawback is that culture of rotifers require exceptionally good management to keep cultures clean and at peak production.

Since the introduction of rotifers (and even before), a large amount of research has been done to identify prey requirements for larval fish. The intent of these studies varies from identifying the impacts of larvae on zooplankton resources in the wild (Dekhnik, *et al.* 1970; Cushing 1983; Kvenseth and Oeiestad 1984), to estimating the year class strength for fish stock assessment (Laurence 1974; Frank and Leggett 1986; Fossum 1988; Duffy, *et al.* 1996), to determining the food intake of larval fish (Boehlert and Yoklavich 1984; Thompson and Harrop 1991; Karamushko and Reshetnikov 1994), and most recently identifying optimal prey densities for the mass rearing of larval fish (Houde 1978; Werner and Blaxter 1980; Ringo, *et al.* 1991; Melard, *et al.* 1996; Puvanendran and Brown 1999). As a result, it is recognized that the quantity of prey required for growth and survival is highly variable and is dependent on fish species, temperature, larval size, foraging methods, prey type, and larval growth rates, as well as other factors that may yet be identified.

A fundamental theory that must be acknowledged in a discussion of prey density is the work of Holling (1965), which can be applied to describe the relationship between prey consumption rate and prey density, which he termed a functional response. Research by Houde and Schekter (1980) addressed both the functional response and a developmental response (the change in prey consumption relative to larval growth) for the larvae of three marine fish species. An asymptote was predicted in prey consumption rates, such that no further increase in consumption is expected with increased prey density, however, Houde and Shekter (1980) did not find this point in their work with lined sole, sea bream and bay anchovy. For commercial scale production identifying the

level at which further increases in prey density do not increase prey consumption and therefore growth or survival will result in efficient production of larvae.

The experiments described in this thesis were designed to test methods that might reduce the quantity of rotifers used in the larviculture of fat snook (*Centropomus parallelus*) and Atlantic cod (*Gadus morhua*). The objective was to minimize prey requirements while maximizing growth and survival, therefore increasing the overall efficiency of the process and reducing waste and cost. The question addressed in these experiments is do larvae really need a high prey density prior to metamorphosis? The prediction is, that because larvae have evolved in an environment that has both temporal and spatial heterogeneity in food resources, they will be adapted to deal with food shortages (most likely in the early stages before the yolk sac is exhausted and before they have become effective predators), and that they will be able to take advantage of prey patches when they are encountered. The experiment with fat snook took place at the Marine Fish Laboratory of the Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil, and was the first of its kind for this species. The work on Atlantic cod builds on previous findings at the Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Chapter two provides a background for the larviculture of fat snook and presents the results of an experiment that investigated the effect of prey density on the growth, survival and behaviour of larval fat snook. Chapter three presents the background information for the Atlantic cod research and describes the results of a 'match mismatch' experiment that investigated the effect of lowering the prey density offered during each

of three stages of larval development. A comparison of the results of the experiment in chapter three with previous research at the Ocean Sciences Centre, led to the discovery of an effect of flow rate of water through the tanks on the growth, survival and behaviour of Atlantic cod larvae. The effect of flow rate on the growth, survival and behaviour of Atlantic cod larvae is investigated in chapter four. Finally, chapter five gives a brief summary of the findings made in these experiments, draws some comparisons between the results found in the cod experiments, and looks at future directions for larval research.

Chapter 2

Defining prey density requirements for larval fat snook (*Centropomus parallelus*)

2.1 Introduction

2.1.1 Natural history of fat snook larvae

Fat snook (*Centropomus parallelus* Poey, 1860) have a range that extends from the southern tip of Florida to the southern extreme of the State of Santa Catarina, Brazil (Rivas 1986; Froese and Pauly 2000). This species is believed to form large spawning congregations from December through April along the southern coastline of Brazil (according to communications with local fishers, and descriptions for its close relative *C. undecimalis*), and they are described as group synchronous batch spawners by Rivas (1986). Females release several large (100 000 eggs per kilogram adult mass) batches of tiny (0.6 – 0.7 mm) eggs that float in the upper region of the water column.

At temperatures of 25°C eggs hatch in approximately 15 hours after being released (personal observation). The larvae hatch without eyes and the mouth and anus are closed (Fig. 2.1). The newly hatched larvae are completely dependent on their endogenous reserves (yolk sac) for the first few days of life (Morrison 1993). The eyes develop within 32 hours at which pigmentation of the retinae begins. By day two or three, the larval jaw begins development, the mouth opens and the digestive tract expands, and pigmentation of the eyes is nearly complete. Feeding commences approximately three days post-hatch, on micro-zooplankton that are most likely less than 100 µm in width since at this time the larvae are only about 2.7 mm in total length.

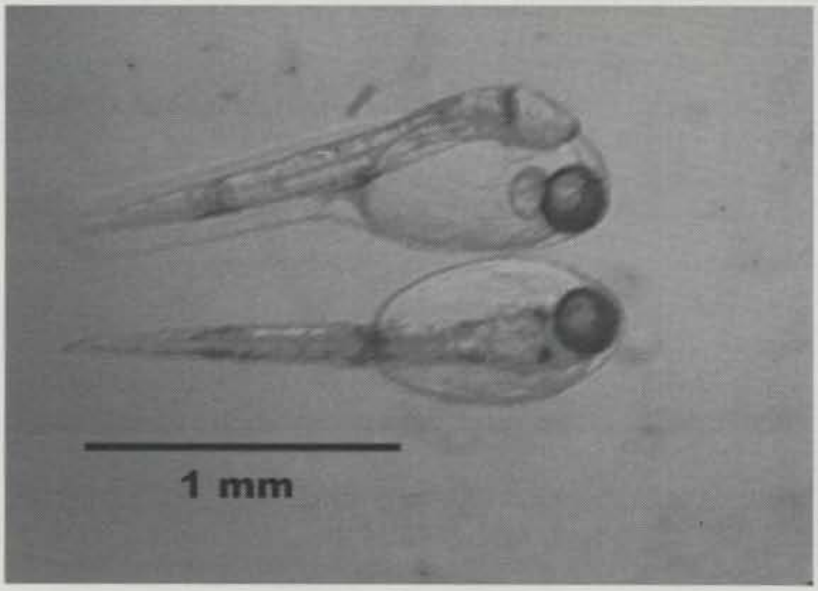


Fig. 2.1. Digital image of fat snook larvae at hatch.

Day five marks the commencement of swim bladder inflation, which requires that the larvae swim to the surface and gulp air to initially inflate their bladders. These visually guided zooplanktivorous carnivores spend approximately 45 days as larvae in the ocean and then move into rivers, estuarine and mangrove habitats as juveniles (Cerqueira 1992).

2.1.2 History of Fat snook aquaculture

Fat snook have been cultured for over 300 years in extensive grow-out ponds in the northeast of Brazil (Cerqueira 1995). Commonly called robalo in Brazil, fat snook has been recognized as a candidate species for aquaculture, because of its: fast growth, high survival, and euryhaline habits. The white flesh is also favoured in the culinary industry (Roberts 1989; Cerqueira 1992; Cerqueira 1995; Boujard, *et al.* 1997). In the wild and at small sizes, *C. parallelus* is commonly mistaken for the much larger conspecific common snook (*C. undecimalis*) by sport fishers, which makes robalo an excellent candidate for the growing number of "pay to fish ponds" in Brazil.

It has only been in the past couple of decades that research has focused on the intensification of culture techniques for fat snook, and only at a handful of locations. The main obstacle has been the inability to consistently produce the large number of juveniles required to make on-growing facilities for this species viable (Cerqueira 1995).

2.1.3 Larviculture work on fat snook to date

As with many r-selected marine fish species (with many small eggs), the small and fragile larval stage has presented a bottleneck to the production of juveniles (Stepien 1976; Houde and Schekter 1980; Dhert, *et al*. 1998). The difficulty has been in obtaining consistent and predictable survival through the first feeding stage up to metamorphosis. Selecting appropriate prey types, sizes and concentrations is vital with respect to the survival and growth of small marine larvae (van Der Meeren and Naess 1993). With the introduction of rotifers (*Brachionus plicatilus*) as a first feeding organism in the late sixties (Lubzens, *et al*. 1989) much higher and more consistent survival was achieved in the larviculture of numerous marine species including fat snook.

Cerqueira (1992) reported the first successful induction of a female fat snook using Human Chorionic Gonadotropin hormone (HCG). Since then research at the Marine Fish Laboratory, Federal University of Santa Catarina (LAPMAR, UFSC) has examined various issues including: rearing salinity concentrations, lighting, and nutritional quality of food supply for larval fat snook, attractants used in pelleted food, and diet composition for juveniles, and various details concerning induced spawning such as oocyte size as an indicator of female ripeness and use of luteinizing hormone releasing hormone analog (LHRHa) as a liquid injection or a slow release pellet. However, to date survival to metamorphosis has been low, and insufficient seed stock has been produced to support viable commercial grow out operations. As part of the ongoing research at LAPMAR and financed by funding from the Canadian International Development

Agency (CIDA) provided through the Brazilian Mariculture Linkage Program (BMLP). this research project was set up as a technology transfer between Brazil and Canada.

After assessing the larviculture protocols at LAPMAR, two areas were chosen as potential areas for improvement. Previous larviculture at the laboratory had been performed in near static systems (< 20 % exchange per day) with new water being added when algae and rotifers were added to the tanks (Seiffert, et al. 2001; Lajonchere 2001). It was felt that given the high temperatures and the high densities of prey offered to the larva that water quality would degrade rapidly. Therefore, experiments would be carried out in a flow through system, later to be compared in an experiment investigating the effect of flow. The high prey densities used at LAPMAR were also higher than those reported for other species, and it was believed that reducing the quantity of rotifers required could create considerable savings in labour and cost, without compromising production, as measured by survival and growth.

This chapter details the findings of one experiment that was planned as a preliminary investigation into the minimal rotifer requirements of larval fat snook. The objective was to determine the lower limit of rotifer requirements for larval fat snook. It was expected that the density of rotifers in the larval tanks could be reduced to densities similar to those used in the larviculture of other marine finfish (<10 prey ml).

2.2 Materials and Methods

2.2.1 Husbandry and General Methodology

Mature (3-4 year old) cultured fat snook broodstock were relocated from $8m^3$ on-growing pens to $10m^3$ black concrete spawning tanks. Spawning tanks were indoors under natural light and were approximately 1m deep with a gravel bottom. The broodstock used in this study were raised to maturity from previous spawnings at LAPMAR. These fish had been fed a commercial pelleted diet (Supra Salmonideos), however, they were put on a laboratory prepared diet prior to spawning (Table 2.1.).

Table 2.1. Ingredient list with percentage composition for Marine Fish Laboratory prepared breeder diet for fat snook.

Ingredients	Composition (%)
Fish Meal	45.0
Fish	24.5
Squid	15.0
Shrimp	15.0
Vitamin Premix	0.4
Vitamin C	0.1

One female (720 g, 405 mm), chosen based on ripeness of the ovaries (mean oocyte diameter greater than 0.34 mm), was injected (inter-muscularly) with 50 µg kg LHRHa to induce egg oocyte release. Two males were induced to release milt by using 25µg kg LHRHa and were released back into the tank. Fertilized eggs were released approximately 36 hours after induction (at 22.5°C). Release of eggs generally takes place in the early morning, between midnight and sunrise.

The buoyant eggs were retained in an overflow collector attached to the outflow of the spawning tank. After 12 hours, more than 90% of the eggs had been collected and they were moved to a 100 litre cylindro-conical fiberglass incubation tank. Temperature was 23.5°C, with light aeration and low water exchange. eggs hatched after approximately 15 hours. Within 12 hours of commencement of hatching, 80% of the eggs had hatched, and the larvae were transferred to 12, black, 140 litre cylindrical fiberglass treatment tanks at an initial stocking density of 27 larvae per litre.

Treatment tanks were maintained at 26.0 ± 1.5°C using 100 watt aquarium heaters connected to a central thermostatic control. From one to five days post-hatch (dph) tanks remained static with no water exchange except for the overflow caused by the addition of algae and rotifers. Zero water exchange for the first five days is necessary due to the relatively undeveloped state of the larvae, which are unable to swim against even the slightest current. Starting on day five, each tank received a constant inflow of 194 ± 20 ml per minute (2 exchanges per day) of filtered (sand-filter) seawater. Approximately 1000 ± 250 lux of light was provided to each tank 24 hours per day by two 40-watt fluorescent bulbs. Tank water was greened twice daily starting on day 2 with *Nannochloropsis oculata* algae, grown in 3000 litre semi-continuous outdoor cultures.

Treatment tanks were equipped with a surface skimmer to remove oily deposits from the surface. Care was taken to ensure that the surface skimmers were functioning properly and cleaned regularly during swim bladder inflation (5 - 9 dph). Each tank had three small aquarium style porous air-stones through which a low airflow was maintained to keep the prey well mixed throughout the tank. One of these air-stones was placed

directly under the mesh sock (150μm) that covered the overflow tube such that larvae were swept away from this area by the upward current.

Rotifers (*Brachionus rotundiformis*) with a mean length and width of 172 μm and 112 μm respectively, were introduced to the treatment tanks twice daily (≈9am and 9pm). Prior to the addition of new rotifers at each feeding period, the density of prey remaining in the tank was estimated from five samples taken at five different locations in the tank. The size of the samples was regulated so that counts were between 5 and 40 rotifers, using 1 or 5 ml glass pipettes. From the prey counts the number of rotifers required to return the tank to the required prey density was added. A second set of samples was taken to ensure that the nominal prey density had been achieved. Rotifers and algae were able to pass through the mesh covering the overflow therefore an estimate of the duration of time that the prey remained in the tank was required. On two occasions prey clearance from treatment tanks was monitored by repeating the five pipette samples every hour over a twelve-hour period.

It should be noted here, that due to failures in the production of algae and rotifers at LAPMAR it was not possible to complete the planned experiments investigating the effect of flow rate and lower prey densities.

2.2.2 Experimental Design

Three replicate tanks of each of 30, 20, 10 and 5 rotifers per ml were randomly assigned to twelve treatment tanks. The four prey densities were chosen based on previous larviculture protocols at the LAPMAR. Successful rearing of fat snook larvae

has been accomplished at this laboratory for many years using prey densities of approximately 30 - 40 rotifers per ml from 1 - 25 days post hatch, and then switching to 10 - 15 rotifers per ml as *Artemia* nauplii are slowly introduced starting on day 25. Although rotifers are offered for 25 days post hatch, my experiment was terminated when the larvae commenced metamorphosis (16 dph). The range of prey densities chosen for this experiment were meant to encompass the densities previously used at LAPMAR as well as adding lower levels approaching those used in the mass rearing of other species such as Atlantic cod (*Gadus morhua*) 4 prey ml. (Puvanendran and Brown 1999), bay anchovy (*Anchoa mitchilli*) 5 prey ml. (Houde and Schekter 1978), gudgeon (*Gobio gobio*) 5.5 prey ml. (Kestemont and Awaiss 1989), yellowtail flounder (*Limanda ferruginea*) 8 prey ml (Rabe and Brown 2000), and white bass (*Morone chrysops*) 10 prey ml. (Densen and Smith 1996).

2.2.3 Data Collection

The day before larvae were transferred to the treatment tanks from the incubator, the initial lengths of ten larvae were measured. Samples of ten larvae from each treatment were also collected for measurements of standard length and myotome height (depth of body excluding fin at the insertion point of the anus) on days 3, 5, 8 and 12. On the final day of the experiment (day 16), 30 larvae from each tank were sampled for length and myotome height measurements. Day 16 was chosen as the final day since 90% of the larvae in all treatments were showing signs of metamorphosis (notochord flexion and discontinuous finfold). Measurements were made from digital images

captured from a Sony CCD camera mounted on a dissecting scope, using the Dazzle Video Creator hardware software (Dazzle Multimedia' 1999). These images (bitmaps) were then opened in the Scion Image freeware program (Release Beta 3b, Scion Corp. 1998) that was calibrated using a calibration slide with 1, 0.1 and 0.01 mm markings. The calibration resulted in a number of pixels per millimetre in the vertical and horizontal directions and gave an accuracy of ±0.0128 mm based on an error of 1 pixel for image formation and 1 pixel for length measurements.

Behavioural observations were made on days 6, 10 and 14 using The Observer event recording program (Version 2.0, Noldus Information Technology, 1990) on a laptop computer. On each observation day, one tank from each treatment was chosen for observation without repetition. Ten larvae were randomly selected and each individual visually followed in the tank for one minute using the focal animal technique (Altman 1974). A single keystroke was pre-assigned to each of six modal action patterns (MAPs) (Table 2.2; Barlow 1977). This data was later summarized into duration and frequency using The Observer program's analysis capabilities.

Survival was calculated based on the difference between the estimated starting number and the absolute final number of larvae remaining in each tank. Other researchers using cold water marine species (Brown and Taylor 1992; Puvanendran and Brown 1999) have collected and counted dead larvae each day in order to estimate a survival curve by back calculating from the final survival. This technique was not possible given the rate at which snook larval corpses degraded at high water temperatures.

Table 2.2. Modal Action Patterns (MAPs) for larval fat snook adapted from Puvanendran (1999).

MAP	Description
Swim	forward movement of larvae by self generated propulsion with caudal area of body and fins
Pause	larvae motionless
Orient	larvae aligns itself headfirst towards a prey item
Lunge	trunk of larvae assumes an S-shape from which the larvae rapidly moves forward towards a prey item
Capture	prey item is taken into mouth
Thrash	larval body undulates from side to side rapidly resulting in backwards motion

2.2.4 Data Analysis

A condition index was used to compare the condition of the larvae in place of measuring dry weight, which was problematic in a humid environment and using small larvae. Due to the high degree of accuracy in measurements (±0.0128 mm) of the digital images, it was possible to obtain estimates of larval robustness that were much more accurate than would have been possible with the available balances. The condition index used was the ratio of body depth to body length, which is a function of larval volume and was used by Koslow, *et al.* (1985) on wild cod larvae (<7.0 mm) and found to be the most sensitive index to changes in the feeding environment.

$$\text{Condition index} = \text{myotome height} \quad \text{standard length} \tag{1}$$

Length-specific growth rate (SGR) was calculated using the following equation:

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

Where L_t is the larval length at time t, L_0 is the length at the beginning of time period t, and t is the length of time period t in days (Buckley *et al.* 1987).

Survival, length specific growth rate and final length data were compared using one way analysis of variance ($P \leq 0.05$). Prior to analysis the data were tested for normality using the Kolmogorov-Smirnov test and observing Q-Q plots of residuals and expected values (SPSS 1999). The data were found to comply with the assumption of normality for the ANOVA analysis.

Behavioural data were also tested in the above manner for normality and it was found that only duration and frequency data for swim and pause MAPs were normally distributed. These data were therefore tested using a two-way ANOVA ($\alpha = 0.05$). Differences between treatments were further investigated with one-way ANOVAs on each day. Orient and capture frequency were compared between prey densities using the Kruskall-Wallis test for non-parametric data (Sokal and Rohlf 1969). Neither lunge nor thrash are analyzed here because lunge was only present in the first observation day, and thrash data was collected for another experiment. They are mentioned here only to complete the repertoire of behaviours observed for larval fat snook.

2.3 Results

2.3.1 Prey clearance

The rate of removal of rotifers from the treatment tanks was observed in two tanks for each prey density. From the clearance curve it was found that 50% of the initial prey density remained after 10 hours, and approximately 40% remained by the next feeding period (Fig. 2.2).

2.3.2 Survival

There was no significant difference in survival for larvae in the four prey densities ($F = 0.165$, $df = 3$, $P = 0.917$; Fig. 2.3). The highest survival was 60.8% in one replicate at 5 prey ml, and the mean survival for this treatment (three tanks) was the highest at 38.8%, while the lowest mean survival (29.4%) was in the 30 rotifers per ml treatment, which also had the tank with the lowest survival at 9.7%.

2.3.3 Growth

There was no significant difference ($F = 1.152$, $df = 3$, $P = 0.386$) in the mean final standard length among fish raised at any of the four prey densities (Fig. 2.4). Length-specific growth rates were not significantly different ($F = 1.129$, $df = 3$, $P = 0.394$) among treatments, and averaged 7.53 % per day over the entire experimental period. The highest length-specific growth rate was during the first five days, (14.37 ± 0.60 % per day) over all treatments (Table 2.3).

Table 2.3. Length specific growth rates for larval fat snook from hatch to metamorphosis

	Length –Specific Growth Rate (% day)					
Prey Density	Day 1	Day 5	Day 8	Day 12	Day 16	Total
5	0	14.52	5.71	4.39	4.78	7.46
10	0	14.13	2.35	8.02	4.84	7.67
20	0	13.89	6.26	4.42	4.92	7.45
30	0	14.94	4.38	5.30	4.76	7.54
Mean	0	14.37	4.67	5.53	4.83	7.53

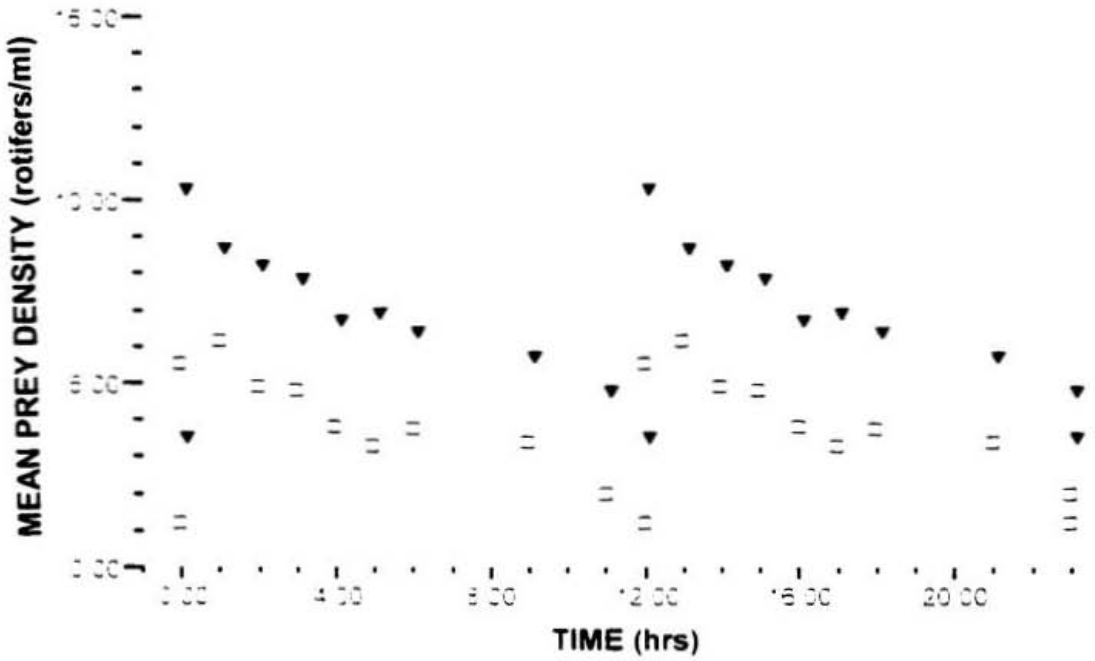


Fig. 2.2. Mean prey density (± 2 S.E.) over time (hrs) for treatment tanks with starting nominal prey densities of 10 (▼) and 5 (□) rotifers per millilitre.

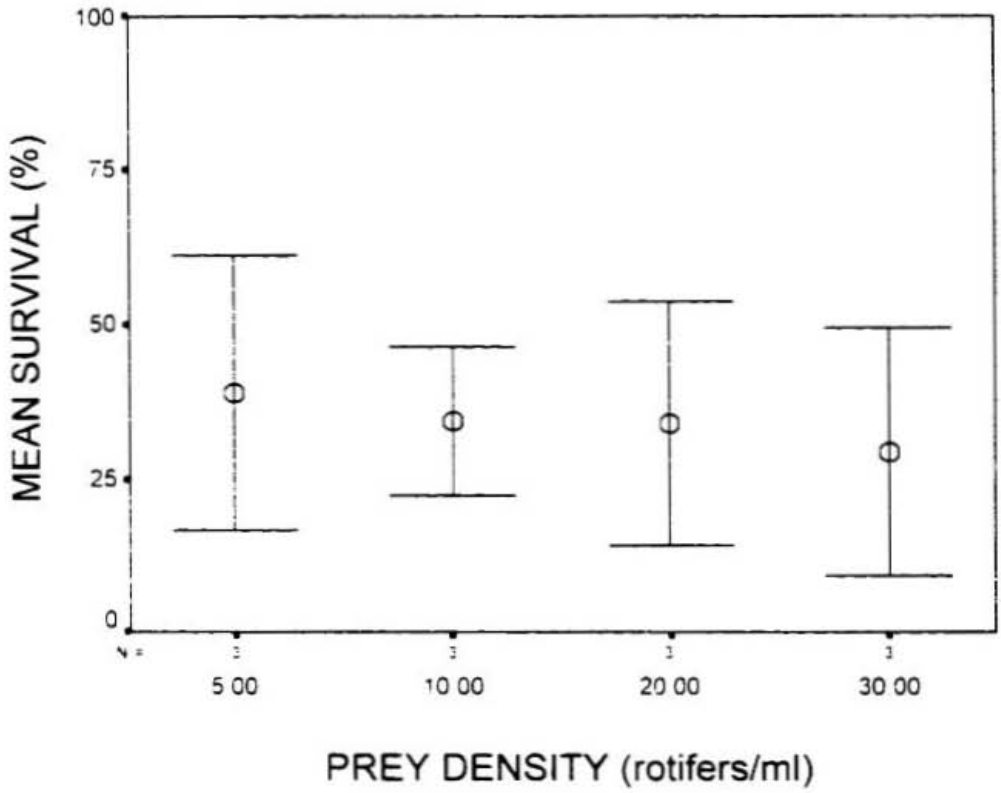


Fig. 2.3. Mean percent survival (± 2 S.E.) on day 16 for larval fat snook reared at four different prey densities (n = 3 per treatment).

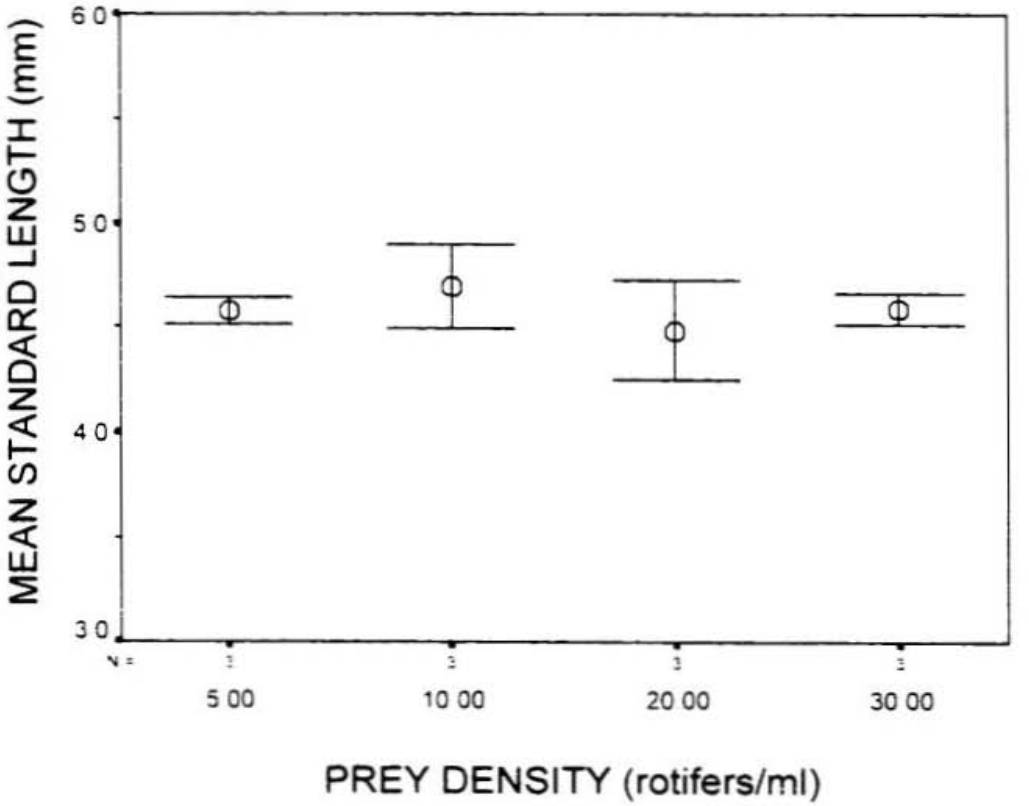


Fig. 2.4. Mean standard length (± 2 S.E.) on day 16, for larval fat snook reared at four different prey densities (n = 30 per sample).

2.3.4 Behaviour

The behaviour of larval fat snook consisted of a series of short swims and pauses punctuated occasionally by foraging events (orient, lunge, and capture). Larvae spent most of their time motionless and approximately 10% of the time swimming. Only a small percentage of the time was spent in orient, lunge, capture, and thrash behaviours.

Prior to day 10 post-hatch the larvae were observed to exhibit a lunge behaviour that was preceded by the larvae assuming a s-curve shape. The lunge and s-curve behaviour were size related and were only observed in larvae less than 3.0 mm in length and was last observed on day 10, when the mean size of larvae was 3.42 mm.

The mean duration of time spent swimming per minute on all days combined was 6.41 sec min and was not significantly different ($F = 2.494$, $df = 3$, $p = 0.061$) among any of the prey densities. As the p-value was close to $\alpha = 0.05$ both a Tukey's HSD and a Student-Newman- Keuls post hoc analysis were performed and no difference was found between any of the treatments. A two-way analysis of variance with prey density and days post-hatch (dph) as fixed factors and an interaction term between prey density and dph, explained 23.7% of the variation in the data. All factors and the interaction term were significant at $\alpha = 0.05$ (Table 2.4). When the mean swim duration of larvae from each treatment was examined on each of observation, it was found that larvae in the 5 prey ml treatment swam significantly longer than larvae in all of the other treatments on day 6 ($F = 5.584$, $df = 3$, $p = 0.002$), while on day 10 there were no significant ($F = 1.546$, $df = 3$, $p = 0.213$) differences between any of the treatments. On day 14 swim duration

decreased with increasing prey density and larvae at 5 prey ml swam significantly ($F = 3.136$, $df = 3$, $p = 0.030$) more than larvae at 30 prey ml (Fig 2.5).

Table 2.4. Summary of two-way ANOVA results for swim and pause duration of fat snook larvae over time and fed at different prey densities.

Behaviour	Source	F-value	df	p
Swim Duration	Prey density	3.652	3	0.014
$R^2 = 0.237$	Day	14.051	2	<0.001
	Prey density * Day	3.113	6	0.006
Pause Duration	Prey density	2.889	3	0.037
$R^2 = 0.198$	Day	13.445	2	<0.001
	Prey density * Day	1.485	6	0.186

There was a significant effect of both day and prey density on the duration of pause, but the lack of interaction between the two factors implies that the effect of prey density differed during development (Table 2.4). On day 6 there was no significant ($F = 2.301$, $df = 3$, $p = 0.090$) difference in pause duration among the prey densities. Likewise, there were no differences on day 10 ($F = 0.630$, $df = 3$, $p = 0.599$) post hatch. On day 14, however, there was a trend of increasing pause duration with increased prey density. The 30 prey ml treatment larvae paused significantly longer ($F = 3.524$, $df = 3$, $p = 0.019$) than did the larvae in both the 5 and 10 prey ml treatments (Fig. 2.6).

The mean frequency of orient for all treatments over all days was 0.514 orients per minute (orients min) and although there were significant differences (χ^2 =12.598, df = 3, P = 0.006) between treatments, a Kruskal-Wallis test on each day found that there was only a significant difference (χ^2 = 14.004, df = 3, p = 0.003) on day 6 post hatch and there was no linear trend in the data to support a change due to prey density. There were no significant differences in orient frequency between treatments for either of the other two observation days (day 10, χ^2 = 1.128, df = 3, p = 0.770; day 14, χ^2 = 5.990, df = 3, p = 0.112) (Fig. 2.7).

Capture frequencies were low at all prey densities and averaged 0.0113 captures min for all treatments over all days. There was no significant difference (χ^2 =2.030, df = 3, P = 0.566) in capture frequency between treatments when all days are combined. There were also no significant differences on any of the observation days when tested separately (day 6, χ^2 = 0.001, df = 3, P = 1.000; day 10, χ^2 =4.800, df = 3, P = 0.187; day 14, χ^2 =3.158, df = 3, P = 0.368) (Fig2.8).

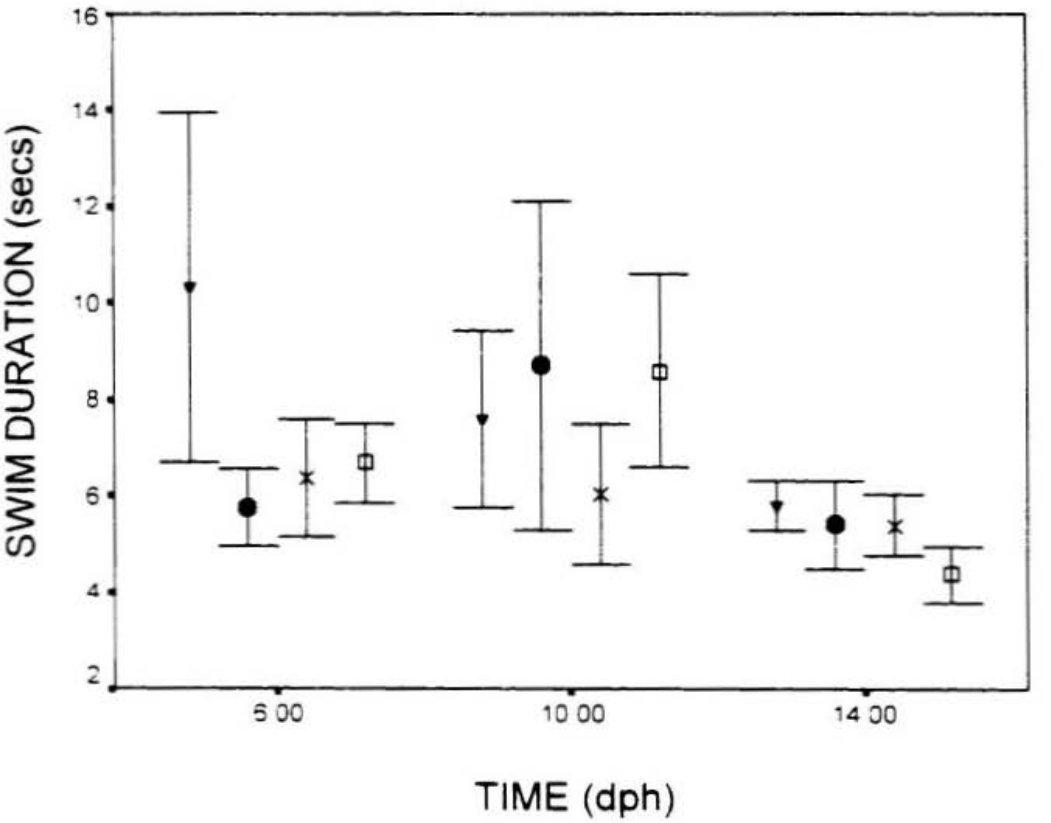


Fig. 2.5. Mean swim duration (± 2 S.E.) over time (days post hatch), for fat snook larvae reared at 4 different prey densities. (▼, 5 rotifers ml; ●, 10 rotifers ml; ×, 20 rotifers ml; □, 30 rotifers ml)

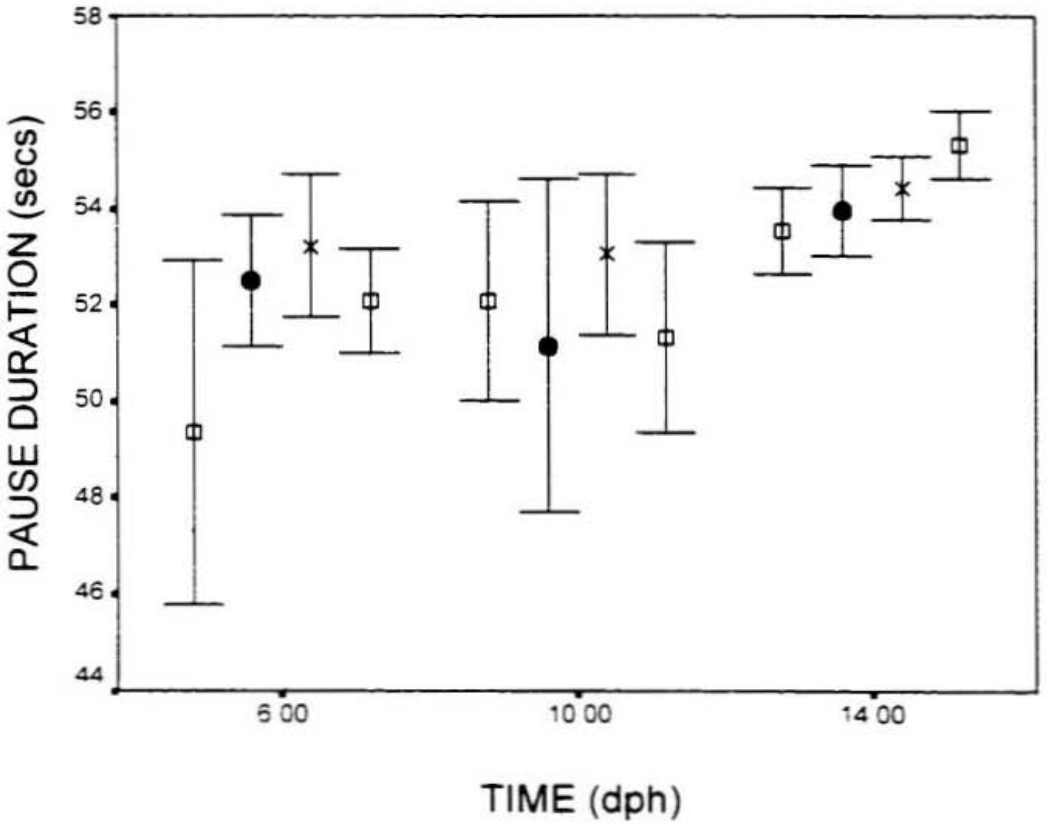


Fig. 2.6. Mean pause duration (± 2 S.E.) over time (days post hatch), for fat snook larvae reared at 4 different prey densities. (▼, 5 rotifers ml; ●, 10 rotifers ml; ×, 20 rotifers ml; □, 30 rotifers ml)

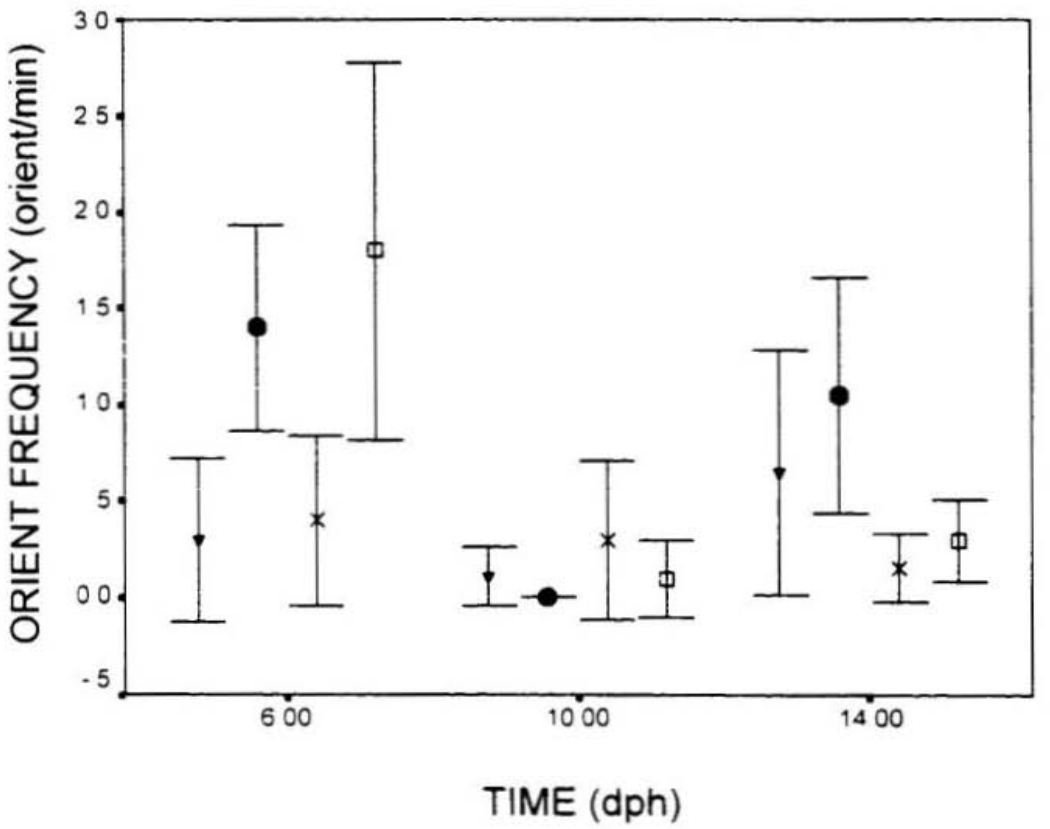


Fig. 2.7. Mean orient frequency (± 2 S.E.) over time (days post hatch), for fat snook larvae reared at 4 different prey densities. (▼, 5 rotifers ml; ●, 10 rotifers ml; ×, 20 rotifers ml; □, 30 rotifers ml)

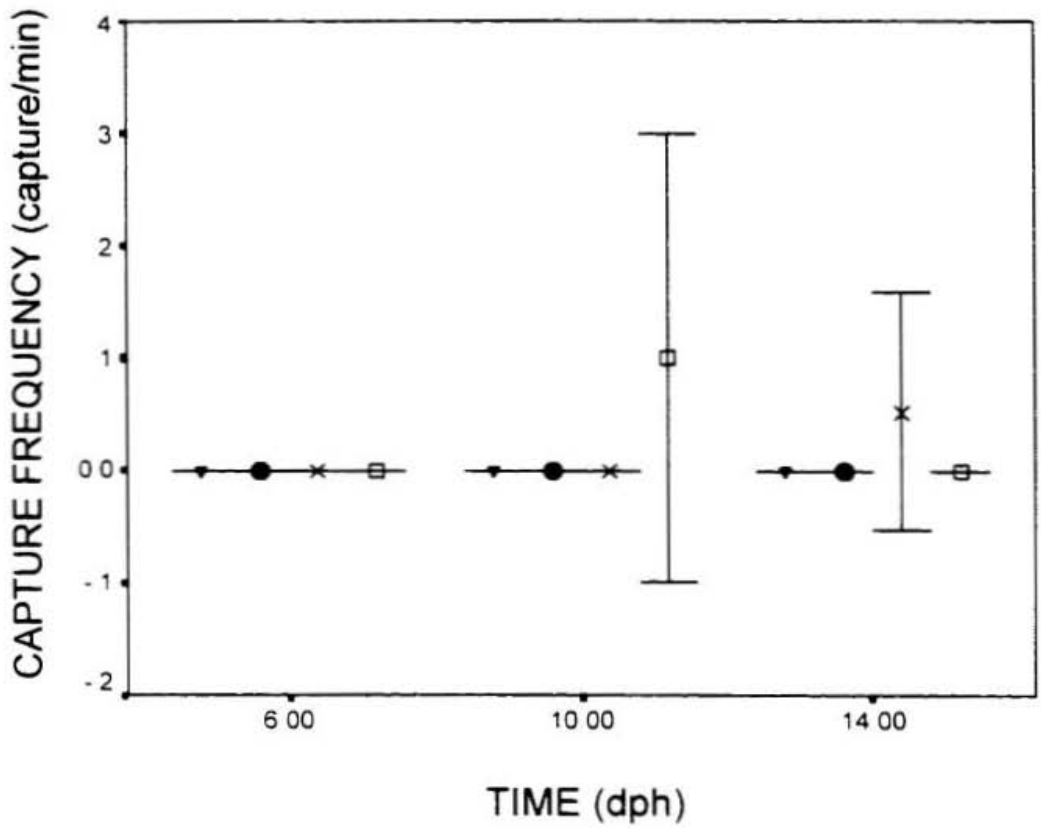


Fig. 2.8. Mean capture frequency (± 2 S.E.) over time (days post hatch), for fat snook larvae reared at 4 different prey densities. (▼, 5 rotifers ml; ●, 10 rotifers ml; ×, 20 rotifers ml; □, 30 rotifers ml)

2.4 Discussion

The poor survival and high costs associated with the larval stage of fat snook have limited the availability of juvenile seed stock required to support commercial on-growing facilities in Brazil (Lajonchere 2001). This research addressed both the poor survival and high costs by attempting to optimize the prey density used in the larviculture of fat snook. The results indicate that a much lower prey density (5 rotifers ml) can be used to attain an equivalent survival to the previously high prey density offered (30 – 40 rotifers ml).

The use of behavioural observations has previously been useful in explaining how differences in light, or prey density affect growth and survival in larval fishes. In studies with Atlantic cod larvae Puvanendran (1999) found that larvae reared in high or "optimal" prey densities (>4000 prey litre), which resulted in higher survival and growth, showed a higher frequency of orients per minute, compared to larvae reared in sub-optimal prey densities. From the behavioural observations on larval fat snook at the four prey densities studied here, the orient frequency data does support the survival and growth data.

The lack of difference in survival and growth across the wide range of prey densities used here suggests that we did not reach the lower limit of prey density that can be used for larval rearing without compromising larval growth or survival. A weak trend towards increasing survival with lower prey densities indicates that further reductions may improve results. Subsequent experiments were attempted as part of this research and although they were all terminated due to lack of rotifers and or algae, some clues may come from the rough data that was collected prior to their termination. One of these

experiments used four treatment prey densities set at 10, 5, 2.5 and 0.5 rotifers per ml, and found an asymptote in both survival and growth with a peak at 2.5 prey per ml.

The overall high survival of larvae from hatch to day 16 was some of the highest survival achieved at LAPMAR. Day 16 was chosen as the end of the experiment since at this point it was clear that metamorphosis was well underway as the dorsal fin was commencing division and notochord had completed flexion (Fig. 2.9). One of the possible explanations for the high survival was the use of a flow-through system. Previous larviculture of fat snook at LAPMAR had been done in near static systems, with most new water only being added with algae and live food, and occasionally to top up tanks or increase volume. Flow-through or open systems have been used with great success and can reduce metabolites, nitrogen as nitrate, nitrite and ammonia, and increase the diversity of bacteria flora, which has been shown to increase survival for the larva of the common snook (Kennedy, *et al.* 1998). Clearly there are conditions in which a flow-through system would not be advantageous particularly where the incoming water quality is questionable, or when insufficient volume of water exists to permit a continuous flow through (Melard, *et al.* 1996).

Possibly the most critical factor that needs to be considered in relation to flow is the effect it has on the duration of prey availability in the larval rearing tank. In this experiment rotifers were enriched with algae, which was also available to the rotifers while they were in the rearing tank, so the role of flow-through, here was to improve

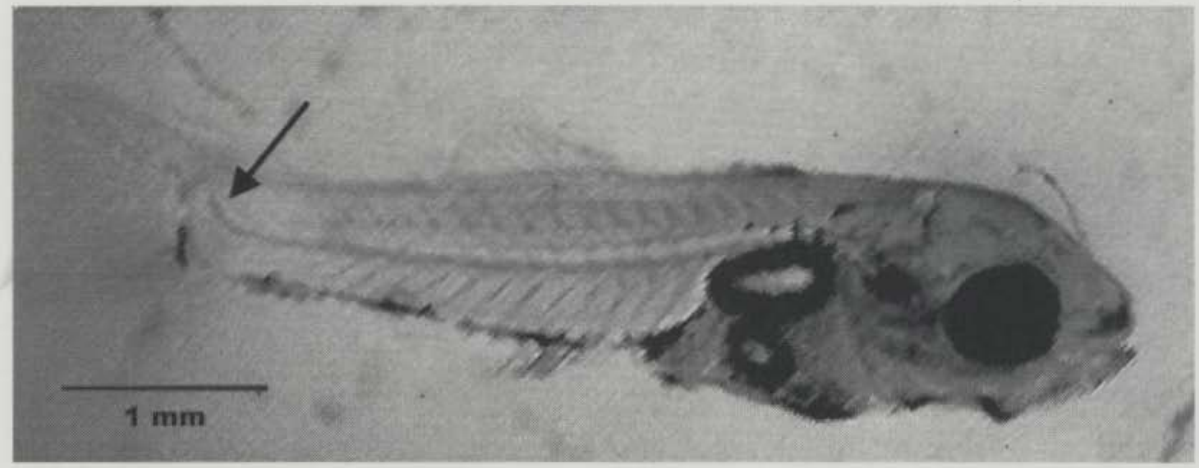


Fig. 2.9. Digital image of a 16 day old fat snook larvae, which has commenced metamorphosis, evident by the discontinuous fin-fold, and notochord flexion, indicated by arrow.

water quality. There was a fairly slow prey clearance rate (50% remained after 10 hours) that probably would not have differed greatly from a static system with respect to its effect on the foraging behaviours of the larvae. This aspect would have been tested if further experimentation with larval fat snook had been possible, however, it will be addressed with larval cod in a later section of this thesis.

As this is the first known behavioural study on the larvae of *Centropomus parallelus*, it is important to attempt to classify the behaviour of this species for future reference. The larval behaviours can be described as short bouts of swimming followed by longer periods of pause. Orientation towards prey and captures were consistently preceded by a pause. This description matches that provided by Browman and O'Brien (1992a and b) for the behaviour of white crappie (*Poxomis annularis*) and golden shiner (*Notemigonus crysoleucas*), and which has also recently been used for the larvae of Atlantic cod (Puvanendran and Brown 1999). The term used to describe this pattern is a saltatory search pattern and it is intermediate to the sit and wait or ambush foraging pattern, and the active or cruise foraging pattern (Browman and O'Brien 1992a). Although the saltatory search pattern applies to the entire larval period for fat snook, there were some changes in the foraging behaviours as the larvae grew.

Prior to attaining approximately 3.0 mm in length, larval fat snook were observed to lunge towards their prey prior to capture. The larvae assumed a sigmoid or s-curve shape, in which the larvae would move its head forward and backward relative to a prey item, preceding the lunge. The s-curve has been observed for other fish larvae (yellowtail, winter flounder and Atlantic halibut: personal observation) and is described

for the larvae of whitefish (*Coregonus wartmanni*) by Braum (1966), yet a clear understanding of the purpose of this behaviour was not found in the literature. The loss of this behaviour may coincide with an ontogenetic change in eye morphology. A similar hypothesis was presented for the losses of fixate, lunge and snap MAPs in a centrarchid species by Brown and Colgan (1984). They found that loss of behaviours from the repertoire of four centrarchid fishes was related to size and not age. In the present experiment, after the larvae reached lengths over 3.0 mm, the lunge and s-curve behaviours were no longer observed, and capture events became more difficult to distinguish from the sudden burst of swimming that invariably followed pause events.

Various researchers have used capture frequency and consumption rate data to support Holling's (1965) hypothesis of a functional response between predator and prey density for larval fish (Houde and Schekter 1980; Werner and Blaxter 1980; Puvanendran and Brown 1999). However, in this experiment capture frequency was highly variable. There are several possible explanations for this: firstly captures were only recorded if the prey item was observed to be ingested. Behaviours that appeared to be captures based on the body movements of the larvae when the prey item was not observed were not included. Secondly, the duration of the observation period was too short to ensure the occurrence of a capture event. A longer observation period could have accommodated the low capture frequencies, however, it would have meant sacrificing the number of larvae observed or the number of replicates and treatments observed since recording of behavioral observations was a lengthy and laborious task. Therefore, in this and the

following experiments. I have chosen to focus on the frequency of orient as an indicator of foraging effort rather than capture frequency.

It is not possible from the experiment conducted here, to accurately define the lower limit of prey density that will minimize live food requirements while optimizing growth and survival. However, I can conclude from this experiment that lower prey densities (5 prey ml) can be used to rear larval fat snook than were previously being used (30 – 40 prey ml). By reducing prey requirements it will be possible to decrease the costs associated with the larviculture of this species. The six-fold decrease in rotifer demand will increase the resources available to ensure that back up cultures of both algae and rotifers are available for future larval production at LAPMAR. Furthermore, it appears that given the high water quality and high temperatures at this lab, the use of a flow-through system will greatly improve overall survival of larval fat snook.

Chapter 3

Behaviour of Atlantic cod larvae under varying feeding conditions: Exploring ways to reduce rotifer requirements for larviculture.

3.1 Introduction

3.1.1 Natural History of Atlantic Cod Larvae

In the wild, Atlantic cod eggs are released into the ocean in several batches from a single adult female each year (group synchronous batch spawners). The buoyant eggs float in congregations in the upper 50 m of the water column (Ellertsen, *et al.* 1984), and hatch after approximately 80 degree-days, with closed mouths and anuses. The free-living larvae (eleutheroembryo) are completely dependent on their endogenous reserves (yolk sac) for the first few days of life (Morrison 1993). The duration that the yolk sac lasts is temperature dependent (Fukuhara 1990; Miranda, *et al.* 1990) and at 10°C the yolk lasts approximately 8 – 10 days (personal observation).

Larval feeding commences at approximately 25 degree-days (days × degrees Celsius, e.g. 2.5 days at 10°C) and in the wild the larvae feed on micro-zooplankton (ciliates, copepod nauplii, copepodites, gastropod larvae, cladocerans) (Tilseth and Ellertsen 1984; Thompson and Harrop 1991; Gronkjaer and Wieland 1997). During feeding, the larvae do not assume a sigmoid or s-curve posture prior to attacking a prey item at any time during development (Ellertsen, *et al.* 1980), unlike the larvae of some other species such as herring (Gallego 1994), winter flounder, halibut and fat snook (chapter 2). Using Cushing's (1983) model of dry weight of food required by growing cod larvae, and the size of larval guts contents, Thompson (1991) estimated that in the

Irish Sea cod must eat 156 - 196 food items per day to maintain the high growth rate (10% body weight per day) observed. He also suggested that as the larvae grow they do not increase their consumption rate to satisfy the increasing requirements for nourishment, but rather increase the size of prey selected. Others have suggested that in order to acquire the high number of food items, the prey must be densely congregated in cloud-like formations or "micro-patches", based on the average number of prey items available as determined from various sampling techniques (Ellertsen, *et al*. 1984; Thompson and Harrop 1991; Gallego 1994). One of the complications in this area of research is that the techniques used for sampling (mainly devices which collect samples from a vertical or horizontal water column) result in calculations of average densities across the water column collected, which eliminates the patchy distribution. Furthermore, the size of prey eaten by larvae at first feeding (30 – 150 μm) is rarely retained in the sampling devices (Houde 1975; Frank, 1986).

Much of our knowledge of the life history of Atlantic cod has only been acquired in the last couple of decades due to a sudden interest in response to failing wild capture harvests in Europe, Iceland and North America. Research has been aimed at predicting year class strength and stock assessments in order to attempt to predict sustainable harvest levels. Recently, interests have been shifting to the growing aquaculture industry and its potential role in revitalizing the cod industry.

3.1.2 The Match Mismatch Hypothesis Revisited (Cod Experiment I)

A detailed look at the ontogeny of larval Atlantic cod by von Herbing *et al.* (1996), identifies the larval stage as the period during which cod undergo the greatest morphological changes. Based on the development of the digestive tract and the origin of nutrients, the larval period can be divided into three stages. The first is the yolk sac or endogenous feeding stage, during which the larvae are completely dependent on the reserves they were provided with as eggs. Stage two is a transition period between endogenous and exogenous nutritional sources. By stage three the endogenous reserves have been completely utilized and the larvae must ingest all of their nutritional requirements.

Until recently, the entire larval stage was treated uniformly with a constant feeding rate up until metamorphosis (Puvanendran and Brown 1999). Feeding of Atlantic cod larvae usually commences at about one to three days post-hatch using approximately 4000 - 5000 rotifers per litre and they are maintained at this prey density until the larvae reach six to seven millimetres in length, when they are gradually switched to *Artemia* nauplii. Based on the suggestions of other researchers (described below) it was considered that lowering the prey density towards the end of the larval period may have little negative impact on the growth and survival of larval cod.

Kestemont and Awaiss (1989) found that feeding rate changes quickly during the first few weeks of life for larvae gudgeon, *Gobio gobio*, in which larvae decreased their feeding rate from 40% of their initial body weight on day one to 12% in the third week. Houde (1978) states that prey densities are probably only limiting during the first 3 or 4

days when larval searching abilities are poorest, and that prey density during the first 5 days has the greatest impact on survival. High prey densities at the early "critical" stage in larval rearing have been found to be vital to the survival of larval fishes (Frank and Leggett 1986; van Der Meeren and Naess 1993). High prey densities are required to initiate first feeding before larval endogenous reserves are depleted. Puvanendran (1999) working with larval Atlantic cod, suggests lowering prey density towards the end of larval rearing in order to reduce the requirements for live food in larviculture. Several authors state that many species of larvae clearly show increased foraging abilities as they develop (Blaxter and Staines 1970; Rosenthal and Hempel 1970; Hunter 1972; Houde and Schekter 1980; Browman and O'Brien 1992; Browman and O'Brien 1992; Miller, *et al.* 1992), which might suggest that prey levels may be less important later in development because the larvae are more efficient foragers (Houde 1978).

The ability for larvae to survive periodic decreases in prey density caused by temporal or spatial heterogeneity in zooplankton abundance in relation to stage of development has been investigated in field studies (Ellertsen, *et al.* 1988), and in the lab for larval Atlantic cod (Goteeitas *et al.* 1996). However, it has not yet been examined in the context of reducing live food requirements in aquaculture.

The objective of this first experiment was to investigate the effect of lowering the prey density offered to larvae during each of the three larval stages (as defined earlier) on the survival, growth and behaviour of Atlantic cod larvae.

3.2 Materials and Methods

3.2.1 General methodology for Cod Experiment I

The larvae used in this experiment came from eggs collected from adult Atlantic cod broodstock from the Newfoundland 3PS stock. The mature adults were over-wintered in ocean pens and transferred to the Ocean Sciences Centre, in early April 1999. Approximately 40 individuals ranging in size from 40 – 60 cm were kept in a 38 000 litre fiberglass tank, 4 meters high and 4 meters in diameter. In the ocean pens and while at the Ocean Sciences Centre the broodstock were maintained on a diet of mackerel, herring, squid, and capelin.

The mature cod spawned naturally (without induction or manipulation) in the spawning tank. The buoyant eggs were collected in a mesh bag in an overflow collector mounted on the outside of the spawning tank. A relatively small batch of eggs (1850 ml) was chosen to increase the probability of the eggs coming from only one or two females. The intention here was to reduce the variability in egg sizes and therefore the variability in larval sizes, to facilitate analysis of growth measurements. Hatching commenced at 69.9 degree-days, and by 78.8 degree-days approximately 80% had hatched and this was considered day 1.

Larvae were transferred to the treatment tanks on day two by which point hatching was greater than 90%. Larvae were gently scooped out of the incubator and carefully decanted into a bucket. The number of larvae in the bucket was estimated from 5 samples after mixing. This procedure was repeated numerous times during the transfer process to increase accuracy of estimates. Based on the results of these counts the

number of millilitres required to stock each experimental tank with 1200 larvae (40 larvae litre) was then transferred.

Treatment tanks were 30 litre glass aquaria with opaque black painted sides. Each tank was equipped with one overflow tube over which a 243µm mesh was attached: this size allowed both algae and rotifers to pass through in order to decrease tank fouling and to remove rotifers prior to the exhaustion of enrichments. Two air-stones were used, one at each end of the tank. One air-stone was located under the overflow tube such that the airflow created an upward flow of water over the mesh sock to deter larvae from approaching the drain. Inflow of water was initially set to 0 mls per minute for the first three days to reduce disturbance. On day four flows were set to 40 ml min (1.9 exchanges day), this was later increased to 80 ml min (3.8 exchanges day) on day 12 post hatch. The water provided to the tanks was filtered (5µm paper filter) seawater and was pumped to a header tank where it was gravity fed to the tanks to ensure consistent flow rates.

Treatment tanks received approximately 770 ±250 lux provided by two 40 watt deluxe daylight fluorescent tubes, 24 hours per day. Temperatures were maintained at 11.8 ±1 °C by submersing the tanks in a water bath, which was thermostatically controlled by an external heating and cooling device.

Tank water was greened with micro algae (*Isochrysis* spp.) twice per day just after feeding. Larvae were fed rotifers (*Brachionus plicatilis* s-type), which were on average 304 µm long by 130 µm wide at the widest point, twice per day at approximately 10 a.m. and 10 p.m. Before feeding, each tank was sampled 4 times with a 10ml pipette at four

locations in the tank. The samples were taken such that an equal amount of all depths were sampled. The number of rotifers in each pipette was counted, and the volume of new rotifers added to the tank was then calculated based on the density of that day's culture and what remained in the tank.

In order to determine the length of time that prey remained in the treatment tanks (duration of prey availability) at the two flow rates and the three prey densities, hourly samples of rotifer density were taken from tanks for periods up to 12 hours. The hourly sampling of prey density was a labourious and lengthy task and was therefore only carried out on 6 occasions, 3 times while flows were set at 40 ml min, twice at 80 ml min, and once after fish had been removed from the tanks with flows still set at 80 ml min. On each occasion 2 to 3 tanks at each prey density were monitored on an hourly or half hour basis for up to twelve hours.

3.2.2 Experimental Design

The duration of the experiment matched the larval period (approximately 28 days to the commencement of metamorphosis) and was divided into three stages as previously described. Three prey densities were used: high (4000 rotifers ml), medium (2000 rotifers ml) and low (500 rotifers ml), based on the results of previous studies at our laboratory which found that 4000 prey ml optimized growth and survival, while 500 prey ml resulted in 100% mortality after 2-3 weeks (Puvanendran 1999). The total number of combinations of three prey densities and three larval stages is $3^3 = 27$, however, as discussed above previous work had suggested a decreasing prey density over

time may be most efficient, so twelve combinations were chosen. Twenty-four tanks were assigned one of twelve treatments (Fig. 3.1) in which the prey density was changed at each stage. For example treatment 4 received high prey density during stage I (1-5 dph), medium during stage II (5-12 dph) and low during stage III (12-28 dph). Space and time limited the total number of tanks available for experimentation to less than 27.

3.2.3 Data Collection

Measurements of standard length (length from tip of snout to the end of caudal fin) and myotome height (depth of body excluding fin at the insertion point of the anus) were made on ten larvae from each tank (20 per treatment) once during stage I, twice during stage II, and three times during stage III. Length and myotome height were measured from digital images captured from a dissecting scope on a Sony CCD camera connected to a personal computer using the Image Pro software (Version 4.0, Media Cybernetics, 1998)

On the same day that morphological measurements were made behavioural observations were also recorded. Ten larvae from each tank were randomly selected and individually observed in the tank for one minute using the animal focal technique (Altman 1974). Behaviours (MAPs, Table 3.1) were recorded using the Observer program (Version 2.0, Noldus Information Technologies, 1990) on a laptop computer, as described in chapter 2.

Mortality was calculated by subtracting the absolute final number of larvae from the estimated starting number. A survival curve was estimated based on the start and

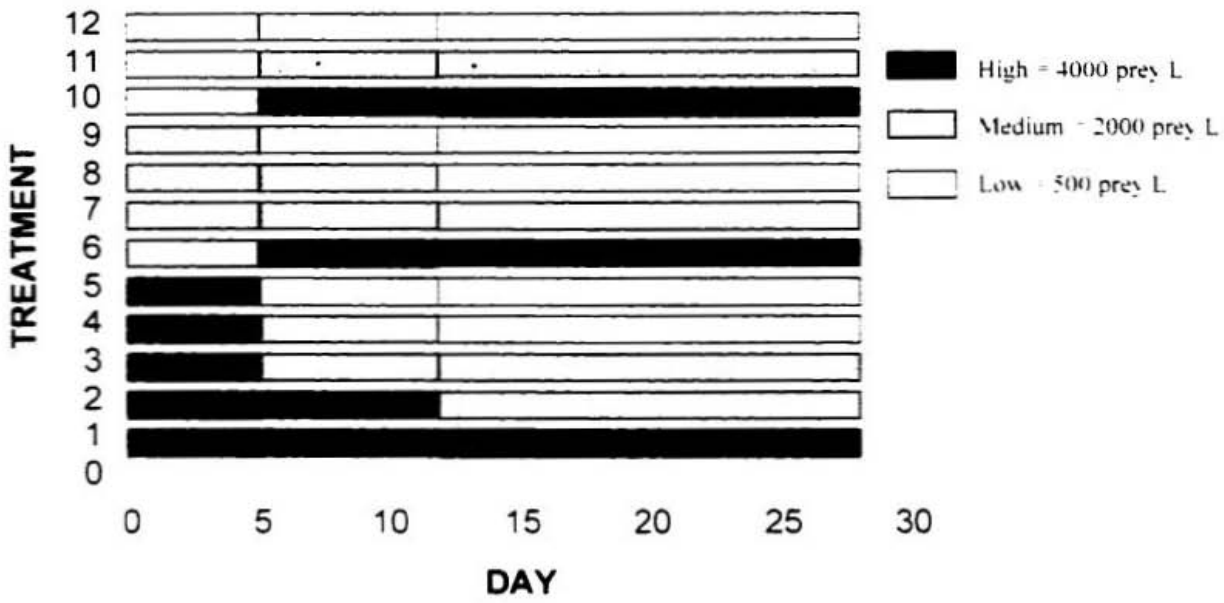


Fig. 3.1. Experimental design for Cod Experiment I, feeding schedule of treatments, prey densities and timing of stages. (stage I, 1-5 dph; stage II, 5 - 12 dph; stage III 12 - 28 dph). Each treatment was performed in duplicate.

Table 3.1. Modal Action Patterns (MAPs) for larval Atlantic cod adapted from Puvanendran (1999).

MAP	Description
Swim	forward movement of larvae by self generated propulsion with caudal area of body and fins
Pause	larvae motionless
Orient	larvae aligns itself headfirst towards prey item
Lunge	trunk of larvae assumes an S-shape from which the larvae rapidly moves forward towards a prey item
Capture	prey item is taken into mouth
Thrash	larval body undulates from side to side rapidly resulting in backwards motion

finish numbers as well as the results of three sets of samples taken during the experiment. These samples consisted of five cores taken from each tank. The coring device was a PVC tube with a volume of 330ml. The tube was briskly dropped through the water and secured onto a cap that had previously been placed on the tank bottom. The core was retrieved, and the larvae were counted by slowly pouring them into a white bucket. The larvae were then returned to the tank. These cores were taken on days 6, 8 and 12 post-hatch. Another attempt to collect cores on day 16 was unsuccessful since by this age the larvae swam too quickly to be collected.

3.2.4 Data Analysis

Due to the large number of treatments it was not possible to make behavioural observations and perform morphological measurements for all tanks on the same day.

For this reason, all tanks in replicate A were monitored on one day and replicate B was completed on the following day. Analysis is performed on the mean of A and B. A condition index was used to compare general fitness of larvae between treatments. The condition index used here was the ratio of body depth to body length, which is a function of larval volume and was used by Koslow *et al.* (1985) on wild cod larvae and found to be the most sensitive index to changes in the feeding environment.

$$\text{Condition index} = \text{myotome height} \quad \text{standard length} \tag{3.1}$$

Length-specific growth rate (L-SGR) was calculated using the following equation:

$$\text{L-SGR} = ([\ln(L_t) - \ln(L_0)] \; t) \times 100 \tag{3.2}$$

Where L_t is the larval length at time t, L_0 is the length at the beginning of time period t, and t is the length of time period t in days (Buckley *et al.* 1987).

Analysis of final survival was performed with a one-way analysis of variance (ANOVA), while comparison between survival curves over time was done with a two-way ANOVA with day and prey density as factors. Two-way ANOVAs were also employed for the analysis of L-SGR, final standard length and final condition index, with prey density and time as factors. Survival, length and condition index were checked for normality using the Kolmogorov-Smirnov test and plots of residuals and predicted values were observed (SPSS 1999).

All statistical analysis on the behavioural data was performed on the mean of ten larvae in each replicate tank. Swim and pause data were normally distributed, however the orient and capture data were not normally distributed and could not be transformed. The assumption of normality however, can be relaxed when the size of the sample is

large (sample size here n = 144, and 1318 larvae were observed: Sokal and Rohlf, 1969). A repeated measures design was applied to the two-way analysis of variance for the behavioural data when compared among treatments and over time.

3.3 Results

3.3.1 Prey Clearance

The results of numerous sampling periods indicate that 100% of the prey was never completely removed from the treatment tanks, even at 80 ml min and the lowest prey density (500 prey ml). Removal rates were substantially different at the two flow rates: at 40 ml min (1.9 exchanges day) 50% of the prey were removed in approximately five hours after which few prey were removed (Fig. 3.2). At 80 ml min (3.8 exchanges day) 50% of the prey was removed after approximately four hours and prey continued to be evacuated until almost all prey were removed (Fig 3.3). A comparison between tanks with and without larvae showed no difference in removal rate of prey, suggesting that larvae only eat a fraction of the total quantity of prey provided.

3.3.2 Survival

A survival curve was constructed from the data obtained from core samples and from initial and final survival estimates. To simplify presentation of the data only treatments (1, 7, and 12) with constant prey density throughout are provided. The

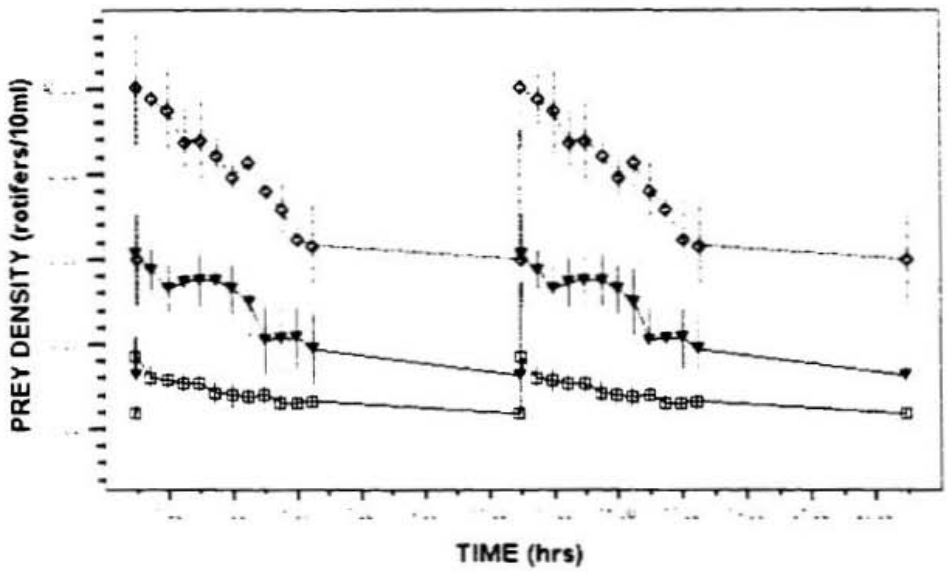


Fig. 3.2. Mean prey remaining over time (± 2 S.E.) at three different prey densities, at a flow of 40 ml min. (◇, 4000 prey litre; ▼, 2000 prey litre; □, 500 prey litre).

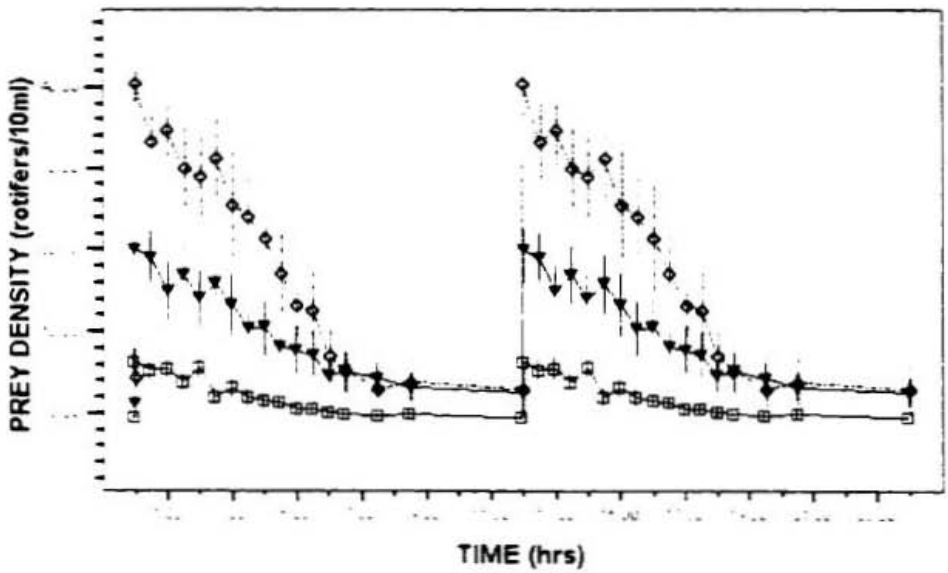


Fig. 3.3. Mean prey remaining over time (± 2 S.E.) at three different prey densities, at a flow of 80 ml min. (◇, 4000 prey litre; ▼, 2000 prey litre; □, 500 prey litre).

average for all constant prey treatments combined shows that: only 15% of larvae had died by day 6 and more than 60% of the larvae died by day 12 which means that nearly 50% of the mortality occurred over the initial 8 days (Fig. 3.4).

Final survival was highest (>14%) for treatments that received high prey density (4000 prey l) during stage II and III, with the exception of tank B-6 that had only 10% survival at the end of the experimental period. The lowest survival was seen in treatment 12, which received low prey density (500 prey l) for the entire experiment (Fig. 3.5). By multiplying the number of days by the respective prey density offered during those days a factor called prey-days was created. A one-way ANOVA using the factor prey-days accounted for 86.2% of the variation in survival ($F = 6.805$, $df = 11$, $p = 0.001$).

3.3.3 Growth

Length-specific growth rate (L-SGR) was not significantly affected by prey density ($F = 0.366$, $df = 2$, $p = 0.694$). There was a significant increase in L-SGR over time ($F = 14.604$, $df = 5$, $p < 0.001$). However, the difference in L-SGR is most apparent when compared before and after day 10, which coincides with the switch from endogenous to exogenous feeding. A t-test shows that there was a significant difference ($t = -8.72$, $df = 142$, $p < 0.001$) between the L-SGR of tanks on the days before the switch (around 10 dph) and afterwards (Fig. 3.6).

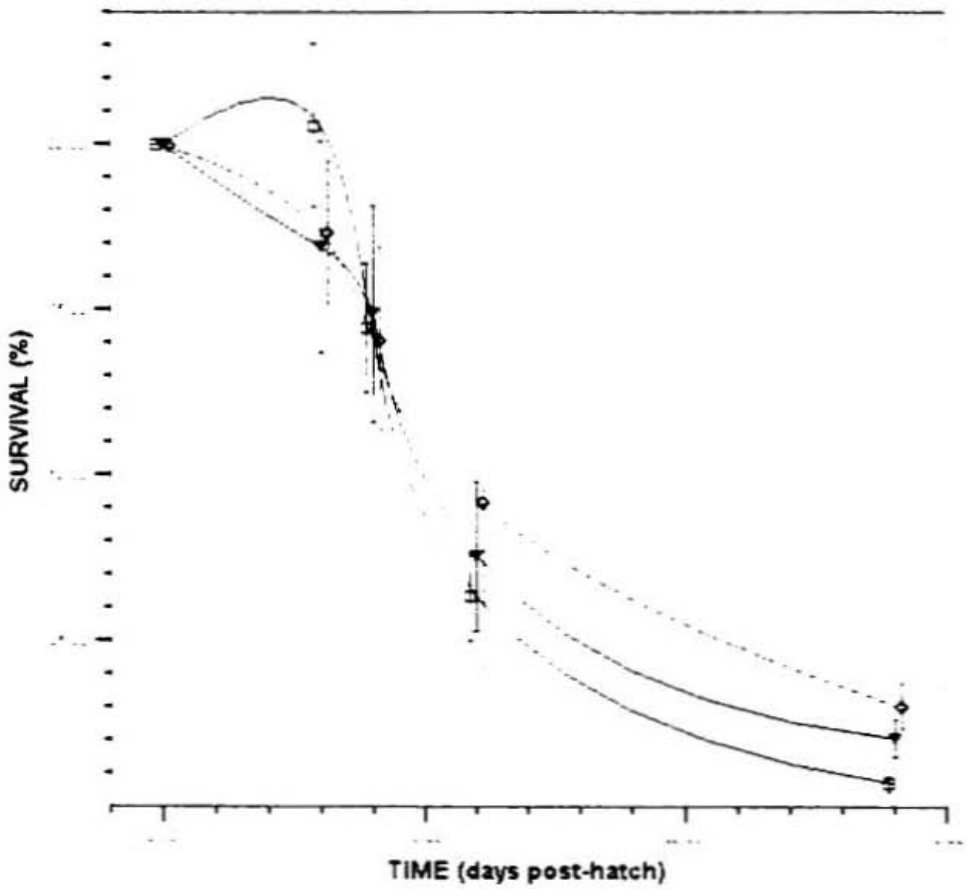


Fig. 3.4. Survival curve for Atlantic cod larvae reared at three different prey densities. (○, 4000 prey litre; ▼, 2000 prey litre; □, 500 prey litre).

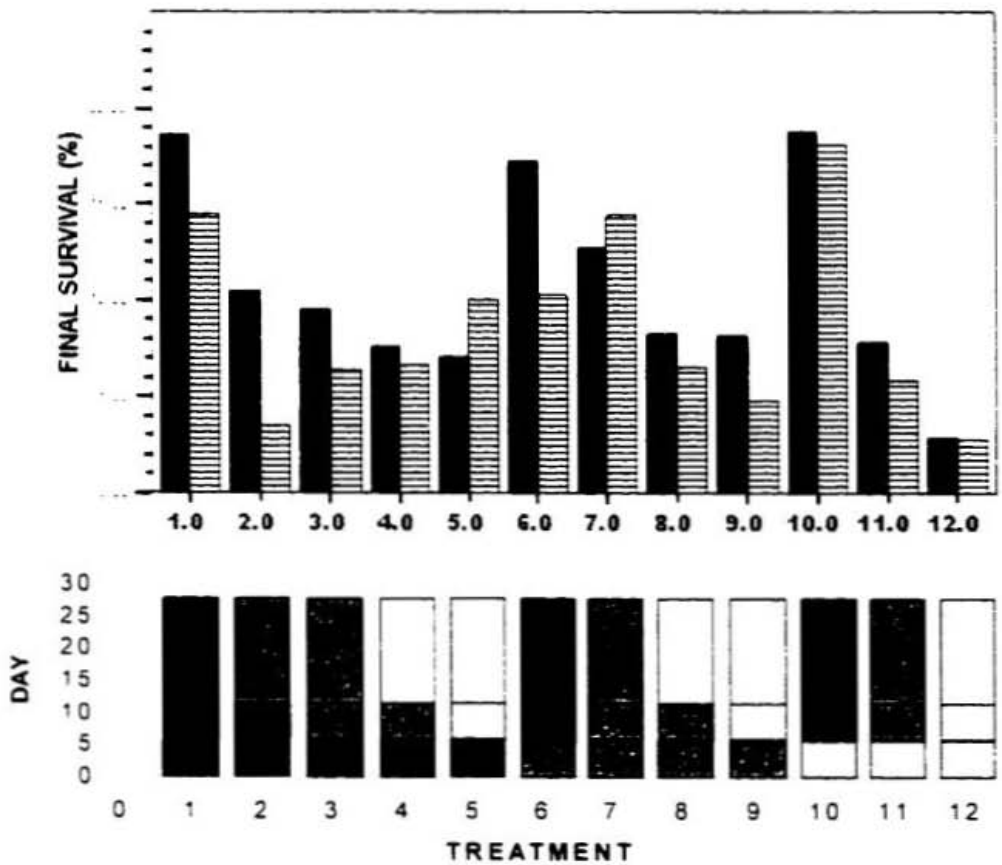


Fig. 3.5. Final survival on day 26 of Atlantic cod larvae for each of the treatments, as summarized below the x-axis from Fig. 3.1 (solid black bars represent replicate A; striped bars represent replicate B).

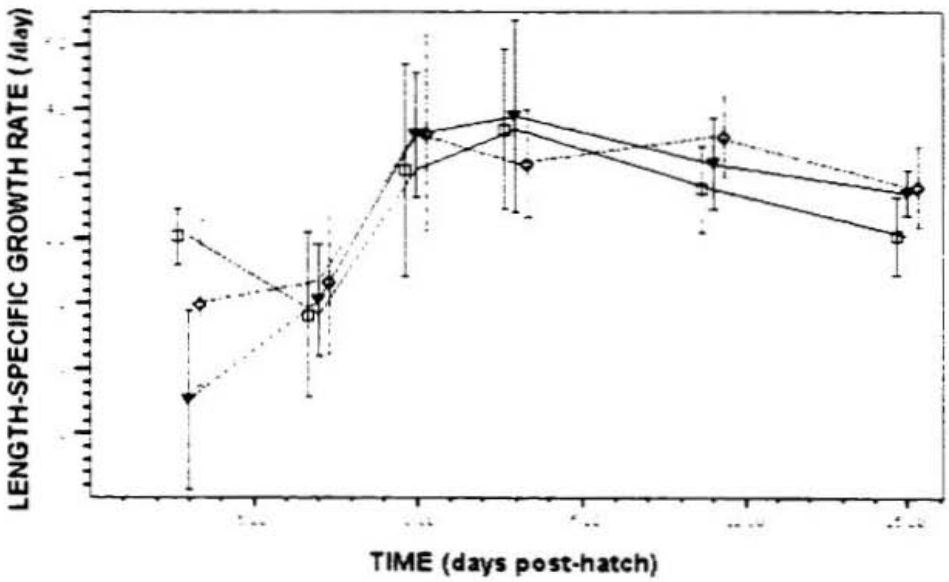


Fig. 3.6. Length specific growth rate per day, over time (dph) for Atlantic cod larvae reared at three different prey densities. Individual points represent the mean of all treatments at that prey density on the day specified, error bars equal ± 2 S. E. (◊, 4000 prey litre; ▼, 2000 prey litre; □, 500 prey litre).

Prey density had a significant effect on the growth of larval cod (Fig. 3.7a; Table 3.2.). By day 19 post-hatch larvae in the 4000 prey l (treatments 1, 6 and 10) and 2000 prey l (treatments 2, 3, 7 and 11) treatments were significantly longer (F=5.665, df=2, P=0.004) in standard length than those in the 500 prey l treatments (4, 5, 8, 9 and 12). This difference widened toward the end of the experiment (F =50.26, df =2, P<0.001). Larvae that were raised in treatments that received low prey density (tanks 4, 5, 8 and 9) during stages II & III, and III, were significantly (F =50.26, df =2, P<0.001) smaller than larvae in all other treatments. One exception to this was treatment 12 (low prey density for all stages) which had a mean final length comparable to treatments with high and medium prey densities for the later two stages (Fig. 3.7a).

The factor prey-days was significant (F = 13.716, df = 11, p < 0.001) suggesting that the cumulative amount of prey supplied to each treatment had an effect of growth, but the R^2 value was low (0.172).

The effects of prey density and age on condition were similar to those for length. Both time period and prey density, had significant effects on the condition of larvae (Fig. 3.7b, 3.8b; Table 3.2), and larvae in treatments with low prey density in stages II & III, and III, were in significantly poorer condition (F = 16.430, df = 2, p < 0.001) by day 19 and the difference remained significant until the last measurement on day 25 (F = 102.64, df = 2, p < 0.001).

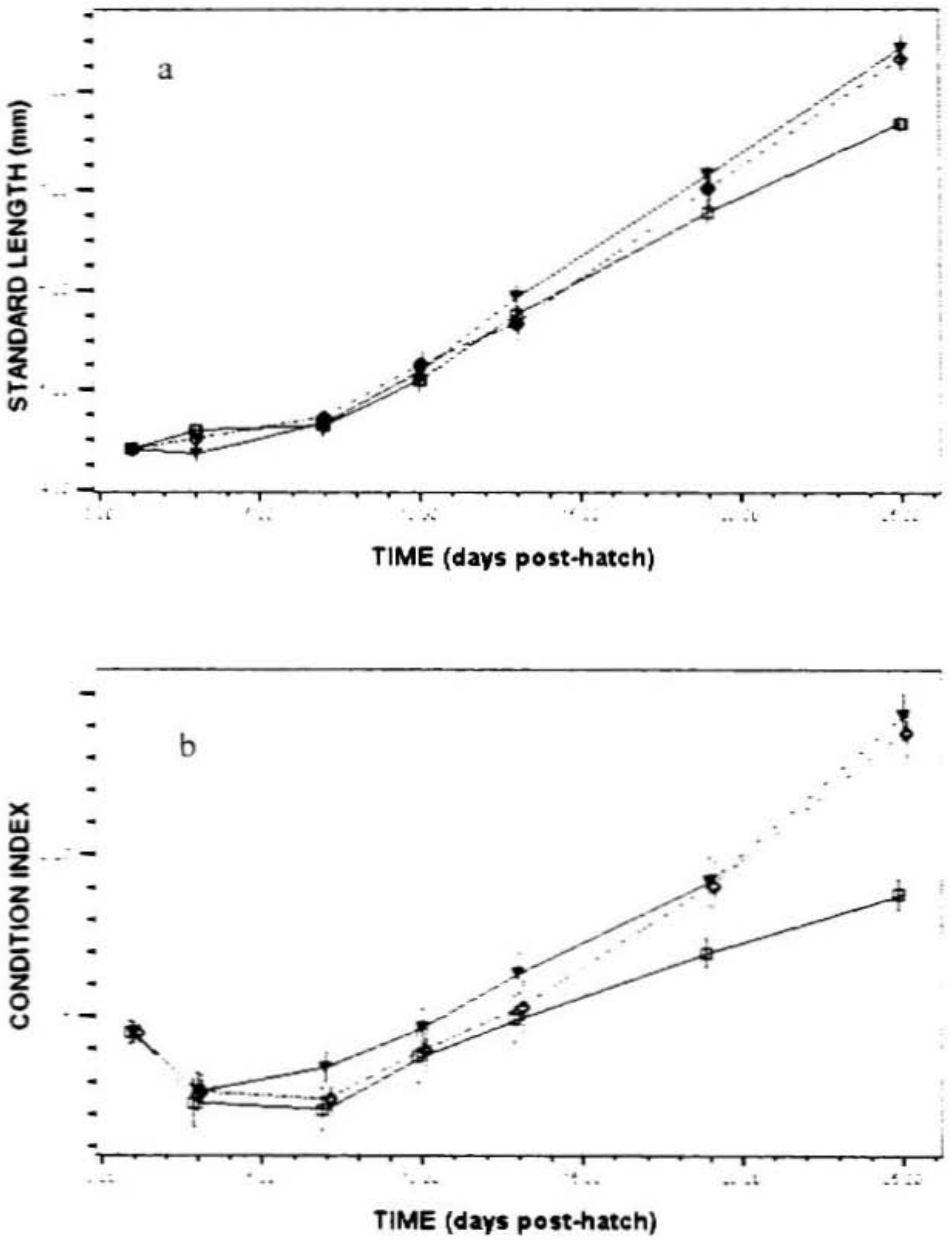


Fig. 3.7. Change in mean (± 2 S.E.) of: (a) standard length, and (b) condition index (mm) over time (days post-hatch) of Atlantic cod reared at three prey densities. Individual points represent the mean of all treatments at that prey density on the day specified, error bars equal ± 2 S. E. (○, 4000 prey litre; ▼, 2000 prey litre; □, 500 prey litre).

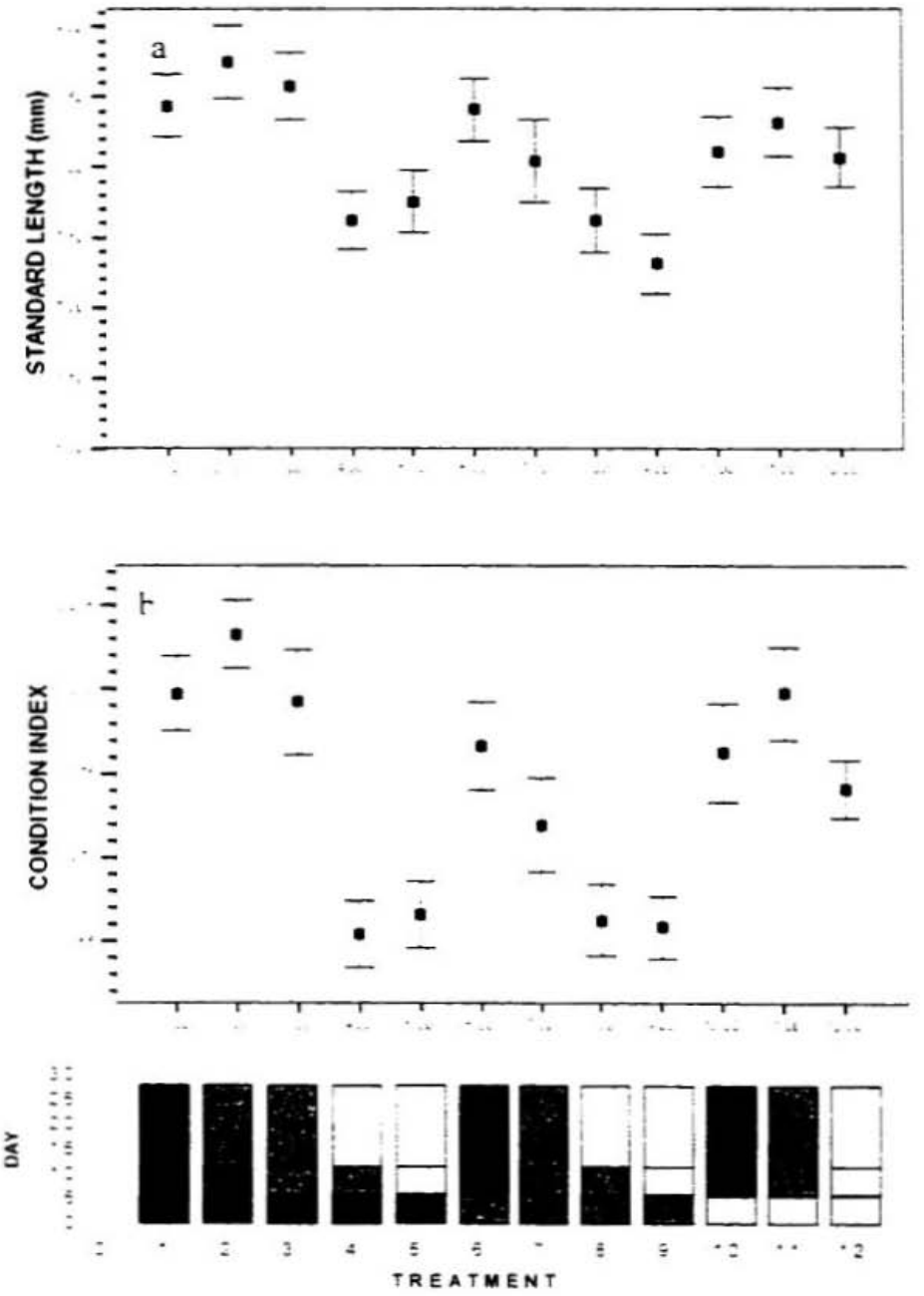


Fig. 3.8. Final length (a) and condition index (b) (± 2 S.E.) of Atlantic cod larvae reared in twelve different treatments as summarized here from Fig. 3.1. (black = 4000 prey ml, gray = 2000 prey ml, white = 500 prey ml) (n = 60 larvae per treatment).

Table 3.2. Results of a two-way ANOVA on the standard length and condition index for Atlantic cod larvae at different prey densities over time.

Measure	Source	df	F- value	p
Standard Length	Time	6	1535.53	<0.001
	Prey density	2	8.093	<0.001
	Time * Prey density	12	8.211	<0.001
Condition Index	Time	6	393.718	<0.001
	Prey density	2	29.941	<0.001
	Time * Prey density	12	14.389	<0.001

3.3.4 Behaviour

A two-way analysis of variance with a repeated measures design found no significant differences in the swim duration, pause duration, orient frequency or capture frequency between treatments, but did show a significant effect of time for all four behaviours (Table 3.3). In order to simplify the results for a general understanding of the trends observed, and because there were no significant differences between treatments, all data have been combined to show the effect of time.

The mean of all treatments shows that the duration of time spent swimming per observation decreased from a mean of 8.45 sec min on day 3 to 5.483 sec min by day 25 post-hatch (Fig. 3.9a). However, the duration of time spent in pause did not show a concomitant increase as would be expected, in fact the duration of pause decreased as well from 50.30 sec min on day 3 to 48.10 sec min on day 25 (Fig. 3.9b). The remaining time was occupied by increases in both the frequency and duration of orient and capture events as the larvae developed. Orient frequency increased from 1.55 orients min on day 3 to 5.41 orients min by day 25, which resulted in the duration of orient increasing from 1.11 sec to 5.75 sec. (Fig 3.10a). Likewise frequency of capture increased from 0 captures min on day 3 to 0.159 captures min by day 25, also resulting in a very small increase in the duration of capture, from 0.00 sec to 0.07 sec (Fig 3.10b).

There is a clear pattern of division between the orient and capture frequencies before and after the switch from endogenous to exogenous feeding which occurred around days 10 – 12. Prior to the switch, orient ($t = -7.368$, $df = 1316$, $p < 0.001$) and

capture ($t = -7.155$, $df = 1040.73$, $p < 0.001$) frequencies were significantly lower (based on the results of a t-test for unequal variances) than afterwards (Fig. 3.10ai and bi).

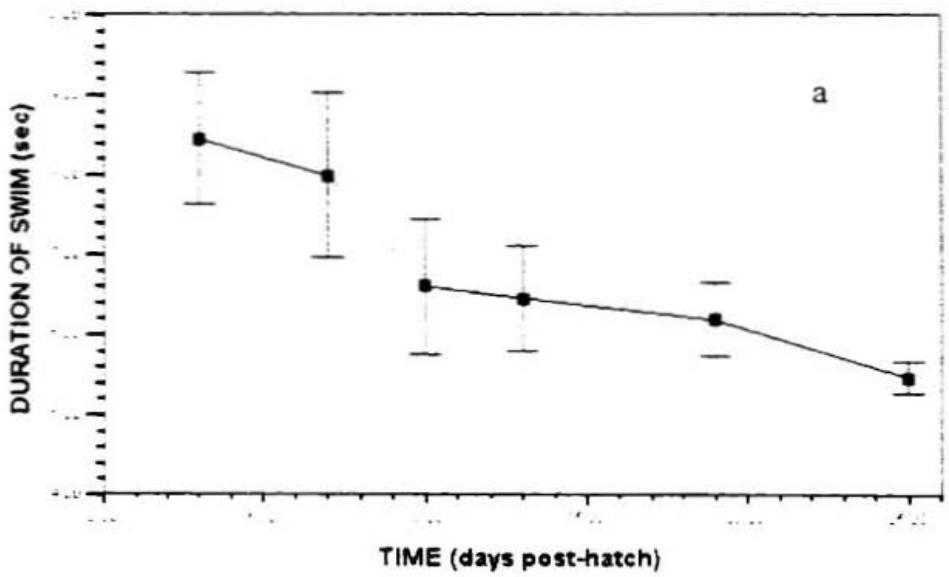
When the mean orient frequency on day 25 (the final day of observations) is plotted for each treatment there is a clear trend with orient frequency being inversely proportional to the density of prey offered during stage III (Fig 3.11). This trend was significant when treatments are grouped by prey density offered during stage III ($F = 5.113$, $df = 2$, $p = 0.016$). Likewise, capture frequency showed a similar inverse relationship to the prey density offered at stage III (Fig 3.12), which was also significant ($F = 8.202$, $df = 2$, $p = 0.002$).

Table 3.3. Results of two-way ANOVA with repeated measures design on the MAPs of larval Atlantic cod at 12 different treatments with varying prey density at three stages of larval development.

MAP	Source	df	F-value	p
Swim duration	Time	5	16.41	<0.001*
	Treatment	11	2.54	0.062
	Time * Treatment	55	1.19	0.258
Pause duration	Time	5	2.81	0.024*
	Treatment	11	2.35	0.079
	Time * Treatment	55	1.18	0.268
Orient frequency	Time	5	20.09	<0.001*
	Treatment	11	2.15	0.103
	Time * Treatment	55	0.87	0.697
Capture frequency	Time	5	15.49	<0.001*
	Treatment	11	1.89	0.145
	Time * Treatment	55	1.59	0.040**

*Significantly different ($p<0.05$)

**Affected by all zero values for the first two days of sampling.



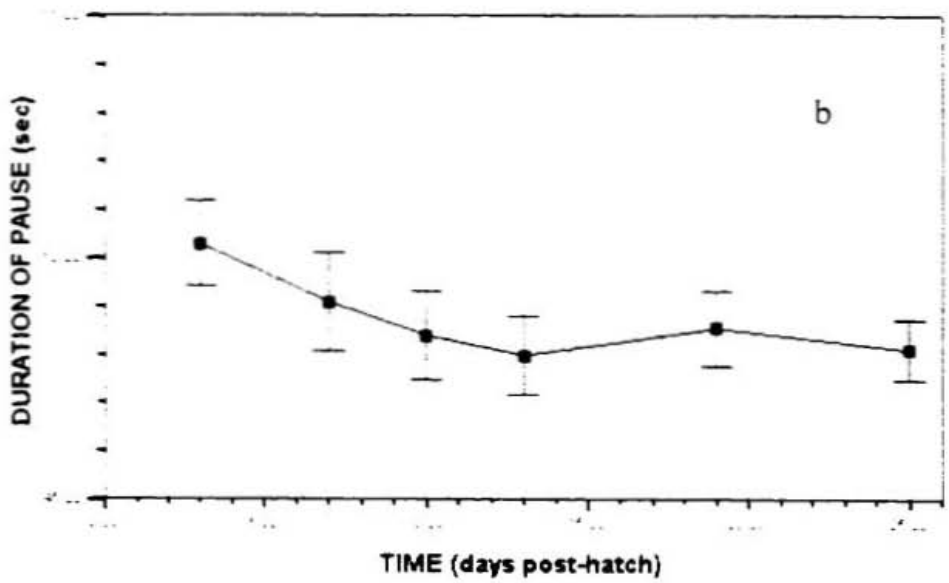


Fig. 3.9. Mean duration (sec) of (a) swim and (b) pause MAP's (± 2 S.E.) of Atlantic cod larvae over days. (n = 240 per sample).

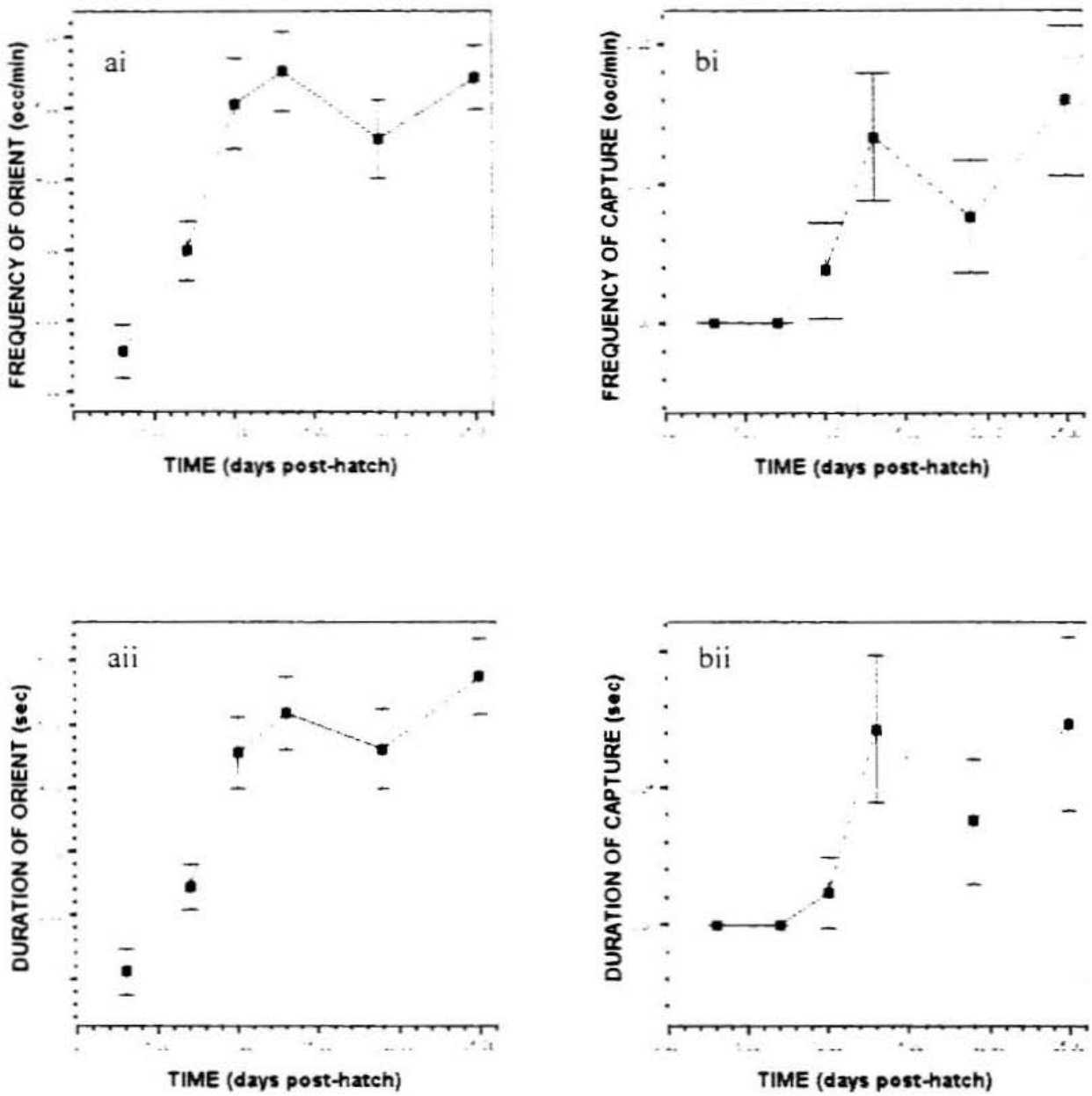


Fig. 3.10. Mean (i) frequency (= min) and (ii) duration (sec) of (a) orient and (b) capture MAPs (± 2 S.E.) for Atlantic cod larvae over time (n = 240 per sample).

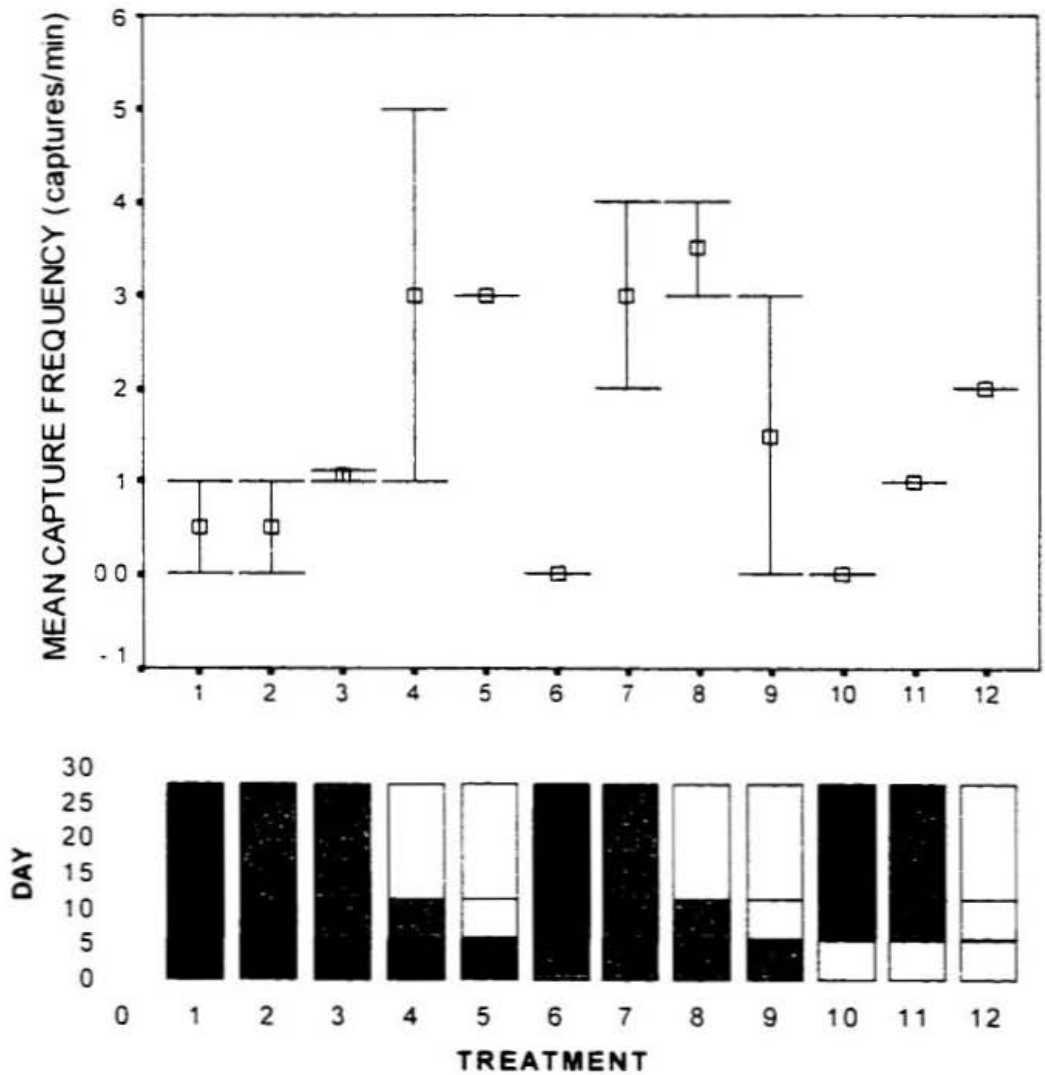


Fig. 3.11. Mean capture frequency (± S.E.) on day 25, for larval Atlantic cod reared in 12 different treatments as summarized here from Fig. 3.1 (black = 4000 prey l, gray = 2000 prey l, white = 500 prey l) n = 10 larvae per treatment.

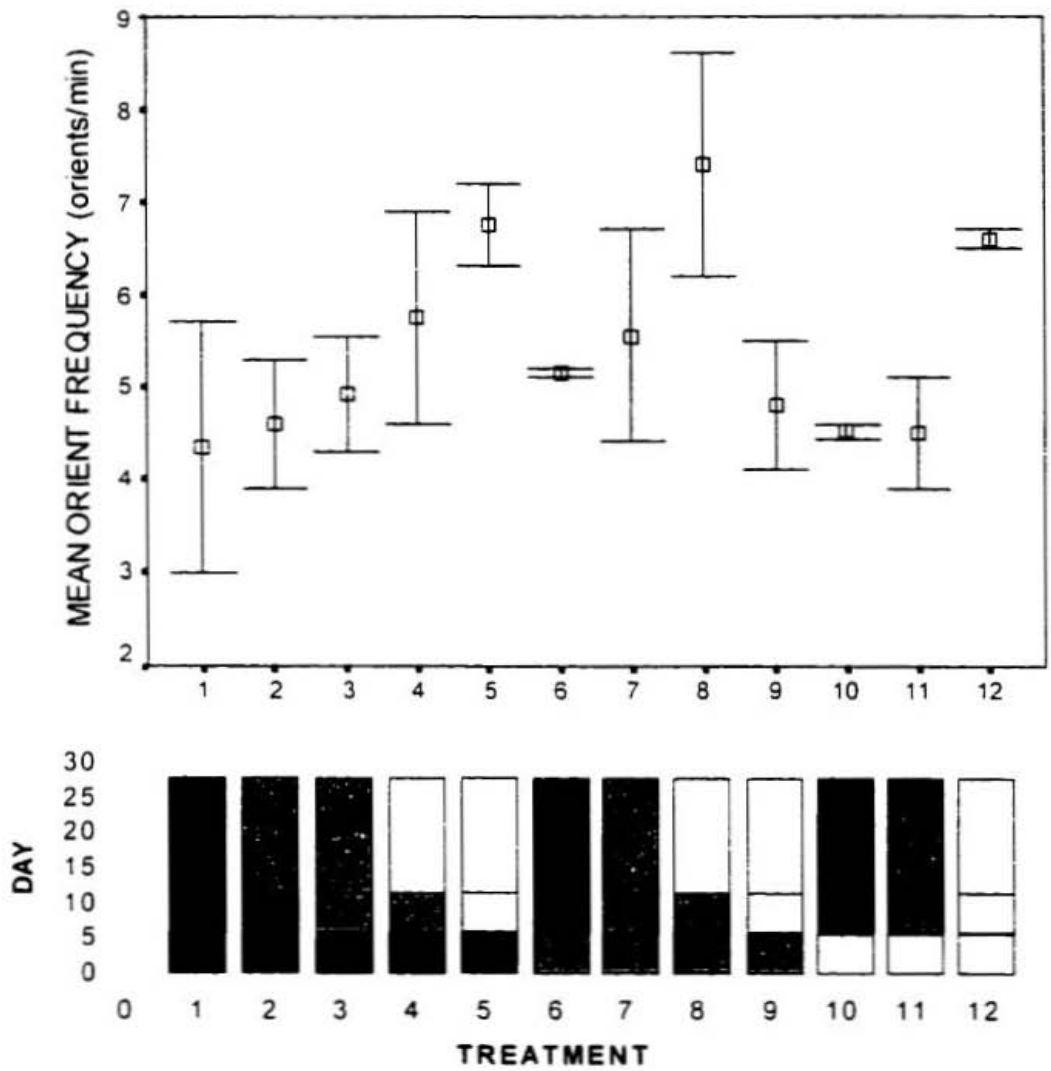


Fig. 3.12. Mean orient frequency (± S.E.) on day 25, for larval Atlantic cod reared in 12 different treatments as summarized here from Fig. 3.1 (black = 4000 prey l, gray = 2000 prey l, white = 500 prey l) n = 10 larvae per treatment.

3.4 Discussion

The results of this experiment reconfirm the "match mismatch" hypothesis presented by Cushing (1972), that a lower prey density or mismatch early in larval development is less detrimental to growth and survival than a mismatch later in the larval period. Survival and growth in this experiment were much lower for treatments that received low prey densities (2000 and 500 prey l) during the later two stages of larval development. The increased growth and survival were not supported by concomitant increases in foraging behaviour (specifically orient and capture) as has been previously observed for Atlantic cod under similar experimental conditions (Puvanendran and Brown 1999). In fact the opposite trend was observed, at higher prey densities both orient and capture frequency decreased. The possible reasons for these finding are discussed below.

The survival curve obtained in this experiment found that for all treatments 60% of the mortality occurred by day 12 post-hatch which coincides with the end of the transition from endogenous to exogenous feeding. Hart and Werner (1987) suggest that these sudden intervals of mortality and changes in behaviour (also observed here) coincide with major advancements in development. Similar mortality results were obtained by Ellertsen *et al.* (1980) who found that the yolk sac was depleted by 10 – 12 dph at 6 C. Although the effect of the yolk may last well beyond the switch to exogenous feeding, a visual inspection of the size of the yolk sac indicated that it was entirely depleted externally by 7 - 9 days post-hatch under the conditions used here.

The coring device used in this experiment permitted an estimate of the population in each tank during the experiment with minimal disturbance to the larvae. When combined with initial and final counts, the data collected from the cores provided relatively accurate survival curves for each prey density. The curves generated support the final survival results, which found that treatment 10, which had a low prey density for the first five days and then switched to high prey density for the remaining time, had the highest survival.

The mortality for the first 6 days was lowest for larvae in treatments with low prey density, while from days 12 to 25; larvae in the high prey density treatments had the lowest mortality. It is not possible to ascertain why larval survival was highest in the low prey density during stage I, however, the larval stocking density combined with high prey density may have exceeded some threshold level, such that dissolved oxygen was limiting, or some metabolic waste lowered water quality, in the high and medium prey densities. Although larval cod have been reared at stocking densities as high as 300 litre (Baskerville Bridges and Kling 2000), the flow rates, which were relatively low during stage I in this experiment, may have counteracted the low stocking densities. Another possibility is the presence of algae, which might have been in greater concentrations in the low prey density tanks since there were fewer rotifers to reduce the standing crop of algae. This is unlikely however, since recent research has shown that larval cod can be reared in the complete absence of algae, although, survival was not reported (abstract only) (Baskerville Bridges 1999).

The objective of this experiment was to investigate the effect of lowering the prey density offered to larval Atlantic cod. This experiment was designed to examine the idea that low prey densities early in larval development are more detrimental than low prey densities later (Rosenthal and Hempel 1970; Houde 1978; Houde and Schekter 1980; Puvanendran 1999). The ability for older larger larvae to accommodate lower prey densities has been justified by the fact that larvae have improved foraging abilities related to: increased size, morphological development, and increased experience (Blaxter and Staines 1970; Drost 1987; Browman and O'Brien 1992a). However, the results of this work support the findings of Gotceitas *et al.* (1996), who found increased survival of cod larvae with low prey density offered early in larval ontogeny.

The results of the behavioural observations do not appear to support the survival and growth data. It was anticipated that increased survival and growth as observed in the treatments that had high or medium prey densities during stages II and III, would be reflected by increased orient and capture frequencies as has been observed in previous experiments (Puvanendran and Brown 1999). However, higher orient and capture frequencies in the low prey density treatments towards the end of the experiment suggest that larvae in the low prey density conditions were searching and capturing more food during the observation period, which was immediately after feeding. This is the exact opposite of what Puvanendran and Brown (1999) found using a similar methodology. This discrepancy led to an investigation of the effect of flow rate and long-term observations of larvae after feeding. These results of which explain the discrepancy

between my work and previous work at this laboratory and will be provided in chapters 4 and 5.

The high number of sampling days particularly around stage II, permitted a clear picture of the changes that occur in the behaviour of larval cod as they go through the transition from endogenous to exogenous feeding. There were distinct and sudden changes in almost all of the behaviours observed as would be expected during a period of rapid development (Hart and Werner 1987). Of particular interest was the two to three day lag in the increase in capture frequency that followed the increase in orient frequency that occurred around day 10 post-hatch. This may reflect a learning period and or the incomplete development of morphological features necessary for capture.

Comparison of the results of this experiment to the previous work of Puvanendran (1999) found that many of the values for frequency and duration of behaviours for larvae reared at the same prey densities differed substantially. For example, the increase in orient frequency from 1-3 orients min to 4.5-5.5 orients min after week 2 was also seen in Puvanendran's ontogenetic observation on larval cod, which increased from 1-2 orients min to 6-8 orients min in the second week. However, Puvanendran's larvae continued to increase their orient frequency to highs of 10-12 orients min by week 4, levels that were not seen in this or the following experiments. Likewise, the frequency of capture in this study was only a fraction of that reported in Puvanendran's (1999) work.

A thorough comparison of the methodologies could find few differences between this experiment and Puvanendran's (1999). One difference was the flow rates. In my experiment flow rates were set at 40 and then 80 ml min, which is equivalent to

approximately 2 and 4 exchanges per day respectively. Puvanendran's experiment had flow rates of approximately 20 exchanges per day. The main effect of flow with respect to foraging behaviours is in relation to the duration of time that prey remains in the tanks. At higher flow rates the rotifers will be removed from the tank much more rapidly.

From the data collected in the prey clearance monitoring, it is possible to plot the duration of time that 50% of the initial prey density (duration of prey availability $^{50\%}$) remains in the tanks verses flow rate (Fig 3.13). The plot assumes that at an infinitely high flow rate the time that prey will remain in the tank approaches zero, and vice versa if flow is slowed towards zero exchanges per day, the duration approaches 12 hours. From this it is possible to estimate that the duration of prey availability $^{50\%}$ is approximately one hour in Puvanendran's experiment. As mentioned earlier larval cod are known to eat approximately 150-200 prey per day, which means if I back calculate using equation 3.3, I find that Puvanendran's larvae must have been capturing prey at a rate of approximately 1.45 rotifers min, given two feedings per day.

$$\frac{\text{Total prey consumed day}}{\text{Duration of prey availability}^{50\%}\text{ (min)}} = \text{Prey consumed min} \qquad (3.3)$$

The same calculations find my larvae feeding at a rate of about 0.5 rotifers minute. When compared to the observed behavioural data, these figures are in close agreement. Thus, given the very low capture rate in this experiment, it is not surprising that the one-minute observation period was unable to result in significant differences between

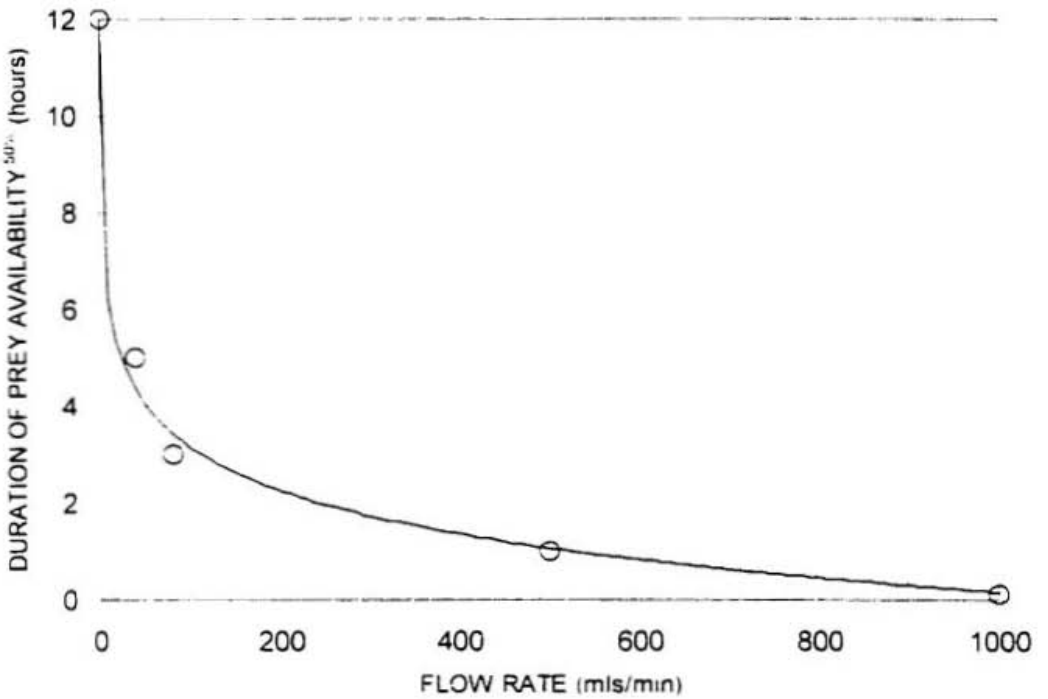


Fig. 3.13. Projected prey clearance curve, with known data points at 40 and 80 ml min, assumed points at 0 and 420 ml min, and a predicted duration of prey availability for Puvanendran's (1999) study at 500 ml min.

treatments for the observed capture frequency. This explanation for the difference between the observed foraging frequency in this study and that of Puvanendran (1999) suggests that larval cod can adjust their foraging strategy to accommodate a variety of prey conditions, an idea that is tested in the second set of cod experiments (Chapter 4).

The results of this experiment reconfirm the 'match mismatch' hypothesis (Cushing 1990), which was recently tested for cod larvae by Gotceitas *et al.* (1996); that larval cod are less affected by a mismatch (low food resources) early in development, with respect to survival and growth. From a larviculture perspective this means that rotifer requirements can be reduced by offering less live food during stage I with no adverse effects on growth or survival. The advantages of reducing the number of rotifers used in larviculture include, decreasing inputs into the tank thus improving water quality, and decreasing demand on live food, which reduces labour and costs associated with production of larvae.

The discovery of a potential relationship between foraging rate of larval cod and the duration of prey availability clearly needs to be investigated more closely. Therefore, the effect of duration of prey availability on foraging behaviours, survival and growth is the focus of the second set of experiments performed with cod larvae (Experiment II), which comprises the fourth and fifth chapters of my thesis.

Chapter 4

Effects of the duration of prey availability on the growth, survival and behaviour of larval Atlantic cod. (Experiment II)

4.1 Introduction

Larval rearing protocols and research methodologies frequently consider the effect of prey density, as well as other parameters, such as prey size and nutritional quality, etc., but rarely do they take into account the duration of time that prey is available to the larvae (Houde and Schekter 1978). Prey availability is an important issue in larval rearing, as the presence of a suitable quantity of prey will help in the initiation of first feeding (Houde 1978). However, frequently, prey density and the duration of prey availability are treated independently. This dissociation exists because prey density is actively controlled at the time of feeding, but the duration of prey availability is not normally controlled for directly, but rather it is controlled indirectly through the frequency of feeding and the flow rate to the rearing tank.

Kestemont and Awaiss (1989) found that increased feeding frequency with live rotifers from once every two days: to one, two and four times per day increased growth rates in the European cyprinid, gudgeon (*Gobio gobio*). In a recent study using yellowtail flounder (*Pleuronectes ferrugineus*) larvae Rabe and Brown (2000) found that feeding more than once per day improved growth and survival compared to feeding once per day. Their work included a plot of prey clearance rates, which gives a much clearer picture of the dynamics of the experimental conditions and makes the work easier to repeat.

The objective of the experiment in this chapter was to investigate the effects of varying the duration of prey availability on the survival, growth and behaviour of larval Atlantic cod, from hatch to the end of feeding with rotifers. Based on the results in Chapter 3 it was hypothesized that orient and capture frequencies would increase as the duration of prey availability decreased. This would provide an explanation for the differences in results between Chapter 3 and a study by Puvanendran and Brown (1999).

4.2 Materials and Methods

4.2.1 General Methodology

Larvae were hatched from eggs collected from broodstock maintained at the Ocean Sciences Centre. Husbandry and egg collection were as reported in Chapter 3, with a few minor differences. Additional broodstock were added to the spawning tank in early spring of 2000. The volume of eggs collected was 2800 ml, and the eggs commenced hatching at 84 degree-days and were more than 80% hatched by 91.3 degree-days (day 1). The same 30 litre opaque glass treatment tanks and set up were employed as in first cod experiment (Chapter 3).

4.2.2 Experimental Design

Three replicate tanks were set up for each flow treatment. Four flow rates (420, 80, 40 and 10 ml min) were chosen for the following reasons. A high flow of 420 ml min equates to approximately 20 exchanges day and was chosen to approximate the flow levels used in a previous experiment by Puvenandran (1999) with cod larvae in the same

tanks, for which data could be compared. Furthermore at these high flows it was felt that prey would be removed in less than an hour as predicted in Chapter 3, thus possibly causing larvae to increase their foraging rate. The mid range flows of 80 and 40 ml min were chosen to be consistent with previous work (Chapter 3) and resulted in approximately 4 and 2 exchanges day respectively. The low flow rate of 10 ml min equates to 0.5 exchanges day and approaches the flow rates used in the 3000 litre commercial scale facilities at the OSC. This was chosen with the intent of being able to draw direct comparisons to the processes that might be occurring in commercial production facilities.

Four additional tanks were used for the long-term observations. Two of these tanks had a flow of 10 ml min and two were at 420 ml min. Observations were made on days 22-24 post hatch.

4.2.3 Data Collection

Standard length and myotome height (as described in section 2.2.3) were measured on ten larvae (30 per treatment) on day 1, 5, 12, 19 and 27 post-hatch. Measurements were made from digital images (as described in sections 2.2.3). Survival was calculated from the final absolute count of larvae left in each tank on day 27 and from initial stocking estimates.

Behavioural observations were conducted on days 4, 8, 16, 20, 23 and 26, following the same format used in first cod experiment (section 3.2.4). However, in addition to the one-minute observations of ten larvae 10-20 minutes after feeding, one-

minute observations were also made on ten larvae prior to feeding and at hourly intervals for six hours after feeding. The objective of these long-term observation sets was to detect any fluctuations in foraging rate over time as prey were removed from the tank due to water exchange.

Tanks at each of the flow rates were randomly chosen for measurement of water quality parameters. Three water parameters were considered important to compare as an assessment of water quality: ammonia, pH, and dissolved oxygen. Dissolved oxygen and pH were tested using electronic devices, while ammonia concentrations were determined with a titration technique followed by an electronic colourimetric comparison to a set of standards (performed at the Ocean Sciences Centre).

4.2.4 Data Analysis

Condition index and length specific growth rate (L-SGR) were calculated as in the previous experiment (sections 3.2.5). A repeated measures two-way ANOVA with day and prey density as fixed factors and an interaction between the two were used for standard length, and conditions index data. Frequency and duration of behaviours were analyzed using a nested two-way ANOVA model, and differences between pre and post-feeding observations were further explored using independent sample t-tests. A repeated measures design was not used for the behavioural data because different replicates were used on successive observation days.

4.3 Results

4.3.1 Water Quality Parameters

Dissolved oxygen was not different across the flow rates and was above 95 % saturation in all tanks. The pH of all tanks was neutral for marine water at 8.1, and did not vary more than 0.2 between tanks. There were however, large differences in ammonia between treatments. Levels of ammonia ranged from 0.527 mg l in the low flow (10 ml min) treatments to 0.001 mg l in the high flow (420 ml min) treatments (Table 4.1).

Table 4.1. Ammonia concentration in mg l for each flow rate. Where more than one tank was assessed, a mean value for that flow rate is provided.

Flow (ml min)	Tank number	Concentration of ammonia (mg l)
10	1	0.612
10	7	0.544
10	12	0.425
10	Mean	0.527
40	15	0.153
80	4	0.085
420	3	0.017
420	10	0.017
420	Mean	0.017

4.3.2 Prey Clearance

As flow rate increases the duration of prey availability decreases exponentially. For the flow rates in this experiment, the prey clearance values confirmed that the shape of the curve presented in figure 3.14 (Chapter 3) was correct (Fig 4.1). The best fit (R^2 = 0.9709) to the shape of this curve was an exponential curve with the following equation:

$$\ln(y) = -0.01111877556 * x - 2.484038 \qquad (4.1)$$

4.3.3 Survival

Final survival was low in all treatments (< 10%), but was highest at lower flows with a mean of 3.7 % in the 10 ml min treatment and 3.2 % in the 40 ml min treatment. For the 80 ml min treatment mean survival was 2.6 % and was lowest (0.9%) at a flow of 420 ml min (Fig. 4.2). A straight line provided the best fit, however, it poorly described the data (R^2 = 0.1516). The high mortality in some tanks was caused by an unidentified mucous-slime (possibly a bacteria or fungus), which trapped larvae resulting in death. The mucous-slime also trapped rotifers resulting in death, which secondarily reduced the prey density in these tanks, further lowering survival of larvae. For this reason a plot of survival in tanks without this unidentified pathogen resulted in a different shaped trend-line (Fig 4.3). The best-fit line to these tanks is a log equation (4.2) with an R^2 value of 0.9316. However, because the exponential equation was the best fit

$$Y = -2.002009451 * \ln(x) - 13.4217022 \qquad (4.2)$$

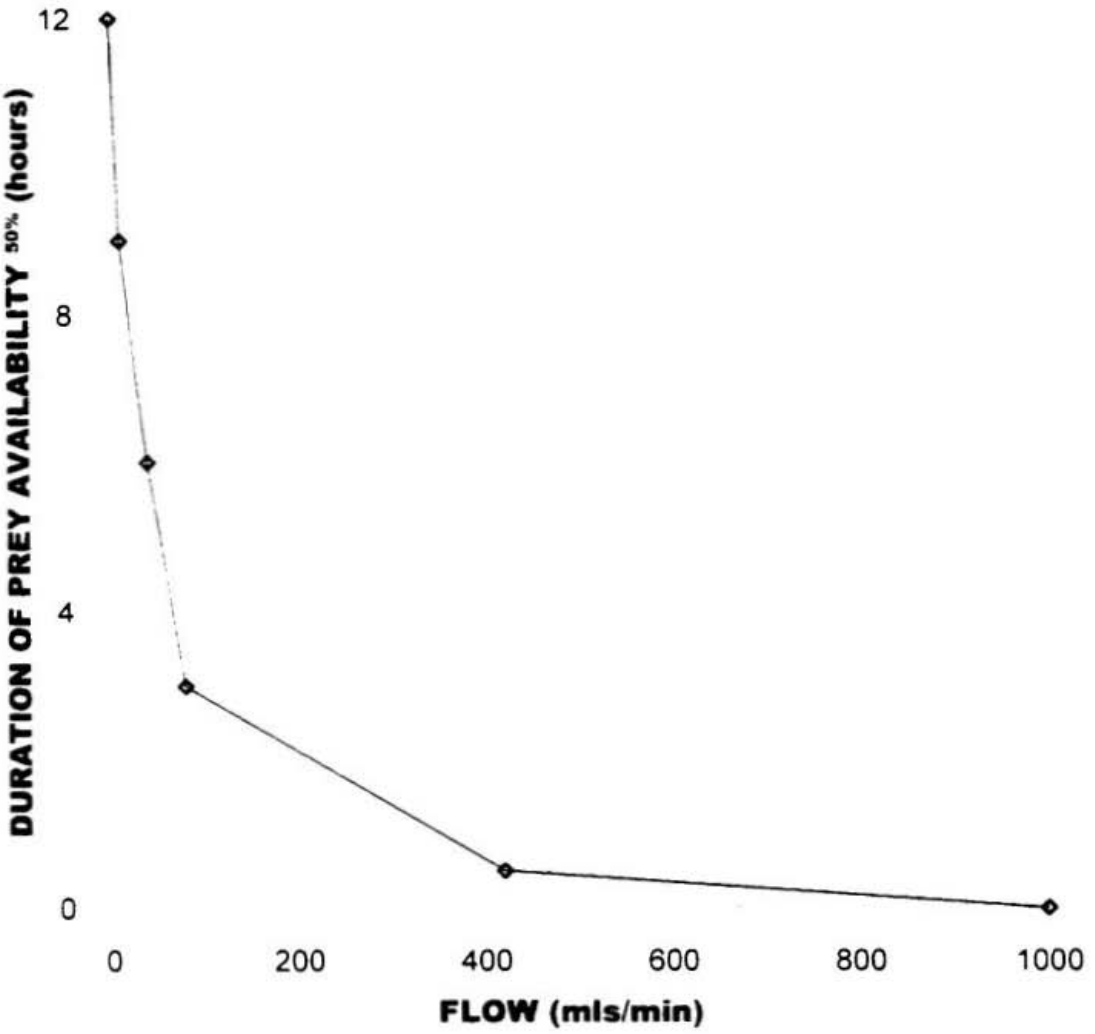


Fig. 4.1. Duration of prey availability [illegible] at four flow rates. Line of best fit is an exponential curve with the equation ln (y) = -0.011118[illegible]556 * x + 2.484038, see text for details.

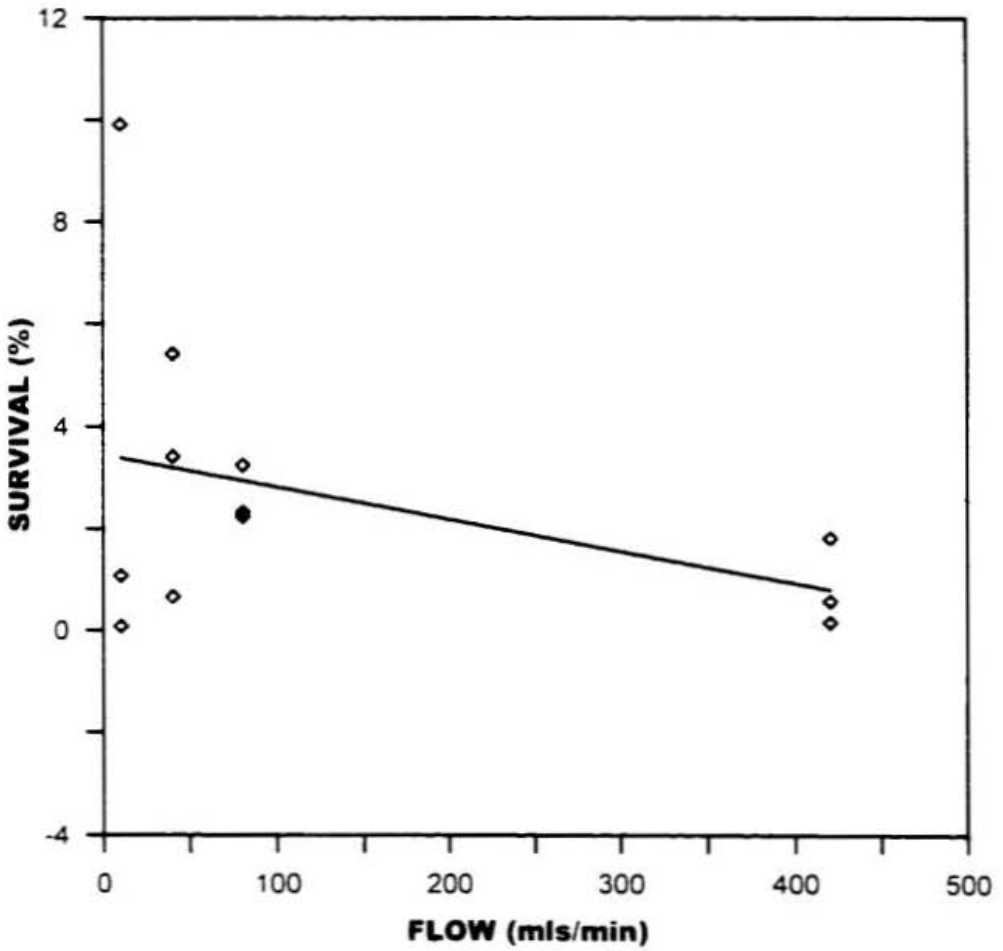


Fig. 4.2. Mean final survival (± 2 S.E.) for Atlantic cod larvae reared at four flow rates.

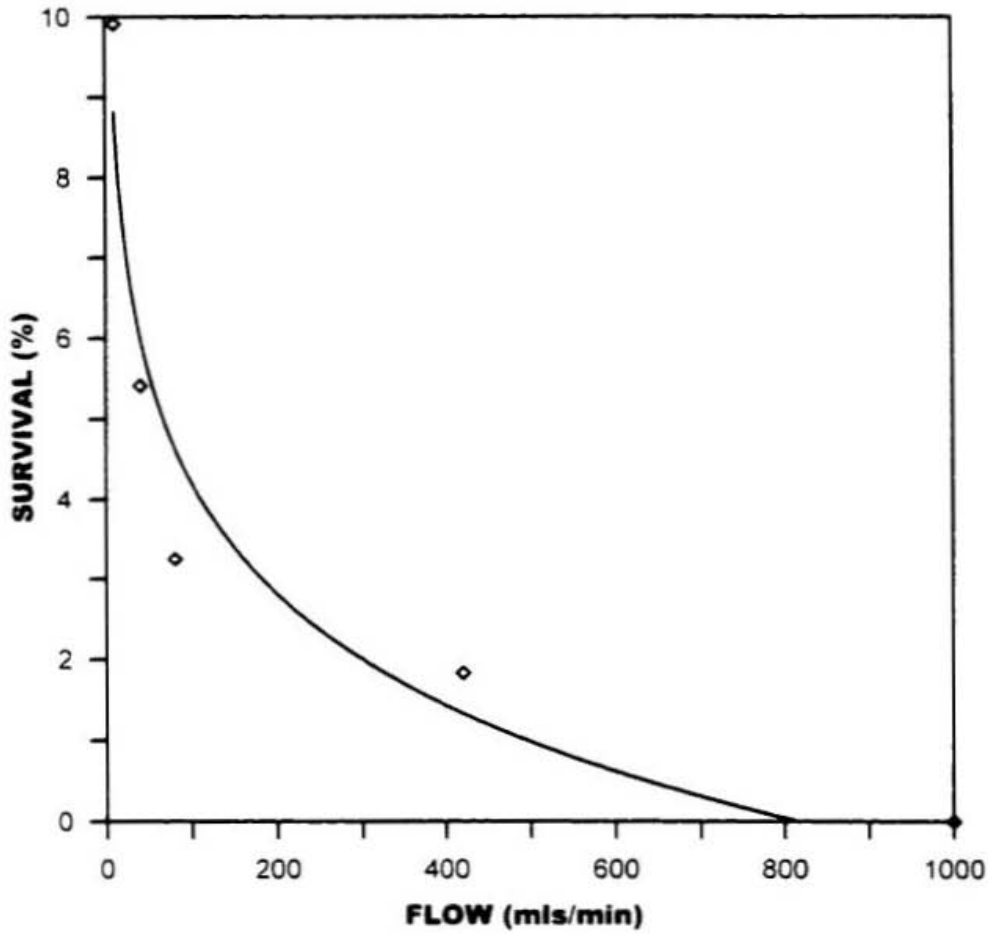


Fig. 4.3. Maximum percent survival of larval Atlantic cod reared at four flow rates. The line of best fit has an exponential equation were ln(y) = -0.00324938055 * x - 1.88911889, see text for details.

for the duration of prey availability [illegible] data, an exponential trend was also tested. An exponential equation (4.3) was still a strong fit to the data ($R^2 = 0.7390$).

$$\ln(y) = -0.00324938055 * x - 1.88911889 \qquad (4.3)$$

4.3.4 Growth

The mean length at hatch of larvae in all treatments was 4.495 ± 1.35 mm (Fig. 4.4). On day 5 there was no significant differences ($F = 1.127$, $df = 3$, $p = 0.349$) in length of larvae in the different treatments (Fig 4.5). At day 12 post hatch there were no significant differences ($F = 2.243$, $df = 3$, $p = 0.088$) in length among treatments, but a trend toward increasing length with decreasing flow rate was evident (Fig. 4.5). By day 19 the larvae in the 420 ml min treatment were significantly ($F = 16.56$, $df = 3$, $p < 0.001$) shorter in length than larvae in all other flow treatments (Fig 4.5). On the final sampling day (27dph) the length of larvae in the high flow treatment (420 ml min) was significantly smaller than all other treatments ($F = 14.42$, $df = 3$, $p < 0.001$), and larvae from the next fastest flow rate (80 ml min) were also significantly smaller ($P = 0.040$ Tukey's HSD) than larvae of the lowest flow (10 ml min) (Fig. 4.5). There was an interaction between age and flow implying that the effect of flow changes over time (Table 4.2). The change appears to be an increase in the effect of flow as the larvae develop. Flow and day, and the interaction, accounted for 89.3% of the variation in standard length of larvae in this experiment.

The same two-way (time and flow) model for condition index was not as comprehensive as the model for standard length, but still accounted for 41.8% of the variation (Table 4.2; Fig. 4.6). There was an interaction between flow and time, however, the trend is not as clear, and the effect of flow on condition is not the same as it was for standard length. There were no significant differences in condition index values on day 5 ($F = 1.143$, $df = 3$, $p = 0.384$), or day 12 ($F = 0.486$, $df = 3$, $p = 0.693$). On day 19 post-hatch the condition of larvae in the high flow (420ml min) was significantly ($F = 12.089$, $df = 3$, $p < 0.001$) lower than larvae in all other treatments. By the final day of measurements (27 dph) the 420 ml min treatment had the lowest condition index, and the 10 ml min treatment had a higher condition index than the 40 ml min treatment ($F = 8.073$, $df = 3$, $p < 0.001$).

Length-specific growth rates ranged between 0.75 % and 3.2 % per day, and larvae reared at lower flow rates tended to have higher length-specific growth rates (Fig. 4.7). Growth rates were higher at all flow rates between days 5 and 19 post hatch, and then decreased towards the end of the experimental period. Larvae in the higher flows had higher L-SGR's over the first five days, while treatments with low flow had higher L-SGR for most of the duration of the experiment. All treatments had a similar L-SGR at the end of the experiment.

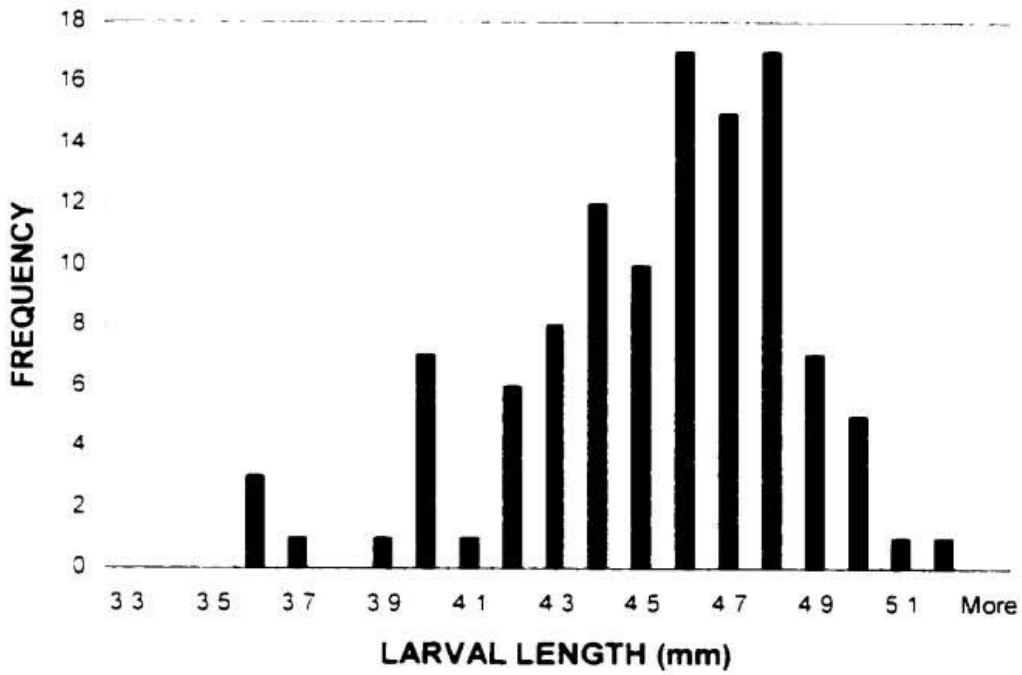


Fig. 4.4. Frequency histogram for larval size at hatch for Atlantic cod used in this experiment.

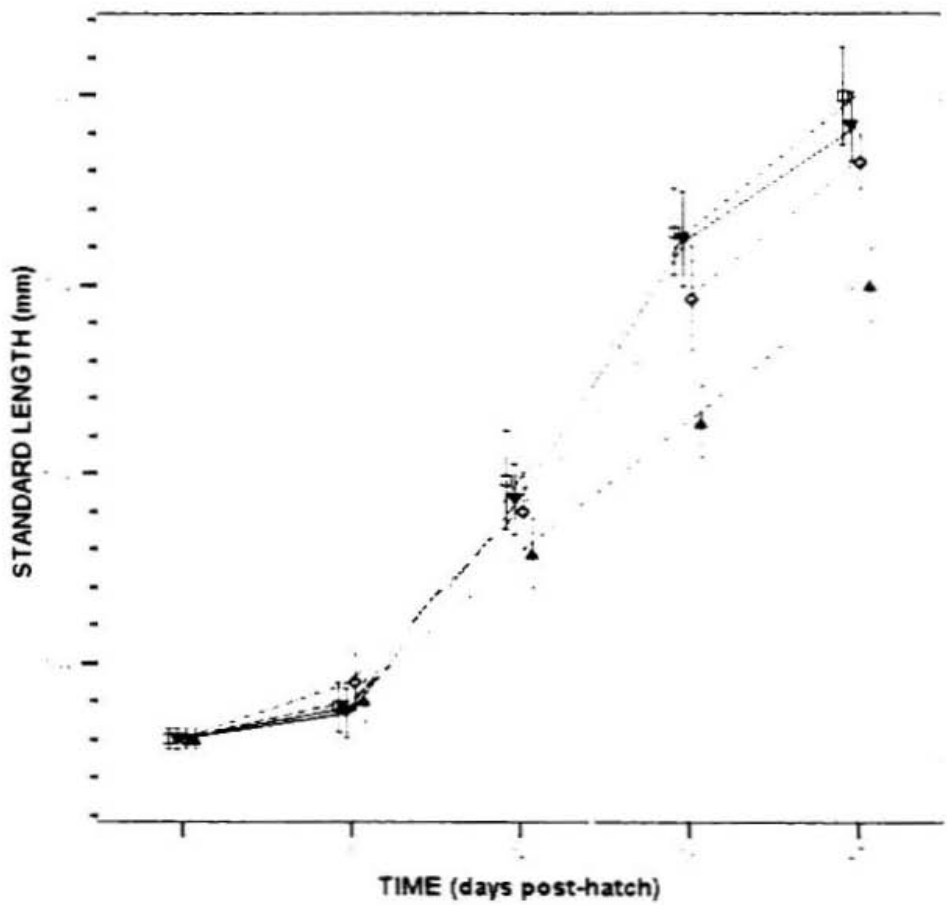


Fig. 4.5. Mean standard length (± 2 S.E.) over time for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ◇, 80 ml min; and ▲, 420 ml min).

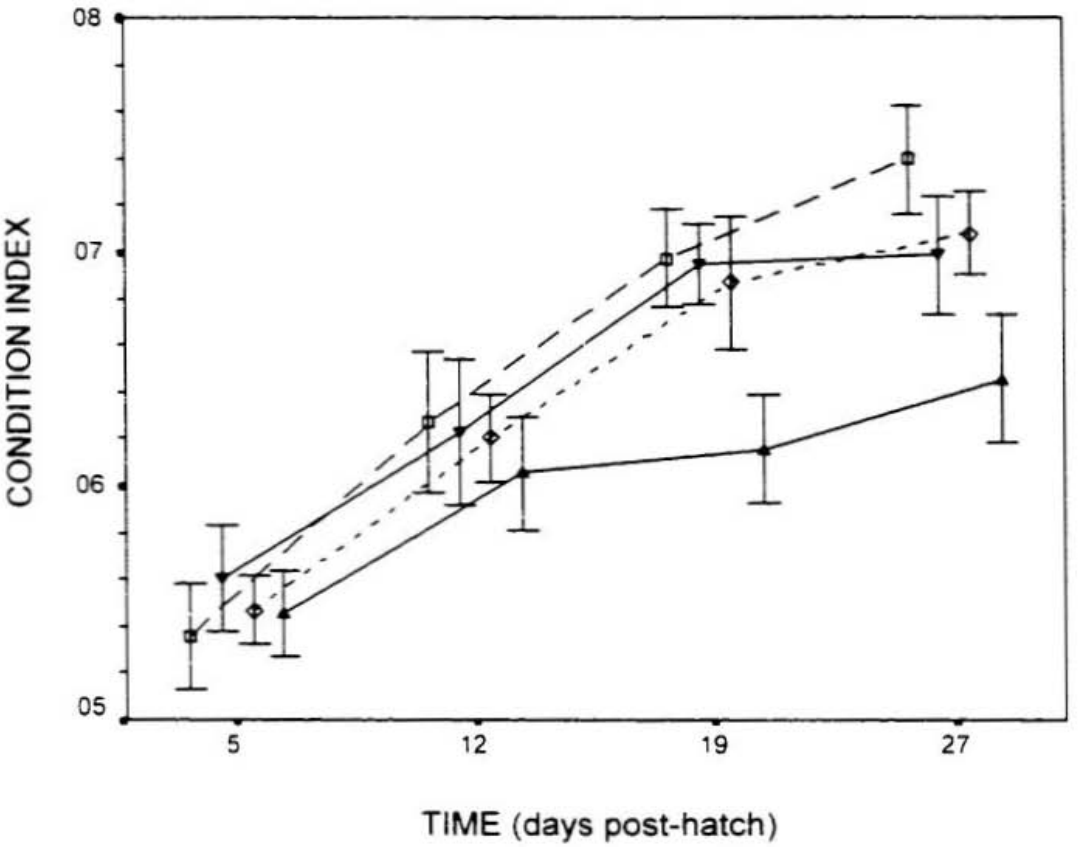


Fig. 4.6. Mean condition index (± 2 S.E.) over time for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ◇, 80 ml min; and ▲, 420 ml min).

Table 4.2. Results of two-way ANOVA's with repeated measures design for standard length and condition index of larval Atlantic cod reared at four flow rates.

Measurement	Source	F	df	p
Standard Length	Day	1259.39	4	<0.001
	Flow	8.04[†]	3	0.008
	Day * Flow	3.979[†]	12	0.009
Condition Index	Day	73.446	3	<0.001
	Flow	8.195	3	<0.001
	Day * Flow	2.132	9	0.026

[†] This data did not meet the assumption of sphericity and therefore the Wilks' test was used which also provides an approximate F value.

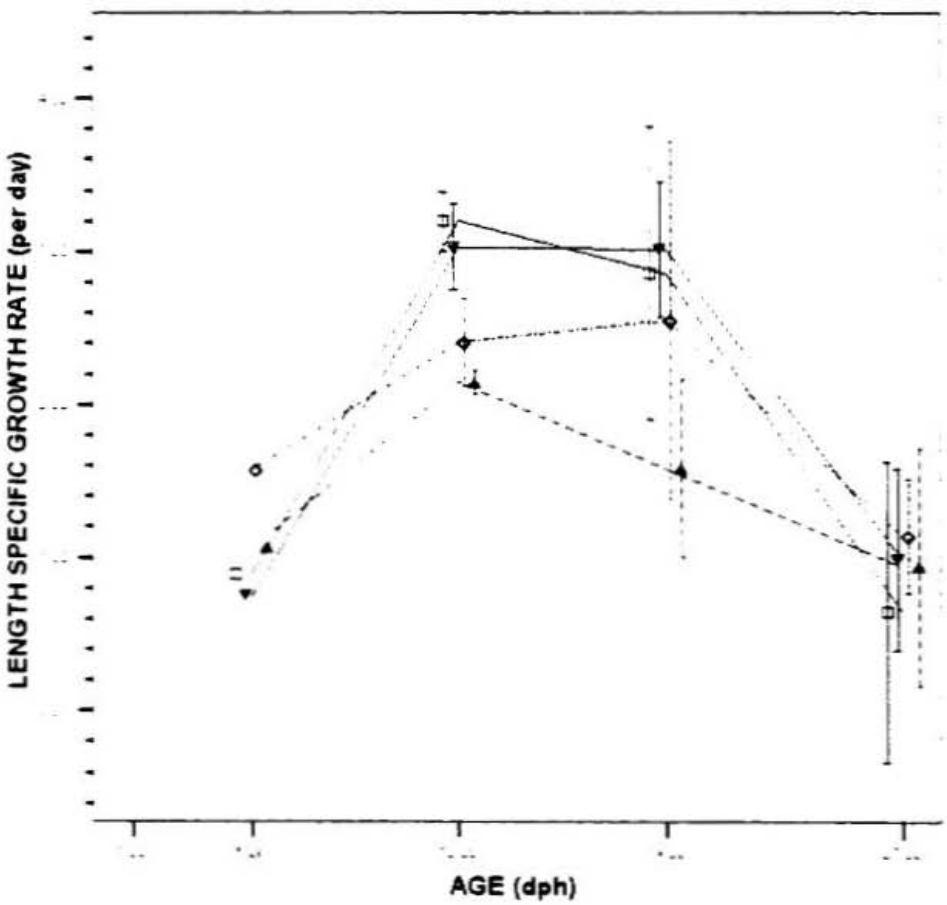


Fig. 4.7. Length specific growth rate (± 2 S.E.) with age (days post-hatch) for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ◇, 80 ml min; and ▲, 420 ml min) (n = 30 per sample).

4.3.5 Behaviour

The effect of the flow treatment was significant for swim duration ($F = 8.670$, $df = 3$, $p < 0.001$), pause duration ($F = 7.799$, $df = 3$, $p < 0.001$), and orient frequency ($F = 5.762$, $df = 3$, $p = 0.001$), but not for capture frequency ($F = 3.391$, $df = 3$, $p = 0.077$; Table 4.3). The effect of flow on swim duration does not follow a trend with increasing flow rate or over time (Fig. 4.8). There was a decrease in pause duration as flow rate increased, with the exception of the 40 and 80 ml min treatments that were between the 10 and 420 ml min treatments but were reversed in order (4.9). Orient frequency showed the inverse of the effect observed for pause duration, with increasing frequency of orient with increasing flow rate (Fig. 4.10). There was no obvious trend in capture frequency across the flow rates (Fig. 4.11). Time (dph) had a significant effect on all of the above-mentioned behaviours, and there was a significant interaction between time and flow for swim duration and orient frequency.

There were significant differences in swim duration, pause duration, orient frequency, and capture frequency between pre and post feeding observations (Table 4.3). Furthermore, there was a significant interaction between pre and post feeding and flow rate for pause duration and orient frequency. These models were able to explain some of the variation in the data (duration of swim, $R^2 = 0.325$; duration of pause $R^2 = 0.232$; frequency of orient, $R^2 = 0.332$; frequency of capture, $R^2 = 0.094$; Table 4.3).

When only post-feeding data is considered, which makes the data set comparable to Experiment I (Chapter 3) and all treatments are combined as was done in the previous experiment, comparisons can be drawn between this experiment and Experiment I

Table 4.3. Results of two-way nested ANOVA's for behaviour of Atlantic cod larvae reared at four flow rates.

Behaviour	Source	F	df	p
Duration of swim	Pre post feeding	4.513	1	0.034
	Time (dph)	26.764	5	<0.001
$R^2 = 0.325$	Flow	8.670	3	<0.001
	Pre post * Flow	1.148	3	0.329
	Time * Flow	5.350	14	<0.001
Duration of pause	Pre post feeding	20.606	1	<0.001
	Time (dph)	11.133	5	<0.001
$R^2 = 0.232$	Flow	7.799	3	<0.001
	Pre post * Flow	3.517	3	0.015
	Time * Flow	1.504	14	0.105
Frequency of orient	Pre post feeding	37.239	1	<0.001
	Time (dph)	19.027	5	<0.001
$R^2 = 0.332$	Flow	5.762	3	0.001
	Pre post * Flow	7.624	3	<0.001
	Time * Flow	2.380	14	0.003
Frequency of capture	Pre post feeding	8.434	1	0.004
	Time (dph)	2.718	5	0.020
$R^2 = 0.094$	Flow	2.291	3	0.077
	Pre post * Flow	0.187	3	0.905
	Time * Flow	1.492	14	0.110

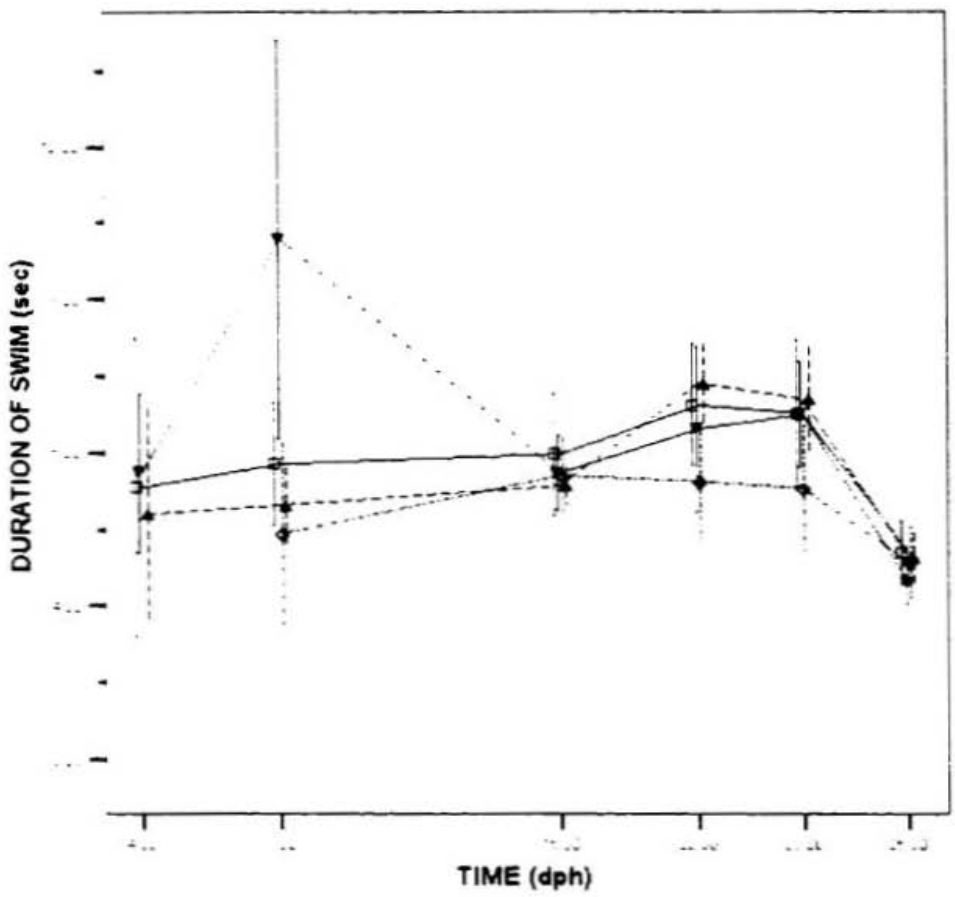


Fig. 4.8. Mean duration of swim (± 2 S.E.) over time (dph) for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ○, 80 ml min; and ▲, 420 ml min).

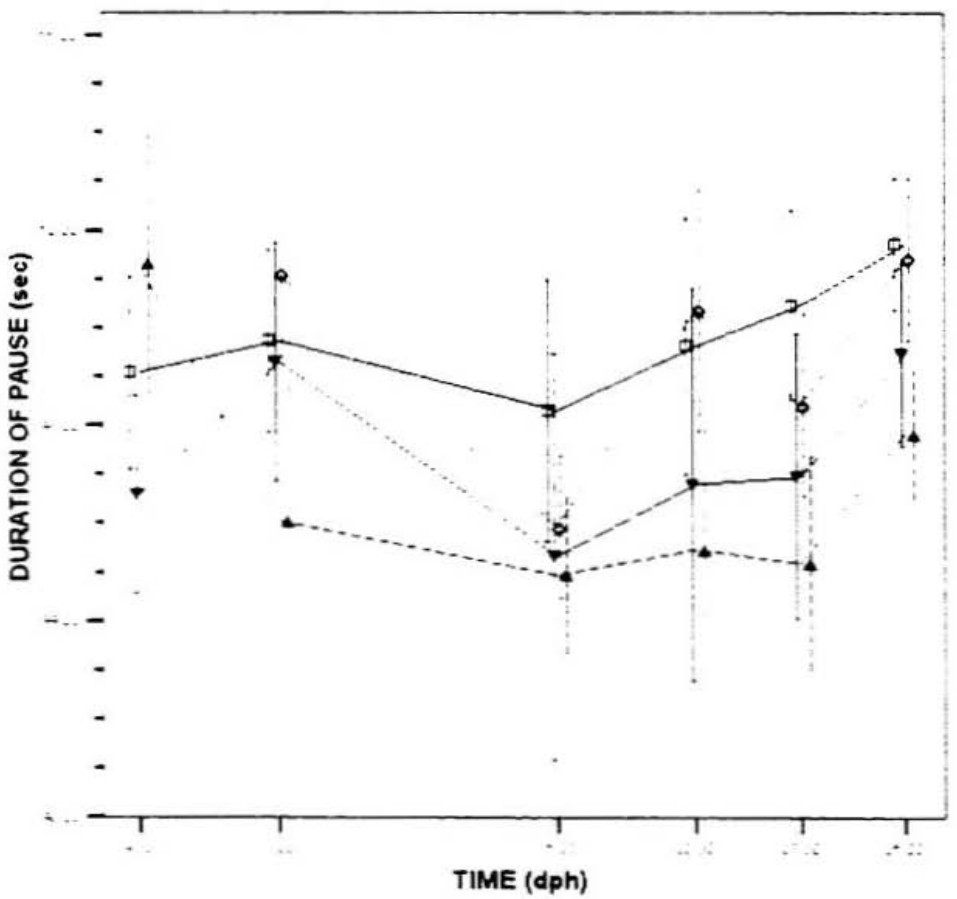


Fig. 4.9. Mean duration of pause (± 2 S.E.) over time for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ○, 80 ml min; and ▲, 420 ml min).

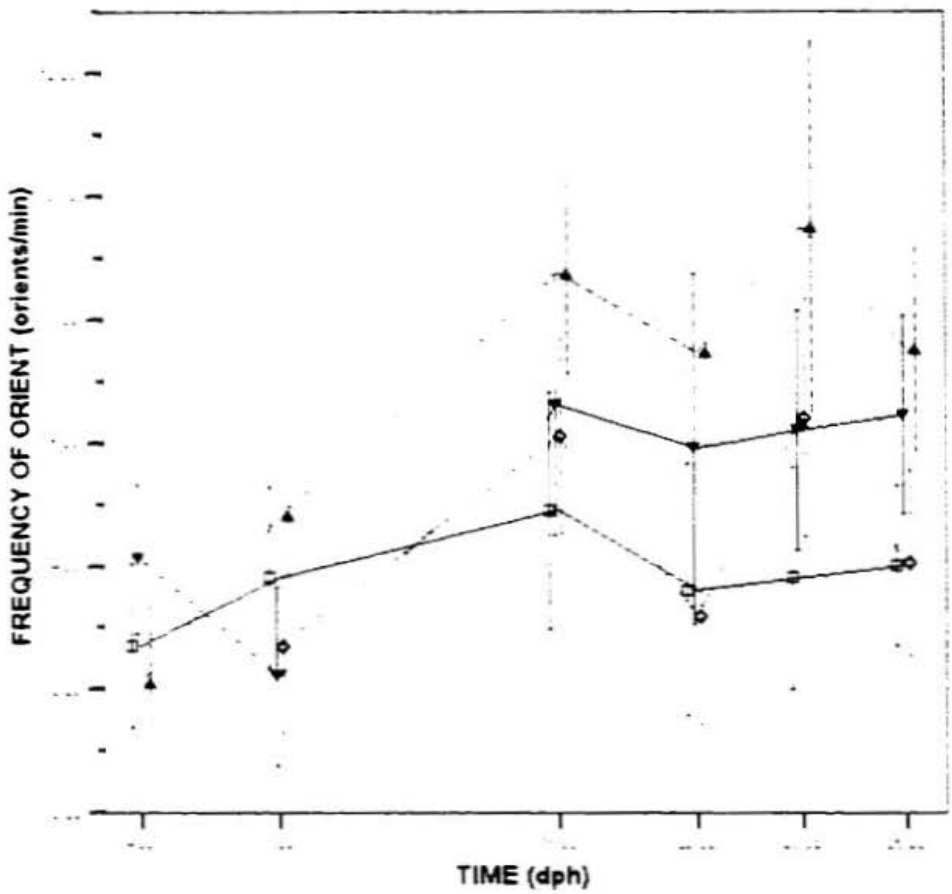


Fig. 4.10. Mean frequency of orient (± 2 S.E.) over time for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ◇, 80 ml min; and ▲, 420 ml min).

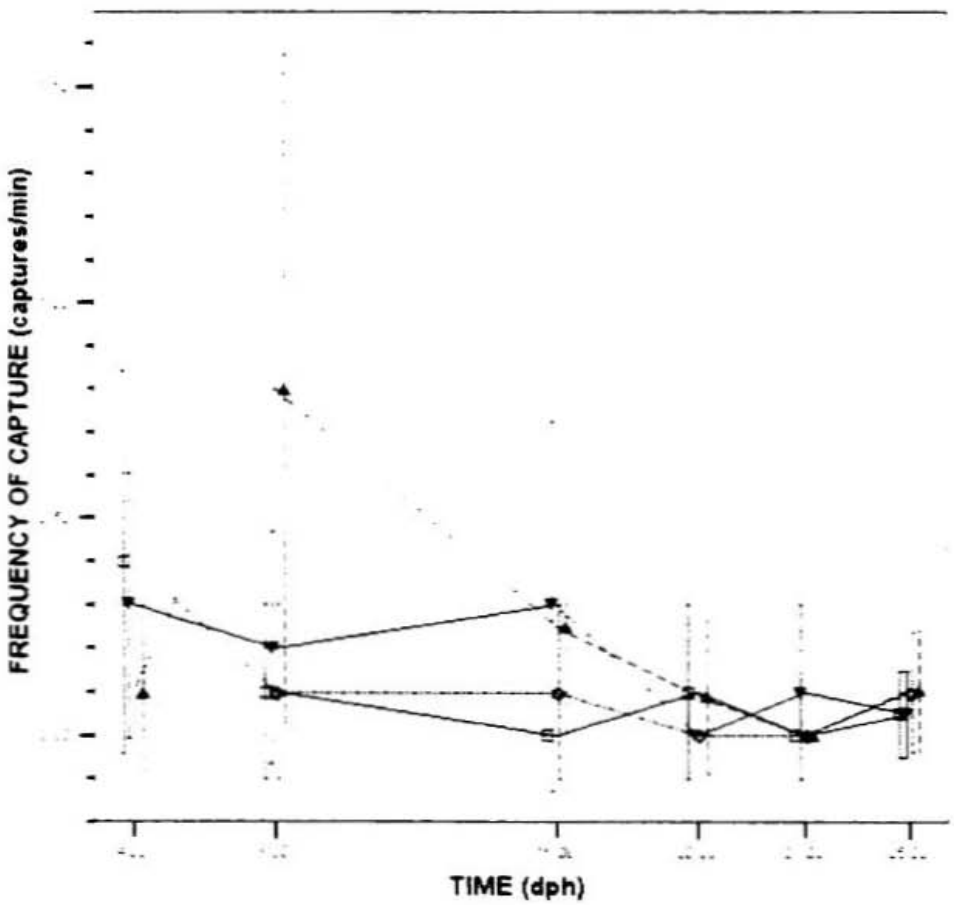


Fig. 4.11. Mean frequency of capture (± 2 S.E.) over time for Atlantic cod larvae reared at four flow rates (□, 10 ml min; ▼, 40 ml min; ◇, 80 ml min; and ▲, 420 ml min).

(Chapter 3). The duration of time spent swimming did not show the same decreasing trend with time as was observed in Experiment 1. However, there is the same sharp decrease on the last day of observations (26 dph) as was seen on day 25 of the first experiment (Fig. 4.12a). Duration of pause likewise shows no trend over time (Fig. 4.122b). Orient frequency increased from a mean of approximately 3 orients min on days 4 and 8 post-hatch to mean values ranging from 5 to 7 orients min for days 16 through 26 post-hatch (Fig. 4.12c). There is also a clear division between the orient frequency on the two days before the switch from endogenous to exogenous feeding (10-12 dph) compared to the four observation days after the switch (Fig 4.12c).

One of the goals of this experiment was to attempt to determine what the larvae were doing at other times beyond the ten minutes immediately after feeding. There were, as anticipated, large differences in the pre and post feeding behaviours (Table 4.3). Both orient ($t = -6.792$, $df = 508.4$, $p < 0.001$) and capture ($t = -2.608$, $df = 445.9$, $p = 0.009$) frequencies were significantly higher after feeding. While pause duration significantly ($t = 4.976$, $df = 518$, $p < 0.001$) decreased after feeding, and swim duration was unchanged ($t = 1.252$, $df = 518$, $p = 0.211$) (Figures 4.13a-d).

The long-term observations were intended to help in the calculation of total prey consumption by larval cod by showing the decay in foraging rate over time, allowing for a more accurate calculation of prey consumption. Due to the low capture rates in all treatments this objective was difficult to realize. However, using orient frequency it is possible to get a sense of the rate of foraging or level of hunger experienced by the larvae. Two tanks were observed before feeding, 10 minutes after feeding and again 1, 2,

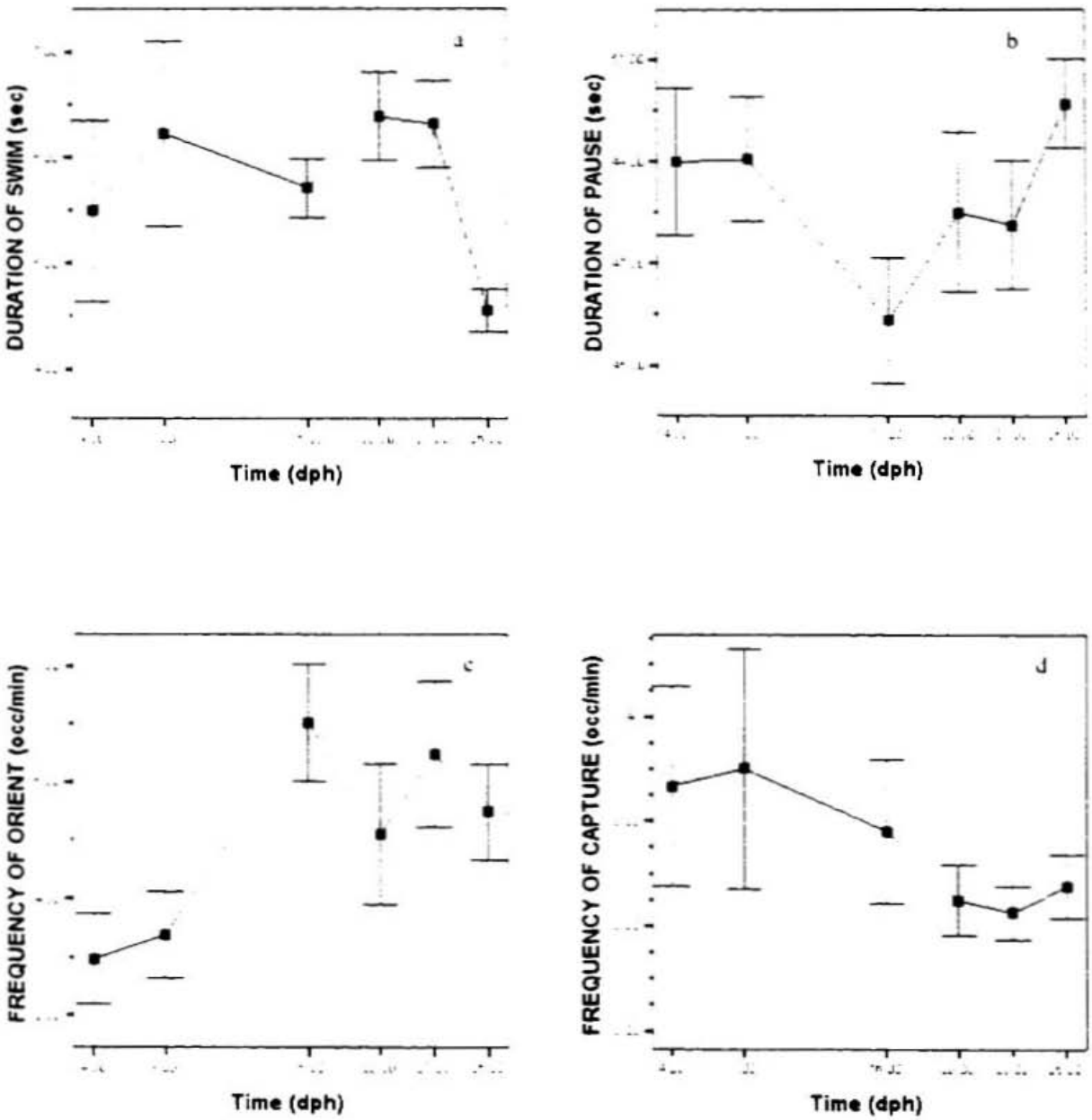


Fig. 4.12. Mean frequency duration (± 2 S.E) of swim (a), pause (b), orient (c) and capture (d) MAP's for all larvae over time.

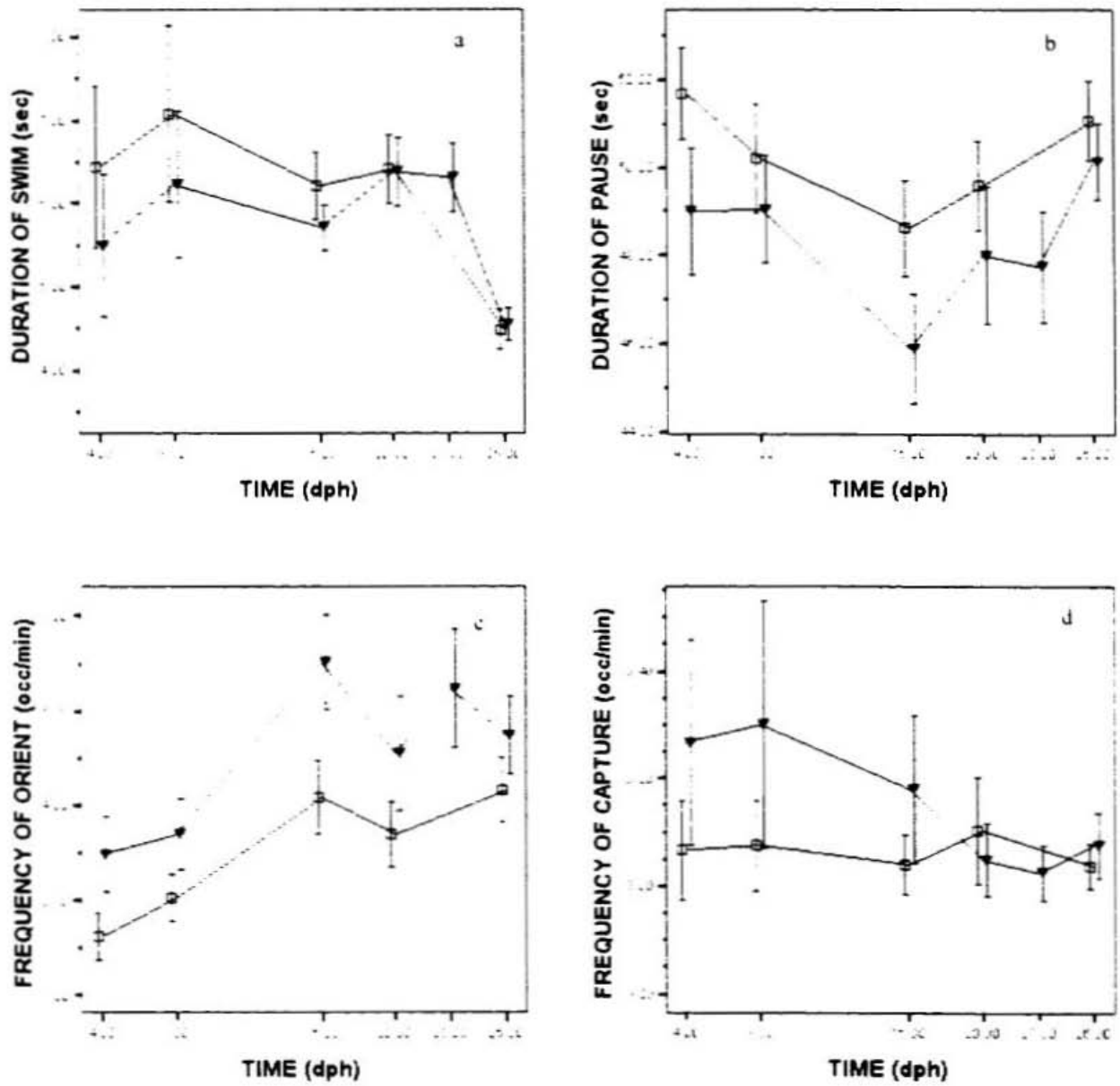


Fig. 4.13. Mean frequency duration (± 2 S.E.) of swim (a), pause (b), orient (c) and capture (d) MAP's for pre and post-feeding observations (□, pre-feeding; ▼, post-feeding).

3, 4, and 6 hours after morning feedings for three days. The mean of three days of observations with ten larvae observed for each day, for each of the high and low flow treatments, shows that larvae in the high flow oriented more frequently then larvae in the low flow at all time periods (Fig. 4.14). The other major difference between the orient frequency of larvae in the high and low flows is the sudden increase in orient rate that occurred immediately after feeding (10 mins) in the high flow (420 ml min) treatment. This increase was short lasted and decreased before the next observation period at one-hour post feeding. The decrease in orient frequency was synchronous with the decrease in prey density that dropped to approximately 1400 prey litre one hour after feeding. Conversely the larvae in the low flow rate (10 ml min) treatment showed a much more gradual increase in orient rate after feeding, and a much slower decrease after about two to three hours post feeding.

4.4 Discussion

The results of this experiment are two-part, and demonstrate firstly, that larval Atlantic cod show increased growth and survival in environments that offer a greater duration of prey availability (regulated by flow rate in this experiment). Secondly, that this increased growth and survival are not the result of increased foraging frequency but rather are due to an extended foraging period. The ability to interpret the increased growth and survival is afforded by the use of the long-term behavioural observations. Although, long-term observations are time consuming they provide a more complete understanding of the dynamics of larval behaviours.

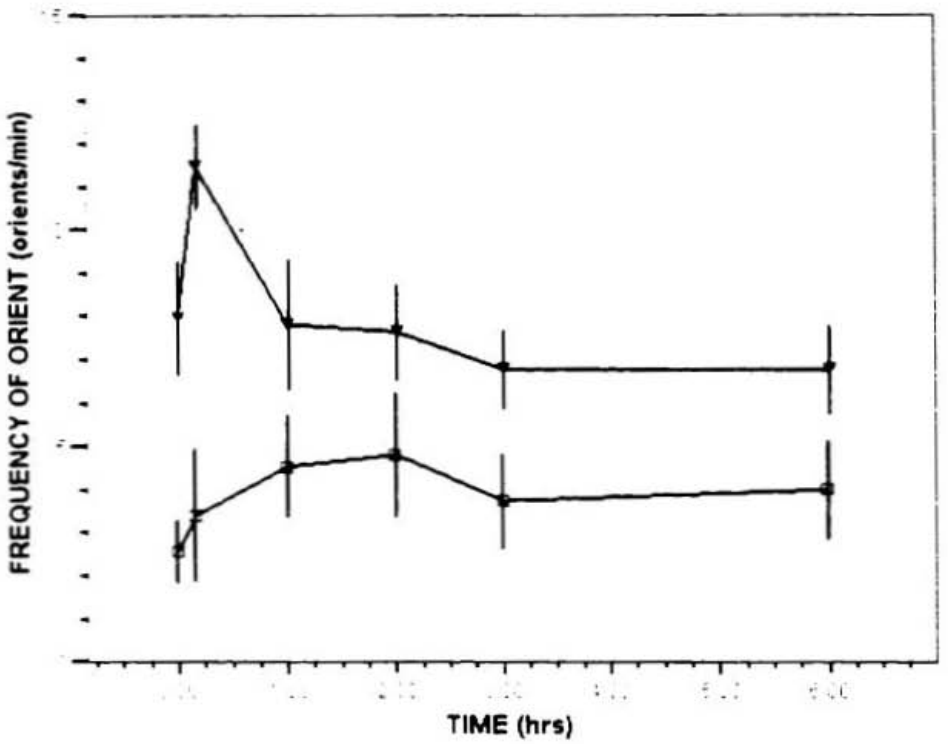


Fig. 4.14. Long-term observations for mean frequency of orient (± 2 S.E.) averaged over three days for Atlantic cod larvae reared at high and low flow (□, low flow = 10 ml min; ▼, high flow = 420ml min) (n = 30).

The standard length and survival data affirm that larvae reared in lower flow perform better than larvae in high flow treatments. Condition index results on the final day did not support these findings for the 80 ml min treatment. However, knowing that condition is a highly sensitive indicator of prey conditions (Koslow, *et al.* 1985) led me to explore these results more thoroughly. What I found was that the survival was lower in the 80 ml min treatment than the 40 ml min treatment, so one possibility is that fewer larvae were competing for the available prey, and since larval cod have a high growth potential irrespective of previous foraging conditions (van Der Meeren and Naess 1993), the low larval density could adequately explain the sudden increase in condition index. However, as discussed in the previous chapter (Experiment I) the presence of larvae were assumed to have little effect on standing crop of rotifers, compared to the effect of water exchange. This argument should be tested in a laboratory setting for larval cod, the results of which would be of great benefit to future stocking and prey density studies.

The length-specific growth rates were in the same range as those reported in the previous chapter and by Puvanendran and Brown (1999) in their work on larvae Atlantic cod, which was preformed in the same tanks and at similar temperatures. In all three of these studies there is a decrease in growth rate around 28-35 days, which I speculate may indicate a reduction in growth rate as the larvae commence metamorphosis.

The results of the pre and post feeding observations were not as anticipated. It was expected that larvae in the low flow treatments would show an increased orient frequency in the pre feeding observation periods since they would still have prey remaining in the tanks. Conversely, I predicted that larvae in the high flow treatments

would have ceased orienting by the next feeding, as there would be no prey left. In fact the exact opposite was seen on some observation days. The explanation for the persistence of the orientation behaviour when food was no longer present is that the larvae in these tanks had not eaten for several hours and were therefore hungry. These hungry larvae were orienting towards small suspended particles adrift in the tank. The larvae took on an erratic pattern of swimming, going from one piece of suspended mater to another and orienting at each multiple times.

The results of the long-term observations provide an explanation for why larvae in the high flow treatments showed increased orient and capture frequencies but not a concomitant increase in survival and growth as was observed by (Puvanendran and Brown 1999). The series of "snapshots" obtained from the long-term observations demonstrated that these increases in foraging frequency lasted only a short time interval in the high flow treatments because prey was removed quickly. The results also suggest that cod larvae can quickly adapt to changing prey conditions, as seen by the sudden increase (spike) in orient rate with increased prey density (at feeding), and that they adjust their orient rate with prey density (orient frequency decreased as the prey were evacuated from the tank). One possible interpretation is that larvae that are hungrier will orient more frequently, even at non-prey items in search for food. With this in mind, it becomes apparent that the long-term observations were vital in explaining why larvae in the lower flow treatments experienced higher growth and survival. The larvae in these lower flow treatments were exposed to prey for longer periods, and thus spread their foraging efforts out over time. While the larvae in the high flow treatment, had either

become adapted to the prey being available for short periods and thus increased their foraging rate immediately after feeding, or they were very hungry (after not feeding for several hours) and thus increased their foraging rate as a result of hunger.

Although low flow increased the duration of prey availability, it also reduced the removal of metabolic wastes and increased the residence time of pathogens in the tanks. The low survival in two of the low flow tanks was likely associated with the high concentration of ammonia in these tanks. The presence of the unidentified mucous-slime pathogen increased as flow rate decreased, although it was not possible to quantify this increase.

From a cod larviculture perspective, the results of this study suggest that not only is the density of prey available to larvae important but also that the duration for which prey is available can effect both survival and growth as a result of changes in the foraging pattern of the larvae. These results support the finding of Rabe and Brown (2000), who found that increased feeding frequency also improved growth and survival for yellowtail flounder (*Pleuronectes ferrugineus*) larvae. The differences in frequency of orient and capture between Puvenandran and Brown's (1999) study and that reported in Chapter 3 can also now be explained by differences in flow rate. Finally, it is likely that these results apply for other species of visually planktivorous larvae, therefore future larviculture protocols should consider both prey evacuation rate and water quality when selecting flow rates.

CHAPTER 5

GENERAL SUMMARY AND FUTURE DIRECTIONS

The objectives of the experiments in this thesis, were to investigate alternative feeding strategies in an attempt to reduce the live food requirements during the larval stage of fat snook and Atlantic cod for aquaculture, without significantly decreasing larval growth or survival. Observations of the behaviours of larvae in the different treatments were used to explain the mechanisms that account for differences in growth and survival, and to increase our understanding of the natural history of the species.

For fat snook larvae, the investigation began with identifying the optimal prey density, which is the lowest prey density that provides the highest growth and survival. A simple experiment comparing four prey densities ranging from 5000 to 30 000 prey litre was designed. The results demonstrate that there were no significant differences in growth or survival within this prey density range. Ensuing experiments exploring lower prey densities, were attempted on four separate occasions, however, all were curtailed due to crashes of the rotifer or algae cultures.

Larval fat snook were shown to have a saltatory foraging strategy, which is characterized by short bursts of swimming followed by pause events during which the larvae search for prey (Browman and O'Brien 1992a, 1992b). Fat snook larvae were also found to use a sigmoid position prior to a lunge towards a prey item as part of their repertoire of behaviours, however, both the sigmoid or s-curve, and lunge were removed from the foraging pattern once larvae exceeded 3.0 mm in standard length.

The larval survival in the fat snook experiment was some of the highest ever attained with this species. It is possible that the use of a flow through system may be in part responsible for this success. However, future studies are needed to investigate this possibility, and also to investigate further potential reductions in the prey requirements for fat snook larvae.

For the cod experiments, much more information was available regarding the optimal culture conditions. Previous experiments, had already defined the optimal lighting as being greater than 1000 lux (Puvanendran 1999), and the optimal prey density at around 4000 prey litre (Puvanendran and Brown 1999). An investigation into the ability of larval cod to tolerate a mismatch (sub-optimal foraging conditions) early in development had also been carried out (Gotceitas, *et al.* 1996). Furthermore, Puvanendran's (1999) work had extensively looked at the ontogeny of foraging behaviours, and the existence of a functional response in relation to prey density. Therefore, much of the foundation for the cod work already existed unlike the fat snook research. For this reason it was possible to begin directly investigating the possibility of fine-tuning the larviculture protocol to maximize growth and survival and minimize live food requirements.

The larval cod experiments looked at two ways of modifying the foraging conditions. The first experiment followed the 'match mismatch' design and looked at the role of three different prey densities at different stages in larval development. The results can be summarized as follows: a lower prey density (500 prey litre) resulted in the highest survival over the first 5 days, and thereafter a high prey density (4000 prey litre)

resulted in the highest survival. The best growth (standard length) and larval condition were achieved at a prey density of 4000 prey litre all the way through. However, at the low flow rates used, 2000 prey litre did not result in significantly lower growth or survival. In contrast to previous behavioural studies with larval cod at our lab (Puvanendran and Brown 1998; Puvanendran 1999; Puvanendran and Brown 1999) orient frequency was not found to increase with prey density, in fact the opposite trend was observed. Comparison to the previous works found that flow rates differed. Thus an investigation of the effect of flow rate was undertaken.

The second experiment manipulated foraging conditions by reducing the duration of time that prey was available in the tank between feedings. This was accomplished by increasing the flow rate (which also had consequences on water quality). The results of the second experiment show that larval survival and growth are increased at lower flow rates, because larvae have longer to forage. The behavioural observations supported these findings and led to the discovery that orient frequency was most influenced by hunger state. It was also observed that hungry larval cod were able to dramatically increase their orient rate (and capture rate) to increase consumption when food is first introduced to the tank, and that their orient rate decreases as the prey density decreases (is evacuated from the tank).

A relationship was found between the frequency of orient and the duration of pause in the second experiment. The relationship is defined by the equation:

$$\text{Orient frequency} = 37.07 - -0.66 * \text{pause duration} \qquad (5.1)$$

and accounted for 70% of the variation in these behaviours. When all cod experimental data was combined which included 2975 observations, the equation varied only slightly from that found in Experiment II:

$$\text{Orient frequency} = 36.45 - -0.64 * \text{pause duration} \qquad (5.2)$$

and still accounted for 60% of the variation in the data (Fig. 5.1). Given the saltatory search behaviour of larval cod, this equation has ecological significance. Larvae increase their frequency of orient in relation to hunger: as the orient frequency increases they spend more time searching and less time in pause. From the experimenter's point of view orient frequency is much more difficult to observe than duration of pause, therefore, knowing that there exists a strong relationship between these behaviours could facilitate future behavioural studies of this species.

One of the puzzles of larval research is the extremely high mortality that occurs around the time of first feeding. Researchers attribute the bulk of this mortality to starvation and predation (Dekhnik, *et al.* 1970; Hunter 1972; Cushing 1976; Houde and Schekter 1978; Buckley, *et al.* 1987). However, Nellen (1986) has an interesting interpretation of the predation theory. He suggests that the advantage of high fecundity for r-selected bony fish, is that they have adapted to nourish themselves through their offspring. In laboratory settings the effects of predation are eliminated and yet the mortality is high. To gain a better understanding of this mortality requires being able to

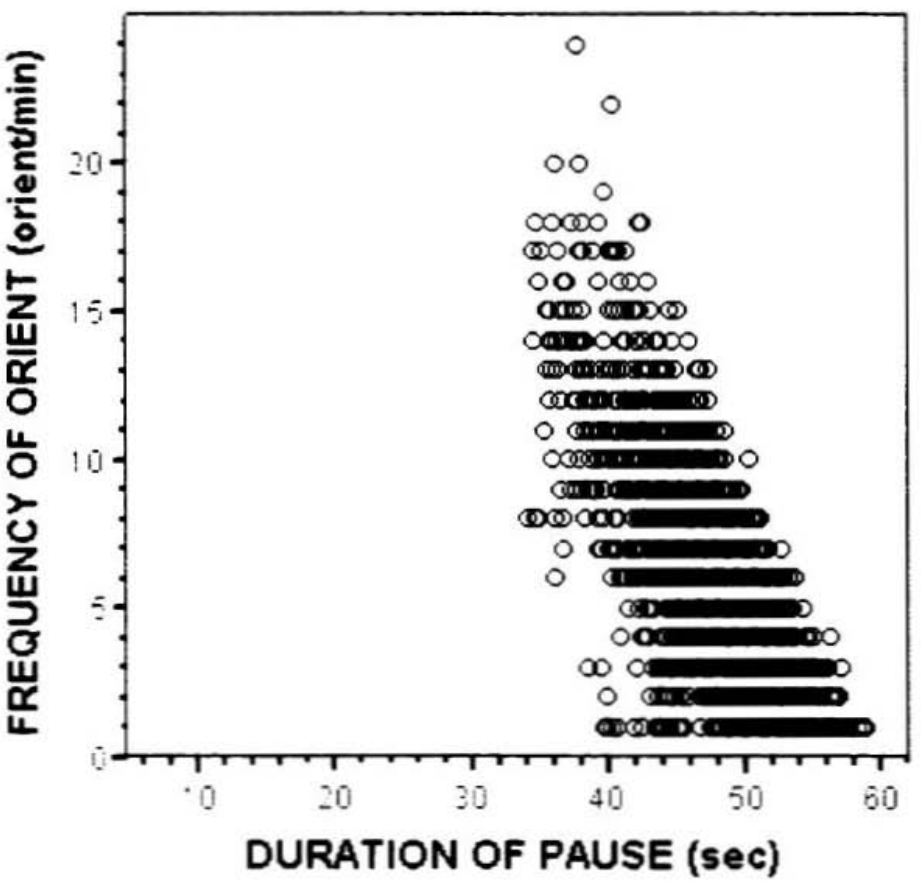


Fig. 5.1. A plot of the relationship between pause duration and orient frequency for Atlantic cod larvae from one minute behaviour observations (n = 2975).

quantify it, so that effects of treatments can be observed. This poses a problem due to the extremely small size of r-selected fish larvae. The tank-coring device used in the first cod experiment was an attempt to overcome this problem, and provided a fairly good picture of the mortality curve for the first 12 days for larval cod. Unfortunately, after approximately 14 days post-hatch the larvae became too fast and were able to avoid the coring device. A solution to the problem of counting tiny larvae in the water column of small and large tanks may not be far away. While performing these studies a sonar system was found that is able to differentiate particles of 0.5 mm. Although they have not yet been tested on larval fish a larger version of the Dynamically Responding Ultrasonic Matrix System™ (DRUMS) produced by Guigné Inc. have been used to enumerate and size fish kept in floating cage pens, and the smaller version may be a useful technology for larval fish research.

The use of behavioural observations in the experiments described in this thesis provided an underlying mechanism to explain the growth and survival results. Although at first it was not clear why larvae in higher prey densities were orienting less frequently towards prey, it became apparent after a series of hourly observation, that frequency of orient is governed by hunger as well as the density of prey items around the larvae. This was the first time that such a long series of hourly observations has been made on larval cod, and it documents the sudden change in foraging rate as larval cod adapt to novel prey conditions when they encounter a patch of prey in the temporally and spatially heterogeneous environment in which they have evolved.

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Appendix A

Implications for Atlantic cod aquaculture

The results of the larval cod experiments provide valuable information for improvements to the larviculture protocols for this species and possibly for other species as well. The discovery that decreased prey density during the endogenous feeding stage actually increased survival means that not only can a reduction in live food be made during this stage but also it will result in improved production.

Secondly, that increased duration of prey availability results in increased growth and survival. This means that prey density and flow rate should not be considered independently. Larviculturists should be aware of the length of time that prey are remaining in the culture tanks at densities that are suitable for larval foraging. Plotting a simple prey clearance curve for different flow rates for each tank and outflow design will greatly improve the understanding of prey dynamics in culture tanks.

The recommendations that come from this research are that a low prey density (500 prey litre) should be applied for the first five days, and then rapidly increased to a higher prey density (4000 prey litre) until the switch to *Artemia* naupli. A moderate flow rate should be employed, such that ammonia levels are kept low, and approximately 50-75% of prey is removed between feedings. Most importantly, however, is that the duration of prey availability is considered when determining the flow. If necessary, increased feeding frequency as opposed to increased prey density is recommended to compensate if increased flow is required to improve water quality.