# THE BEHAVIOUR, GROWTH, AND SURVIVAL OF WITCH FLOUNDER AND YELLOWTAIL FLOUNDER LARVAE IN RELATION TO PREY AVAILABILITY

JESSICA RABE

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# THE BEHAVIOUR, GROWTH, AND SURVIVAL OF WITCH FLOUNDER AND YELLOWTAIL FLOUNDER LARVAE IN RELATION TO PREY AVAILABILITY

By

©Jessica Rabe

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

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# ABSTRACT

Laboratory experiments were conducted to examine the effects of varying prey availability on the behaviour, growth, and survival of larval witch flounder (*Glyptocephalus cynoglossus*) and yellowtail flounder (*Pleuronectes ferrugineus*). The performance of larvae in relation to prey availability can provide insight into larval behavioural ecology, highlight factors that promote survival at sea, and aid in construction of appropriate feeding strategies during larviculture. In the first study, witch flounder larvae were exposed to a range of prey densities (250-16000 prey per liter) and their behaviour was recorded during feeding trials. Larvae were also reared at a range of prey densities (2000-8000 prey per liter) and their growth and survival were monitored. The foraging behaviour of witch flounder was not as affected by variation in prey availability as are other species. Larvae appear to have low prey requirements as they exhibit low foraging activity. Growth and survival of witch flounder in culture is relatively robust to changes in prey availability. The ability to forage when prey is more or less abundant may be a response to the extended larval period of this species. In the second study, yellowtail flounder larvae were reared at a constant high prey density (8000 prey per liter) and fed at high prey density at different feeding frequencies (1, 2, and 4 per day). Larval behaviour, growth and survival were monitored. The consumption rate of larvae was highest in the treatments fed 1 and 2 times per day. Growth and survival of larvae fed 2 and 4 times per day were similar to that of larvae fed continuously. It is concluded that two feedings per day at high prey density is adequate for the culture of yellowtail flounder. Pulse feeding may be an efficient strategy for larval rearing.

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# CHAPTER 1

# INTRODUCTION AND OVERVIEW

## 1.1 Introduction

The focus of this thesis is the behaviour, growth, and survival of the larvae of two flatfish species, witch flounder (*Glyptocephalus cynoglossus*) and yellowtail flounder (*Pleuronectes ferrugineus*), in relation to prey availability. This research has both academic and applied goals. Information regarding the early life history of fishes is interesting from a behavioural ecology perspective, and is also useful to fisheries scientists and the developing aquaculture industry.

Fisheries biologists hypothesize that starvation is one of the major factors contributing to mortality during the larval period (Hjort, 1914; Hunter, 1981). Because of the link between larval survival and year class strength (Cushing, 1975; Hunter, 1981; Lasker, 1981), examining the relationship between larval fish and their prey can provide insight into recruitment variability. Furthermore, the first-feeding stage of the fish life cycle is typically the bottleneck to commercial aquaculture (Tilseth, 1990). The experiments described in this thesis can therefore aid in constructing appropriate feeding regimes for these species.

## 1.2 Witch flounder

Witch flounder is a commercially important member of the family Pleuronectidae found in the deeper waters of the north Atlantic (Bowering, 1976). Despite its commercial importance, little is known about the life history of this species. What is known is largely the result of field observations conducted by fisheries scientists for stock assessment purposes. Because of its commercial importance and recent decline in stock abundance (Bowering, 1987), witch flounder has been identified as potential candidate for aquaculture in the Canadian Atlantic Provinces. However, this species exhibits a life history characteristic that may pose a challenge to larviculture: a protracted larval period. Witch flounder have one of the longest larval stages among flatfishes (Evseenko and Nevinsky, 1975; Miller *et al.*, 1991; Osse and Van den Boogaart, 1997), which may last longer than four months in the wild (Bigelow and Schroeder, 1953). As mortality during the larval period is typically high in the wild and in culture, this long larval period may prevent the successful development of witch flounder aquaculture. For this reason, the focus of my research is the larval stage.

The experiments presented here are the first reported on witch flounder culture in Atlantic Canada. In Chapter 2, I describe some preliminary results. I was able to make some interesting comparisons between witch flounder larvae from two different source populations under different experimental conditions. Although I am primarily interested in the larval stage, I also monitored the growth rates of juveniles from both populations. In Chapter 3, I present the results of a feeding experiment on witch flounder larvae. This is one of the first larval rearing experiments described for this species, and the first that focuses on witch flounder behaviour. Because this species has such an interesting life history, I discuss the results primarily in terms of the behavioural ecology of witch flounder larvae in Chapter 3. However, the relevance of these results to aquaculture is discussed in Appendix A.

### 1.3 Yellowtail flounder

Yellowtail flounder is a shallow-water member of the family Pleuronectidae found in the western North Atlantic (Pitt, 1970). Yellowtail flounder has historically supported a moderate commercial fishery, but stocks have been depleted in recent years (Bowering and Brodie, 1991). Like witch flounder, yellowtail flounder has been identified as a candidate species for aquaculture because of its consumer acceptability and reduced availability. Research on yellowtail flounder aquaculture has been conducted at the Ocean Sciences Centre, Memorial University of Newfoundland for the past five years. In the 1998 season, the laboratory produced nearly 20,000 yellowtail flounder juveniles. While we are clearly successful at rearing yellowtail flounder, it is necessary to improve our rearing efficiency. Therefore, the experiment conducted on yellowtail flounder, described in Chapter 4, is aimed at refining the rearing techniques for this species.

### 1.4 Objectives

This thesis focuses on the interactions of marine fish larvae and their prey. In Chapter 2, preliminary results on witch flounder culture are presented. In Chapter 3, the effects of prey density on witch flounder performance are examined. This is a critical first step in understanding the foraging ecology and aquaculture potential of this species. The goal of this experiment is to gain information on the feeding behaviour of witch flounder larvae, as limited information is available on the early life history of this species. In Chapter 4, the effects of feeding frequency on yellowtail flounder performance are examined. This experiment was possible because more is known about the prey requirements of yellowtail flounder larvae. The experiments presented in this thesis are related to the foraging of larval fishes and are relevant to both ecology and aquaculture.

# CHAPTER 2

# PRELIMINARY RESULTS OF WITCH FLOUNDER CULTURE

## 2.1 Introduction

The witch flounder (*Glyptocephalus cynoglossus*) is a member of the family Pleuronectidae that once formed a significant component of the Newfoundland commercial flatfish catch (Bowering and Brodie, 1991). Due to the recent collapse of commercial fish stocks and the interest in generating economic development in Newfoundland, a research program was initiated at the Ocean Sciences Centre, Memorial University of Newfoundland, to investigate the potential of witch flounder as a species for aquaculture. Witch flounder was chosen as a candidate species because it is a local species that consistently fetches a high price. However, it exhibits undesirable biological characteristics for culture such as a long larval period. For this reason, the present research was focused primarily on the larval stage. This is the first stage in the fish life cycle and is typically the bottleneck to commercial production (Tilseth, 1990).

## 2.2 Witch flounder biology

Witch flounder are right-sided flatfish that can grow to a size of 65-70 cm and live to 25 years in Newfoundland waters (Bowering, 1976). Western Atlantic populations are found from North Carolina (USA) to Labrador. However, this species is only commercially abundant north of Cape Cod, Massachusetts (USA; Burnett *et al.*, 1992). Witch flounder is primarily taken as by-catch in other offshore fisheries, such as those for

Atlantic cod (*Gadus moruha*) and American plaice (*Hippoglossoides platessoides*). However, there is a limited inshore fishery which targets this species in the deep-water bays of Newfoundland (Bowering, 1976). Witch flounder is also found in the eastern Atlantic Ocean where it supports moderate yield fisheries in the North Sea, Irish Sea, and around Iceland (Burnett *et al*., 1992). The individuals in these populations differ dramatically from those in western populations in that they do not grow to such a large size (Steinarsson *et al*., 1989). The biological information on witch flounder presented in this chapter is drawn primarily from western Atlantic populations and Newfoundland populations in particular.

Witch flounder inhabit relatively deep waters. In Newfoundland catches of highest abundance occur at depths of 185-366 meters (Bowering, 1976), although the species has been caught at depths up to 1,500 meters (Pechenick and Troyanovsky, 1970) and as shallow as 20 meters (Bowering, 1976). It is found on the muddy bottoms which are typical of the continental slope bordering the fishing banks of Newfoundland and Labrador (McKenzie, 1955). Commercially exploited populations also exist in the Gulf of St. Lawrence and the Gulf of Maine where the maximum depth is not as great (Powles and Kohler, 1970; Bowering and Brodie, 1984; Burnett *et al*., 1992). Adult witch flounder feed on benthic polychaete worms that are associated with soft bottoms. These annelids make up 75% of their diet (Langton and Bowman, 1981). Witch flounder has been caught at temperatures of 2-6°C around Newfoundland (Bowering, 1976) and according to Bowering (1989), temperature is more important than depth in regulating habitat choice.

In Newfoundland, witch flounder is managed as four separate units and a total allowable catch has been in place since 1974 (Bowering, 1976). However, there are thought to be more than four distinct spawning stocks of witch flounder. Six stocks have been identified on the basis of genetic variability (Fairbairn, 1981), while seven have been identified on the basis of meristic characters (Bowering and Misra, 1982).

Witch flounder are thought to grow slowly compared to other flatfish species (Burnett *et al.*, 1992). The slowest growing Newfoundland populations are found in the Gulf of St. Lawrence and the fastest are found on the Northeast Newfoundland Shelf. This suggests that growth rate is not entirely dependent on temperature (Bowering, 1976). Indeed, the life history characteristics of this species, such as late age at maturity (Bowering, 1976) and slow growth rate, fit expectations of density-dependent population growth models and suggest that it is resource limited and competition is high. It has been suggested that the slow growth in the Gulf of St. Lawrence population is due to increased competition for food with redfish, *Sebastes marinus* (Bowering, 1976).

The spawning season of witch flounder is extensive and ripe fish have been found throughout the year (Bowering, 1990). Peaks of spawning intensity vary throughout the species range. For example, in Labrador, peak spawning occurs from March-May (Evseenko and Nevinsky, 1975), and in the Gulf of Maine it occurs in July-August (Bigelow and Schroder, 1953). The age and length at which 50% of the population reaches sexual maturity is approximately 5 yr and 28 cm for males and 9 yr and 45 cm for females, but varies throughout the species range (Bowering, 1976).

Witch flounder exhibit intermediate fecundity and produce eggs that number in the hundreds of thousands. The fecundity varies with fish size, population, and year (Bowering, 1978). The size of eggs ranges from 1.10-1.45 mm with a mean of 1.27 mm (Evseenko and Nevinsky, 1975). Egg size and morphology is very similar to that of Atlantic cod and the eggs of these species cannot be differentiated until the last stages of egg development (Brander and Hurley, 1992). Witch flounder is thought to have one of the longest larval periods of the flatfish (Miller *et al.*, 1991; Osse and Van den Boogaart, 1997). Its Pacific relative, the rex sole (*Glyptocephalus zachirus*), also has a long pelagic phase (Pearcy *et al.*, 1977).

The long larval period and slow growth rates of this species reported from field observations have previously dissuaded researchers from studying the potential of witch flounder for aquaculture. However, preliminary results (Bidwell *et al.*, 1997) suggest that the survival of witch flounder larvae in culture is high despite the long larval period. Furthermore, slow growth rates in the field, if they result from prey resource limitation, will not necessarily translate into slow growth in culture, as it will be possible to provide adequate food for growth.

### 2.3 Project overview

In 1996 Deborah Bidwell, a graduate student of Dr. Hunt Howell of the Coastal Marine Laboratory, University of New Hampshire, began preliminary work with witch flounder larvae. She was able to rear five larvae successfully to metamorphosis. Because this is such an accomplishment for a rearing attempt with a new, unfamiliar species, the

Ocean Sciences Centre began a program to investigate the potential of witch flounder culture in cooperation with the Coastal Marine Laboratory. This chapter serves to describe the history of the witch flounder project and to present some of my preliminary results.

In 1997, I traveled to New Hampshire to assist Ms. Bidwell in the collection of witch flounder gametes. Unfortunately few eggs were collected, and efforts during the 1997 season were directed towards maintaining the larvae, and experiments were not conducted. In the 1997 season, witch flounder broodstock were also collected from Fortune bay, Newfoundland.

It was possible to obtain eggs from the Gulf of Maine again in the 1998 season. As before, few eggs were collected. These eggs were used in an experiment determining the effects of prey density on the behaviour, growth and survival of witch flounder larvae. This experiment forms the basis of Chapter 3 and is also discussed in Appendix A. Some of these eggs were also used to determine the effects of different temperatures on witch flounder growth (Section 2.4.3). In 1998, eggs were also obtained from the broodstock collected in the previous year. These eggs were used to compare the growth of witch flounder larvae obtained from the Gulf of Maine and from the Newfoundland collected broodstock under different environmental conditions (Section 2.4.3).

The growth rates of the juveniles from both the 1997 and 1998 year-classes were also monitored (Sections 2.4.4, 2.4.5). While the focus of my thesis is larval rearing, juvenile growth rates are an important determinant of aquaculture potential.

## 2.4 Preliminary results

This chapter will describe some preliminary results of witch flounder culture. I have set up the discussion below as a series of experimental trials or descriptions (Sections 2.4.1-2.4.5), presented in chronological order. This chapter is a venue for presenting results which are meaningful, but which cannot stand as chapters on their own. Future research directions and potential difficulties of working with witch flounder are discussed. Because experiments were not set up in the traditional sense, the data presented is preliminary. Statistical analyses were not performed and information such as methodology is not provided in detail.

### 2.4.1 Preliminary results of larval rearing

Introduction

My first attempt at larval rearing was in 1997. I traveled to New Hampshire to collect witch flounder gametes. I returned to the Ocean Sciences Centre in September with very few (≈2000) eggs. Because few eggs were collected, the aim of the 1997 season was to gain experience working with this species, and experiments were not conducted.

Materials and Methods

Witch flounder were stripped at sea (by Deborah Bidwell) in the Gulf of Maine in early September, 1997. I transported the fertilized eggs to the Ocean Sciences Centre. A 250L cylindro-conical upwelling tank was used for egg incubation and first-feeding. Eggs were incubated at 12° C and hatched in approximately 10 days over a period of one day.

The rearing protocol was modified from that used for Atlantic cod and yellowtail flounder in our laboratory (Puvanendran and Brown, 1999; Puvanendran, unpublished data). Larvae were fed rotifers enriched with *Isochrysis* twice a day at 4000 prey per liter (p/L) from day 1. After 30 days, *Artemia*, enriched with Algamac (Bio-Marine, USA) or protein selco (INVE, Belgium), was added at 2000 p/L in addition to rotifers. The light intensity at the water surface was 200 lux. Although cod of Grand Banks origin and yellowtail flounder prefer high light intensities (Puvanendran and Brown, 1998; Puvanendran, unpublished data) witch flounder larvae were observed feeding at this low light intensity and it was not increased. Larvae were moved to a flat-bottomed 3000L tank on day 40. The light intensity at the surface of this tank was 1000 lux. *Artemia* was added four times a day at 2000 p/L. Rotifers were also added four times a day at 4000 p/L although gut color of larvae indicated that mainly *Artemia* was being consumed. An artificial diet (pellet size 100-200 μm) was offered to the larvae from day 50. However, it was not until day 100 that live food was reduced for weaning. Microalgae-enriched water was used until day 100. The average rearing temperature was approximately 10° C with a range of 8.4°-13.2°C. Larvae were measured every few days from hatching to week 17 when 50% of the larvae had settled. The sample sizes were small (1-4 fish) due to the limited number of animals.

## Results

Only 50% of the approximately 2000 eggs hatched. Some larvae were observed feeding on day 1 post-hatch. Larvae grew and survived well under this protocol. The total length of the larvae increased from 5.9 to 59 mm over the 17 week study period (Figure 2.1). The survival was 75% from hatching to the age of 4 months.

Weaning was difficult. It was not until day 70 that larvae were observed to ingest an artificial diet and weaning was not complete until day 160. A wide variation in larval size was also observed. Larvae suffered from an infection that was not possible to diagnose (Atlantic Veterinary College, Prince Edward Island). This killed approximately 10% of the larvae. A cloudy white growth would develop on the head or muscle of larvae and they could survive for 1-3 weeks in this condition. However, it would increase in size and eventually death would occur.

## Discussion

The growth and survival of witch flounder was high during this first attempt at rearing the species at the Ocean Sciences Centre. Most of the mortality occurred during the egg incubation stage which can be attributed to handling stress during egg transportation. These positive results indicated that the potential exists for the development of commercial witch flounder culture.

Some difficulties of witch flounder culture became apparent as a result of this preliminary work. First, it is very difficult to obtain witch flounder gametes. For this reason, effort was subsequently directed at obtaining witch flounder broodstock. Second, because of the long pelagic phase of the witch flounder life cycle and the wide variation in growth rates observed, the first-feeding period is long and weaning to an artificial diet

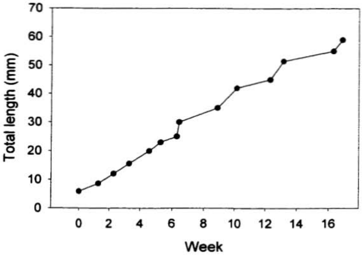

Figure 2.1. Total length (mm) of witch flounder larvae over age (weeks post-hatch). Values are means (n=1-4 per sample). Error bars not shown due to variable sample size.

is difficult. Although witch flounder larvae are relatively large, their larval digestive system may not be adequate to digest an artificial diet. A wide variation in size-at-age means that some larger larvae may be competent to consume a dry diet, while smaller larvae continue to require live prey. Furthermore, a long first-feeding period is expensive, as live food production is labor-intensive. Weaning difficulties may have been the result of using too small a particle size. In 1998, a larger diet was used and larvae accepted the diet easier. With future research directed at weaning, it is likely that the live food requirements of witch flounder larvae can be reduced.

### 2.4.2 Broodstock collection

Witch flounder are fragile, deep-water fish. The fragility of this species poses several problems for broodstock collection. Witch flounder are caught around Newfoundland by Danish seine fishermen. This gear type is relatively good for broodstock collection purposes because it does not drag the catch as much as other gear types. Furthermore, on the south coast of Newfoundland, Danish seiners make day trips. Therefore, it is possible to catch the fish and bring them back to the lab in minimal time.

I contacted Danish seine fishermen and accompanied them on a trip in November, 1997 out of Harbor Breton, Fortune Bay. On the last tow, I collected 30 fish to bring back to the laboratory. I collected fish that did not have any visible signs of net damage. I did not touch them with a net, but instead picked them up with gloved hands. Fish were maintained in 3 plastic tubs on board. Water from the deck hose was used to keep water flowing into the tubs. The trip to shore lasted approximately one hour. On land, fish were transferred to a tank inside a cargo truck. The drive to the Ocean Sciences Centre lasted approximately 9 hours. Throughout the trip, oxygen concentration was monitored and recirculating pumps were used to increase oxygen availability when the oxygen concentration fell below 80% in the tank. At the laboratory, fish were transferred to a 2x2 meter tank. Presently, approximately15 of those fish are still alive.

I attempted to collect fish again in the summer of 1998. I had hoped to collect pre-spawning fish and use their eggs in my experiments. None of the fish that I collected in July survived. This may be due to the stress associated with spawning. It may also be due to the higher temperatures encountered at the surface. November may be a better time for fish collection because there is likely a lesser difference between surface and bottom temperatures than in the summer.

### 2.4.3 Growth of witch flounder larvae from two source populations and the effects of light and temperature

#### Introduction

Light is an important environmental variable for aquaculture and it has been shown to affect the behaviour, growth, and survival of fish larvae (Batty, 1987; Mookerji and Rao, 1993; Puvanendran and Brown, 1998). In the 1997 season, witch flounder larvae grew and survived well at a light intensity of 200 lux. This light intensity is low, and both Atlantic cod (Grand banks origin) and yellowtail flounder have been shown to prefer higher light intensities (>1500 lux, Puvanendran and Brown, 1998; Puvanendran, unpublished data). The eyes of witch flounder larvae are pigmented and are relatively large at hatching which may explain their ability to forage at low light intensities. However, to gain insight into the light requirements of this species, a simple trial was set up to determine if witch flounder larvae grew and survived differently under high (2000 lux) and low (200 lux) light. Larvae from the broodstock of Newfoundland origin were used in this comparison.

There is also evidence that individuals from different areas within a species' range will perform differently when reared in a common environment if they are genetically adapted to local conditions (Conover and Schultz, 1997). This has important implications for broodstock selection as a grower would prefer to use animals from the faster growing source population. The collection of eggs from witch flounder broodstock as well as from the Gulf of Maine provided an opportunity to compare the growth of witch flounder larvae from two source populations: Newfoundland (NF) and New Hampshire (NH). This comparison was conducted at two temperatures to determine if temperature affected the relative performance of larvae from both populations.

## Materials and Methods

Eggs were collected from witch flounder broodstock at the Ocean Sciences Centre (NF) and were stripped at sea in the Gulf of Maine (NH; by Deborah Bidwell). The NH eggs were shipped by courier on ice to the Ocean Sciences Centre. The NF larvae were obtained 12 days earlier than the NH larvae. Because the NF broodstock were not expected to spawn so soon after being captured they were not checked regularly for ripeness. This is unfortunate as over 100 mL eggs were collected, although only <5 mL were viable.

Egg incubation and rearing methodology (feeding schedule, tank set-up, etc.) is similar to that described in Chapter 3. The NH eggs hatched over a period of 1 day while the NF eggs hatched over a 2 day period. There were 2 replicate tanks for each treatment. The treatments used were: NF fish raised at high (2000 lux) and low (200 lux) light at ambient (12-14°C) temperatures, NF fish raised at 7°C, NH fish raised at low light at ambient temperatures (the 4000 p/L treatment of Chapter 3), and NH fish raised at 7°C. The ambient temperature tanks were flow-through systems, while it was only possible to use static systems at the low temperature. All fish were fed enriched rotifers and/or *Artemia* three times per day at 4000 p/L (see Chapter 3 for enrichment products). Larvae were measured for standard length only. NH larvae were measured weekly and NF larvae were measured every 1-2 weeks.

This experimental trial was terminated at week 7. Many of the NF larvae died as a result of a visible infection. A white growth would develop within the muscle of the larvae. This white growth was different from that observed in the 1997 larvae. Diagnostic laboratories (Atlantic Veterinary College, Prince Edward Island) could not determine the cause of the mortality. As the NH larvae were younger than the NF larvae, the NH larvae were moved to larger tanks at week 5 as it was assumed that the infection may have resulted from the larvae out-growing their tanks. The surviving NF larvae were moved to a 3000L tank.

## Results

All larvae at 7°C died within 10 days. Larvae reared at ambient temperatures performed well. Larvae from NH were slightly larger than NF larvae at hatching (5.62 mm ± 0.12 mm S.E., 4.89 mm ± 0.055 mm, respectively). Larvae from both populations reached similar sizes at the end of the experiment at ambient temperatures. NF larvae also grew similarly under both high and low light. However, NF larvae underwent a lag period of growth between weeks 2-4 and were smaller than NH larvae at week 4 (Figure 2.2).

## Discussion

The mortality of the larvae at 7°C is consistent with the results of Bidwell (1999) who found that witch flounder larvae do not initiate feeding at 10°C or below. However, the static systems used at the colder temperature clearly may have contributed to the high mortality in this treatment. Witch flounder larvae from NH and NF populations appear to grow similarly at the warmer, ambient temperatures. This finding was surprising because the NF larvae were presumed to suffer from poor egg quality, as the eggs were likely over-ripe, and the broodstock did not feed for an extended period in captivity. This may explain the smaller size of the NF larvae at hatching. It is unclear what caused the decrease and subsequent increase in growth rates of the Newfoundland larvae. It may be related to the infection they suffered. Some infected larvae may have been included in the week 4 measurements, reducing the mean standard length.

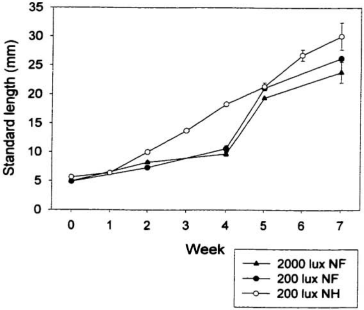

Figure 2.2. Mean standard length (mm) of witch flounder larvae from two source populations reared at different light intensities over age (weeks post-hatch). NF=Newfoundland origin, NH=New Hampshire origin. Symbols are means of the two means for each replicate ± S.E.

### 2.4.4 Growth of witch flounder late-stage larvae and juveniles from two source populations

The growth comparison between fish from the Newfoundland and New Hampshire source populations was continued once larvae were in the later stages of the larval period.

Materials and Methods

Late stage larvae from both NH and NF populations were moved to experimental tanks in early November, when the rearing experiment (Chapter 3, Part B) was completed. Weaning to an artificial diet (pellet size 300-500 μm) was initiated and weaning was complete when the experiment began on December 14. On day 0 of the experiment, NF larvae were 20 weeks, 3 days post-hatch and NH larvae were 18 weeks, 5 days post hatch.

Four 220L circular tanks were used in the experiment. The water flow was adjusted such that water entered at the bottom of the tank and flowed in a circular pattern to facilitate tank cleaning. There were 60 fish per tank. Most of the fish in both populations were still pelagic when the experiment began. Two replicate tanks were used per population. The light intensity was approximately140 lux at the center of each tank and ranged from 60-190 lux over the surfaces of the tanks. A photoperiod of 18L:6D was used. Fish were fed to excess with a commercial diet (a mixture of pellet sizes 600-1200 and 1100-1900 μm). The diet manufacturer varied depending on availability. At the start of the experiment the temperature was approximately 10°C. However, for some extended periods the temperature was reduced to <5°C (Figure 2.3).

Fish were measured for standard length and wet weight every few weeks until week 18. On each sampling day, 20 fish per tank were measured. An effort was made to measure pelagic and settled fish in proportion to their occurrence. In addition, fish were scored for progression of eye migration during the metamorphosis period. A simple index of eye migration stage was used. Fish were scored as pre-crest, crest, post-crest, and incomplete eye migration. Pre-crest refers to the migrating eye not yet reaching the dorsal fin margin. Crest refers to the stage at which the migrating eye is aligned with the dorsal fin margin. Post-crest refers to the eye having migrated past the dorsal fin margin to the prospective occular side. Incomplete refers to abnormal eye migration. Migration may not initiate or may not proceed past a certain point. In witch flounder, this was often manifested as the migrating eye stopping at the dorsal fin margin.

## Results

At the start of the experiment, NH fish were larger and more developed than NF fish. The mean standard length of NH larvae was 5.77 ± 0.07 cm on day 0. At this time 42% of NH fish were scored as post-crest, 20% were scored as pre-crest, 23% were scored as crest, and 15% were scored as incomplete eye migration. The mean standard length of NF fish on day 0 was 5.62 ± 0.02 cm. At this time 5% of NF fish were scored as post-crest, 52.5% were scored as pre-crest, 32.5% were scored as crest, and 10% were scored as incomplete eye migration.

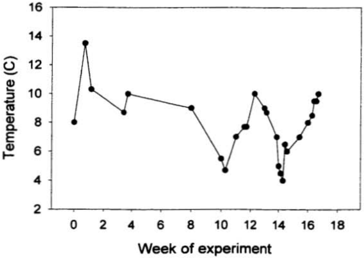

Figure 2.3. Temperature (°C) in the experimental tanks over the study period.

Both populations grew at similar rates. However, the NF fish suffered from a lag in growth between weeks 4-6. After this period they resumed their previous growth rates, and at the end of the experiment NF fish were smaller than NH fish (Figure 2.4). The average length-specific growth rate (L-SGR; see Chapter 3 for equation) for the NH fish was 0.33 % $day^{-1}$. The average L-SGR for the NF fish was 0.26 % $day^{-1}$. The absolute growth rate (see Chapter 3 for equation) for the NH fish was 0.022 cm $day^{-1}$ over the study period. The absolute growth rate for the NF fish was 0.019 cm $day^{-1}$ over the study period. The average weight-specific growth rate (W-SGR) for the NH fish was 1.19 % $day^{-1}$ and for the NF fish was 1.14 % $day^{-1}$. The survival was similar for both populations. The NH fish suffered 8.3% mortality and the NF fish suffered 10.8% mortality. Typically, it was the slower developing, pelagic fish that died.

At the end of the experiment <5% of fish in both populations were still pelagic larvae. The approximate bottom coverage was <25% in all tanks. The smallest size at which a fish was scored as post-crest was 4.8 cm SL. The largest fish scored as pre-crest was 6.6 cm SL. Fish within the size range of 4.4-7.3 cm were scored as crest. The mean size of NH fish scored as crest was 5.66 cm while the mean size of NF fish scored as crest was 6.17 cm. The process of settlement was also protracted and roughly coincided with the eye moving from the crest to the prospective occular side. During this time individuals were semi-pelagic, the proportion of time spent on the bottom of the tank increasing with development.

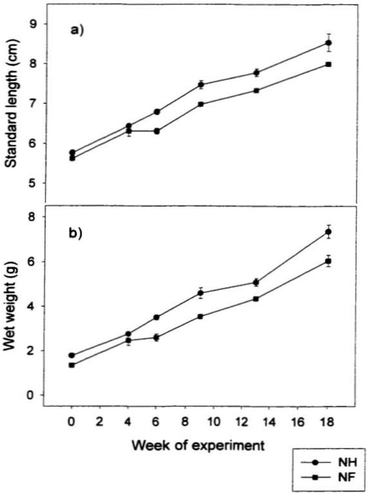

Figure 2.4. Mean a) standard length (cm) and b) wet weight (g) of late-stage larval and juvenile witch flounder over the 18 week study period. NH=New Hampshire origin, NF=Newfoundland origin. Values are means of the two means for each replicate ± S.E.

## Discussion

The results suggest that NF and NH fish grow at similar rates. However, it is unclear what caused the lag in growth of the NF fish during weeks 4-6. This occurred when the larvae were between the lengths of 6-7 cm. This size range corresponds to the transition from a late stage pelagic larva to a newly settled benthic juvenile. Reasons for the growth difference are not obvious as the fish did not appear to suffer from stress, and it may be related to the transition period.

Data on the stage of eye migration was collected to obtain a preliminary estimate of the size at which witch flounder larvae undergo metamorphosis, as the timing of metamorphosis is often defined by eye migration stages for flatfish (eg. Chambers and Leggett, 1987). As larvae were scored as crest between such a large size range (4.4-7.3 cm), a simple character such as stage of eye migration may not be useful to identify the stage of transition from a larva to a juvenile for this species. There is evidence that the processes of transformation varies between NH and NF populations as NH larvae scored as crest were smaller than NF larvae. Future work should be directed at more precisely studying the transition to a juvenile, and determining if the size at settlement varies between populations. Because the transformation from larva to juvenile is protracted for witch flounder, this species may be a useful model for those interested in this stage of fish development.

The weight-specific growth rates (≈1.15% day) reported in this preliminary study are relatively slow compared to other species that are considered aquaculture candidates. Witch flounder also do not feed actively compared to other species. One possible cause of the low prey consumption is that the artificial diet offered to the fish is very different in texture, smell, and/or behaviour from their diet in the wild. In the wild, the adult witch flounder diet is composed of 75% polychaete worms (Langton and Bowman, 1981). Because the diet of this species is not naturally diverse, it may be less likely to consume and perform well when fed an artificial diet compared to species that are used to consuming a wider range of prey. The variable temperature regime used may also have contributed to the sub-optimal growth rates. Other species such as yellowtail flounder have performed well in the experimental system used in this trial (D. Boyce, personal communication). However, witch flounder may have unique environmental requirements and research involving light regime, temperature, tank design, and diet would be valuable.

### 2.4.5 Growth of 1997 year-class juveniles

#### Introduction

Growth rates of fish are an important determinant of aquaculture potential. For this reason, the growth of the juveniles from the 1997 year-class was monitored at the same time as the growth of the NF and NH fish from the 1998 year-class.

Prior to the initiation of measurements, the 1997 year-class did not perform well. Due to a tank shortage, they were moved from a 3000L tank to a shallow (50cm) 2x2 meter tank in February, 1998. The temperature in this tank was maintained around 6°C through the rest of the winter. The fish grew slowly during this period. Furthermore, all of the fish which had not settled by the time of tank change (15%) died in the new tank. In the summer, when temperatures reached over 12°C, many of the juveniles contracted furunculosis (Atlantic Veterinary College, Prince Edward Island) and died. The fish also contracted a stomach disorder that could not be diagnosed by laboratories (Atlantic Veterinary College, Prince Edward Island). Fortunately, it was possible to save 157 juveniles using feed coated in oxytetracycline.

## Materials and Methods

In November 1998, juveniles from the 1997 year-class were moved to the same experimental system used for the comparison of NF and NH late stage larvae and juveniles from the 1998 year-class. Fish were size graded by eye into "small" and "large" groups and stocked into two 220L tanks. There were 88 fish in the large group and 69 fish in the small group. The approximate bottom coverage was between 50-75% in both tanks during the experimental trial. The light intensity at the center of the large cohort tank was 120 lux (range: 75-180). The light intensity at the center of the small cohort tank was 40 lux (range 35-50 lux). The temperature regime was the same as that for the juveniles from the 1998 year-class (Figure 2.3). Fish were fed to excess at 1.25% body weight per day. There was always extra food in the tanks under this feeding regime. Fish were measured for standard length and wet weight every few weeks. On each sampling day, 20 fish per tank were measured.

### Results

Fish in both size classes appear to grow similarly (Figure 2.5). The L-SGR of the larger fish was 0.16% day$^{-1}$ and the W-SGR was 0.67% day$^{-1}$. The L-SGR of the smaller fish was 0.21% day$^{-1}$ and the W-SGR was 0.63% day$^{-1}$.

### Discussion

Fish in the small and large size classes appear to grow similarly. Thus, the lower light intensity over the tank containing the small size cohort likely did not inhibit growth. As witch flounder occupy a deep habitat in the wild, it is probable that the juveniles are capable of foraging in light-limited conditions. Because of the poor health in the fish history, these growth rates should be interpreted with caution.

## 2.5 Summary and Conclusions

Preliminary results on witch flounder culture are very positive. It is possible to raise larvae to metamorphosis with relatively high survival. Future work should be directed towards juvenile growth as well as larval and juvenile health. The witch flounder juveniles in this study had fairly slow growth rates and some suffered from bacterial disease. Larvae in both years suffered high mortality due to an infection that was not possible to diagnose. Examinations of the effects of different diets and temperatures on juvenile growth would be particularly useful. Continued research on witch flounder culture is desirable as these positive results suggest potential for the development of commercial witch flounder aquaculture.

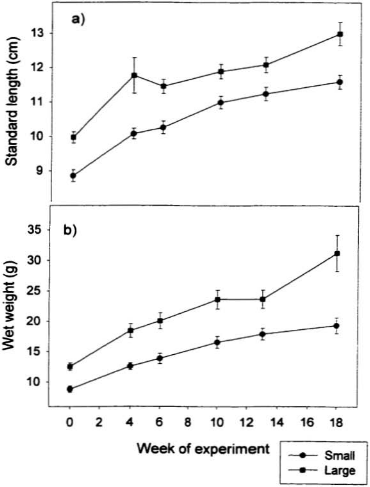

Figure 2.5. Mean a) standard length (cm) and b) wet weight (g) of the small and large groups of witch flounder juveniles from the 1997 year-class over the 18 week study period. Symbols are means (n=20) ± S.E.

# CHAPTER 3

# THE BEHAVIOUR, GROWTH, AND SURVIVAL OF WITCH FLOUNDER LARVAE IN RELATION TO PREY AVAILABILITY

## 3.1 Introduction

Starvation is one of the major causes of larval fish mortality at sea (Hjort, 1914; Hunter, 1981) and it is generally accepted that larval survival has a significant effect on fisheries recruitment (Cushing, 1975; Hunter, 1981; Lasker, 1981). The foraging behaviour of larvae and the availability of prey are therefore important factors determining larval success. Data collected at sea suggests that fish larvae encounter prey on a scale which is spatially and temporally variable (Arthur, 1977; Owen, 1989), and it is likely that larvae have evolved behavioural and physiological responses to this variation in prey availability (Brown *et al.*, 1997). In this paper, the effects of prey density on the behaviour, growth, and survival of witch flounder (*Glyptocephalus cynoglossus*) larvae are examined. Behavioural observations are a powerful experimental tool because they can illustrate underlying causes of variation in larval growth and survival.

Prey density has been shown to affect the behaviour, growth, and survival of many species of marine fish larvae (Houde and Schekter, 1980; Werner and Blaxter, 1980; Puvanendran and Brown, 1999). While many species respond to variations in prey density, the nature of this response is often species-specific (Houde and Schekter, 1980). The foraging success of a larva at different prey densities will depend on its morphology and physiology, including such factors as visual acuity, swimming speed, assimilation efficiency, and metabolic rate. Given that the shape and size of larvae change with development, their response to prey density may change with time. The performance of larvae (measured in terms of behaviour, growth, and/or survival) at different prey densities can be used to make assumptions about the foraging environment they encounter in the wild, illustrate how they respond to variations in prey availability, and ultimately determine the susceptibility of the species to starvation.

Witch flounder are interesting study organisms because they display a life history characteristic which is very different from most species of flatfish. Most flatfish larvae undergo metamorphosis at a relatively small size and early age (see Miller *et al.*, 1991; Osse and Van den Boogaart, 1997). Witch flounder have a protracted larval period that may last for longer than four months (Bigelow and Schroeder, 1953; Chapter 2, Section 2.4.4). Because this species commits itself to the pelagic environment for longer than many other species, witch flounder larvae may have developed the ability to cope with variations in prey availability and may not be as susceptible to starvation compared to other species.

The results of this experiment will be used to discuss the development of behaviour of witch flounder larvae. Of particular interest is the foraging behaviour of the larvae. The sequence of events in an individual predation event will be described to determine if this conforms to any model of larval foraging strategy, and to determine if this strategy changes with time. The effects of prey density on foraging behaviour and the importance of prey density throughout ontogeny will be determined. The growth and survival results will be used to support these observations. The results will be compared to those for yellowtail flounder to determine if differences in feeding behaviour between these species can be related to any of their life history characteristics. Finally, the susceptibility of witch flounder larvae to mortality at sea will be discussed.

### 3.2 Materials and Methods

Eggs and milt of adult witch flounder in spawning condition were collected aboard a commercial fishing vessel in the Gulf of Maine. The eggs from approximately 10 females were fertilized with milt from an equal number of males. Egg stripping and fertilization was performed by Deborah Bidwell. Fertilized eggs were shipped by courier on ice to the Ocean Sciences Centre, Logy Bay, Newfoundland. Upon arrival the eggs were stocked into two 250L cylindro-conical upwelling incubators at 12°C. Larvae hatched on the seventh day following fertilization. At this point 10 larvae were sampled for morphometric measurements (defined below) and this was identified as day zero (week 0) of the experiment.

The number of larvae was estimated by taking sub-samples from the egg incubators at hatching. Approximately 1900 larvae hatched. The experiment was conducted in two parts in order to obtain the most information possible from the limited number of larvae available. In Part A of the experiment, larvae were exposed to a range of prey densities and their behaviour was recorded during feeding trials. In Part B, a second group of larvae were reared at a range of prey densities and their growth and survival were monitored. Larvae were divided in the following manner: 400 larvae were used for

Part A, 1170 larvae were used for Part B, and the remaining 330 larvae were used in a separate experiment (described in Chapter 2, Section 2.4.3).

### Part A-Behaviour

Experimental design

Larvae were stocked into a 250L cylindro-conical upwelling tank on day zero. This tank served as a general rearing tank for larvae that were used in feeding trials. The light intensity at the surface was 200 lux and continuous lighting (24h) was used. Preliminary results with witch flounder larvae showed that this light intensity and light regime resulted in good growth and survival (Chapter 2, Section 2.4.1). The temperature in this tank ranged from 4-14°C and the mean temperature was approximately 12°C.

Feeding began on day 1 post-hatch. Rotifers (*Brachionus plicatilis*), enriched with culture selco (INVE, Belgium) and/or *Artemia franciscana* nauplii, enriched with DHA selco (INVE, Belgium) or Algamac (Bio-Marine, USA) were used as prey for the larvae. Larvae were fed three times daily at 4000 prey per liter (p/L). The prey density in this rearing tank ranged from 0-4000 p/L throughout the day. Microalgae (*Isochrysis* and *Nannochloropsis*) was added to this tank twice daily.

Data Collection

Behavioural observations were conducted every 3-4 days from weeks 2-8 beginning on day 8. Prior to feeding, larvae were arbitrarily selected and removed from the general stock tank, placed in 2L glass bowls containing the appropriate density of prey, and allowed to acclimate for five minutes. The prey densities used in the feeding trials were 250, 500, 1000, 2000, 4000, 8000, and 16000 p/L. Ten larvae were observed at each prey density and two larvae were placed together in the same bowl for observation. Larvae were not observed to interfere with each other during observation periods. Behavioural observations on individual larvae lasted for two minutes using the focal animal technique (Altman, 1974). The daily order of the observations of prey density trials was varied over the study period. The light intensity was 200 lux during the observations. On observation days, 12 larvae were non-lethally sampled for standard length and returned to the rearing tank.

Larval behaviours were categorized into Modal Action Patterns (MAPs, Barlow, 1968). During observation periods the frequency and duration of the following MAPs were recorded using an event recorder (Tandy 102): swim, pause, turn, shake, sink, orient, fixate, and lunge. For simplicity, these behaviours are grouped as follows: locomotory behaviours: swim and turn; inactive behaviours: pause, sink, and shake; foraging behaviours: orient, fixate, and lunge. Lunges on prey were scored as success or miss to produce the variable capture success [=success/(success+miss)] when it was possible for the observer to determine the outcome of a lunge (Table 3.1).

### Data Analysis

The frequency and duration of the behaviours were summarized using the Observer software package (Noldus Information Technology, Wageningen, Netherlands,

Table 3.1. Definition of Modal Action Patterns (MAPs) observed in developing witch flounder larvae, after Barlow (1968).

| MAP | Definition |
|---|---|
| Locomotory MAPs | |
| Swim: | Forward movement of the larva through water column resulting from undulations of the caudal region. |
| Turn: | A rapid lateral turn initiated by the head, usually results in rotating the body approximately 180°. |
| Inactive MAPs | |
| Pause: | Larva is motionless (similar to "non-swimming" of Munk, 1995). |
| Sink: | Larva is motionless and descends through water column, often head first. |
| Shake: | Rapid lateral undulations of the entire larval body. |
| Foraging MAPs | |
| Orient: | The head movement towards a prey item (similar to "orientation" of Brown and Colgan, 1985). |
| Fixate: | The larva is stationary and bends its caudal region into an "S" shape position, typically follows orient (Laurel, 1998). |
| Lunge: | The larva moves towards prey from the fixate position in an attempt to capture prey (similar to "bite" of Laurel, 1998). |
| Success: | Lunge that results in prey capture. |
| Miss: | Lunge in which prey capture was unsuccessful. |

version 2.0). All statistical analyses were performed using Minitab 9.2. (Minitab Inc., State College, PA). The effects of prey density and larval size on behavioural data were analyzed using analysis of covariance (ANCOVA), with size as the covariant ($\alpha$=0.05). For each behavioural response variable, the mean value for each of the 10 individual larvae per prey density was calculated for each observation day and this value was used in the analysis. Means for each treatment were weighted by the inverse of the standard deviation (S.D.) around that mean in the ANCOVA (P. Pepin, personal communication). In cases where the S.D. for a treatment was zero, the mean S.D. for that MAP (for all prey density-size combinations) was used to weight the mean for that treatment, in order to include in the analysis those larvae which did not perform during observation periods.

For most MAPs, a linear model was adequate to describe the data. However, a second order polynomial was fitted to the swim and pause duration data. Those MAPs that dropped out of the behavioural repertoire or ceased to vary between treatments (swim, turn, pause, sink, shake) posed a problem for the statistical analysis. For the turn, pause, sink and shake duration analyses, only data for the size range prior to the near decrease or disappearance of that MAP was used, in order to satisfy the assumptions of the ANCOVA. For the swim duration analysis, only data for the size range prior to the larvae spending most of their time swimming was used. The behavioural response variables were also logarithmically transformed to satisfy ANCOVA assumptions. Plots of residuals and predicted values were examined for heteroscedasticity and normality for each model and model assumptions were satisfied.

The orient frequency data could not be easily fitted to a linear or polynomial equation and was analyzed differently. A one-way analysis of variance (ANOVA) was used to determine the effects of prey density on orient frequency within the size range where orient frequency was variable between treatments (10.5-20.8 mm). A Tukey test was then used to determine which treatment means differed. This analysis ignores the effects of size on orient frequency.

## Part B-Growth and Survival

### Experimental Design

Six 33L rectangular glass tanks were used for the experiment. The sides of the tanks were painted black and all tanks were kept in a water bath to minimize temperature fluctuations. The tanks were supplied with filtered (25μm) seawater. Each tank was fitted with two air stones that provided light aeration to promote a homogeneous distribution of prey. The light level at the water surface was 200 lux and continuous lighting (24h) was used. The temperature ranged from 10-15°C and the mean temperature was approximately 12.5°C.

On day zero, newly hatched larvae were transferred to the experimental tanks. Larvae were stocked at 195 (≈6/L) per tank. Three replicated treatments were chosen: 2000, 4000, and 8000 p/L (the 4000 p/l treatment is the 200-lux NH treatment discussed in Chapter 2, Section 2.4.3). The use of a greater number of treatments and replicates was desired; however, this design was chosen due to space limitations. Previous results in our laboratory for other North Atlantic fish larvae indicated that this range of prey densities and replication level was sufficient to observe the effects of prey density on growth and survival (Laurel, 1998; Puvanendran and Brown, 1999; Puvanendran, unpublished data).

Rotifers (*Brachionus plicatilis*) and/or *Artemia franciscana* nauplii were used as prey for the larvae. The live prey was enriched with commercial products as indicated in Part A. Prey densities in the tanks were adjusted three times a day (approximately 10AM, 4PM and 10PM). Microalgae (*Isochrysis* and *Nannochloropsis*) was added to the experimental tanks prior to each feeding. Prey densities were determined by sampling a 5 mL aliquot from different depths within the tanks (below surface, mid-depth and above bottom) before each feeding. The total number of prey items in each sample was counted and the prey density was adjusted as required.

At week 5, the larvae were transferred to larger 65L orange tubs as the larvae had grown in size. The rearing experiment was stopped at week 12 when the larvae were too large to continue feeding *Artemia* and weaning to an artificial diet was initiated. At this point most larvae had begun eye migration and all larvae were pelagic, spending little time on the bottom of the tanks.

### Data Collection

Larvae were sampled weekly for growth. Standard length (SL, measured from tip of snout to posterior end of notochord) was measured every week. For weeks 0-3, larvae were measured to the nearest 0.1 mm using a dissecting microscope. Standard length was measured to the nearest 1 mm using a ruler after week 3. On weeks 0, 1, 5, 8, and 12 larvae were lethally sampled (killed by an overdose of MS-222) for SL, body height (BH, myotome height posterior to anus), head depth (HD, measured posterior to eye), eye diameter (ED, measured perpendicular to body axis), and dry weight (DW). For all other weeks, live larvae were measured for standard length only. Lethally sampled larvae were kept in beakers on ice and measured immediately after death to prevent shrinkage due to osmotic loss. Larvae were rinsed in 3% ammonium formate (to remove salts), placed on pre-weighed aluminum foils (weighed to nearest 0.001 mg), dried at 55°C for at least 48 hours, and re-weighed. When larvae were lethally sampled, five larvae were measured from each tank. When larvae were non-lethally sampled for SL only, ten larvae were measured per tank (except for weeks 2-3 where n=5 per tank).

The absolute growth rate was calculated according to the equation:

$(L_t - L_0)/t$

and the length-specific growth rate (L-SGR) was calculated according to the equation:

$\mathrm{SGR}=(\ln(L_t)-\ln(L_0)/\mathrm{t})\mathrm{x}100$,

where $L_t$ is the mean final length (mm), $L_0$ is the mean initial length, and $t$ is the period of growth (days) (Busacker *et al*., 1990).

All tanks were examined for mortalities twice daily from day 14. The small larval carcass decomposes too quickly to be observed prior to this time. At the end of the experiment, the number of surviving larvae in each treatment was recorded.

## Data Analysis

For each growth measurement (SL, BH, HD, ED, DW), a mean value was calculated for each replicate and this value was used in the analysis. These growth measurements were analyzed by treatment using ANCOVA with week of the experiment as the covariant ($\alpha$=0.05). Dry weight data was logarithmically transformed to satisfy the assumptions of ANCOVA. An ANOVA was used to test for differences in survival at weeks 2, 5, and 12.

## 3.3 Results

### Part A-Behaviour

Ontogeny of Behaviour

The behaviour of the larvae changed throughout the study period. Initially larvae divided their time between periods of inactivity (60-80% of total time) and locomotory activities (20-40% of total time, Figure 3.1). As the larvae grew, they became more active, spending most of their time performing locomotory activities (>90%), and very little time inactive (<5%). For example, a sequence of MAPs for a small larva (9.4 mm) at 500 p/L occurred as follows: swim (0.9), pause (3.8), sink (1.4), turn (0.6), pause (6.0), turn (1.0), swim (0.9), pause (0.7), orient (0.5), etc (numbers in parentheses indicate duration of activity in seconds). A MAP sequence for a larger larva (24.4 mm) at 2000 p/L was as follows: swim (61.2), orient (0.1), fixate (0.6), lunge (0.3), swim (16.2). This shift from inactivity to locomotion occurred between the sizes of 10.5-16.2 mm. Throughout the study period larvae spent between 2-10% of the total time observed performing foraging activities (Figure 3.1).

The behaviours used by the larvae also changed throughout the study period. Turn was used when larvae were small (<10 mm) and rapidly dropped out of the behavioural

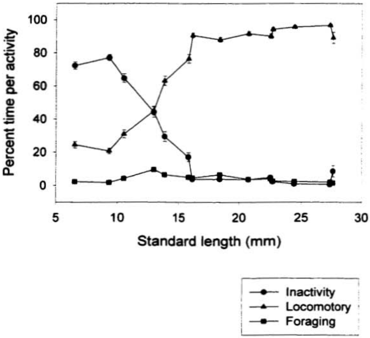

Figure 3.1. Mean percentage (%) of time witch flounder larvae spent performing the inactive, locomotory, and foraging MAPs over standard length (mm) during two minute observation periods. Values are means (n=70 larvae per length) ± S.E.

repertoire and disappeared by the time larvae reached 10.5 mm (Figure 3.2). Pause, sink, and shake all decreased when larvae reached 16.2 mm (Figure 3.3). At this point the shake behaviour did stop altogether, while both pause and sink persisted at low frequency. The durations of the locomotory and inactive MAPs were significantly affected by size, but not prey density (Tables 3.2, 3.3). At the end of the study period (27.6 mm) there was a slight increase in pause and concomitant decrease in swim duration. This was due to some larvae settling during the observation periods.

### Foraging behaviour

The total time engaged in foraging was initially low (2%), increased rapidly to a peak of 10% at 13.8 mm, and then decreased slowly back to its initial low frequency. The variation in total time spent foraging was largely due to variation in orient duration. The duration of the fixate and lunge MAPs (<2% of total time per MAP) was relatively constant over the observation periods while the orient duration ranged from 1-7% of total time (Figure 3.4).

The frequencies of the foraging behaviours were highly variable and many larvae did not forage during the observation periods. The frequency of orient changed throughout the study period. Orient frequency reached a peak between the sizes of 10.5 and 20.8 mm (Figure 3.5). Within this size range there was a significant effect of prey density on orient frequency where orient frequency increased with increasing prey density (Tables 3.4, 3.5). After this size interval, the frequency of orient was low for all sizes and treatments. The frequencies of fixate and lunge varied from 0-4 per two minute

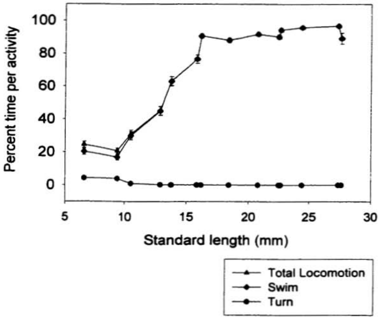

Figure 3.2. Mean percentage (%) of time witch flounder larvae spent performing the locomotory MAPs over standard length (mm) during two minute observation periods. Values are means (n=70 larvae per length) ± S.E.

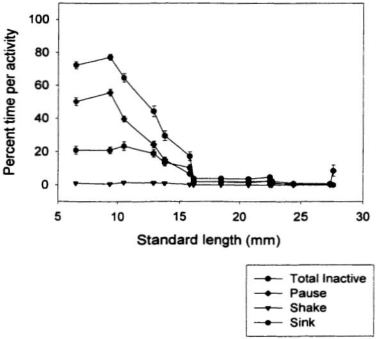

Figure 3.3. Mean percentage (%) of time witch flounder larvae spent performing the inactive MAPs over standard length (mm) during two minute observation periods. Values are means (n=70 larvae per length) ± S.E.

Table 3.2. Summary of ANCOVA results for locomotory MAPs of witch flounder larvae at different prey densities. Each model was run until the larval size indicated in parentheses. Size$^2$ is the square of larval size; for some MAPs a polynomial term was used in the model.

| MAP | Source | df | F | p |
|---|---|---|---|---|
| Swim duration<br>(20.8 mm) | Size | 1 | 19.2 | 0.000 |
| | Size$^2$ | 1 | 6.02 | 0.018 |
| | Prey density | 6 | 0.43 | 0.853 |
| | Size*Prey density | 6 | 0.20 | 0.974 |
| | Size$^2$*Prey density | 6 | 0.13 | 0.992 |
| | Error | 42 | | |
| Turn duration<br>(13.8 mm) | Size | 1 | 56.6 | 0.000 |
| | Prey density | 6 | 1.09 | 0.398 |
| | Size*Prey density | 6 | 0.82 | 0.570 |
| | Error | 21 | | |

Table 3.3. Summary of ANCOVA results for inactive MAPs of witch flounder larvae at different prey densities. Each model was run until the larval size indicated in parentheses. $\text{Size}^2$ is the square of larval size; for some MAPs a polynomial term was used in the model.

| MAP | Source | df | F | p |
|---|---|---|---|---|
| Pause duration | Size | 1 | 26.4 | 0.000 |
| (20.8 mm) | $\text{Size}^2$ | 1 | 11.3 | 0.002 |
| | Prey density | 6 | 0.88 | 0.516 |
| | Size*Prey density | 6 | 1.16 | 0.347 |
| | $\text{Size}^2$*Prey density | 6 | 1.14 | 0.356 |
| | Error | 42 | | |
| Sink duration | Size | 1 | 79.8 | 0.000 |
| (18.4 mm) | Prey density | 6 | 0.73 | 0.631 |
| | Size*Prey density | 6 | 1.35 | 0.257 |
| | Error | 42 | | |
| Shake duration | Size | 1 | 13.0 | 0.001 |
| (16.2 mm) | Prey density | 6 | 0.28 | 0.940 |
| | Size*Prey density | 6 | 0.25 | 0.955 |
| | Error | 35 | | |

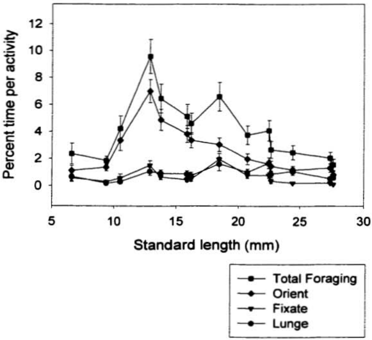

Figure 3.4. Mean percentage (%) of time witch flounder larvae spent performing the foraging MAPs over standard length (mm) during two minute observation periods. Values are means (n=70 larvae per length) ± S.E.

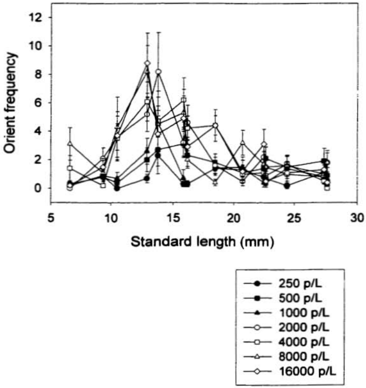

Figure 3.5. Orient frequency of witch flounder larvae during two minute observation periods at different prey densities over standard length (mm). Values are means (n=10) ± S.E.

Table 3.4. Summary of ANOVA results for orient frequency of witch flounder larvae at different prey densities between the sizes of 10.5-20.8 mm.

| MAP | Source | df | F | p |
|---|---|---|---|---|
| Orient frequency | Prey density | 6 | 4.71 | 0.001 |
| | Error | 42 | | |

Table 3.5. Summary of Tukey's pairwise comparison for orient frequency of witch flounder larvae at different prey densities between the sizes of 10.5-20.8 mm. Significant differences occur where 0 lies outside the range of listed confidence intervals for each test. *Significant difference (p<family error rate of 0.05).

| Prey Density | 250 | 500 | 1000 | 2000 | 4000 | 8000 |
|---|---|---|---|---|---|---|
| 500 | -0.549<br>0.156 | | | | | |
| 1000 | -0.487<br>0.219 | -0.290<br>0.415 | | | | |
| 2000 | -0.733*<br>-0.028 | -0.539<br>0.168 | -0.599<br>0.106 | | | |
| 4000 | -0.802*<br>-0.097 | -0.606<br>0.099 | -0.668<br>0.037 | -0.422<br>0.283 | | |
| 8000 | -0.746*<br>-0.041 | -0.550<br>0.155 | -0.612<br>0.093 | -0.366<br>0.339 | -0.296<br>0.409 | |
| 16000 | -0.790*<br>-0.085 | -0.594<br>0.111 | -0.656<br>0.049 | -0.410<br>0.295 | -0.340<br>0.365 | -0.396<br>0.309 |

observation throughout the study period. Larvae at higher prey densities tended to perform more fixate and lunge MAPs compared to larvae at lower prey densities, especially within the 13.8-16.2 mm size range (Figures 3.6, 3.7). This trend was not statistically significant (Table 3.6). The frequencies of fixate and lunge increased slightly throughout the study period and there was a significant effect of size on the frequency of these foraging MAPs (Figures 3.6, 3.7; Table 3.6). The capture success of the larvae ranged from 0-1 and was unaffected by prey density or size (Figure 3.8; Table 3.6).

### Part B-Growth and Survival

At hatching, the mean standard length of the larvae was 5.62 mm (± 0.12 mm S.E.). ANCOVA results show that the standard length, dry weight, body height, head depth, and eye diameter of the larvae were not dependent on prey density (Table 3.7). Larvae in all treatments grew similarly (Figures 3.9-3.13; see Table 3.8 for growth equations). The average absolute growth rate from week 0-12 for all treatments was 0.53 mm $d^{-1}$. The average specific growth rate (SGR) from week 0-6 was 3.67 % $d^{-1}$ and from week 0-12 was 2.60 % $d^{-1}$.

The survival results were not corrected for lethally sampled fish (20 per tank). The survival in all treatments was similar. At week 5, when larvae were moved to larger tanks, the mean survival was 28.7% for the 2000 p/l treatment, 25.1% for 4000 p/l, and 31% for 8000 p/L. At the end of the experiment (week 12), the mean survival was 14.1% for 2000 p/L, 4.6% for 4000 p/L, and 8.9% for 8000 p/L (Table 3.9). Survival was not dependent on prey density at week 2, 5, or 12 (Table 3.10).

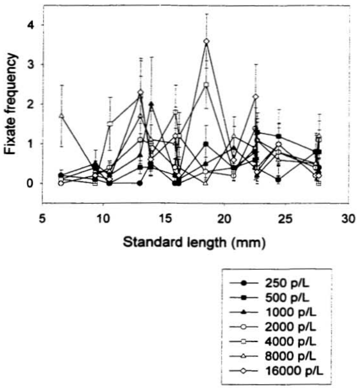

Figure 3.6. Fixate frequency of witch flounder larvae during two minute observation periods over standard length (mm) at different prey densities. Values are means (n=10) ± S.E.

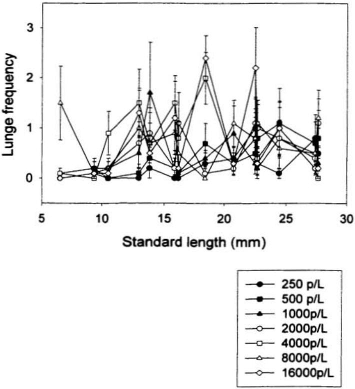

Figure 3.7. Lunge frequency of witch flounder larvae during two minute observation periods over standard length (mm) at different prey densities. Values are means (n=10) ± S.E.

Table 3.6. Summary of ANCOVA results for the foraging MAPs fixate, lunge, and capture success of witch flounder larvae at different prey densities.

| MAP | Source | df | F | p |
|---|---|---|---|---|
| Fixate frequency | Size | 1 | 6.94 | 0.010 |
| | Prey density | 6 | 0.67 | 0.676 |
| | Size*Prey density | 6 | 0.52 | 0.794 |
| | Error | 84 | | |
| Lunge frequency | Size | 1 | 15.8 | 0.000 |
| | Prey density | 6 | 0.83 | 0.547 |
| | Size*Prey density | 6 | 0.62 | 0.714 |
| | Error | 84 | | |
| Capture success | Size | 1 | 0.38 | 0.541 |
| | Prey density | 6 | 0.87 | 0.523 |
| | Size*Prey density | 6 | 0.43 | 0.856 |
| | Error | 66 | | |

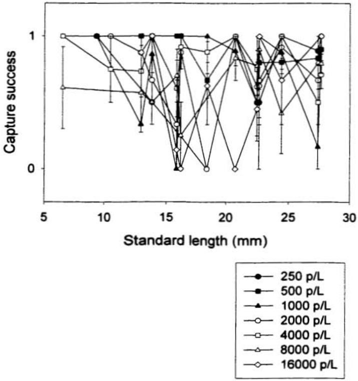

Figure 3.8. Mean capture success [success/(success+miss)] of witch flounder larvae during two minute observation periods at different prey densities over standard length (mm). Values are means (n=0-10 as only lunges for which the outcome of the attack was scored are included) ± S.E.

Table 3.7. Summary of ANCOVA results for growth response variables for witch flounder larvae reared at different prey densities.

| Response variable | Source | df | F | p |
|---|---|---|---|---|
| Standard length (mm) | Age | 1 | 7041.4 | 0.000 |
| | Prey density | 2 | 0.85 | 0.434 |
| | Age*Prey density | 2 | 0.75 | 0.478 |
| | Error | 66 | | |
| Dry weight (mg) | Age | 1 | 2640.4 | 0.000 |
| | Prey density | 2 | 0.06 | 0.942 |
| | Age*Prey density | 2 | 0.21 | 0.811 |
| | Error | 18 | | |
| Body height (mm) | Age | 1 | 582.1 | 0.000 |
| | Prey density | 2 | 0.03 | 0.974 |
| | Age*Prey density | 2 | 0.28 | 0.762 |
| | Error | 18 | | |
| Head depth (mm) | Age | 1 | 2903.1 | 0.000 |
| | Prey density | 2 | 0.16 | 0.855 |
| | Age*Prey denisty | 2 | 0.60 | 0.560 |
| | Error | 18 | | |
| Eye diameter (mm) | Age | 1 | 2016.7 | 0.000 |
| | Prey density | 2 | 0.05 | 0.949 |
| | Age*Prey density | 2 | 0.06 | 0.941 |
| | Error | 18 | | |

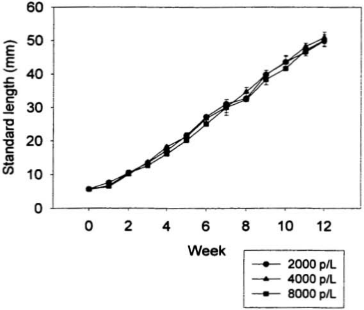

Figure 3.9. Mean standard length (mm) of witch flounder larvae reared at different prey densities over time (weeks post-hatch). Values are means of the two means for each replicate ± S.E.

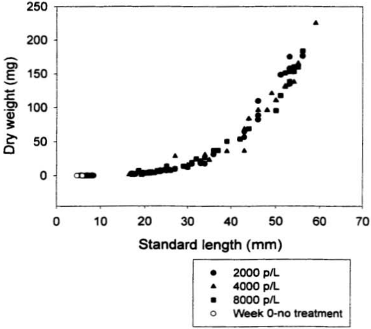

Figure 3.10. Relationship between standard length (mm) and dry weight (mg) of witch flounder larvae reared at different prey densities. Symbols are individual larvae.

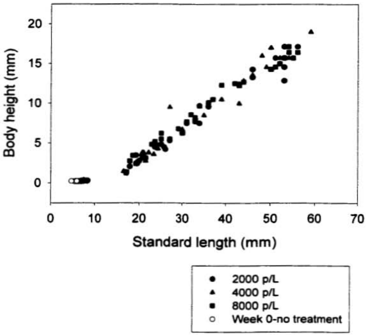

Figure 3.11. Relationship between standard length (mm) and body height (mm) of witch flounder larvae reared at different prey densities. Symbols are individual larvae.

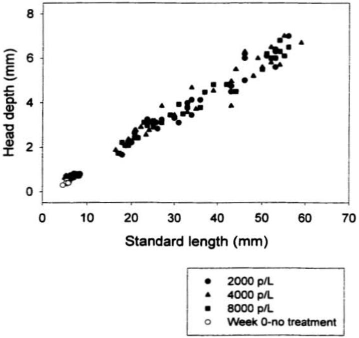

Figure 3.12. Relationship between standard length (mm) and head depth (mm) of witch flounder larvae reared at different prey densities. Symbols are individual larvae.

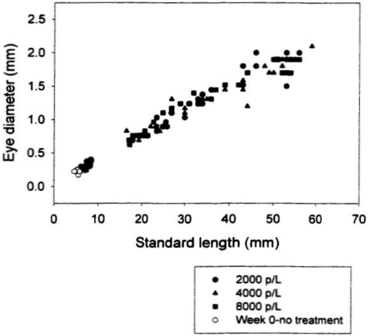

Figure 3.13. Relationship between standard length (mm) and eye diameter (mm) of witch flounder larvae reared at different prey densities. Symbols are individual larvae.

Table 3.8. Regression equations (in the form y=a+bx) describing changes in growth response variables with age for witch flounder, where y=response variable (indicated if transformed) and x=week.

| Response variable | a | b | $R^2$ |
|---|---|---|---|
| Standard length | 1.86 | 4.06 | 98.9 |
| Dry weight<br>log DW | -0.14 | 0.19 | 99.1 |
| Body height<br>log BH | 0.07 | 0.10 | 96.3 |
| Head depth | 0.21 | 0.47 | 99.2 |
| Eye diameter | 0.15 | 0.14 | 99.0 |

Table 3.9. Survival of witch flounder larvae reared at different prey densities. Lethally sampled larvae (20 per tank) are not included in calculations.

| Prey density | 2000 | | 4000 | | 8000 | |
|---|---|---|---|---|---|---|
| Replicate | A | B | A | B | A | B |
| #larvae day 0 | 190 | 190 | 190 | 190 | 190 | 190 |
| #larvae week 2 | 68 | 73 | 71 | 47 | 66 | 83 |
| #larvae week 5 | 53 | 59 | 59 | 39 | 53 | 68 |
| #larvae week 12 | 23 | 32 | 10 | 8 | 26 | 9 |
| %survival (± S.E.) week 2 | 36.15 (1.28) | | 30.26 (6.15) | | 38.20 (4.36) | |
| %survival (± S.E.) week 5 | 28.72 (1.54) | | 25.13 (5.13) | | 31.03 (3.85) | |
| %survival (± S.E.) week 12 | 14.10 (2.31) | | 4.62 (0.51) | | 8.97 (4.36) | |

Table 3.10. Summary of ANOVA results for survival of witch flounder larvae reared at different prey densities at weeks 2, 5, and 12.

| Time period | Source | df | F | p |
|---|---|---|---|---|
| Week 2 | Prey density | 2 | 0.87 | 0.503 |
| | Error | 3 | | |
| Week 5 | Prey density | 2 | 0.61 | 0.600 |
| | Error | 3 | | |
| Week 12 | Prey density | 2 | 2.75 | 0.210 |
| | Error | 3 | | |

### 3.4 Discussion

Witch flounder grew and survived in all treatments used in this study. This experiment is the first examining the behaviour of witch flounder larvae. Because larval behaviour can be influenced by many factors other than prey density, including temperature (Hunter, 1981), light, (Puvanendran and Brown, 1998), prey type (Drost, 1987), and turbulence (MacKenzie and Kiørboe 1995; Browman, 1996), as well as other factors such as tank design, it is important to recognize that these results are a preliminary step towards understanding the behavioural ecology of witch flounder larvae.

#### Ontogeny of witch flounder behaviour

Locomotory and inactive behaviours

Three types of prey search behaviour have been described for larval fishes. Cruise searchers swim continuously while scanning for prey. At the opposite end of the spectrum, ambush foragers are stationary and wait for prey to enter their search space. The saltatory strategy is an intermediate search behaviour where larvae scan for prey within their visual field, but only during the motionless periods that punctuate swimming events. If prey is not located, larvae will swim a short distance and scan again (O'Brien *et al.*, 1990; Browman and O'Brien, 1992).

The shift from time spent inactive to time spent performing locomotory activities suggests that the witch flounder foraging strategy changed from a saltatory search strategy to a cruise search strategy during the study period. When larvae are small (<10 mm) foraging includes many turns and brief periods of swimming which serve as repositioning acts. The search strategy of larger larvae is remarkably different. Swimming is not interrupted by pauses and turns; larger larvae swim slowly, continuously cruising for prey. By the time that larvae reach 16.2 mm, most of their time is spent swimming (>90%) and little is spent inactive (<5%).

During this apparent shift from a saltatory to cruise strategy, the behaviours used by the larvae also change. The turn and shake behaviours disappeared from the behavioural repertoire, while the frequency of the pause and sink behaviours decreased. The decrease and/or disappearance of turn at 10.5 mm, and sink and shake, around 16.2 mm, were rapid. However, the general shift from pause to swim, the remaining two dominant behaviours, was more gradual. The disappearance of MAPs from the behavioural repertoire during ontogeny has been reported previously and has been related to size increases (Brown and Colgan, 1985). In witch flounder, the change in search strategy and disappearance or decrease of behaviours occurs gradually between the sizes of 10.5-16.2 mm and coincides with a dramatic change in larval morphology.

The most obvious change in witch flounder morphology that can be expected to contribute to changes in behaviour during this 10.5-16.2 mm size interval, is that larvae begin to increase in body height as well as length. This height increase is largely a result of finfold growth. However, myotome height also increases in this size range (Figure 3.12). By the time that larvae are 20 mm SL, their form has changed dramatically. They are no longer long and thin, but have grown in length and height, resulting in a ribbon-like form.

This shape change likely necessitates behavioural changes. Increased height along the entire body axis will increase the resistance acting on the larvae, requiring more energy to perform certain motions. A probable result of this shape change is that any lateral rotation, such as a rapid turn motion, becomes difficult and this could account for the decline in the turn behaviour during this time. Later stage larvae that have increased in body depth swim at a constant slow speed, searching for prey. Once their shape has changed, stopping and re-starting could become energetically costly, necessitating the switch to a cruise-searching mode coincident with the disappearance of the resting activities pause and sink. When larvae are startled they are able to swim rapidly in one direction, but the rapid and successive changing of directions is difficult due to the increased body height.

Other authors have reported ontogenic changes in swimming behaviour which were related to morphological developments. The swimming behaviour of anchovy larvae is a useful model because it has been extensively studied. Swimming in yolk-sac anchovy larvae consists of continuous bouts of energetic swimming followed by resting periods. First-feeding larvae change to an intermittent beat and glide swimming pattern (Hunter, 1972). The energetics of swimming in anchovy has been modeled by Weihs (1980) and this change seems to be an adaptive energy sparing mechanism to cope with the low Reynolds number encountered by the smaller yolk-sac larvae. Further speculation concerning the energetics of witch flounder swimming behaviour is not warranted. However, it seems reasonable that the ontogenic changes in locomotory and inactive

MAPs of the larvae are related to morphological development as these changes occur simultaneously.

The nature of the shake, sink, and pause behaviours is not straightforward. These MAPs disappear or decline around the size of 16.2 mm. Sinking has been reported in other species, such as the snapper (*Pagrus auratus*) and, like the pause MAP, has been interpreted as a resting behaviour. In snapper, it occurs in yolk-sac larvae and feeding larvae during nighttime periods of inactivity (Pankhurst *et al.*, 1991). Sinking is typically observed only in the early stages of other species such as the black sea bream, *Acanthopagrus schlegeli* (Fukuhara, 1987). However, Kawamura and Ishida (1985) note that sinking occurs in both yolk-sac larvae and larger feeding larvae of the Japanese flounder, *Paralichthys olivaceus*, which have just attacked a prey item. Observations of sinking in later stage witch flounder larvae were not related to feeding events. The sink, shake, and pause behaviours are typical of early stage yellowtail flounder larvae (Puvanendran, unpublished data) and the same is probably true of witch flounder, as they only occur at high frequency during the early stage. The persistence of these behaviours in witch flounder is likely the result of some smaller, slower growing individuals being included in the observations. When the behavioural observations were terminated, a few small, slow growing individuals remained in the stock tank.

### Foraging behaviour

Orient was the only foraging MAP statistically affected by prey density. The increase in orient frequency with prey density is in agreement with expected encounter rates. The change in orient frequency with size is interesting as this MAP was only affected by prey density within a limited size range. Orient frequency was initially low, increased between the mean size interval of 10.5-20.8 mm SL across treatments in response to prey density, and finally decreased to low levels. The initial low frequency of orient followed by an increase associated with greater larval size can be explained by changes in swimming speeds and encounter rates (Mittelbach, 1981). However, the decrease in orient frequency across treatments later in the study period is puzzling as larger larvae are generally competent swimmers (Rosenthal and Hempel, 1971; Laurence, 1972; Houde and Schekter, 1980) and are expected to exhibit a high prey encounter rate. This decrease in orient frequency may be due to improved foraging ability associated with greater visual acuity. Miller *et al.* (1993) showed that the visual angle - the smallest angle which a stimulus may subtend the eye and remain resolvable (Neave, 1984) - decreases during the development of three species of fish larvae. Thus, the eye develops such that larvae can likely detect prey items in their periphery without turning the head and orienting. In this case, the orient frequency decreases as most orient MAPs would be recorded only when the larva completes its foraging routine with a fixate and lunge.

A trend of increased consumption at higher prey densities was detected as the frequencies of fixate and lunge tended to increase with prey density. However, differences between treatments were not significant. This effect of prey availability on foraging behaviour was first described as a functional response by Holling (1965) and has been reported for many species of larval fish (Houde and Schekter, 1980; Werner and Blaxter, 1980; Puvanendran and Brown, 1999).

Capture success data is useful to support hypotheses of larval foraging ability. Many species of larvae show increases in capture success with age (Chitty and Houde, 1981; Hunter, 1981; Tucker, 1989) and variable capture success at different prey densities (Puvanenderan and Brown, 1999). The results of this experiment suggest that witch flounder capture success is highly variable and is unaffected by size or prey density. Unfortunately, it was difficult for the observer to determine the outcome of lunges on prey in the experimental set-up used. This was due to the low light intensity (200 lux). Furthermore, at higher prey densities, it was especially difficult to determine the outcome of lunges and more data was collected for capture success at low prey densities. Finally, many fish did not lunge during the observation periods. Because few larvae attacked prey and it was difficult to determine the outcome of the attacks, the sample size for capture success data is small. For these reasons, little emphasis is placed on the capture success data, as it may be unrepresentative of the capture ability of witch flounder.

### Comparative evidence and behavioural ecology

The foraging behaviour of witch flounder is affected by variations in prey density. This finding is not surprising, as many studies report effects of prey density on the behaviour, growth and survival of different species of larvae (Laurence, 1977; Houde and Schekter, 1980; Werner and Blaxter, 1980; Munk and Kiørboe, 1985; van der Meeren and Naess, 1993; Puvanendran and Brown, 1999). However, the main finding of this study is that witch flounder are not affected by changes in prey availability in the same manner as are other species of larvae observed under similar laboratory conditions. The typical pattern among fish larvae - that they increase their prey consumption rate with increased prey density (Houde and Schekter, 1980) - was supported by this study as the lunge frequency of witch flounder larvae was often higher at higher prey densities. However, while this trend exists, differences in lunge frequency and assumed prey consumption at different prey densities were not statistically significant. Thus, the results for witch flounder are unusual in that the effects of prey density on foraging behaviour were not as strong as results that have been reported for other species, and statistically significant results were only detected over a limited size range.

The results from the growth and survival experiment lend support to the conclusion that witch flounder foraging behaviour is not strongly affected by prey density. Statistically significant differences in growth and survival were not found between prey density treatments. Although the experimental treatment and replicate levels were not exhaustive, this experimental design has resulted in informative differences in growth and survival for other cold water marine fish larvae such as Atlantic cod (*Gadus moruha*; Puvanendran and Brown, 1999), redfish (*Sebastes* sp.; Laurel, 1998) and yellowtail flounder (*Pleuronectes ferrugineus*; Puvanendran, unpublished data) reared under similar laboratory conditions. While the use of a low number of replicates may have prevented the statistical detection of moderate differences in performance between treatments, the rearing experiment was supported by the results of the behaviour experiment. Furthermore, an important result of this study is that the foraging behaviour of witch flounder larvae is very different from that of other species reared in laboratory conditions.

The ecological implications of these results can be illustrated by a comparison of the behaviour of witch flounder and yellowtail flounder at different prey densities. As it is always difficult to extrapolate laboratory observations to the field, the ecological significance of these results is largely restricted to a discussion of comparisons between laboratory derived results for witch flounder and yellowtail flounder. Both species are members of the family Pleuronectidae, and the larvae co-occur in the water column around Newfoundland (Bonnyman, 1983). Furthermore, both species have been reared under similar conditions in our laboratory. Witch flounder and yellowtail flounder have similar larval specific growth rates (3.67 % $d^{-1}$, 3.65 % $d^{-1}$ respectively, from week 0-6; yellowtail flounder data from Puvanendran, unpublished data). However, the size and age of yellowtail flounder at metamorphosis is more typical of other flatfish species. Yellowtail flounder undergo metamorphosis around the length of 16 mm which occurs approximately between weeks 6-8 in the laboratory. Witch flounder have a longer larval period and metamorphose at a larger size. Unfortunately, data on witch flounder larvae from the field is scarce. Bigelow and Schroeder (1953) suggest that the witch flounder larval period may last up to 4-6 months and point out that they grow to a larger size than other flatfish larvae found in the Gulf of Maine. Evseenko and Nevinsky (1975) report that witch flounder begin metamorphosis at the size of 22.5 mm, as the right and left sides begin to appear differently at this time. Bonnyman (1983) found a late stage larva measuring 46.5 mm in January over the Grand Banks, after a peak of spawning in late May. This suggests that some larvae may over-winter in the water column. In the laboratory, the transition from a pelagic, largely transparent larva to a benthic, scaled and pigmented juvenile occurs between the sizes of 44-73 mm and the ages of 4-7 months (see Chapter 2, Section 2.4.4). Because of the extended development time for this species, it is not meaningful to pinpoint the exact stage of transition from larva to a juvenile using a character such as stage of eye migration. It is clear, however, that this species is very different from most other flatfish in that the larvae are in the water column for a much longer time and are much larger than other species during the pelagic phase (Miller *et al.*, 1991; Osse and Van den Boogaart, 1997).

In similar rearing experiments on yellowtail flounder in our laboratory it was found that the larvae have specific requirements for high prey densities. While larvae reared at 4000-16000 p/L had similar rapid growth rates, only larvae reared at 8000 p/L had greater than 20% survival at week 6. This difference in survival was related to the foraging behaviour of the larvae. The number of prey captured by yellowtail flounder, quantified by gut content analysis, was highest at 8000 p/L compared to all other prey densities. The consumption rate (measured as lunges per minute) of yellowtail flounder larvae also increased dramatically with age compared to witch flounder. For first-feeding yellowtail flounder larvae the consumption rate was 0-1 $\text{min}^{-1}$, and increased to 6-12 $\text{min}^{-1}$ at week 6, depending on prey density (Puvanendran, unpublished data).

The comparison between witch flounder and yellowtail flounder illustrates two important points. First, witch flounder are able to find prey when it is both more or less abundant, demonstrated by the lack of a strong effect of prey density on foraging, growth and survival compared to yellowtail flounder. Second, witch flounder do not have high prey requirements, illustrated by the gradual increase in average consumption rate from

0.2 to 0.6 prey items per two minutes over the study period (weeks 2-9), compared to the increase from 0-1 to 6-12 prey items per minute for yellowtail flounder (weeks 1-6).

This comparative evidence suggests that witch flounder is both behaviourally and physiologically adapted to variations in prey availability. The foraging behaviour of witch flounder is largely unaffected by changes in prey density compared to yellowtail flounder. This indicates that witch flounder is not dependent on high prey availability for feeding, but can find prey and thus survive, when prey is more or less abundant. The lack of a strong effect of prey density on the foraging ability of witch flounder is not solely a result of the greater size of this species. Redfish are ovoviviparous and larvae are large at extrusion (6-8.9 mm; Penny and Evans, 1985). Laurel (1998) found that the foraging behaviour, growth, and survival of redfish larvae in the laboratory is strongly affected by variations in prey availability.

It is remarkable that witch flounder can grow as rapidly as yellowtail flounder given their lower lunge frequency and assumed prey consumption. Two possible mechanisms that may explain this phenomenon are a high assimilation efficiency and low metabolic requirements of witch flounder. Witch flounder may be able to assimilate more energy for growth and metabolism from prey compared to yellowtail flounder. For most of the study period witch flounder larvae were larger than yellowtail flounder. This size difference alone implies that its digestive system is larger, more developed, and more efficient than that of yellowtail flounder. Increases in digestive efficiency with body size have been reported (Govoni *et al.*, 1986; Klumpp and von Westernhagen, 1986). Laurence (1977) notes that the prey requirements relative to body weight of winter flounder are initially high. Although the larvae are small, their digestive system is inefficient and they require more prey per unit body weight compared to larger larvae. The prey requirements decrease relative to body weight as winter flounder grow because the digestive system becomes more efficient (Laurence, 1977). Witch flounder larvae probably have lower prey requirements per unit body weight compared to yellowtail flounder larvae due to their larger body size. Furthermore, the length of the witch flounder gut appears proportionally longer compared to that of yellowtail flounder (personal observation). Therefore, for larvae of the same size, witch flounder have a longer, presumably more efficient digestive system compared to yellowtail flounder. These differences in size may contribute to lower prey requirements and higher assimilation efficiency of witch flounder.

The swimming speed and general activity level of witch flounder is also different from yellowtail flounder. Yellowtail flounder are cruise searchers (personal observation) which swim continuously, searching for prey. When witch flounder overlap in size with yellowtail flounder larvae they are saltatory searchers and spend a considerable amount of time inactive. Even when their search strategy changes and they constantly cruise for prey, witch flounder larvae swim slowly through the water column. Yellowtail flounder are much faster swimmers. This higher activity could explain the higher prey requirements of yellowtail flounder larvae compared to witch flounder larvae. Higher activity imparts a greater need for prey, which will cause larvae to be more susceptible to starvation in the absence of prey (Hunter, 1981). The difference in activity levels between these flatfish species parallels differences between anchovy and mackerel larvae observed by Hunter (1981). Anchovy larvae swim slower than mackerel larvae for all developmental stages and anchovy also consume less oxygen per dry weight compared to mackerel (Hunter, 1972; Hunter and Kimbrell, 1980). Hunter (1981) suggests that these striking differences in cruising speed are indicative of major differences in life history strategies because they imply differences in metabolic requirements. Measurements of swimming speed and oxygen consumption would be useful to further compare the metabolic requirements of witch flounder and yellowtail flounder larvae.

The ecological significance of the weak effects of prey density on foraging, and the absence of effects on growth and survival found in this study can be understood given the length of the larval period of witch flounder. Witch flounder have an extended larval period and are committed to being in the variable environment of the water column for much longer than other species. The abundance of zooplankton prey for fish larvae can vary over four orders of magnitude during the year, typically reaching a peak in the warmer months and decreasing dramatically in the winter (Myers *et al.*, 1994). Therefore, witch flounder larvae will necessarily encounter periods of both high and low plankton abundance during the long larval period and must be able to cope with variations in prey availability to ensure some survival during this time. This is a very different strategy from other species that have shorter larval periods and likely rely on a match of spawning with plankton production to promote larval survival. Indeed, the match-mismatch hypothesis of Cushing (1972) and the body of work that has followed it can be applied to most species that show strong responses of larval growth and survival to prey availability, such as yellowtail flounder.

### Witch flounder and predation

Starvation and predation are two factors which contribute to the high mortality rates of fish larvae in the wild (Hunter, 1981). I have argued that witch flounder larvae are not as susceptible to starvation as are other species because they can find prey when it is both more or less abundant and because they have low prey requirements. It is important to speculate on the susceptibility of the larvae to predation, as this factor will also contribute to mortality in the wild. Research in fisheries recruitment has been directed toward understanding the effects of factors such as food availability and temperature, which can affect growth rates and change the length of the larval period in the wild. This is because a longer larval period will increase the duration of susceptibility of the larvae to size-specific predation, reducing overall survival, "the stage duration hypothesis" (Houde, 1987). The growth results from the rearing experiment indicate that the length of the long larval period of witch flounder cannot be decreased by increasing prey availability from 2000-8000 p/L. There must be some mechanism other than rapid growth to metamorphosis by which witch flounder can decrease its susceptibility to predation, as the larval period is long even when prey is readily available.

It can be expected that predation pressure on witch flounder larvae is high because of the long larval period and large size at metamorphosis (Bailey and Houde, 1989). These factors will cause witch flounder to be susceptible to larval-specific predation for longer than other species and make it a more attractive prey item due to its large size (Litvak and Leggett, 1992). In order to promote some survival through the long larval period, witch flounder may have evolved a predator avoidance strategy. Witch flounder larvae have a fluorescent yellow band around the perimeter of the fin-fold (personal observation). This band is only apparent when the larvae are viewed alive against a black background, which may explain why it has not yet been reported. When larvae reach the size of approximately 15 mm, the band develops. I suggest that this band is a predator avoidance strategy that acts by resembling the tentacles of ctenophores, thereby warning potential predators not to attack. This is similar to the hypothesis of Fraser and Smith (1974) that a pigmented, free-trailing intestinal loop on rare "exterilium" fish larvae acts to discourage potential predators because it resembles a poisonous coelenterate. Moser (1981) speculates that strategies of mimicry may be adaptations characteristic of prolonged pelagic life. A strategy of mimicry such as this may help protect witch flounder larvae from the impact of predation on mortality during the long larval period.

### Why a long larval period?

I have argued that witch flounder has evolved strategies to avoid both starvation and predation during the extended larval period. The question remains as to why witch flounder have a long larval period, one of the longest among flatfishes (Miller *et al.*, 1991; Osse and Van den Boogaart, 1997). Because witch flounder is different from other species, the long larval period is probably a derived trait that has evolved as a response to some limitation on population growth. A possible answer to this question is that witch flounder need to be large at settlement, and require the long larval period to grow to a large size. A larger size might confer competitive ability on the newly settled recruits. Witch flounder are characterized as slow-growing (Burnett *et al.*, 1992) and slow growth rates suggest high competition for resources according to life history theory (Bell, 1997). Thus, the larger size at settlement may be imperative for the newly settled juveniles to compete with the larger, benthic members of the population.

### Limitations of experimental protocol

In the interpretation of data, it is assumed that the behavioural response of witch flounder larvae observed during the two-minute observation periods is an accurate representation of the response of the larvae to the experimental prey density. The results indicate that witch flounder larvae respond to variations in prey density, but this response is not as dramatic as seen in other species. This could mean 1) that the two-minute observation period was not sufficient to observe a response or 2) that witch flounder larvae do not respond as strongly to changes in prey density as do other species. Many methods have been used to estimate the behavioural response of larvae to different prey densities including the focal animal technique (Puvanendran and Brown, 1999), counts of prey remaining after feeding (Houde and Schekter, 1980), gut content analysis, and bioenergetic models (Laurence, 1977). Unfortunately, none of these methods are perfect. A comprehensive research effort would involve all methods. Collecting many types of data to answer the same question will provide a clearer understanding of the larva's behavioural and physiological response to variations in prey density. For example, because many species of larvae increase their prey consumption with increasing prey density (Houde and Schekter, 1980), researchers may conclude that higher prey densities are required by the larvae. However, this extra prey may not be assimilated. This is exemplified by herring larvae (*Clupea harengus*) passing live *Artemia* through the anal pore when fed at high prey densities (Werner and Blaxter, 1980). For this reason, growth rates at different prey densities are necessary to fully interpret behavioural results. Gut content analysis was not an option in this study because of the limited number of animals. A one-minute observation period has been used previously to examine the effects of prey density on foraging behaviour (eg. Laurel, 1998; Puvanendran and Brown, 1999). Preliminary observations on witch flounder larvae indicated that they fed less frequently than other species. For this reason, a two-minute observation period was used. The behavioural results are supported by the results of the rearing experiment where witch flounder grew and survived equally well at the range of prey densities tested. Estimating the response of fish larvae to variations in prey availability is not a straightforward process and requires more refined methodology. For these reasons, these interpretations offer reasonable, but preliminary, insight on the effects of prey availability on witch flounder larvae. Future research should be directed at determining the behavioural and growth response of larvae to prey densities lower than 250 p/L. Clearly, witch flounder are different from other species as they did not respond strongly to the wide range of prey densities used in this experiment. It is possible that a "critical" prey density for this species may be found at lower prey densities.

## Conclusion

The behavioural ecology of witch flounder larvae is very different from the larvae of other species. The results presented here illustrate that witch flounder foraging behaviour was largely unaffected by variations in prey availability and comparisons with yellowtail flounder indicate that it has low prey requirements. Because of the long larval period, witch flounder is likely not dependent on a "match" of spawning with plankton production. Instead, it will necessarily encounter periods of both high and low plankton abundance. Two physiological mechanisms have been proposed which this species may use to grow and survive in a variable prey environment: high assimilation efficiency and low activity. Comparative evidence suggests that witch flounder is both behaviourally and physiologically adapted to a fluctuating prey environment and is therefore less susceptible to starvation as are other species. The results of this study on witch flounder indicate that this species has evolved behavioural, physiological and morphological mechanisms to promote survival throughout its extended larval period.

# CHAPTER 4

# THE EFFECTS OF FEEDING FREQUENCY ON THE BEHAVIOUR, GROWTH, AND SURVIVAL OF YELLOWTAIL FLOUNDER LARVAE

## 4.1 Introduction

Marine fish larvae are often fed at high prey densities during larviculture. Higher prey densities serve to increase the encounter rate between predator and prey and an increase in consumption rate with prey density has been observed for many species (Houde and Schekter, 1980; Munk and Kiørboe, 1985; Klumpp and von Westernhagen, 1986). Given the rapid growth of larvae, it is assumed that they must maintain optimal feeding rates in order to grow and survive. Therefore, a great deal of attention is invested in the monitoring and maintenance of prey densities. Greater feeding rates typically result in rapid growth and development, as well as greater condition of the larvae, and ultimately high survival.

While it is recognized that larvae require high prey densities to feed efficiently, the required frequency of exposure to high prey availability has received little research interest (but see Houde and Schekter, 1978). However, this is a relevant issue as laboratory and field evidence suggests that larvae may not require the constant exposure to high prey densities often used in larviculture. In this paper, a new strategy for feeding larval fish is tested. Yellowtail flounder (*Pleuronectes ferrugineus*) larvae were offered live prey at high density constantly and at different feeding frequencies (1, 2, and 4 per day). It is possible to test this strategy with yellowtail flounder as previous research has determined the prey density that maximizes the ingestion rate of this species. A goal of this experiment was to determine if larvae require the constant high levels of prey often used in rearing tanks, or if "pulses" of high prey availability are sufficient to sustain rapid growth.

This feeding strategy has an ecological basis. Data from field observations suggests that fish larvae encounter prey on a scale which is spatially and temporally variable (Arthur, 1977; Owen, 1989). It is reasonable to assume that larvae are both behaviourally and physiologically adapted to these conditions of varying prey availability (Brown *et al.*, 1997). For example, northern anchovy (*Engraulis mordax*) and herring (*Clupea harengus*) larvae decreased their search area at high prey density, in an apparent attempt to stay within a patch of prey (Hunter and Thomas, 1974; Gallego, 1994). Houde and Schekter (1978) were interested in the idea that patches of high prey density in the sea contribute to recruitment and compared the survival of larvae fed in simulated high-density patches to the survival of larvae fed continuously at high prey density. They found that only three hours of exposure to high prey density is required by sea bream (*Archosargus rhomboidalis*) larvae in order to obtain the same high survival observed when reared under constant high prey density.

Continuous feeding may be harmful as well as unnecessary. There is evidence that prolonged feeding periods and high rations decrease digestive efficiency as they increase evacuation rate (Boehlert and Yoklavich, 1984). Johnston and Mathias (1994) showed that apparent digestibility increased with gut retention time in larval walleye (*Stizostedion vitreum*). As gut evacuation time is shorter in continuously feeding fish (Canino and Bailey, 1995) it is predicted that larvae in the constant prey treatment will have reduced absorption efficiency compared to pulse-fed larvae. This evidence suggests that some species of marine fish larvae are adapted to the patchy distribution of prey in the wild and they consume prey when it is available in rearing tanks, having lost any satiation response. Furthermore, feeding and digestion require energy. For example, an increase in oxygen consumption was observed in sea bream juveniles offered higher rations (Guinea and Fernandez, 1997). Because the consumption of excess food decreases absorption efficiency and increases the energy required for digestion, and because live food is expensive, continuous feeding may not be an optimal strategy for larval rearing.

The effect of feeding frequency on the growth of fishes has been examined on a number of different marine and freshwater species with the goal of improving the efficiency of rearing techniques (Grove *et al.*, 1978; Tsevis *et al.*, 1992; Kayano *et al.*, 1993; Goldan *et al.*, 1997). This work has largely focused on juvenile fishes. It is possible to obtain precise estimates of food eaten and weight gained due to the dry, artificial diet and large size of juveniles compared to larvae. For this reason, a body of work on juvenile rations and food conversion exists. This is not possible to the same extent with larvae, in part because of the complexities of larval feeding energetics. For example, the ration, or prey required by the larvae, varies with the density of prey available, as it requires more energy to pursue and capture prey at low prey densities (Laurence, 1977; Houde and Schekter, 1978). As it was not possible to weigh the prey offered to the larvae, behavioural observations on feeding larvae were used to determine if larvae fed at different frequencies consumed different amounts of prey.

It has been suggested that the undifferentiated digestive system of larval fish is adapted to exploit high-density patches of prey (Govoni *et al.*, 1986; Klumpp and von Westernhagen, 1986). Although absorption efficiency decreases at high feeding rates, Bohlert and Yoklavich (1984) have calculated, using the ingestion rates of Werner and Blaxter (1980), that the increase in prey consumed at high prey density more than compensates for the overall decrease in assimilation efficiency in larval herring. Larvae may therefore maximize growth rate rather than growth efficiency. However, in aquaculture, as in many industries, it may be desirable to favor efficiency rather than absolute growth (Klumpp and von Westernhagen, 1986). Live food for fish larvae is costly, and maintaining high prey densities may be wasteful. Therefore, a goal of this experiment is to determine if it is possible to reduce the amount of prey offered to the larvae, without significantly decreasing growth and survival. Pulse feeding may be a more efficient strategy to deliver the required amount of live food to the larvae. The objectives of this study were to determine 1) if feeding frequency affects growth and survival of yellowtail flounder larvae and 2) if the observations on growth can be explained by differences in larval behaviour.

## 4.2 Materials and Methods

### Experimental Design

Eggs and milt were collected from captive broodstock maintained at the Ocean Sciences Centre, Logy Bay, Newfoundland. Fertilized eggs were incubated in a 250L cylindro-conical upwelling tank. The eggs hatched over a 24-hour period. Larvae were sampled for initial morphometric measurements (described below) once most (>90%) of the eggs had hatched and this day was considered day zero of the experiment.

Larvae were reared in rectangular 33L glass tanks. The sides of the tanks were painted black. All tanks were kept in a water bath and were supplied with filtered (25μm) seawater. Two air stones were used per tank to provide gentle aeration and promote a homogeneous distribution of prey. The light intensity at the water surface was approximately 750 lux in the center of each tank and continuous lighting (24h) was used. The temperature ranged from 11.5°-14.5°C throughout the experiment and the mean temperature was approximately 12.5°C. The experiment lasted 7 weeks as this is the approximate midpoint of the transformation from the larval to juvenile stage in yellowtail flounder under these rearing conditions.

Larvae were stocked into experimental tanks on day one post-hatch. The stocking density used was 60 larvae per liter. Feeding began on day 2 and all treatments were fed at 8000 prey per liter (p/L), adjusted three times daily (around 10AM, 4PM, and 10PM), from day 2-10 in order to establish feeding in all tanks. On day 11, the different feeding treatments were initiated. Rotifers (*Brachionus plicatilis*), enriched with culture selco (INVE, Belgium) and/or *Artemia franciscana* nauplii, enriched with DHA selco (INVE, Belgium) or Algamac (Bio-Marine, USA) were used as prey for the larvae. Larvae were fed rotifers until the end of week 4. Weaning to *Artemia* was complete by the end of week 5.

The four treatments used in this study were feeding once (1X, at 10 AM), twice (2X, every 12 hours: at 10 AM and 10 PM), four times (4X, every 6 hours: at 10 AM, 4

PM, 10 PM, and 4 AM), and continuously (by automatic feeder) at 8,000 p/L. Two replicate tanks were used per treatment. An automatic feeder consisted of a reservoir for live prey and a solenoid attached to a timer. Aeration within the reservoir kept the prey evenly distributed. The prey density of 8000 p/L was chosen because yellowtail flounder can capture the most prey items per 15 minute period at this prey density compared to both lower and higher prey densities, quantified by gut content analysis. Furthermore, although the growth of yellowtail flounder larvae is similarly rapid at prey densities from 4000-16000 p/L, the survival of this species is highest at 8000 p/L (Puvanendran, unpublished data).

Prey densities were determined by sampling a 5 mL aliquot from different depths within the tanks (below surface, mid-depth and above bottom) prior to the 10 AM, 4 PM, and 10 PM feedings. The total number of prey items in each sample was counted and the prey density was adjusted as required. Microalgae (*Isochrysis* and *Nannochloropsis*) was also added to all tanks at 10 AM, 4 PM, and 10 PM. Due to the 24 hour experimental feeding schedule, prey densities were not counted prior to the 4 AM feeding. The prey densities in the continuously fed tanks were monitored at the same time as the 10 AM, 4 PM, and 10 PM feedings. However, for all other feedings for this treatment it was assumed that prey densities had decreased as expected.

When the feeding treatments were initiated (day 11) the flow rates in all tanks were increased such that within two hours after feeding the prey availability had dropped to <4,000 p/L and within six hours it had been reduced to <1,000 p/L. The automatic feeder was programmed to deliver prey to the continuously fed tanks every two hours to make up for prey lost in the outflow. To ensure that the prey densities decreased as desired, prey densities were measured hourly for 6 hours following the 10 AM feeding, four times over the course of the experiment and prey densities for the measured 6 hour period were averaged and extrapolated over the 24 hour day (Figure 4.1).

Because the nutritional content of live prey changes with time in enrichment medium, an attempt was made to control for differential enrichment of live food. This was especially important because of the different feeding schedules of the live food (rotifers enriched 4 times daily, *Artemia* fed once daily) and larvae. Rotifers were collected from live food culture tanks around 10 AM and maintained in microalgae for 6-24 hours prior to feeding, depending on which fish treatment was being fed. For most feedings, *Artemia* was taken directly from enrichment tanks. However, for the 4 AM feeding *Artemia* was maintained in microalgae for 6 hours. Microalgae was also added to *Artemia* in the automatic feeder reservoir. The volume of microalgae added was approximately 20% of the rotifer or *Artemia* volume.

### Data collection

Larvae were sampled weekly to measure growth. Five larvae from each tank were sampled for standard length (mm) and then pooled for a measurement of dry weight (mg) and ash weight (mg). Larvae were kept on ice and measured immediately after death to prevent shrinkage due to osmotic loss. A dissecting microscope was used to measure larvae to the nearest 0.1 mm. Larvae were rinsed in 3% ammonium formate (to remove salts), placed on preweighed aluminum foils (weighed to nearest 0.001 mg), dried at 55°C

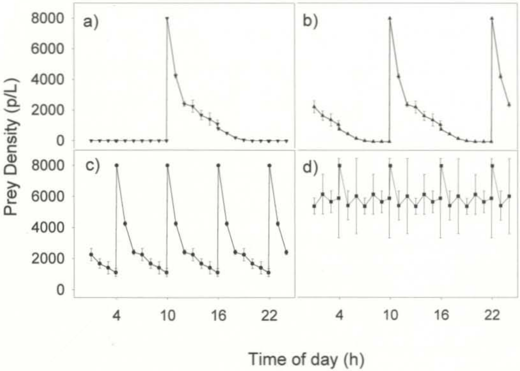

Figure 4.1. Prey densities (prey per liter) in experimental treatments (± S.E.) over a typical 24 period: (a) one feeding per day, (b) two feedings per day, (c) four feedings per day, (d) continuously fed. Prey densities were measured in all tanks between 10-16h and extrapolated over the 24h period.

for at least 48 hours, and reweighed. Larvae were then burned in a muffle furnace at 400°C for 12 hours and ash weights were measured. On day zero, four sets of 5 larvae each were sampled from the incubation tank. On the final sampling day (day 49, week 7), three sets of 5 larvae each were sampled from each tank, except for one of the 1X replicates where only two fish remained. Fish were also sampled for chemical analysis (30 per tank) which will not be discussed here.

Dead larvae were removed twice daily from day 14. The small larval carcass decomposes too quickly to be observed prior to this time. At the end of the experiment, the number of surviving larvae in each treatment was recorded.

Behavioural observations on larvae were conducted once weekly during weeks 1 and 2, before different feeding regimes began, and twice weekly during weeks 3-7. All tanks were observed following the first (10 AM) meal of the day. The order of tanks observed was changed systematically on each observation day. Observations on larvae began 5 minutes following the adjustment of prey densities to 8000 p/L. The focal animal technique (Altman, 1974) was used to observe ten arbitrarily chosen larvae in each tank. Each larva was observed for one minute. During each observation, the frequency and duration of the following Modal Action Patterns (MAPs; Barlow, 1968) were recorded using an event recorder: swim, shake, sink, pause, orient, fixate, and lunge (Table 4.1). The lunge:orient ratio was calculated as this variable can be used as a measure of how likely a larva is to attack a prey item upon encounter. Only 2 larvae remained in one of the 1X replicates during week 7. Observations on these larvae are not included in the analysis.

Table 4.1. Definition of the Modal Action Patterns observed in developing yellowtail flounder larvae, after Barlow (1968).

| MAP | Definition |
|---|---|
| Swim: | Forward movement of the larva through water column resulting from undulations of the caudal region. |
| Pause: | Larva is motionless (similar to "non-swimming" of Munk, 1995). |
| Sink: | Larva is motionless and descends through the water column, often head first. |
| Shake: | Rapid lateral undulations of the entire larval body. |
| Orient: | The head movement towards a prey item (similar to "orientation" of Brown and Colgan, 1985). |
| Fixate: | The larva is stationary and bends its caudal region into an "S" shape position, typically follows orient (Laurel, 1998). |
| Lunge: | The larva moves towards prey from the fixate position in an attempt to capture prey (similar to "bite" of Laurel, 1998). |

## Data analysis

The frequency and duration of the behaviours were summarized using a behavioural software package (Observer, Noldus Information Technology, Wageningen, Netherlands, version 2.0). All statistical analyses were performed using Minitab 9.2. (Minitab Inc., State College, PA). The effects of feeding frequency and age on growth indices and behavioural data were analyzed using analysis of covariance (ANCOVA), with week as the covariant ($\alpha$=0.05). For each response variable (growth or behavioural measurement), the mean value for each replicate tank was calculated and this value was used in the analysis. When significant results were obtained from the ANCOVA model, planned one-tailed pairwise comparisons between treatments were performed to determine which treatments differed ($\alpha$=0.10; in a one-tailed test the $\alpha$-value can be doubled). Three planned comparisons were chosen, as three treatment degrees of freedom were available. In these three comparisons, all treatments were compared to the continuous fed treatment (1X *vs*. constant, 2X *vs*. constant, 4X *vs*. constant). Regression equations describing the change in response variable with age were calculated for each treatment when significant results were obtained from the model. For response variables which were unaffected by treatment or the interaction of age and treatment, only one regression equation is provided which describes the change in that response variable with age.

In order to fit the assumptions of ANCOVA it was necessary to logarithmically transform some response variables. Dry weight and ash free dry weight data were logarithmically transformed. MAP frequency data were transformed according to the equation Log (MAP frequency + 1) when zero values occurred. It was not necessary to transform standard length, swim duration, orient frequency, and lunge:orient ratio data to satisfy ANCOVA assumptions. Plots of residuals and predicted values were examined for heteroscedasticity and normality for each analysis. For all tests the assumption of heteroscedasticity was met. However, in some instances the residuals differed from a normal distribution. In all cases the error df was greater than 30 and/or the p-value was highly significant, in which case the non-normality of residuals would not affect the p-value (D. Schneider, personal communication). Therefore, non-normality of the residuals was not considered to affect the interpretation of the results.

For most response variables, a linear model was adequate to describe the results. However, a second-order polynomial was used to analyze the orient frequency data. The sink, shake, and pause MAPs are characteristic of early stage yellowtail flounder larvae and are not expected to be affected by feeding frequency (Puvanendran, unpublished data). Therefore, they were not analyzed statistically.

A one way analysis of variance (ANOVA) was used to test for differences in survival between treatments. The number of larvae alive prior to the final morphometric sampling (week 7) was used to determine percent survival. The percent survival was calculated from day 0-49 and day 14-49, after the different feeding regimes were established.

### 4.3 Results

Growth: The standard length of the larvae was significantly affected by the interaction of age and feeding frequency (Table 4.2, Figure 4.2a). Pairwise comparisons of standard length-at-age between treatments show that the growth rate of larvae in the 1X treatment was significantly lower than the growth rate of larvae fed continuously (Table 4.3). Regression equations illustrate that the growth rate of larvae in the 1X treatment was nearly 25% slower than that of larvae in other treatments (Table 4.4). No significant differences were found between growth rates in the 2X and continuous, or 4X and continuous treatments (Table 4.3).

Dry weight of the larvae was not significantly influenced by feeding frequency (Table 4.2). However, by week 7, larvae in the 1X treatment weighed less than larvae in all other treatments (Figure 4.2b). A similar pattern was seen with ash free dry weight (Table 4.2, Figure 4.2c).

Survival: Survival results were not corrected for larvae removed for growth measurements or chemical analysis. A total of 75 larvae were removed per tank, prior to the final sampling day. Survival from day 0-49 was low in all tanks (0.11-6.66%) and was not dependent on feeding frequency. Survival from day 14-49 was high and variable (8.0-55.44 %), and was not dependent on feeding frequency (Tables 4.5, 4.6). One of the 1X replicates suffered unusually low survival to day 14 (1.39%) and day 49 (0.11%).

Behaviour: Orient frequency increased early in the study, peaked during weeks 3-4, and then declined later in the study for all treatments (Figure 4.3a). The frequency of orient in the 1X and 2X treatments was always higher than in the 4X and constant

Table 4.2. Summary of ANCOVA results for standard length (mm), dry weight (mg), and ash free dry weight (mg) response variables for yellowtail flounder larvae reared at different feeding frequencies.

| Response variable | Source | df | F | p |
|---|---|---|---|---|
| Standard length (mm) | Age | 1 | 1197.8 | 0.000 |
| | Treatment | 3 | 0.89 | 0.456 |
| | Age*Treatment | 3 | 3.55 | 0.021 |
| | Error | 48 | | |
| Dry weight (mg) | Age | 1 | 2101.0 | 0.000 |
| | Treatment | 3 | 0.67 | 0.574 |
| | Age*Treatment | 3 | 1.04 | 0.385 |
| | Error | 48 | | |
| Ash free dry weight (mg) | Age | 1 | 1962.4 | 0.000 |
| | Treatment | 3 | 0.62 | 0.607 |
| | Age*Treatment | 3 | 1.84 | 0.477 |
| | Error | 48 | | |

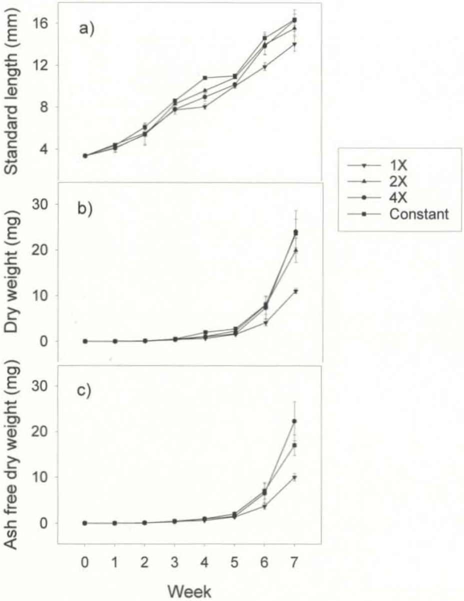

Figure 4.2. Mean of: (a) standard length (mm), (b) dry weight (mg), and (c) ash free dry weight (mg) of yellowtail flounder larvae reared at different feeding frequencies over age (weeks post-hatch). Symbols are means of the two means for each replicate ± S.E.

Table 4.3. Summary of ANCOVA results for one-tailed pairwise comparisons between treatments for standard length and age. All treatments are compared to the constant prey treatment. 1X=one feeding per day, 2X=two feedings per day, 4X=four feedings per day.

| Constant treatment *vs.* | Source | df | F | p |
|---|---|---|---|---|
| 1X | Week | 1 | 818.0 | 0.000 |
| | Treatment | 1 | 0.15 | 0.701 |
| | Week*Treatment | 1 | 11.2 | 0.003 |
| | Error | 24 | | |
| 2X | Week | 1 | 753.3 | 0.000 |
| | Treatment | 1 | 0.38 | 0.546 |
| | Week*Treatment | 1 | 0.11 | 0.739 |
| | Error | 24 | | |
| 4X | Week | 1 | 558.3 | 0.000 |
| | Treatment | 1 | 1.18 | 0.289 |
| | Week*Treatment | 1 | 0.01 | 0.926 |
| | Error | 24 | | |

Table 4.4. Regression equations (in the form $y=a+bx$) describing the change in response variables with age, where $y$=response variable (indicated if transformed) and $x$=week. The equation $y=a+bx+cx^2$ is used for orient frequency. An asterix (*) indicates which treatments differ significantly from the constant treatment. 1X=one feeding per day, 2X=two feedings per day, 4X=four feedings per day, Constant=continuously fed.

| Response variable | Treatment | a | b | $R^2$ |
|---|---|---|---|---|
| Standard length | 1X* | 2.57 | 1.57 | 96.8 |
| | 2X | 1.97 | 1.93 | 96.4 |
| | 4X | 1.54 | 2.00 | 94.4 |
| | Constant | 2.36 | 1.98 | 97.4 |
| Fixate frequency<br>log (fixate F+1) | 1X* | -0.800 | 0.131 | 70.1 |
| | 2X* | -0.035 | 0.109 | 85.7 |
| | 4X | 0.009 | 0.060 | 47.2 |
| | Constant | -0.013 | 0.061 | 82.3 |
| Lunge frequency<br>log (lunge F+1) | 1X* | -0.112 | 0.136 | 72.6 |
| | 2X* | -0.059 | 0.112 | 86.0 |
| | 4X | -0.004 | 0.062 | 50.0 |
| | Constant | -0.027 | 0.063 | 84.9 |
| Lunge:orient ratio | 1X* | -0.192 | 0.139 | 83.5 |
| | 2X* | -0.198 | 0.128 | 92.4 |
| | 4X | -0.117 | 0.096 | 70.3 |
| | Constant | -0.077 | 0.074 | 75.5 |
| Dry weight (mg)<br>log DWT | ---- | -1.78 | 0.437 | 96.8 |
| Ash free dry weight (mg) | ---- | -1.86 | 0.443 | 96.7 |
| Orient frequency<br>log (orient F)<br>c=-0.0368 | ---- | 0.084 | 0.310 | 36.9 |
| Swim duration | ---- | 46.1 | 1.82 | 60.6 |

Table 4.5. Survival of yellowtail flounder larvae reared at different feeding frequencies. Survival was back-calculated to day 14 and quantified at the end of the experiment. Lethally sampled larvae (75 per tank) are not included in calculations. 1X=one feeding per day, 2X=two feedings per day, 4X=four feedings per day, Constant=continuously fed.

| Treatment | # larvae day 0 | # larvae day 14 | # larvae day 49 | % survival day 0-49 | % survival day 14-49 |
|---|---|---|---|---|---|
| 1X (a) | 1800 | 129 | 46 | 2.56 | 35.65 |
| 1X (b) | 1800 | 25 | 2 | 0.11 | 8.00 |
| 2X (a) | 1800 | 244 | 119 | 6.61 | 48.77 |
| 2X (b) | 1800 | 262 | 120 | 6.67 | 45.80 |
| 4X (a) | 1800 | 142 | 43 | 2.39 | 30.28 |
| 4X (b) | 1800 | 312 | 110 | 6.11 | 35.25 |
| Constant (a) | 1800 | 101 | 32 | 1.77 | 31.68 |
| Constant (b) | 1800 | 193 | 107 | 5.94 | 55.44 |

Table 4.6. Summary of ANOVA results for survival of yellowtail flounder larvae reared at different feeding frequencies. Survival calculated from days 0-49 and 14-49.

| Time period | Source | df | F | p |
|---|---|---|---|---|
| Day 0-49 | Treatment | 3 | 2.03 | 0.252 |
| | Error | 4 | | |
| Day 14-49 | Treatment | 3 | 1.55 | 0.333 |
| | Error | 4 | | |

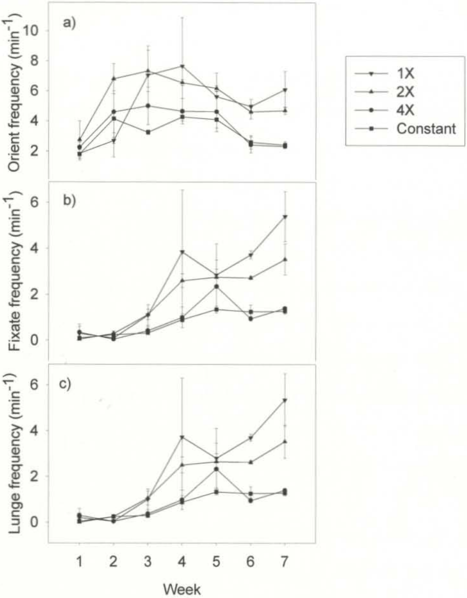

Figure 4.3. Mean (a) orient frequency ($\text{min}^{-1}$), (b) fixate frequency ($\text{min}^{-1}$), and (c) lunge frequency ($\text{min}^{-1}$) of yellowtail flounder larvae reared at different feeding frequencies over age (weeks post-hatch). Symbols are means of the two means for each replicate ± S.E.

treatments after week 2. However, orient frequency was not significantly affected by feeding frequency or age (Table 4.7). The frequency of the foraging MAPs fixate and lunge were similar and increased throughout the study period (Figure 4.3b,c). The frequencies of fixate and lunge were significantly affected by the interaction of age and feeding frequency (Table 4.7). Pairwise comparisons show that the 1X and 2X treatments had a significantly greater increase in fixate and lunge frequency with age compared to the constant treatment. The frequencies of fixate and lunge were not significantly different between the 4X and constant treatments (Tables 4.8, 4.9). Regression equations show that the increase in fixate and lunge with age was approximately twice as great for the 1X and 2X treatments compared to the 4X and constant treatment (Table 4.4). The lunge:orient ratio was significantly affected by the interaction of age and feeding frequency (Table 4.7, Figure 4.4). Pairwise comparisons show that the 1X and 2X treatments had a significantly greater increase in lunge:orient ratio with age compared to the constant treatment. The lunge:orient ratio of the 4X treatment was not significantly different from that of the constant treatment (Table 4.10).

Swim duration increased during the study period. There was a significant effect of age on swim duration (Table 4.7, Figure 4.5). The frequencies of the pause, sink, and shake MAPs decreased throughout the study period (Figure 4.6) and larvae spent <3% of their time in these activities after the different feeding frequencies began.

Table 4.7. Summary of ANCOVA results for behaviour response variables for yellowtail flounder larvae reared at different feeding frequencies. $Week^2$ is the square of larval age in weeks; for orient frequency a polynomial term was used in the model.

| Response variable | Source | df | F | p |
|---|---|---|---|---|
| Orient frequency | Week | 1 | 39.8 | 0.000 |
| | $Week^2$ | 1 | 35.9 | 0.000 |
| | Treatment | 3 | 0.65 | 0.588 |
| | Week*Treatment | 3 | 0.19 | 0.900 |
| | $Week^2$*Treatment | 3 | 0.03 | 0.992 |
| | Error | 43 | | |
| Fixate frequency | Week | 1 | 113.1 | 0.000 |
| | Treatment | 3 | 0.25 | 0.860 |
| | Week*Treatment | 3 | 4.27 | 0.010 |
| | Error | 47 | | |
| Lunge frequency | Week | 1 | 125.7 | 0.000 |
| | Treatment | 3 | 0.40 | 0.751 |
| | Week*Treatment | 3 | 4.76 | 0.006 |
| | Error | 47 | | |
| Lunge:orient ratio | Week | 1 | 209.8 | 0.000 |
| | Treatment | 3 | 0.79 | 0.505 |
| | Week*Treatment | 3 | 3.84 | 0.015 |
| | Error | 47 | | |
| Swim duration | Week | 1 | 89.9 | 0.000 |
| | Treatment | 3 | 1.99 | 0.129 |
| | Week*Treatment | 3 | 0.48 | 0.698 |
| | Error | 47 | | |

Table 4.8. Summary of ANCOVA results for one-tailed pairwise comparisons between treatments for fixate frequency and age. All treatments are compared to the constant treatment. 1X=one feeding per day, 2X=two feedings per day, 4X=four feedings per day.

| Constant treatment *vs.* | Source | df | F | p |
|---|---|---|---|---|
| 1X | Week | 1 | 56.0 | 0.000 |
| | Treatment | 1 | 0.36 | 0.556 |
| | Week*Treatment | 1 | 7.51 | 0.012 |
| | Error | 23 | | |
| 2X | Week | 1 | 124.4 | 0.000 |
| | Treatment | 1 | 0.11 | 0.747 |
| | Week*Treatment | 1 | 10.1 | 0.004 |
| | Error | 24 | | |
| 4X | Week | 1 | 36.3 | 0.000 |
| | Treatment | 1 | 0.06 | 0.806 |
| | Week*Treatment | 1 | 0.00 | 0.969 |
| | Error | 24 | | |

Table 4.9. Summary of ANCOVA results for one-tailed pairwise comparisons between treatments for lunge frequency and age. All treatments are compared to the constant treatment. 1X=one feeding per day, 2X=two feedings per day, 4X=four feedings per day.

| Constant treatment *vs.* | Source | df | F | p |
|---|---|---|---|---|
| 1X | Week | 1 | 63.7 | 0.000 |
| | Treatment | 1 | 0.62 | 0.439 |
| | Week*Treatment | 1 | 8.61 | 0.007 |
| | Error | 23 | | |
| 2X | Week | 1 | 133.8 | 0.000 |
| | Treatment | 1 | 0.23 | 0.638 |
| | Week*Treatment | 1 | 10.4 | 0.004 |
| | Error | 24 | | |
| 4X | Week | 1 | 41.2 | 0.000 |
| | Treatment | 1 | 0.07 | 0.791 |
| | Week*Treatment | 1 | 0.00 | 0.957 |
| | Error | 24 | | |

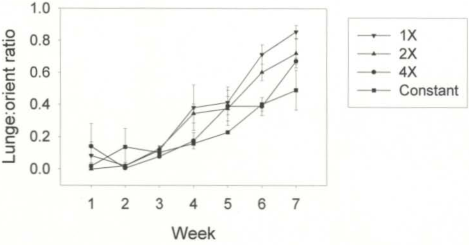

Figure 4.4. Mean ratio of lunge to orient of yellowtail flounder larvae reared at different feeding frequencies over age (weeks post-hatch). Symbols are means of the two means for each replicate ± S.E.

Table 4.10. Summary of ANCOVA results for one-tailed pairwise comparisons between treatments for lunge:orient ratio and age. All treatments are compared to the constant treatment. 1X=one feeding per day, 2X=two feedings per day, 4X=four feedings per day.

| Constant treatment *vs.* | Source | df | F | p |
|---|---|---|---|---|
| 1X | Week | 1 | 95.6 | 0.000 |
| | Treatment | 1 | 1.48 | 0.236 |
| | Week*Treatment | 1 | 8.86 | 0.007 |
| | Error | 23 | | |
| 2X | Week | 1 | 156.8 | 0.000 |
| | Treatment | 1 | 2.82 | 0.106 |
| | Week*Treatment | 1 | 11.06 | 0.003 |
| | Error | 24 | | |
| 4X | Week | 1 | 61.4 | 0.000 |
| | Treatment | 1 | 0.18 | 0.679 |
| | Week*Treatment | 1 | 0.98 | 0.332 |
| | Error | 24 | | |

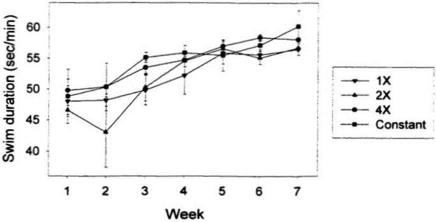

Figure 4.5. Mean swim duration (sec/minute) of yellowtail flounder larvae reared at different feeding frequencies during one minute observation periods over age (weeks post-hatch). Symbols are means of the two means for each replicate ± S.E.

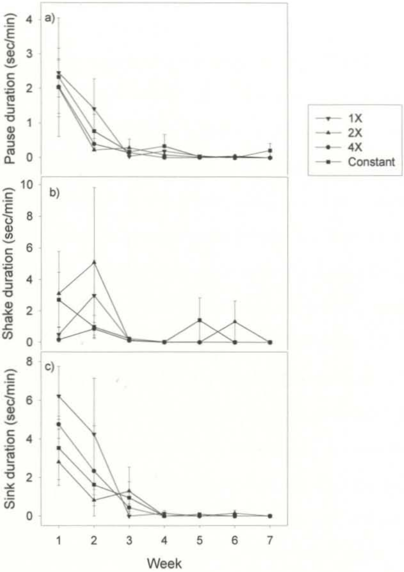

Figure 4.6. Mean (a) pause duration (sec/minute) (b) shake duration (sec/minute) and (c) sink duration (sec/minute) of yellowtail flounder larvae reared at different feeding frequencies during one minute observation periods over age (weeks post-hatch). Symbols are means of the two means for each replicate ± S.E.

## 4.4. Discussion

Yellowtail flounder larvae grew and survived in all treatments used in this study. The results demonstrate that yellowtail flounder larvae do not require constant, high prey densities. Larvae grew and survived when offered prey at high prey density only once per day. However, a feeding frequency of 2X caused an increase in growth rate compared to 1X. Increasing feeding frequency above 2X did not result in a further increase in growth rate. Therefore, larvae in the 4X and continuous treatments were fed to excess.

The survival in all treatments was low and unaffected by feeding frequency. The low survival can be attributed to poor egg quality and the relatively low light intensity used in the experiment. The eggs used in this study were collected late in the spawning season when temperatures were high and many curled larvae were observed at hatching. Most mortality occurred before the experimental feeding regimes were established, suggesting that the high mortality was due to a general failure to initiate feeding and not due to the different treatments. This is supported by the higher percent survival observed when survival is calculated only from weeks 2-7. Furthermore, yellowtail flounder larvae prefer high light intensities (>1500 lux; Puvanendran, unpublished data). Unfortunately it was not possible to increase the light intensity above 750 lux in the experimental system used, and this likely also contributed to the low survival.

The behavioural data can aid in interpreting the observation that larvae in the 2X, 4X, and constant treatments had similar growth rates and survival despite wide differences in amount of prey offered to the larvae. The increase in lunge frequency with age for the 2X treatment is nearly twice as high as that for the constant treatment (Table

4.4). This increase in instantaneous consumption rate in the 2X treatment likely enabled these larvae to obtain an adequate ration when prey was available and grow and survive as well as larvae in the 4X and constant treatments.

Larvae reared in the 1X and 2X treatments did exhibit a higher lunge frequency and therefore likely consumed more prey during the observation periods compared to larvae reared in the 4X and constant treatments. However, larvae reared in the 4X and constant treatments may have actually consumed more prey per day than larvae in the 1X and 2X treatments, if it is assumed that these observed lunge frequencies were maintained in all treatments when prey was present at high density. The lunge frequency of larvae in the 1X and 2X treatments was between 2-4 times higher than that of larvae reared in the constant treatment (Figure 4.3c). Therefore, it would require between 6-12 hours of feeding at this rate for larvae in the 1X and 2X treatments to consume the same amount of prey as larvae in the constant treatment, assuming a constant ratio of lunges to captures across treatments. Clearly, prey densities of 8000 p/L were not maintained for this extent of time in the 1X and 2X treatments. Of course, larvae will feed when prey densities are <8000 p/L, however it is not possible to estimate the amount of prey consumed during these times. It is only presumed that larvae in the 1X and 2X treatments consumed less prey than larvae in the 4X and constant treatments over a 24-hour period. Large differences in growth between the 2X, 4X and constant treatments were not detected, and it is assumed that the extra prey likely consumed by larvae in the 4X and constant treatments was not assimilated for growth.

The lunge frequency data is interesting because it differs from an accepted model of consumption rate for larval fish. Previous descriptions of predation describe the consumption rate of larvae at different prey densities as a functional response, which was first described by Holling in 1965 (Houde and Schekter, 1981). The consumption rate of many species of larval fish has been shown to increase towards an asymptote with increasing prey density (Houde and Schekter, 1981; Munk and Kiørboe, 1985; Klumpp and von Westernhagen, 1986; Miller *et al.*, 1992). The theoretical interpretation of larval feeding behaviour assumes that the observed consumption rate is a physiological and/or behavioural response to the prey density alone. However, in this experiment, yellowtail flounder larvae fed at the same prey density, in the same foraging environment of 8000 p/L, did not exhibit the same consumption rate. Instead, they altered their consumption rate in response to feeding history or degree of hunger, and therefore prey density is not the sole determinant of consumption rate. Clearly, feeding history is an important determinant of the foraging response.

In his study on cod larvae, Munk (1995) also observed an apparent behavioural response to hunger that was independent of prey density. Larval cod decreased their search effort as they became satiated. In his study, the time spent swimming and searching for prey decreased as the number of prey items in the gut increased. The results for yellowtail flounder differ in that significant differences in the time spent swimming between treatments were not detected. This may be a result of the different prey search behaviours of cod and yellowtail flounder. Cod are saltatory searchers, which search for prey during the motionless periods that punctuate swimming events. If prey is not located, larvae will swim a short distance and search again (Browman and O'Brien, 1992; Puvanendran *et al.*, 1999). For a saltatory searcher, swimming duration can thus be used as an indicator of search effort. Yellowtail flounder are cruise searchers and spend most of their time swimming (personal observation). The time that yellowtail flounder spend performing foraging behaviours therefore makes up little of the total observation period relative to swimming duration. Differences in the total time spent swimming between treatments are therefore expected to be small, and detecting them may be limited by the observer's reflexes.

Larvae reared in different feeding frequencies exhibited different patterns of foraging. A larva may orient towards a prey item upon encountering it. The larva may then follow through its foraging routine by fixating and lunging, in an attempt to attack and consume the prey. The increase in lunge:orient ratio with age was greater in the 1X and 2X treatments. This suggests that larvae reared at low feeding frequencies are more likely to attempt to capture a prey item upon encounter, compared to larvae at high feeding frequencies. This difference in behaviour can be attributed to differing degrees of hunger experienced by larvae in the treatments.

The lunge:orient ratio may be indicative of prey discrimination or selectivity. Larvae that encounter and orient toward many prey items but only attempt to capture a fraction of them may be exhibiting selection in their choice of prey. The data suggests that larvae in the 4X and constant treatments are more selective than the larvae in the 1X and 2X treatments, as they have a lower lunge:orient ratio throughout the study period. This apparent prey selectivity is in accordance with foraging theory which states that prey discrimination should occur only when resources are abundant (Emlen, 1966; MacArthur and Pianka, 1966; Werner and Hall, 1974). Both prey size and species selectivity have previously been reported for other species of larvae and it has been shown that when prey density is low larvae are less selective than when resources are abundant and prey density is high (Houde and Schekter, 1980; Khadka and Rao, 1986; van der Meeren, 1991; Munk, 1995). The yellowtail flounder results differ from previous studies as they suggest that when larvae with different feeding histories are fed at the same high prey density, larvae which are fed less frequently are less selective than larvae which are fed continuously. Of course, while these results suggest differences in selectivity between treatments in accordance with foraging theory, it is difficult to determine for what the larvae are actually selecting. All larvae were fed the same prey type, either rotifers or *Artemia*, with a very brief period (4 days) of cofeeding. It can only be speculated that the larvae at higher feeding frequencies are selecting for larger, energetically profitable prey, out of the general rotifer or *Artemia* population. That this pattern represents some selectivity is supported by the fact that larvae reared at high feeding frequencies, which are presumed to be somewhat satiated, continue to orient towards and therefore demonstrate interest in prey. If the lunge:orient ratio is indeed a measure of selectivity, it demonstrates that larvae can respond to the scarcity of resources, or patchiness of their respective environments. This is interesting because it suggests that prey density is not the sole determinant of prey selectivity.

The lunge:orient ratio results differ from a model of prey selectivity for larval fish. Griffiths (1975) described vertebrate larvae as "number maximizers" which consume different prey types in proportion to their presence in the environment. Larval guppies (*Lebistes reticulatus*) appear to fit this model as they consume different types of prey in proportion to their abundance even at very high prey density (50,000 p/L; Lair *et al.*, 1994). According to Griffiths' theory, larvae in the same foraging environment should exhibit the same degree of selectivity. However, yellowtail flounder in the same "environment" of 8000 p/L altered their apparent selectivity as a response to feeding history. Larvae reared in the 1X and 2X treatments appear to behave as number maximizers, while larvae fed more frequently exhibit a degree of selectivity as they have a lower lunge:orient ratio. Griffiths (1975) did not incorporate hunger into his model, and these results suggest that feeding history will affect prey selection.

The pattern of orient frequency observed is interesting and merits comment. The orient frequency follows a similar pattern with age for all treatments. Initially orient frequency is low; it then rises to a peak at weeks 3-4 and then decreases. The initial low frequency of orient may be explained by a low encounter rate of the larvae due to their small size and slow swimming speed (Mittelbach, 1981). Similarly, an increase in orient frequency at week 2 can be explained by an increase in swimming speed and encounter rate. However, the decrease in orient frequency following week 4 is puzzling as larger larvae are competent swimmers (Rosenthal and Hempel, 1971; Laurence, 1972; Houde and Schekter, 1980) and are expected to exhibit a high prey encounter rate.

This decrease in orient frequency may be explained by an improvement in visual ability. Miller *et al.* (1993) showed that the visual angle - the smallest angle which a stimulus may subtend the eye and remain resolvable (Neave, 1984) - decreases during the development of three species of fish larvae. Thus, the eye develops such that larvae can detect prey items in their periphery without turning the head and orienting. In the case of yellowtail flounder, the orient frequency likely decreases because most orient MAPs would be recorded only when the larva completes its foraging routine with a fixate and lunge.

The growth results are similar to those of Houde and Schekter (1978) who showed that there was a minimum time period of high prey availability (3 hours) that resulted in survival rates of sea bream larvae that approached those observed when larvae were reared at a constant high prey density. However, the yellowtail flounder data differs from the results of Brown *et al*. (1997), who examined the effects of feeding frequency on growth and consumption rate of lumpfish (*Cyclopterus lumpus*) larvae. Their results demonstrated that lumpfish larvae grow faster when fed in discrete meals compared to continuously. Their behavioural results were similar to those for yellowtail flounder in that larvae fed two and three meals per day had a higher instantaneous consumption rate during observation periods compared to larvae fed constantly. The different results, that lumpfish growth is inhibited by continuous feeding and yellowtail flounder growth is not, may be explained by differences in digestive physiology between the two species.

Lumpfish spawn in the spring around Newfoundland, and larvae are larger and more developed at hatching compared to yellowtail flounder. Because of their advanced state of development, the digestive system of larval lumpfish is likely more similar to that of adults compared to yellowtail flounder. The alimentary canal of altricial larvae, such as yellowtail flounder, is relatively undifferentiated (Govoni *et al*., 1986) and a stomach cannot be detected in this species until day 36 (Baglole *et al.*, 1997). Digestion in lumpfish probably occurs within the alimentary canal by extracellular digestion and membrane transport, as this is the adult mode of digestion (Govoni *et al.*, 1986). In altricial larvae, digestion occurs involves pinocytosis and intracellular digestion (Govoni *et al.*, 1986), which presumably occurs in the undifferentiated digestive system of yellowtail flounder. If indeed digestion occurs within the alimentary canal of larval lumpfish, it is not difficult to imagine that assimilation efficiency would be decreased by the continuous passage of prey through the digestive system under constant feeding conditions. Digestion probably does not occur in the alimentary canal of yellowtail flounder and it may be possible that pinocytosis and intracellular digestion can occur as prey passes through the larval gut. These differing results between species support the idea that the undifferentiated digestive system of many species of marine fish larvae is adapted to exploit patches of prey (Checkley, 1984; Govoni *et al.*, 1986; Klumpp and von Westernhagen, 1986).

All treatments were maintained equally during the first 10 days of feeding. This was an effort to establish feeding in all tanks. If the different treatments were initiated on day 1, larvae in the 1X and 2X treatments may have been at a disadvantage as they had less opportunity to learn how to feed. Day 10 is after the period of high mortaility associated with start-feeding in yellowtail flounder at the relatively high temperatures used in this study (Puvanendran, unpublished data) and larvae surviving after this time were presumed to be successful foragers. Initiating the treatments on day 11 was an effort to ensure that differences in lunge frequency were due to differences in consumption rate, and not due to differences in foraging success. To further ensure that capture success was similar between treatments, all larvae were fed at a high prey density that enabled them to capture the greatest amount of prey per unit time. It was not possible for the observer to determine the outcome of all foraging events, and capture success was not recorded. However, because all larvae were allowed to learn how to feed, and were fed at a high prey density that promotes high capture success, it is probable that that the higher lunge frequency observed in the 1X and 2X treatments is not due to low capture success, but due to a higher consumption rate.

The results of this study demonstrate that yellowtail flounder larvae do not require constant high prey densities during larviculture. Feeding larvae twice a day at high prey density provided an adequate ration as similar growth and survival was observed between the 2X and continuously fed treatments. This may be explained by a behavioural response of increased consumption rate in larvae fed twice a day, or a physiological response of decreased assimilation efficiency in larvae fed continuously. The results support the idea that larvae may be able to obtain an adequate ration when prey is distributed in patches in the sea. Furthermore, the behavioural data suggests that larvae exhibit plasticity in responding to their foraging environment. The similar growth between larvae in the 2X and constant treatments demonstrates that there is potential to reduce the live food and labor cost in larviculture. It is not necessary to maintain constant high prey densities in the rearing of yellowtail flounder, provided that larvae are offered high prey density at least twice a day. Because different species vary both physiologically and behaviourally, it may not be possible to universally apply these findings to the larviculture of other species.

# CHAPTER 5

# SUMMARY AND FUTURE RESEARCH

The experiments described in this thesis focused on the behaviour, growth, and survival of witch flounder and yellowtail flounder larvae in relation to prey availability. The objective of the experiments was twofold. First, the results can be used to design rearing protocols for the larviculture of these species. Second, the results can be interpreted in an ecological context. They can be used to provide insight into causes of survival at sea or to test assumptions about foraging theory.

In the first experiment, witch flounder larvae were exposed to a wide range of prey densities and their behaviour was recorded during feeding trials. Larvae were also reared at an intermediate range of prey densities and their growth and survival were monitored. The main findings of this study were that the foraging behaviour of witch flounder larvae is not as strongly affected by prey density as are other species. Furthermore, comparative evidence suggests that larvae have low prey requirements. The lack of a strong effect of prey density on foraging behaviour suggests that fish culturists do not have to be as vigilant in monitoring prey densities for witch flounder larvae as they do for other species. Finally, this indicates that witch flounder may not be as susceptible to starvation in the wild compared to other species. This is in agreement with witch flounder ecology. Witch flounder has an extended larval period and must be able to cope with the wide variations in prey availability they are likely to encounter at sea.

Witch flounder behaves very differently from other species in culture. Because of its unusual life history and low prey requirements, the construction of an energy budget for this species would be interesting. Instantaneous consumption rates toward the end of the study period rarely exceeded 1/min. Measuring the oxygen consumption of witch flounder would be a simple first step in this direction. Rearing the larvae at prey densities <2000 p/L would also be valuable.

In the second experiment yellowtail flounder were exposed to high prey density at different feeding frequencies. The main result of this experiment was that larvae grew and survived similarly whether they were offered prey at high prey density twice a day or continuously. This shows that larvae are not dependent on constant high prey availability to maintain rapid growth. This finding is important for aquaculture as it suggests there is a potential to reduce the cost of live food and labor associated with first-feeding. Furthermore, it supports the hypothesis that patches of high prey availability contribute to survival at sea as larvae grew well when prey availability varied temporally.

An improvement in the foraging ability of witch flounder and yellowtail flounder larvae was suggested by the behavioural observations. For witch flounder this was manifested as a decrease in orient frequency with age as well as a disappearance of the minimal effects of prey density on foraging behaviour, once the larvae reached a larger size. Larvae appeared to reach a size where prey density no longer affected their foraging ability. For yellowtail flounder, a similar decrease in orient frequency was detected. For both species, it appeared that later stage larvae did not need to orient towards as many prey items before encountering one suitable for capture, as their prey capture abilities had improved. Future work examining the orient, lunge, and capture success behaviour of larvae in relation to different types of prey, perhaps on a magnified scale, would be useful to determine if the decrease in orient frequency is indeed evidence of prey capture ability. If it is, this suggests that the larvae are aware of their own abilities to capture prey and vary their attacks accordingly.

The results of the yellowtail flounder experiment suggested that the larvae were foraging in accordance with foraging theory. The ratio of lunge to orient between treatments indicated that larvae fed less frequently were less selective in their prey types. Because the experiment was not set up to test foraging theory, data was not collected in a way that would unequivocally support this hypothesis. Future experiments could be designed to test this hypothesis, perhaps by using a variety of prey types.

The results of the yellowtail flounder experiment also suggested that the assimilation efficiency of the larvae was affected by feeding frequency. This is because larvae fed at higher feeding frequencies likely consumed more prey per day compared to larvae at lower feeding frequencies, although they did not grow faster. Differences in assimilation efficiency could be determined by allowing larvae to feed on labeled prey and sampling for label uptake on a time scale of hours. It is expected that larvae reared with continuous exposure to high prey density will incorporate labeled prey at a slower rate than larvae fed less frequently.

The use of behavioural observations was a valuable component to the design of the experiments presented here. Due to space limitations, it was only possible to use a low number of replicates in each growth and survival experiment. Thus, the interpretations were susceptible to criticism, as statistical analysis was unable to detect minute differences between treatments. However, the behavioural results upheld and elaborated on the growth and survival results, lending support to the tank experiments.

The behavioural results for witch flounder indicated that prey density has a weak effect on foraging and does not affect foraging ability once the larvae reach a certain size. It would not have been possible to obtain this information without the use of behavioural observations, especially given the small number of animals available for the study. Behavioural results on yellowtail flounder larvae showed that larvae fed less frequently were able to respond to limited prey availability by increasing their consumption rate when prey was available. This likely enabled them to grow as fast as larvae reared at high feeding frequency. With this behavioural approach, it was possible to apply the results of both studies to the aquaculture and ecology of witch flounder and yellowtail flounder.

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# APPENDIX A

# IMPLICATIONS FOR WITCH FLOUNDER AQUACULTURE

The results of experiments conducted on witch flounder larvae indicate that this species performs well in a culture situation. Therefore, there is potential for the successful development of commercial witch flounder culture. A goal of the experiment presented in Chapter 3 was to determine the optimal rearing prey density for witch flounder larvae. The results of Part A demonstrated that the foraging behaviour, and in particular the lunge frequency (an indicator of consumption rate) of witch flounder larvae was largely independent of prey density. However, larvae did have a higher lunge frequency at prey densities ≥2000 p/L compared to prey densities <2000 p/L for the early part of the study period. In Part B, larvae grew and survived equally well when reared at 2000-8000 p/L. The results from both Part A and Part B suggest that a prey density of 2000 p/L is adequate for rearing witch flounder larvae. Furthermore, the growth and survival results demonstrate that there is no deleterious effect of growth at higher prey densities for this species as has been seen with redfish (Laurel, 1998). Preliminary results also indicate that light intensity has little effect on witch flounder larval growth and survival as larvae performed similarly under high and low light.

Witch flounder larvae seem to be fairly robust to fluctuations in prey availability and light intensity in culture. Future experimental work should include rearing witch flounder larvae at prey densities <2000 p/L to determine exactly how little prey this species requires during the first-feeding stage. It may also be appropriate to direct future research towards juvenile growth and survival. My preliminary results with juvenile witch flounder suggest that this species grows slowly during this stage and is susceptible to disease. Improvements in juvenile husbandry with particular emphasis on tank design and diet may overcome these difficulties.

The witch flounder project at the Ocean Sciences Centre was highly productive as all aspects of the fish production cycle were addressed. Broodstock were collected, and the growth of two year-classes of larvae and juveniles were monitored. The results presented in this thesis are very encouraging and indicate that witch flounder is a strong candidate for aquaculture.