THE USE OF ENDOGENOUS AND EXOGENOUS RESOURCES DURING THE EARLY DEVELOPMENT OF ATLANTIC REDFISH (Sebastes SPP.)

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THE USE OF ENDOGENOUS AND EXOGENOUS RESOURCES DURING THE EARLY DEVELOPMENT OF ATLANTIC REDFISH (*Sebastes* spp.)

by

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Abstract

Atlantic redfish (*Sebastes* spp.) are a commercially exploited groundfish in the NW Atlantic, yet little is known about the early life history of these species. Their ovoviviparous reproductive strategy and restriction to deep benthic environments during spawning makes studying embryogenic and larval stages difficult. Proper management of exploited fish species, however, depends upon a comprehensive understanding of early development as it is during the egg and larval stages that recruitment variability is considered to be largely determined. I conducted two separate investigations describing the use of endogenous and exogenous resources in larval redfish in an attempt to both provide insight into recruitment processes as well as understand the evolutionary success of these species.

The first study examined changes in lipid and fatty acid profiles in developing pre-extruded larvae. During development within the female there was nearly a 50% reduction in total lipid, suggesting that lipids are an important source of energy and that metabolism of these resources occurs prior to parturition. Triacylglycerol was preferentially catabolised over polar lipids unlike other Atlantic groundfish during embryogenesis such as cod (*Gadus morhua*) and halibut (*Hippoglossus hippoglossus*). High variability in these lipid reserves suggests that sensitivity to mismatches in prey after parturition likely varies between broods, assuming increased endogenous resources reduces risk of starvation.

The second study investigated behaviour, growth and survival of larval redfish reared under prey densities of 0, 500, 1500 and 4500 prey L^{-1} in laboratory conditions.

Some larvae lived to day 18 in the 0 prey L^{-1} treatment despite possible handling stress from collection and transporting. Growth, survival and condition of larvae varied with prey concentration but were highest in the 1500 prey L^{-1} treatment. The significantly lower prey bite:orient ratios in the 4500 prey L^{-1} treatment suggest that larvae may have become confused at higher prey densities. A possible confusion effect may have explained the significant reduction in growth and survival of larvae reared in the highest prey treatment. Although the prey densities used in this experiment are higher than those reported in the field, comparisons with other rearing experiments suggest that prey availability may not be as limiting to redfish as for other commercially important species such as Atlantic cod.

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Chapter 1
General Introduction

1.1 Redfish Ecology

Redfish (*Sebastes* spp.) are long-lived, commercially important groundfish species in the NW Atlantic. They have often been reported to live 40 or 50 years but in some instances fish have been reported to be in excess of 80 years (Scott and Scott 1988). Three species of redfish currently reside off the Newfoundland coast: the deepwater beaked redfish (*Sebastes mentella*), the Acadian beaked redfish (*Sebastes fasciatus*), and the Atlantic golden redfish (*Sebastes marinus*). *S. marinus* is the largest of the three species and can be identified based on its external morphology (Scott and Scott 1988). *S. faciatus* and *S. mentella*, collectively known as the beaked redfish, are not easily distinguished from one another unless internal morphological differences in the swim bladder are used (Ni 1981). Bottom-trawl surveys around the Flemish Cap and the Gulf of St. Lawrence have shown *S. mentella* and *S. faciatus* to be the most abundant redfish species with the former being the most numerous of the two (Ni 1981; Konstantinov 1985; St-Pierre and Lafontaine 1995). Because of their morphological and life history similarities, however, these three species of redfish have been traditionally managed as one stock by the Department of Fisheries and Oceans (DFO) in Atlantic Canada.

The ovoviviparous reproductive strategy of *Sebastes* spp. makes them unique among co-occurring groundfish species in the NW Atlantic. The evolution and ecological significance of live-bearing in fishes has been reviewed by Wourms (1981), but very little

is known about Atlantic redfish. Although the energetic costs to the female are unknown, live-bearing in fishes may contribute to increased larval survival by providing a thermostatic environment devoid of predators during vulnerable periods in development. Larvae of live-bearing species may also benefit by being able to use dead broodmates as an additional energy source during embryogenesis (Boehlert et al. 1986). Lastly, it has been suggested that redfish may use this reproductive strategy to time the release of their young in response to ideal feeding conditions (Runge and de Lafontaine 1996). However, studies have yet to determine if such a behavior actually exists during times of varying food availability.

On the Flemish Cap, *S. mentella* spawns from April to early June while spawning of *S. fasciatus* peaks in June and continues into late August (Penney 1987). In the Gulf of St. Lawrence, however, peak spawning of the beaked redfish (*S. mentella* and *S. fasciatus*) overlap between April and June (St-Pierre and de Lafontaine 1995). The fecundity of *S. mentella* has been estimated to vary between 1500-70000 oocytes per female depending upon age while estimates are slightly higher for *S. fasciatus* (St-Pierre and Lafontaine 1995). Although these fecundities are relatively low compared to most oviparous marine fish, late spring spawning *Sebastes* spp. comprise more than 80% of the icthyoplankton off the northeast coast of Newfoundland (Anderson and Akenhead 1981; Anderson 1994) while ichthyoplankton surveys in the Gulf of St. Lawrence estimate representation at greater than 96% (Runge and de Lafontaine 1996). The dominance of larval *Sebastes* spp. during spawning also coincides with a spring bloom of planktonic copepods, namely *Calanus finmarchicus*. In the form of eggs and early nauplii stages,

these *Calanus finmarchicus* serve as the preferred prey item to newly extruded redfish larvae (Bainbridge and McKay 1968; Anderson 1994).

Despite the commercial importance of redfish, the early life history of these species is virtually unknown. Understanding the reproduction and early life stages of commercially exploited marine fish species is crucial for effective fisheries management. Hjort (1914) first proposed that recruitment was largely determined as a result of successful feeding in larval fish during the switch between endogenous to exogenous feeding. Termed the 'critical period' (May 1974), studies have shown that high mortalities occur when suitable amounts of food are not available after endogenous energy reserves are exhausted (e.g. Blaxter and Hempel 1963; Lasker et al. 1970).

The critical period concept is an underlying component of the match/mismatch hypothesis (Cushing 1990). It is widely hypothesized that larval populations fluctuate as a result of interannual variability in planktonic blooms during fish spawning (Cushing 1990; Anderson 1994; Runge and de Lafontaine 1996; Goteeitas et al. 1996). That is, the release of fish larvae into the water column either does (match) or does not (mismatch) coincide with a bloom of preferred planktonic prey species, which in turn partly determines the strength of a particular year class. The degree to which a match or mismatch in prey potentially affects redfish recruitment is dependent on the larva's access to (1) endogenous resources (i.e. yolk reserves) and (2) exogenous resources (i.e. suitable prey).

1.2 Endogenous Resources

Endogenous energy reserves, namely lipids, provide both metabolic and structural requirements for developing embryos and pre-feeding fish larvae. Proteins (Cetta and Capuzzo 1982) and free amino acids (Rønnestad et al. 1992) have also been shown to be an important energy source in some species although these biochemicals are considered to be of lesser importance than lipid metabolism in developing marine fish.

Lipids are stored in the lipoprotein-yolk and, if present, single or multiple oil globules found within the yolk-sac. Lipid and fatty acid profiles of the yolks of eggs and larvae vary between and within fish species by type as well as by absolute and relative amounts. Certain lipid classes are typically used for metabolism (e.g. triacylglycerol and sterol/wax esters) while other classes are often used as structural components in biomembranes e.g. phospholipids (Sargent 1995). Larvae provided with more metabolic lipid reserves may therefore be less sensitive to mismatches in prey as a result of having lower susceptibility to delayed feeding. Hart and Werner (1987) found that white sucker larvae (*Catostomus commersoni*) had higher survival during the transition to exogenous food than pumpkinseed larvae (*Lepomis gibbosus*). This was attributed to larger egg sizes and yolk reserves in white sucker larvae than pumkinseed larvae. In a review by Miller et al. (1988), larvae with larger yolk sacs were generally found to have greater flexibility in the time in which feeding switches from endogenous to exogenous sources. This was evidenced by longer times to first feeding as well as greater variability in the timing of first feeding in larger yolk-sac larvae. Larger eggs (i.e. higher lipid

content) also typically produce larger and faster growing larvae (Moodie et al. 1989; Brown and Taylor 1992) which in turn should positively affect survival through the increased foraging ability of larger, more developed larvae (Hunter 1981; Brown and Colgan 1985). Thus inter- and intraspecific variability in the endogenous resources of fish larvae can have pronounced effects on behaviour, growth and ultimately survival. Therefore it is important to record these developmental changes, noting especially changes in lipid/fatty acid profiles over time as well as variability in these profiles between specimens. Gathering baseline biochemical data for pre- and post-feeding larvae can be further used to calibrate condition indices (Fraser 1989; Lochmann et al. 1995) as well as determine larval nutritional requirements (Watanabe 1982).

1.3 Exogenous resources

Fish larvae must obtain food for survival after endogenous food supplies are exhausted. Successful feeding is largely determined by the foraging ability of the fish larvae within its prey environment. Foraging ability is dependent on ecological factors (e.g. temperature, light and turbulence) as well as size and species characteristics and development. Changing larval morphology and physiology (e.g. mouth gape and visual acuity) and behaviour (e.g. swimming and searching ability) during growth and development is also of great importance (Hunter 1981). We can therefore expect different responses to matches and mismatches in prey for different larval fish species. By measuring physiological and behavioural responses to changing prey fields over development, one can assess optimal and sub-optimal foraging conditions which may affect survival between years.

Our current understanding of larval redfish feeding ecology is based solely on field experiments using gut content analysis and estimated prey concentrations. No studies to date have successfully carried out experiments on captive redfish larvae, but this is not surprising considering the life history of these species. Redfish are extremely slow growers, growing only to 19 cm by 5 years and recruited into the population at 7-10 years of age on the Flemish Cap (Lilly 1987). Adult redfish are typically found at depths in excess of 400 meters (Lilly 1987), and this, coupled with their live-bearing nature, makes larvae retrieval logistically difficult, time consuming and expensive. Penny and Evans (1985) were able to keep larvae alive for 12 days in aquariums, but insignificant growth and high mortality caused them to discontinue experiments. Methodologies for related species such as *Sebastes marmoratus*, however, have been successfully implemented for the aquaculture industry in Japan (Boehlert and Yamada 1991).

1.4 Objectives

This thesis will cover early life history aspects of Atlantic redfish from the embryonic stages to the beginning of juvenile metamorphosis. The first experiment will elucidate the roles of endogenous resources in pre-extruded larvae by way of lipid and fatty acid analysis. The second experiment will focus on foraging behaviour, growth and survival of larvae after parturition. While each experiment addresses questions independent of one another, speculation into the ecological significance of these results, specifically issues of survival variability, will be emphasised in the last chapter of this thesis.

CHAPTER 2

CHANGES IN LIPID AND FATTY ACID COMPOSITION OF PRE-EXTRUDED REDFISH THROUGHOUT EMBRYOGENESIS

2.1 Introduction:

Lipids have been shown to be an important energy source as well as key components of membranes for developing marine fish eggs and pre-feeding larvae. Free amino acids also serve as an energy source to some species but are generally of secondary importance to the metabolism of lipids. One notable exception, however, is the turbot (*Scopthalamus maximus*) in which free amino acids account for the majority of fuel for energy metabolism in early development (Rønnestad et al. 1992).

The polarity of each lipid determines whether it is classed as either polar or neutral. Neutral lipids are considered to be the most common source of energy, namely in the form of waxy esters or triacylglycerols (TAG). High percentages of TAG are often found in fish eggs with long incubation times for the provision of energy prior to exogenous feeding (Sargent 1995). Polar lipids, however, are considered to be more important for cellular structure than energy. In the form of phospholipids, polar lipids are essential for biomembrane formation because of their hydrophilic head group and two hydrophobic fatty acid tails.

Recent evidence gathered on a wider range of marine teleost eggs and larvae has led researchers to reconsider the role of neutral and polar lipids. For example, halibut

eggs undergo a lengthy developmental period, yet have more than twice the percentage of phospholipids to neutral lipids (Falk Petersen et al. 1986; Falk-Petersen et al. 1989). Similarly, cod (*Gadus morhua*) have been shown to have nearly 10 times the amount of phospholipids to neutral lipids during egg and early larval stages (Fraser et al. 1988). These studies suggest that phospholipids may have a dual function in both energy production and biomembrane formation . Therefore it would appear difficult to predict lipid profiles of previously undescribed species of marine teleost eggs and larvae.

Conversely, the fatty acid signatures show a fairly consistent trend among marine teleost eggs and larvae. Polyunsaturated fatty acids (PUFA) comprise a high portion of the fatty acids present in both the phospholipids and the neutral lipids, particularly the (n-3) PUFAs 22:6(n-3) (DHA) and 20:5n-3 (EPA). Together these (n-3) PUFAs both serve in the development of neural cell membranes as well as provide energy for developing marine fish larvae (Sargent 1995).

Nevertheless, speculation into lipid and fatty acid compositions of undescribed species should be conducted with caution. Despite the tremendous amount of lipid data gathered for marine teleosts within the last two decades, research has focused almost entirely on species with an oviparous mode of reproduction. Few, if any, studies have collected lipid and fatty acid data on developing larvae in ovoviviparous species i.e. species in which fertilization and embryonic development is internal. Energetic studies within some ovoviviparous species have shown that maternal contributions to the embryo can continue after oogenesis (MacFarlane et al. 1993; Boehlert et al. 1986; Boehlert et al. 1984). As a consequence we may expect to find lipid and fatty acid consumption patterns

to be vastly different in species employing an ovoviviparous reproductive strategy.

Redfish (*Sebastes* spp.) are ovoviviparous teleosts and are an important commercial groundfish species in the North Atlantic. *S. mentella* (deep-water redfish) and *S. fasciatus* (Acadian redfish) are the most commonly exploited species in deep water (>400 m) off the Newfoundland coast, yet very little is known about the early life history of these fish. The aim of this study is to gather baseline data and describe changes in lipid and fatty acid composition of pre-extruded larval redfish for the first time. Comparisons between other oviparous marine teleosts are conducted in an effort to further elucidate 1) the roles of specific lipids and fatty acids in development and 2) the maternal-fetal relationships associated with ovoviviparity.

2.2 Materials and Methods:

2.2.1 Larval Collection

Redfish larvae were gathered from adult females collected from bottom trawl surveys aboard a DFO research vessel off the south coast of Newfoundland in NAFO area 3N. Adult females were identified as either *S. fasciatus* or *S. mentella* using extrinsic gas-bladder musculature as described by Ni (1981). Of the 12 female redfish collected, two were identified as *S. fasciatus* and 10 were identified as *S. mentella*. Larvae or eggs from each female were extruded onto foil sheets and separated into one of five developmental stages based on morphometric and pigmentation characteristics using a dissecting scope and digital calipers (See Table 2.1 for developmental definitions). Although the presence of two developmental stages was commonly present within a brood, only one was selected to represent each female. Serum and blood were gently

Table 2.1: Description of developing pre-extruded redfish stages based on eye diameter, total length and pigmentation patterns.

Developmental Stage	Definition
D1	Larvae are unhatched (egg diameter ~1.6 mm) but eyes are clearly visible through the chorion.
D2	5.0-5.5 mm larvae with large yolk sacs. Eyes are 0.29-0.31 mm in diameter and no pigmentation is visible along the body.
D3	6.0-6.5 mm larvae with large yolk sacs. Eyes are larger than D2 larvae (0.38-0.42 mm) but pigmentation is still absent along the body.
D4	6.5-6.8 mm larvae with noticeably reduced yolk sacs from D2 and D3 stages. Pigmentation is present along the dorsolateral surface of the gut and melanophores are punctate in shape. Eyes are 0.5-0.51 mm in diameter.
D5	6.8-7.2 mm larvae with vestiges of yolk sacs remaining. Pigmentation is present along the dorsolateral surface of the gut as well as dorsally over the brain. Further pigmentation is seen along the dorso- and ventro-midlines. Melanophores are either stellate or branched in shape, often connected within postanal areas. Eyes are slightly larger (0.52-0.53 mm) than larvae in D4.

rinsed from the larvae or eggs using filtered seawater before being placed into 25 cl vials containing chloroform. Sample vials contained ten larvae or eggs from an individual female and in no instances were females used for multiple sampling. However, it should be noted that only one sample was gathered for both developmental stages 1 and 5. Vials were stored in chloroform at –20° C until lipid and fatty acid analysis.

Dry weights were calculated from samples of redfish larvae preserved in ethanol collected on a previous sampling trip. Larvae were placed on pre-weighed foils and placed in a drying oven for a period of 24 h at 65° C. Ten larvae were used for each foil and a total of five foils were measured for each developmental stage. Foils were weighed to the nearest 0.0001 mg using an electronic microbalance.

2.2.2 Lipid/Fatty Acid Analysis

The extraction of lipids was carried out using the methods described by Folch et al. (1957) by homogenizing samples in a 20 vol of chloroform:methanol (2:1) and rinsing with water. Individual classes of lipids were quantified in triplicate by thin layer chromatography with flame ionization detection (TLC/FID) using silica gel covered quartz rods (Chromarods-SIII) and a MARK V Iatroscan (Parrish 1987). Samples (0.5 μg) were spotted on rods and developed twice in hexane-diethyl ether-formic acid (99:0.1:0.05) for 20 and 25 min respectively, followed again by the same solvent at a ratio of 79:20:1 for 40 min. Finally, samples were developed twice in a 100 % acetone solvent for a 12 min and then 10 min period. Chromatograms were combined and peak areas were calculated using T-data scan software (RSS Inc., CA, USA). Peaks of sample chromatograms were identified using chromatograms of TLC/FID run standards to give

absolute amounts of each lipid class. Total lipid of each sample was then determined by summing the lipid classes and correlating these with gravimetric lipid weights (Daniel et al. 1993).

Fatty acid methyl esters (FAME) were quantified on a Varian 3400 GC equipped with an Autoinjector. The GC was fitted with an Omegawax 320 column (30 m, 0.32 mm i.d., 0.25 μm film thickness; Supelco, Inc.) with hydrogen used as a carrier gas. Each FAME was prepared from ~ 1 mg of the total lipids using the catalyst BF_3/Methanol. This procedure follows the method of the American Oil Chemists' Society (Ce 1b-89) with exception that hexane replaced iso-octane. The GC oven was initially set for 65° C for 0.5 min ramping to 195° C at 40° C min^{-1} and held there for 15 min. Final oven temperatures reached 220° C at a rate of 2° C min^{-1} and were held for 0.75 min. Individual FAME were derived using Varian Star Integrator software (Supelco standard).

2.3 Results:

2.3.1 Lipid Classes

Dry weight decreased during embryogenesis although this was most pronounced between D4 and D5 (Fig. 2.1). The total lipid content during the late egg stage was ~18 % of the dry weight and fell to just under 16 % at the latest larval developmental stage. Initially, TAG had the highest presence within the lipid classes at 58 % of the total lipid composition and 10.6 % of the dry weight (Table 2.2, 2.3). Throughout development TAG was catabolized and phospholipids (PL) became more highly represented (Fig 2.2). Absolute amounts of PL, however, remained constant throughout development as amounts of total lipid and TAG reserves depleted (Table 2.4; Fig. 2.3). In the last

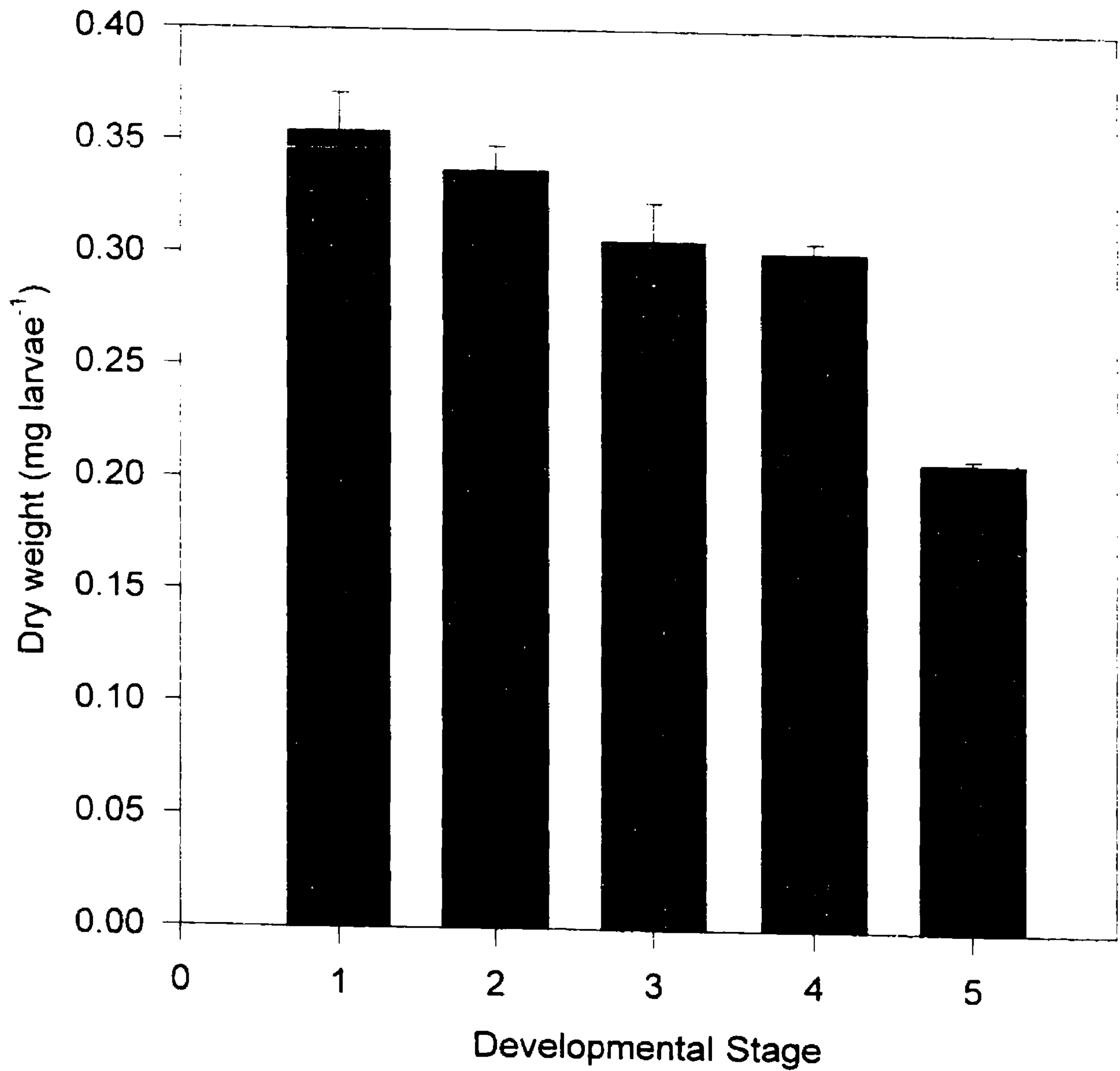


Fig. 2.1: Changes in dry weight (DW) throughout the development of pre-extruded redfish larvae. Values are means (± 2 SE) of five sample comprised of ten pooled larvae.

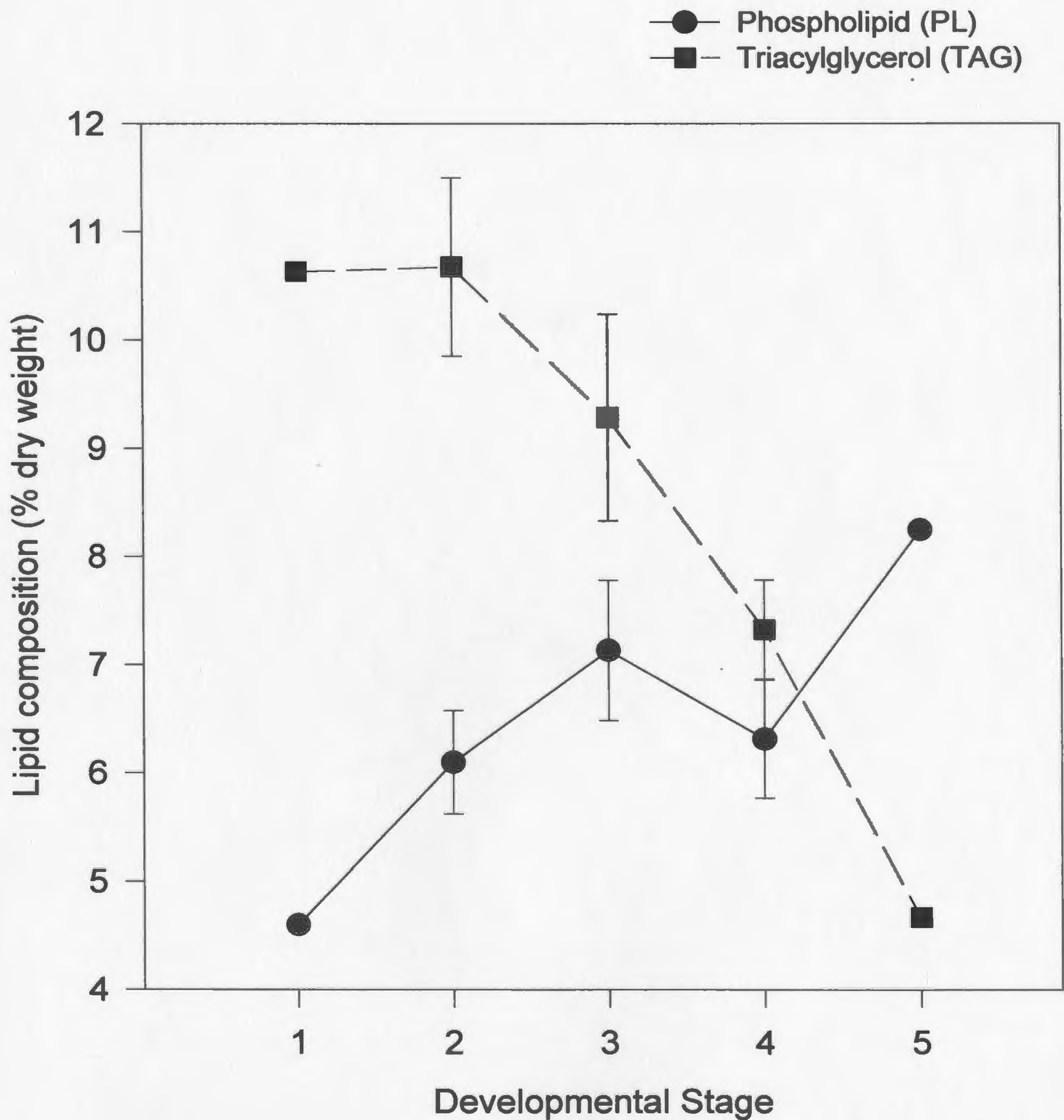


Fig. 2.2: Changes in the average percent dry weight composition of triacylglycerol (TAG) and phospholipid (PL) througout the development of pre-extruded redfish larvae. Data presented as mean (± 1 SE) of 1-5 samples of 10 pooled eggs or larvae.

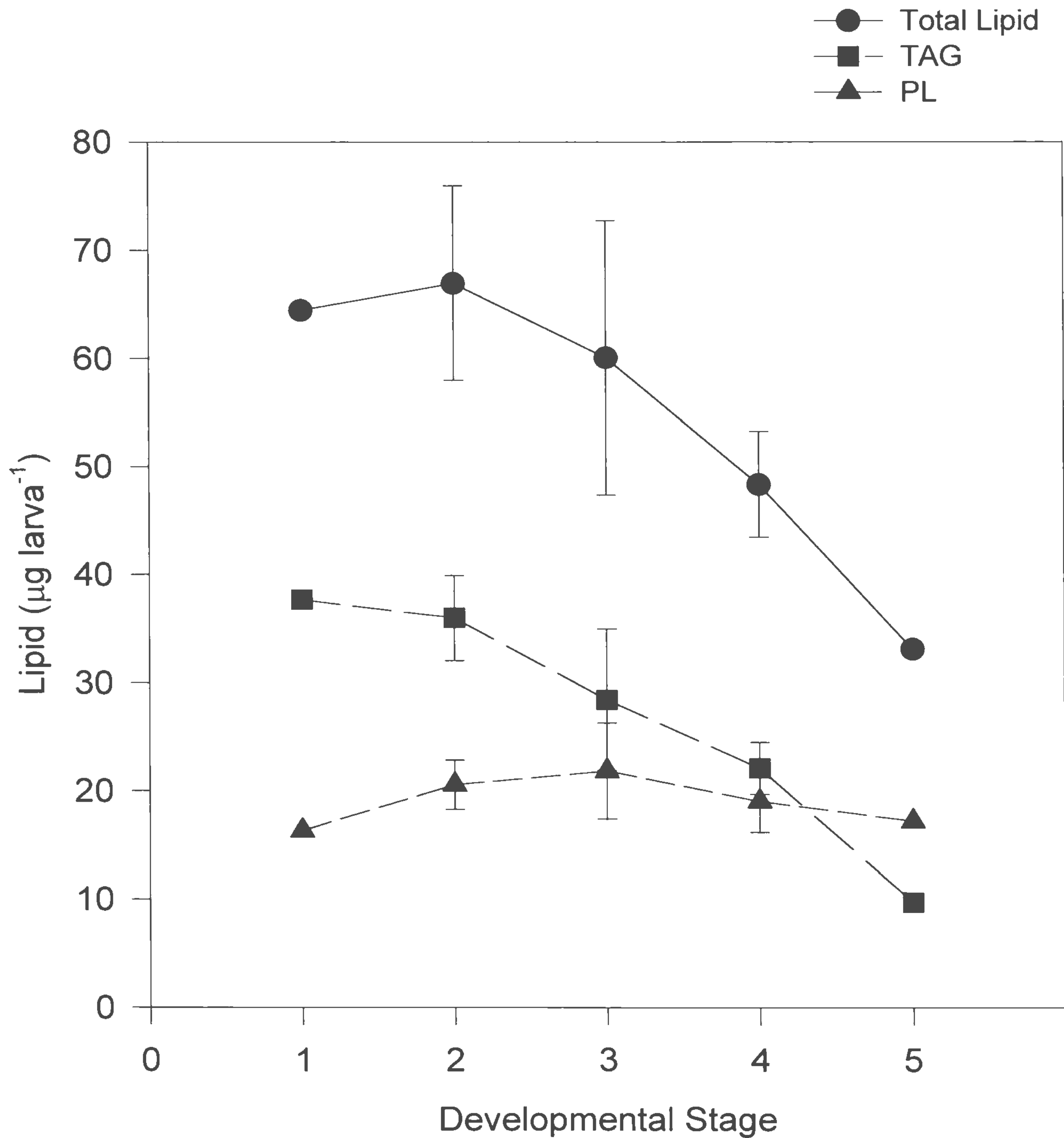


Fig 2.3: Changes in absolute amounts of total lipid, triacyglycerol (TAG) and phospholipids (PL) throughout the development of pre-extruded redfish larvae. Data presented as mean (± 1 SE) of 1-5 samples of 10 pooled eggs or larvae.

Table 2.2: Lipid composition of redfish larvae[a] throughout pre-extruded development expressed as a percentage of dry weight (DW) ± SD. Values based on samples (n) of 10 pooled eggs or pre-extruded larvae.

HC:hydrocarbons; SE:sterol esters; KET:ketones; TAG:triacylglycerols; FFA:free fatty acids; ALC:alcohols; ST:sterols; DAG:diacylglycerols AMPL:acetone-mobile polar lipids; PL:phospholipids

Stage		Total % DW	HC % DW	SE % DW	KET % DW	TAG % DW	FFA % DW	ALC % DW	ST % DW	DAG % DW	AMPL % DW	PL % DW
1	$\bar{x}$	18.192	0.051	1.410	0.000	10.631	0.173	0.000	0.489	0.253	0.597	4.599
(n=1)	SD	na	na	na	na	na	na	na	na	na	na	na
2	$\bar{x}$	19.857	0.117	1.333	0.000	10.676	0.147	0.031	0.696	0.188	0.574	6.098
(n=2)	SD	± 2.665	± 0.045	± 0.134	± 0.000	± 1.165	± 0.096	± 0.043	± 0.221	± 0.064	± 0.397	± 0.675
3	$\bar{x}$	19.615	0.109	1.186	0.000	9.285	0.094	0.021	0.986	0.221	0.582	7.134
(n=5)	SD	± 4.138	± 0.055	± 0.250	± 0.000	± 2.135	± 0.025	± 0.047	± 0.286	± 0.083	± 0.238	± 1.454
4	$\bar{x}$	16.042	0.138	0.783	0.000	7.325	0.120	0.014	0.920	0.130	0.472	6.312
(n=3)	SD	± 1.628	± 0.023	± 0.060	± 0.000	± 0.795	± 0.079	± 0.025	± 0.105	± 0.057	± 0.116	± 0.943
5	$\bar{x}$	15.863	0.105	0.784	0.124	4.667	0.167	0.000	1.225	0.159	0.389	8.247
(n=1)	SD	na	na	na	na	na	na	na	na	na	na	na

[a] Development stage 1 is an egg stage.

Table 2.3: Lipid composition of redfish larvae [a] throughout pre-extruded development expressed as a percentage of total lipid ± SD. Values based on samples (n) of 10 pooled eggs or pre-extruded larvae.

HC:hydrocarbons; SE:sterol esters; KET:ketones; TAG:triacylglycerols; FFA:free fatty acids; ALC:alcohols; ST:sterols; DAG:diacylglycerols AMPL:acetone-mobile polar lipids; PL:phospholipids

Stage		Total μg/larva	%HC	%SE	%KET	%TAG	%FFA	%ALC	%ST	%DAG	%AMPL	%PL
1	$\bar{x}$	64.46	0.28	7.75	0.00	58.44	0.95	0.00	2.69	1.39	3.28	25.28
(n=1)	SD	na	na	na	na	na	na	na	na	na	na	na
2	$\bar{x}$	66.97	0.61	6.73	0.00	53.86	0.72	0.17	3.46	0.93	2.78	30.76
(n=2)	SD	± 8.99	± 0.31	± 0.23	± 0.00	± 1.37	± 0.39	± 0.24	± 0.65	± 0.20	± 1.63	± 0.73
3	$\bar{x}$	60.08	0.53	6.06	0.00	47.27	0.52	0.08	4.99	1.23	2.90	36.43
(n=5)	SD	± 12.68	± 0.16	± 0.60	± 0.00	± 2.36	± 0.24	± 0.19	± 0.91	± 0.65	± 0.94	± 1.48
4	$\bar{x}$	48.38	0.86	4.90	0.00	45.70	0.72	0.10	5.74	0.81	2.96	39.21
(n=3)	SD	± 4.91	± 0.06	± 0.35	± 0.00	± 2.62	± 0.45	± 0.17	± 0.49	± 0.34	± 0.73	± 2.10
5	$\bar{x}$	33.11	0.66	4.94	0.78	29.42	1.05	0.00	7.72	1.00	2.45	51.99
(n=1)	SD	na	na	na	na	na	na	na	na	na	na	na

[a] Development stage 1 is an egg stage.

Table 2.4: Lipid composition of redfish larvae throughout pre-extruded development expressed as μg/larva [a] ± SD. Values based on samples (n) of 10 pooled eggs or pre-extruded larvae.

HC:hydrocarbons; SE:sterol esters; KET:ketones; TAG:triacylglycerols; FFA:free fatty acids; ALC:alcohols; ST:sterols; DAG:diacylglycerols AMPL:acetone-mobile polar lipids; PL:phospholipids

Stage		Total μg/larva	HC μg/larva	SE μg/larva	KET μg/larva	TAG μg/larva	FFA μg/larva	ALC μg/larva	ST μg/larva	DAG μg/larva	AMPL μg/larva	PL μg/larva
1	$\bar{x}$	64.460	0.181	4.996	0.000	37.670	0.612	0.000	1.734	0.896	2.114	16.296
(n=1)	SD	na	na	na	na	na	na	na	na	na	na	na
2	$\bar{x}$	66.965	0.395	4.497	0.000	36.003	0.497	0.103	2.3460	0.632	1.935	20.563
(n=2)	SD	± 8.987	± 0.153	± 0.453	± 0.000	± 3.927	± 0.325	± 0.146	± 0.747	± 0.216	± 1.339	± 2.276
3	$\bar{x}$	60.080	0.333	3.634	0.000	28.439	0.289	0.065	3.019	0.678	1.782	21.850
(n=5)	SD	± 12.676	± 0.167	± 0.765	± 0.000	± 6.539	± 0.078	± 0.145	± 0.877	± 0.255	± 0.728	± 4.454
4	$\bar{x}$	48.377	0.416	2.361	0.000	22.090	0.361	0.043	2.774	0.391	1.423	19.035
(n=3)	SD	± 4.908	± 0.068	± 0.181	± 0.000	± 2.399	± 0.239	± 0.074	± 0.317	± 0.173	± 0.349	± 2.846
5	$\bar{x}$	33.11	0.219	1.636	0.258	9.741	0.348	0.000	2.556	0.331	0.811	17.214
(n=1)	SD	na	na	na	na	na	na	na	na	na	na	na

[a] Development stage 1 is a pre-hatch stage so numbers are reported as μg/egg rather than μg/larva

developmental stage, PL represented 52 % of the total lipids and accounted for 8.3 % of the dry weight whereas TAG showed a 50 % reduction from D1.

Like TAG, sterol esters (SE) also became less represented as the larvae developed. SE accounted for 7.8 % of the total lipids at D1 and later fell to 5 % at D5 (Table 2.2). At the same time sterols (ST) increased in percentage of total lipid from 2.7 % at D1 to 7.7 % at D5 (Fig 2.4, Table 2.2).

2.3.2 Fatty Acids

The predominant fatty acids in all the developmental stages of pre-extruded redfish were 16:0, 16:1n-7, 18:1n-9, 20:1n-9, 20:5n-3 and 22:6n-3 (Table 2.5). Of these fatty acids, hexadecanoic acid (16:0) was the most highly represented saturated acid while *cis*-9-octadecenoic acid (18:1 n-9) and docosahexanoic acid (22:6n-3) were the major monounsaturated and polyunsaturated fatty acids respectively.

Slight changes appeared in the fatty acid composition of larvae throughout development. Total PUFA increased ~ 4.6 % after D1 and remained elevated throughout D2-D5. Total monounsaturates declined throughout development from 38.2 % to 31.8 %, this trend being most supported by the decline of 18:1n-9 from 16.7 % to 13.3 %. Increases in both 18:0 and 16:0 resulted in an overall increase of total saturates from 17.7 % in D1 to 23.3 % in D5.

2.4 Discussion:

There is a general decrease in lipid as a percentage of dry weight during development, although this decrease is not evident from the transition of larvae from D1

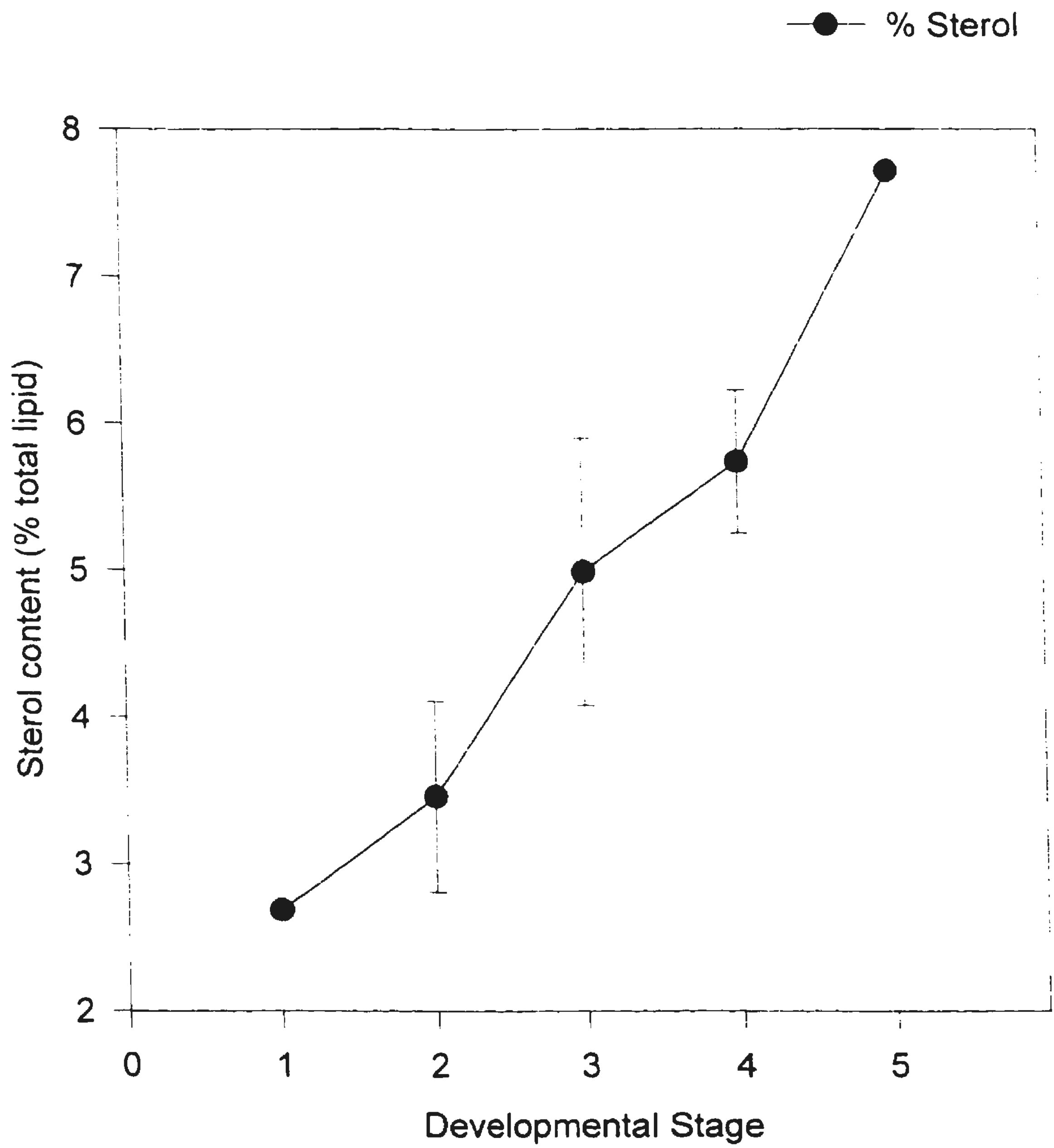


Fig 2.4: Changes in total percent lipid composition of sterols (ST) throughout the development of pre-extruded redfish larvae. Data presented as mean (± 1 SE) of 1-5 samples of 10 pooled eggs or larvae.

Table 2.5: Fatty acid composition of total lipids throughout development of pre-extruded redfish larvae. Data are expressed as a mean percentage of total fatty acid composition ± SD. Values based on samples (n) of 10 pooled eggs or pre-extruded larvae.

Fatty Acid	**DEV I (n=1)**	**DEV II (n=2)**	**DEV III (n=5)**	**DEV IV (n=3)**	**DEV V (n=1)**
14:0	2.4	2.5 ± 0.3	2.5 ± 0.6	2.8 ± 0.8	2.3
16:0	12.3	13.6 ± 0.2	15.8 ± 2.2	13.6 ± 0.8	17.1
18:0	2.9	3.4 ± 0.2	3.7 ± 0.3	3.4 ± 0.2	3.9
Total saturates	17.7	19.5 ± 0.3	22.1 ± 2.4	19.7 ± 1.6	23.3
16:1n-7	8.9	8.1 ± 0.5	7.8 ± 0.5	8.9 ± 0.8	8.9
18:1n-5	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
18:1n-7	4.3	4.6 ± 0.1	4.3 ± 0.4	4.8 ± 0.1	4.9
18:1n-9	16.7	15.0 ± 0.5	13.9 ± 2.8	15.1 ± 1.9	13.3
20:1n-7	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
20:1n-9	6.8	6.0 ± 0.3	5.3 ± 0.9	6.9 ± 1.6	4.7
22:1n-11	1.6	1.1 ± < 0.1	< 1.0	< 1.0	< 1.0
Total monounsaturates	38.2	34.7 ± 0.4	32.9 ± 2.9	35.6 ± 3.3	31.8
16:2	< 1.0	1.1 ± < 0.1	0.7 ± 0.7	< 1.0	1.2
18:2n-6	1.3	1.4 ± < 0.1	1.3 ± 0.2	1.3 ± < 0.1	1.2
20:4n-6	< 1.0	< 1.0	< 1.0	< 1.0	1.1
20:5n-3	11.0	11.8 ± 0.7	11.8 ± 1.2	11.9 ± 0.9	11.7
22:5n-3	1.4	1.3 ± < 0.1	1.0 ± 0.6	1.3 ± 0.2	1.2
22:6n-3	17.6	20.4 ± 1.4	19.7 ± 2.3	18.3 ± 2.0	19.9
Total n-3	30.0	33.43 ± 0.7	32.5 ± 3.5	31.41 ± 2.7	32.8
Total PUFA	31.3	35.9 ± 0.7	34.7 ± 4.0	33.1 ± 2.2	36.2

to D2. Tocher et al. (1985) also noticed fluctuations in lipid content/dry weight of herring larvae throughout development, especially after hatch in which there existed a two-fold increase of % lipid content. This was attributed to the loss of the chorion, the outermost layer of the egg which is known to comprise a significant portion of the dry weight in oviparous fish. Similar findings have also been attributed to chorion loss for a lipid investigation in Atlantic cod, another oviparous fish species (Fraser et al. 1988). Although the trend is still apparent within developing redfish despite being ovoviviparous, the percent lipid gain is marginal (~9 % relative gain) compared with the 100 % gain reported for herring. Likely this is due to a thinner chorion present in developing redfish eggs. Protection from predators and the physical environment is provided by the mother in the genus *Sebastes*, and it has been suggested that less energy is needed towards developing a protective chorion (Boehlert et al. 1984).

From the late egg stage (D1) to the latest pre-extruded stage (D5), there was nearly a 50 % reduction in total lipid mass from a high initial total lipid concentration of ~ 19 % DW. Fraser et al. (1988) found a 19 % reduction of total lipid in Atlantic cod whereas red drum were shown to have a 30 % loss during development (Vetter et al. 1983), each having an initial concentration of ~ 9.9 % and 2.4 % respectively. These data demonstrate the heavy reliance that developing redfish larvae have on available lipid reserves, and may indicate lesser use of other possible energy reserves such as carbohydrates, free amino acids, protein or glycogen for catabolism.

The increase in phospholipids suggests that this lipid class is not a source of metabolic energy during egg and larval development of redfish. Phosphatidylcholine

(PC), a major phospholipid within other cold-water marine fish larvae, is suggested to be a primary source of energy for developing cod larvae (Fraser et al. 1988; Rainuzzo et al. 1992), halibut (Falk-Petersen et al. 1986), and plaice and herring (Tocher et al. 1985). Freshwater and anandromous fish such as African catfish (Verreth et al. 1994) and Atlantic salmon (Cowey et al. 1985) have also been shown to catabolize significant amounts of PC during egg and early larval development. Although individual phospholipid concentrations were not determined in this study, the increase in relative amounts of phospholipid classes through development suggests that these lipids were not primary sources of energy for redfish larvae. Instead, the steady decrease of TAG from D1 to D6 makes this lipid class a more likely candidate for an energy source.

The use of TAG as an energy reserve during larval development is well documented (e.g. Cowey et al. 1985; Fraser et al. 1987), but the degree of TAG catabolism varies among species. In general, species with long developmental periods (e.g. 20 weeks) have higher TAG reserves than species with shorter developmental periods e.g. 20 days (Sargent 1995). No published data are available on gestation times for *S. mentella* or *S. fasciatus*, but gestation times of other *Sebastes* spp. have been documented to be between 1-2 months (Boehlert and Yoklavich 1984; Yamada and Kusakari 1991). Higher TAG compositions have also been documented in fish larvae possessing an oil globule. Redfish larvae have a discrete oil globule within the lipoprotein yolk sac, and it has been shown that these oil globules are rich in neutral lipids such as sterol/wax esters and TAG (Wiegand 1996).

The fatty acid composition of redfish larvae appears to be consistent with other marine fish species. Docosahexanoic acid (DHA-22:6n-3) and eicosapentanoic acid (EPA-20:5n-3) have long been recognized as important essential fatty acids in larval fish development. Both DHA and EPA are found in high amounts in phospholipids as well as the neutral lipids, so they have a dual function in both metabolism and biomembrane formation. Throughout redfish development, DHA and EPA levels roughly follow a 2:1 ratio typical of other marine fish species such as Atlantic cod (Fraser et al. 1988; Uvlund et al. 1988) striped bass (Harrell 1995) and Atlantic herring (Tocher and Sargent 1984).

The use of TAG as opposed to PL classes such as phosphatidylcholine for metabolism is also reflected in the fatty acid data. Triacylglycerol is typically higher in monounsaturates than phospholipids while n-3 PUFAs such as DHA and EPA are generally proportionally higher in phospholipids (Wiegard 1996). Both DHA and EPA percentages of total fatty acid are conserved through the developmental stages while it would appear that the predominant monounsaturated species, 18:1n-9 and 20:1n-9, were catabolized. This can be seen in the total monounsaturates as they decrease from 38.2 % in D1 to 31.8 % in D5 while at the same time total n-3 PUFAs remain fairly steady at ~ 32 % through development and may actually be increasing as a percentage of total fatty acids. Palmitic acid, 16:0, a saturated acid found in high amounts both in TAG as well as the phospholipid class phosphatidylcholine (Bell 1989), also increases throughout larval redfish development. Thus it would appear that TAG is providing an energetic substrate for metabolism as well as the essential fatty acids needed for the synthesis of certain phospholipid classes.

The increase in phospholipids throughout larval development often occurs in marine larvae as phospholipids become incorporated in the larval body (Tocher et al. 1985). In a study by Kaitaranta et al. (1981) the proportion of polar lipids (phospholipids) increased throughout roe maturation in Baltic herring (*Clupea harrengus*) and rainbow trout (*Salmo gairdneri*). A similar trend is reported for turbot (*Scophthalmus maximus*) although only two developmental periods were compared i.e. hatch and first-feeding (Rainuzzo et al. 1992). Phospholipids are important components of biomembranes and certain classes such as phosphatidylethanolamine (PE) and phosphatidylserine (PS) are particularly abundant in neural tissues such as the brain and retina. Analysis of these tissues in cod (Tocher and Harvie 1988; Bell and Dick 1991), rainbow trout (Bell and Tocher 1989; Tocher and Harvie 1988), and herring (Bell and Dick 1993) have shown these phospholipids classes to be present in high amounts along with high concentrations of DHA. The amounts of DHA in these phospholipids were also shown to increase in the development of herring as rods within the eye appeared (Bell and Dick 1993). Redfish eyes grew more than 75 % in diameter from D2 to D5 and were typically greater than 0.6 mm in diameter at extrusion. Ratios of eye diameter to standard length of redfish larvae are calculated to be ~ 10.6 % based upon morphometric data collected by Penney (1987). This is large relative to other North Atlantic groundfish species such as cod (*Gadus morhua*) and yellowtail flounder (*Pleuronectes ferrugnineus*) where ratios are 6.7 % and 6.4 % respectively at hatch (Puvanendran unpub. data). Although morphometric data for Atlantic halibut larvae (*Hippoglossus hippoglossus*) is scarce, eyes of this species at hatch has been

characterized as nonfunctional (Haug 1990). These data suggest that redfish have high demands for phospholipids as structural components in biomembranes throughout development. Smaller and perhaps less developed eyes in Atlantic halibut and cod, on the other hand, may allow for increased metabolism of structural phospholipids, namely phosphatidylcholine, during embryogenesis in these species.

An increase in sterols (ST) is correlated with an increase in body size and developmental stage of redfish larvae. This is not surprising considering the role of ST as a structural class of lipids. Like certain phospholipid classes, ST are incorporated into biomembranes and typically correlated with larval size and mass (Fraser 1989). As a result ST have been used to account for size dependency when using lipid content to evaluate larval condition in marine fish (e.g. Fraser 1988, 1989; Hakanson 1993).

Because redfish are ovoviviparous, caution should be exercised when speculating into the sources of essential fatty acids during embryogenesis. Unlike oviparous eggs which must draw upon a finite source of energy provided during oogenesis, ovoviviparous larvae may be supplied with additional nutrients from maternal serum during gestation. Termed matrotrophic viviparity (Wourms 1981), several ovoviviparous fish species previously thought to provide no additional embryonic nutrition (i.e. lecithotrophic viviparity) have recently been found to do otherwise. Among these have been several species from the genus *Sebastes* (Boehlert and Yoklavich 1984; Boehlert et al. 1986; Dygert and Gunderson 1991; MacFarlane et al. 1993). Although confirmation of matrotrophy in redfish is beyond the scope of this study, it is interesting to note that increased levels of phospholipids have been found in the serum of another *Sebastes*

species, *S. flavidus*, during gestation (MacFarlane et al. 1993). Because these levels of phospholipids in female serum were higher relative to males during the same period, the authors suggest that *S. flavidus* may be matrotrophic. Therefore, increases in phospholipids of redfish larvae during gestation may be the result of maternal contribution rather than synthesis from yolk reserves.

Two of the lipid samples used in this study (1-D3 and 1-D4 stage) were collected from *S. fasciatus* females, but these data were pooled with samples gathered from *S. mentella*. These two species co-occur and share spawning grounds between April and July of each year in the North Atlantic. Externally, beaked redfish are nearly identical to each other although a method using extrinsic gasbladder musculature has been developed to distinguish between these species (Ni 1981). Larvae of the two species are also extremely similar, although subtle differences in morphology and pigmentation between late stage (D5) larvae of *S. mentella* and *S. fasciatus* have been noted (Penney 1985). In addition to being morphologically similar, the early foraging and swimming behaviour of the two species of larvae was shown to be identical (See Chapter 3). The integrity of these two species is thought to be maintained by temporal mismatches in copulation processes in the fall (St-Pierre and de Lafontaine 1995), and the degree of hybridization, if any, remains unknown. While variation was noted in absolute amounts of total lipid in D3, both the high and low outliers were collected from the same species i.e. *S. mentella*. It should also be noted that these outliers resulted in little variance; standard deviations in lipid class and fatty acid composition rarely exceeded 5 % which in turn increases the reliability of the D1 and D5 stages where only one sample was analyzed. Therefore,

while it's recognized that the statistical power of a large sample size of both *S. mentella* and *S. fasciatus* may allow for the qualitative separation of these species, combining both species in this study should not misrepresent overall trends in lipid and fatty acid composition throughout larval development.

The variation in amounts of total lipids between larval samples within D3 is interesting. This may be an indicator of larval dry weight differences from different females assuming higher lipid contents are associated with larger larvae for each developmental stage. Unfortunately, larval dry weights were calculated from preserved samples of larvae from females not used in the study, so individual differences between samples could not be determined.

Nevertheless, variation in total lipid reserves suggests quality differences which could have subsequent effects on larval survival after parturition. Redfish in the North Atlantic are noted for their high degree of interannual variability in recruitment, evidenced by changes in larval abundance (Anderson 1994) and year-class strength (e.g. Lilly 1987). In fact, interannual variation is characteristic of *Sebastes* species in the Pacific as well, suggesting that recruitment mechanisms may be more proximately influenced by the biology of these species rather than the environment. Support for this is provided by Moser and Boehlert (1991) who purport that qualitative differences in larvae could be the result of *Sebastes* species with embryos that depend on additional supplies of nutrients during development. They suggest that in years with little food availability for adult females energy reserves may be conserved rather than used for embryonic development. This could subsequently result in poor year classes of fish, assuming larvae

poorly provisioned in endogenous resources are more susceptible to mortality e.g. due to starvation and/or size-dependent predation. Although there is substantial variation in total lipid reserves in larval redfish, the influence on the survival or developmental rate, either pre- or post-parturition, remains undetermined.

CHAPTER 3

BEHAVIOUR, GROWTH AND SURVIVAL OF REDFISH LARVAE IN RELATION TO PREY AVAILABILITY

3.1 Introduction:

Fisheries biologists hypothesize that the survival success of marine fish is largely determined at the larval stage, primarily through the effects of starvation (e.g. Cushing 1975) or predation (e.g. Hunter 1981; Bailey and Houde 1987). These two components of mortality are not necessarily exclusive; starved larvae are considered to be more vulnerable to predators because reduced growth rates prolong exposure to size-selective predation (Smith 1985; Gamble and Hay 1989). Successful feeding of fish larvae, therefore, should increase survival directly by reducing starvation as well as indirectly by decreasing risk to predation.

The availability of preferred prey is known to affect ingestion and growth rates of larval fish in the laboratory (e.g. Houde and Schekter 1980; Munk and Kiørboe 1985) as well as the field (Anderson 1994). The temporal overlap of larval fish and their preferred prey is subsequently thought to affect year class strength. Termed 'match/mismatch' by Cushing (1972, 1990), the hypothesis assumes that fish spawn at a fixed time along with seasonal peaks in plankton blooms. Years with synchronized release of larvae and prey ('match') should result in higher larval growth and survival than unsynchronized years ('mismatch').

Redfish (*Sebastes* spp.) are likely sensitive to a match or mismatch in prey

because they meet the assumptions of the Cushing's hypothesis. Larval redfish have a strong preference for *Calanus* copepods (Anderson 1994; Runge and de Lafontaine 1996), a prey species which has been shown to have a variable yearly and seasonal release (Anderson 1990). In contrast, redfish spawn intensively for a 1-2 week period in late April and early May with little variability between years (Anderson 1984; Penny and Evans 1985).

Although redfish larvae are likely susceptible to matches and mismatches of prey in the spring, the response to these changes in prey availability is for the most part unknown. In a field study by Anderson (1994), larval redfish were found to be in poorer condition in a year with lower concentrations of preferred prey. While field studies are valuable in our understanding of larval foraging ecology, they are unable to investigate the underlying dynamics of foraging behaviour. Field studies are also unable to account for small-scale prey patchiness and may misrepresent the prey environment by missing smaller plankton in coarse mesh sampling nets (Frank and Leggett 1986).

To date, there have been no successful attempts on conducting experiments with redfish larvae in the laboratory. Likely this is due to the difficulty of acquiring larvae for experimentation; redfish are ovoviviparous and are found in deep water (>300 m) during spring spawning (Ni and McKone 1983). Penny and Evans (1985) attempted to rear redfish larvae in the laboratory after collecting ripe female redfish at sea but were unable to achieve substantial larval growth and survival. In their work, they used *Artemia* nauplii, a prey which may have been too large for the redfish larvae to ingest. *Artemia* nauplii at hatch average >400 μm in length, yet gut content analysis from wild caught

larvae shows redfish prefer to feed on prey items ~150 μm in width, usually in the form of copepod eggs (Runge and de Lafontaine 1996).

Therefore, the objectives of this study are to measure larval redfish growth, behaviour, and survival under controlled laboratory conditions. Specifically, I address the questions of how prey availability (1) influences growth and survival and (2) shapes patterns of foraging behaviour of redfish larvae. The ecological significance of these findings is discussed through comparisons with other Atlantic species.

3.2 Materials and Methods:

3.2.1 Larval Collection

The spawning of beaked redfish, *Sebastes mentella* and *S. fasciatus*, overlaps temporally and spatially off the Newfoundland coast although *S. fasciatus* tends to spawn later (Anderson 1984). The larvae of these two species are nearly identical to each other morphologically at extrusion although slight differences in pigmentation patterns appear to be distinct enough to allow for separation (Penny 1987).

Adult redfish were collected from bottom trawl surveys aboard a DFO research vessel off the Newfoundland coast in NAFO area 3N. Larvae were extruded from ripe females into 10 l plastic containers filled with seawater at ambient surface temperature. A subsample of 10 larvae was removed and measured for total length with digital calipers. Batches of larvae from females were mixed and transferred to large insulated holding containers with slight aeration if subsamples were viable (>6.5 mm total length). Penny and Evans (1985) estimated the total length of redfish larvae at time of extrusion to average 6.0 to 8.9 mm with some variance between years. A total of five batches of

larvae were gathered in this manner: three were extruded from *S. mentella* females and two from *S. fasciatus*. Larvae were kept on board for a maximum of 36 hr before being transferred to onshore facilities for experimentation.

3.2.2 Experimental Design

Larvae were stocked at ~1 larvae L^{-1} in 300 l conical, upwelling containers with 24 h illumination (750 lux) and constant slight aeration. Tanks were provided with filtered seawater at a rate of 1 L min^{-1}. Four prey density treatments were set-up: 4500, 1500, 500 and 0 prey L^{-1}. These prey levels were chosen because they fell within ranges found acceptable for other Atlantic species reared in the laboratory (Puvanendran and Brown 1998). Limited numbers of larvae prevented the 0 prey L^{-1} treatment from being replicated, however all other prey treatments were conducted with two tank replicates. Temperatures were maintained between 10-12° C using heated sea-water provided by a counter-current exchange.

Two strains of the rotifer *Brachionus plicatilis* were used at different times during the 35 d experiment. The smaller strain (~180 x 140 µm) was used exclusively for the first two weeks, with the larger strain (~300 x 200 µm) introduced at a 1:1 ratio on day 17. Larvae were switched exclusively to the larger strain of rotifer on day 20 for the duration of the experiment. An even mixture of microalgae (*Nannochloropsis gaditana* and *Isochrysis* spp.) was also supplied to containers three times a day.

Rotifer densities were maintained by taking four 5 ml aliquot samples three times a day (two within 5 cm of the surface and two taken in the middle of the water column):

prey were added during these times as needed to maintain densities. Mean densities never fluctuated more than 25 % of nominal densities during the experiment.

Standard length (SL), myotome height (MH), presence of prey in the gut and degree of metamorphosis were measured every five days in a non-lethal manner. Five fish were sampled from each tank and placed within a beaker of seawater on ice. Larvae were placed on a petri dish on ice under a stereomicroscope with an ocular micrometer. After measurements the larva was returned to a separate beaker for acclimation within its original tank. Periodic observations of released fish showed this to be an effective non-lethal means of measuring larvae.

As larvae metamorphose into juvenile fish they undergo a series of morphometric and physiological changes. The degree of metamorphosis was determined by the amount of curvature in the notochord in the caudal region (flexion) as well as the development of head spines. These measures have also been employed by Anderson (1994) as criteria for determining the degree of metamorphosis of redfish larvae in the field. Larvae showing slight curvature of the notochord without any head spines were considered "pre-flexed." The development of head spines and increased flexion of the notochord classified the larvae as "flexed." Otherwise larvae were considered "non-flexed" if signs of metamorphosis were not apparent.

Behavioural observations were conducted every second day within 1-hr of adjusting prey densities. Observation periods for each larva lasted 1-min using the focal animal technique (Altman 1974) and the order of observations within tanks was advanced each observational day. Behavioural events were categorized into separate Modal Action

Patterns (MAPs: Barlow 1968: Table 3.1) and recorded on a portable keyboard (Tandy 102). Five fish per treatment were observed in this manner before data were uploaded and summarized using a behavioural software package (Observer: Noldus Information Technology. Waganingen. Netherlands).

3.2.3 Data Analysis:

Analysis of covariance (ANCOVA) was used to compare slopes of behavioural and morphometric data with age in days as the explanatory variable ($\alpha = 0.05$). All data were tested for normality on Minitab 10.2 using histograms of residuals and normal probability plots. Data which could not meet the assumptions of normality were randomized 5000 times if p-values were within 0.10 of the set significance level 0.05 (Manly 1991). P-values derived from the randomization tests were then used to determine significance. Behavioural data were next pooled into six observational periods which roughly corresponded with weeks: three days of observations comprised one "week" e.g. week 1 = days 1-6. week 2 = days 7-12. etc. Significant differences between treatments at subsequent observational periods were determined using Tukey pairwise comparisons with a family error rate of 0.05.

Behavioural data were also plotted against mean size (myotome height) taken within ± 2 days of observations. and analysis of variance (ANOVA) was conducted within 0.05 mm size classes e.g. 0.60-0.64. 0.65-0.69. etc. Significant differences between treatments at these size classes were determined using Tukey pairwise comparisons with a family error rate of 0.05.

Table 3.1: Operational definitions of the Modal Action Patterns (MAPs) observed in redfish larvae.

MAP	Definition
Swim	A quick undulation in the caudal region of the larva resulting in forward movement.
Motionless	No observable movement of the larva.
Orient	A head or eye movement towards a prey item.
Fixate	The larva is stationary and bends its caudal region into an 'S' shape position; typically follows orient.
Bite	The larva lunges forward from the fixate position in an attempt to ingest a prey item.

3.3 Results:

Preliminary observations of larvae collected from females of the two species used in this study the previous summer showed no differences in foraging behaviour (unpub. data). Any mixing of larvae from *S. mentella* and *S. fasciatus* was therefore concluded to have an insignificant effect on data collected during the experiment.

Larvae were observed attacking prey items on day 1 in all prey treatments. Successful feeding was confirmed on day 5 during the first morphometric sampling period by the presence of prey items in the guts of ~10 % of larvae sampled. The percentage of larvae with prey items in the gut increased over time in all prey treatments until day 25 in which 100 % of the larvae sampled had rotifers in the gut. Differences in gut fullness could not be determined without dissecting larvae.

Individual foraging MAPs of orient, fixate and bite were significantly different between treatments (Table 3.2). Not surprisingly larvae in the 0 prey L^{-1} treatment displayed the least number of foraging MAPs in the three weeks during which they were sampled. Foraging activity in the 0 prey L^{-1} treatment was directed only at pieces of detritus in the water column, and in no instances were any attempts at ingestion recorded. Subsequent statistical analyses of bite frequency as well as bite ratios to other behavioural patterns were therefore conducted independently of the 0 prey L^{-1} treatment. It should also be noted that bite:orient frequencies were calculated only from larvae which performed 2 or more orients during their observational period. This ensured that larvae had at least one opportunity to make the decision to bite at a prey item during the 1 min observation. Larvae orienting only once may not have had sufficient time to bite if the

Table 3.2: Summary of ANCOVA results on activity and the foraging MAPs of larvae at varying prey concentrations. (α=0.05)

MAP	Source	df	F	P
Swim Frequency	Covariates (day)	1	243.88	<0.0001
	Treatment	3	0.80	0.493
	Error	521		
Motionless Duration	Covariates (day)	1	2.25	0.134
	Treatment	3	8.71	<0.0001
	Error	521		
Orient Frequency	Covariates (day)	1	36.58	<0.0001
	Treatment	3	11.42	<0.0001
	Error	521		
Fixate Frequency	Covariates (day)	1	14.23	<0.0001
	Treatment	3	18.08	<0.0001
	Error	521		
Bite Frequency*	Covariates (day)	1	27.35	<0.0001
	Treatment	2	27.26	<0.0001
	Error	490		
Bite:Orient Ratio*	Covariates (day)	1	23.78	<0.0001
	Treatment	2	21.08	<0.0001
	Error	490		
Total Foraging Frequency	Covariates (day)	1	35.12	<0.0001
	Treatment	3	16.36	<0.0001
	Error	521		

* 0 prey L^{-1} treatment not included in ANCOVA.

prey item was encountered at the end of an observational period.

By week 5, larvae in the 4500 prey L^{-1} treatment oriented significantly more than larvae in 500 prey L^{-1} (Table 3.3a; Fig. 3.1a). Significantly higher biting frequency in 1500 prey L^{-1}, however, was recorded in week 4 between the 4500 prey L^{-1} treatment and later in week 5 between the 500 prey L^{-1} treatment (Table 3.3b; Fig. 3.1b). Ratios of bite:orient showed that larvae in the 1500 prey L^{-1} treatment were much more likely to strike (bite) after encountering (orient) a prey item than larvae in the highest prey treatment (Table 3.2; Fig. 3.2a). Consequently, the degree of total foraging activity (MAPs orient, fixate and bite) was also highest for larvae in 1500 prey L^{-1} (Tables 3.2 and 3.4; Fig. 3.2b).

Measuring the time spent swimming was limited by the observer's reflexes because redfish larvae have an abrupt swimming behaviour consisting of short bursts (< 0.2 s). Therefore, only the frequency of swimming events could be confidently analyzed. Occasionally a larva would swim rapidly for several seconds but this was rare, occurring in less than 5 % of observations. Within the first 12 days of the experiment, larvae reared in 0 prey L^{-1} swam more frequently (not significant) than other prey treatments (Fig. 3.3a). After day 12, however, larvae in the 0 prey L^{-1} treatment swam very little and spent significant more time remaining motionless (Table 3.5; Fig. 3.3b). The significant drop in motionless duration for larvae in 1500 prey L^{-1} (Tukey's pairwise comparison < 0.0193) is the result of more time spent foraging. Swimming frequency of larvae in the food treatments did not differ from each other, but all showed similar increased swimming activity through time.

Fig. 3.1: Foraging frequency of redfish larvae (*Sebastes* spp.) in different prey concentrations over time: a) orienting frequency and b) bite frequency. Each week is comprised of three days of observations taken every other day. Values are means for each treatment per observational period + 1 SE; n = 30 larvae per week.

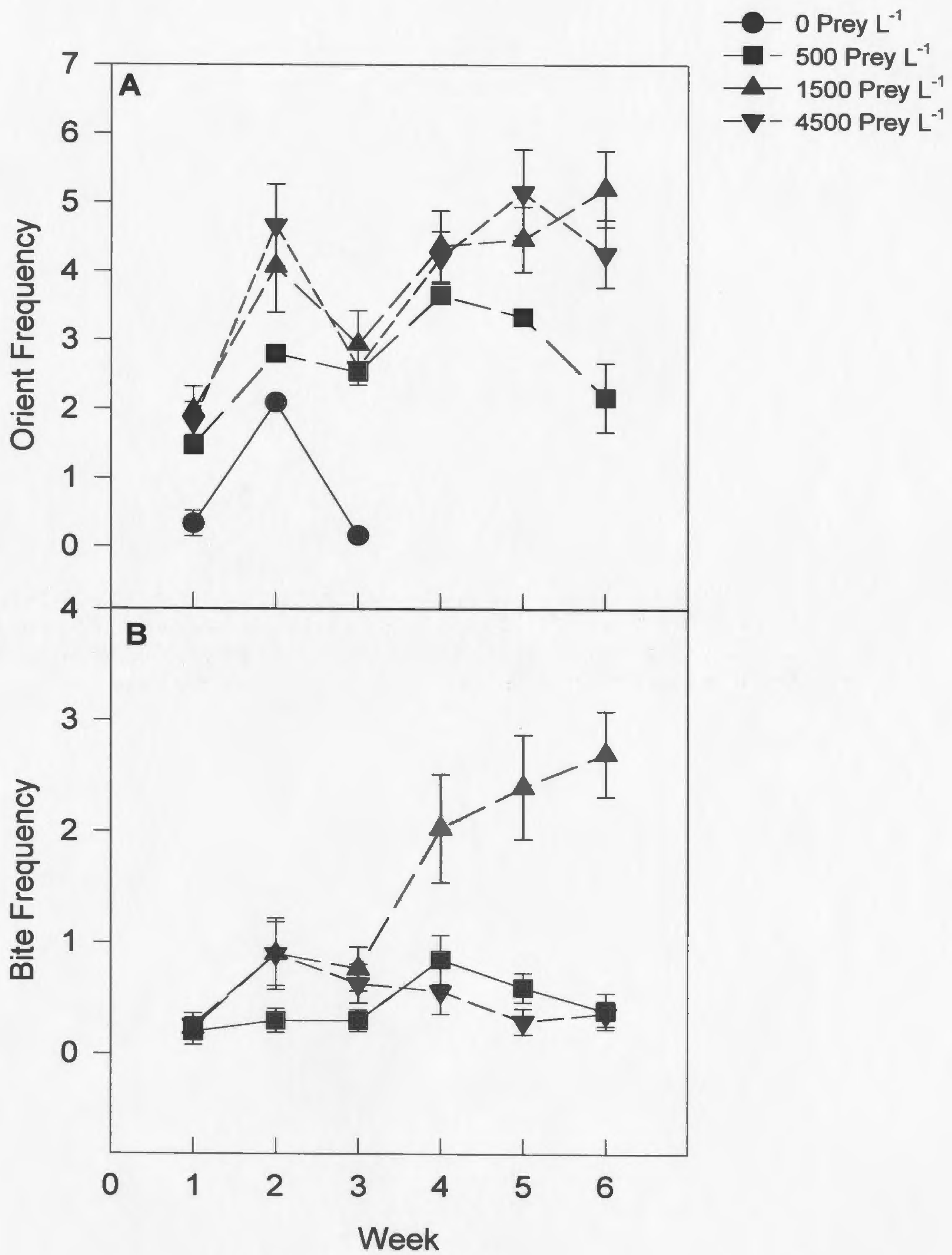

0 Prey L-1
500 Prey L-1
1500 Prey L-1
4500 Prey L-1
A
B
Orient Frequency
Bite Frequency
Week
0
1
2
3
4
5
6
7

Fig. 3.2: Foraging frequency of redfish larvae (*Sebastes* spp.) in different prey concentrations over time: a) bite:orient ratios expressed as a percentage and b) total foraging frequency (orient + fixate + bite). Each week is comprised of three days of observations taken every other day. Values are means for each treatment per observational period ± 1 SE; n = 30 larvae per week.

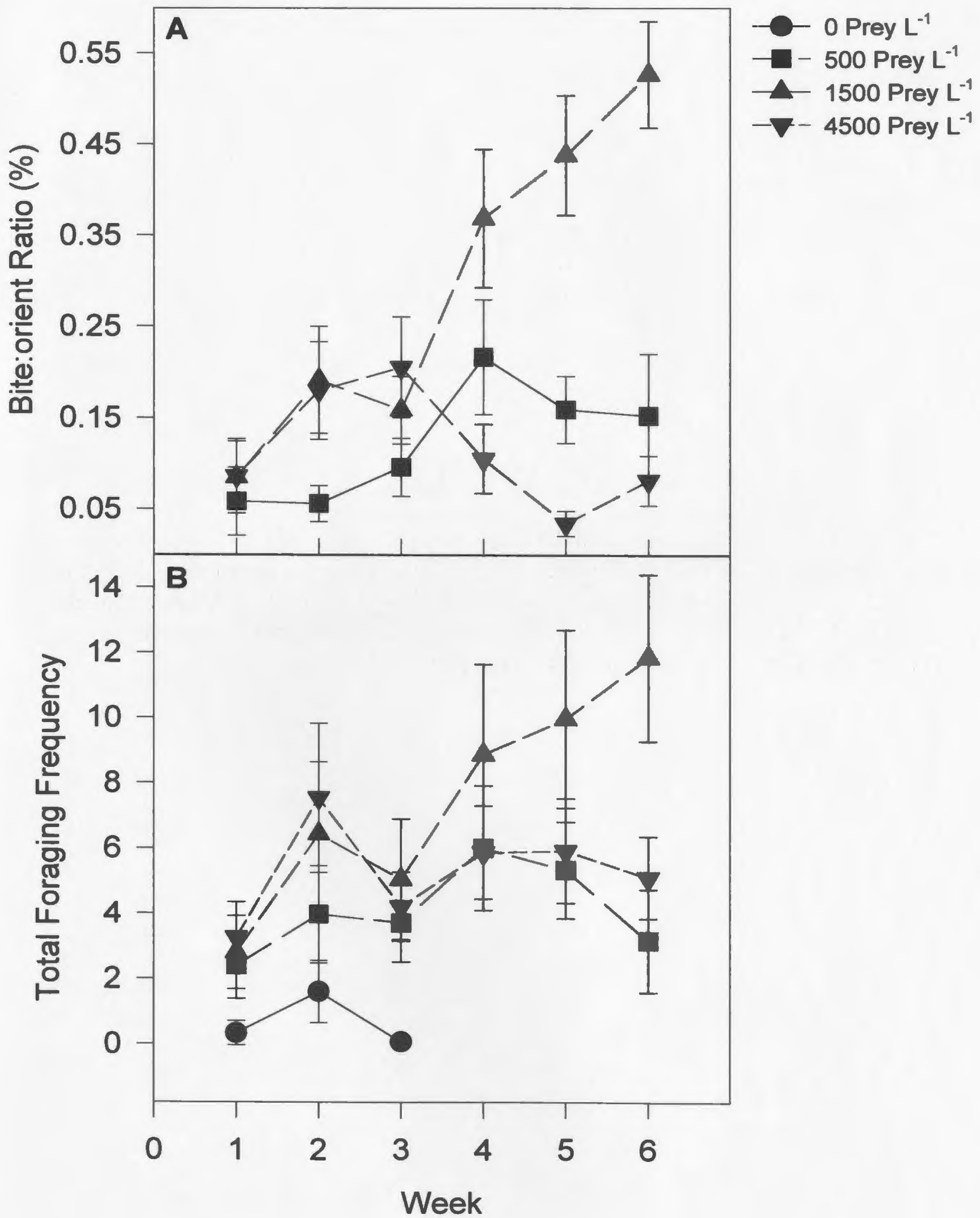
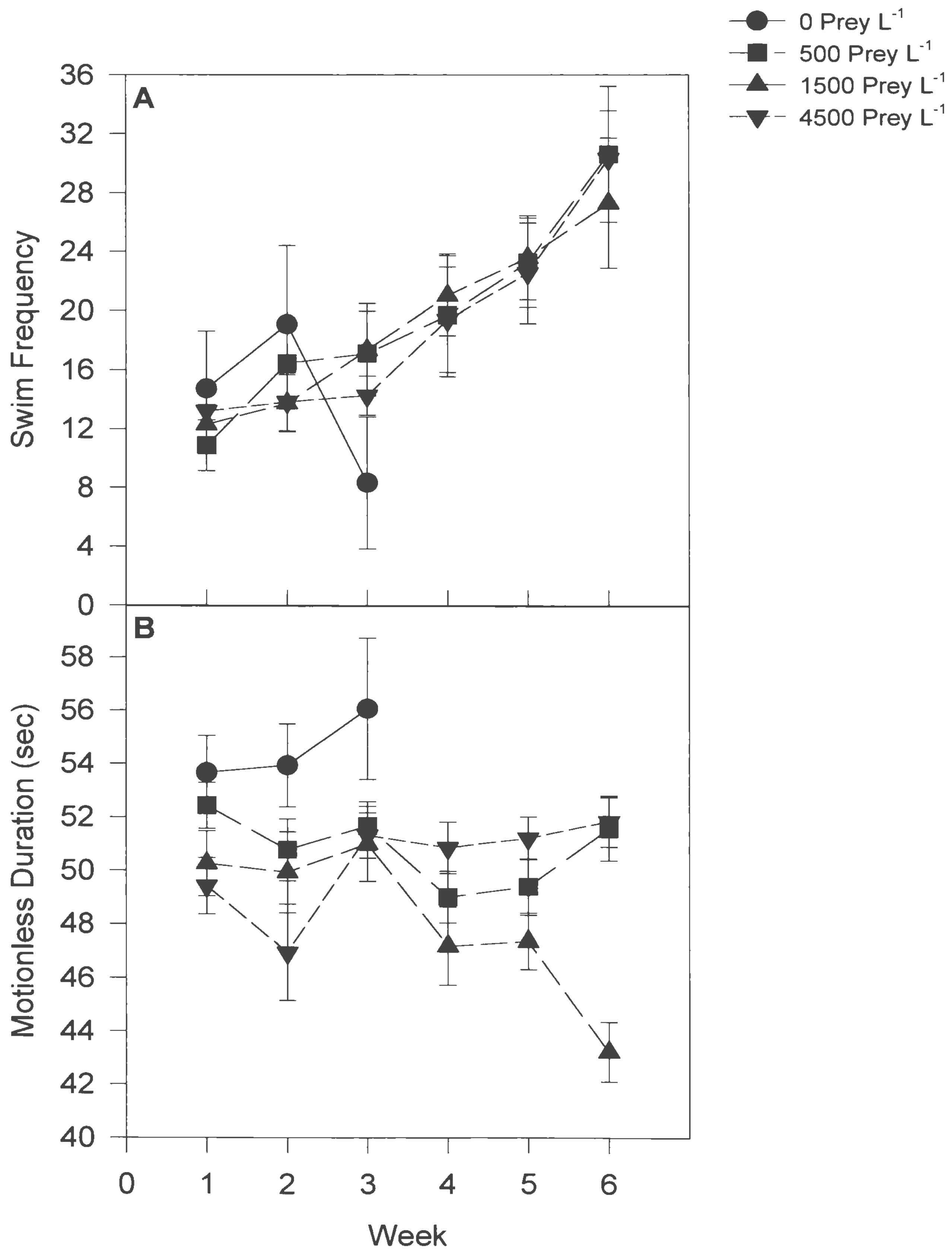

A
B
0 Prey L-1
500 Prey L-1
1500 Prey L-1
4500 Prey L-1
Bite:orient Ratio (%)
0.55
0.45
0.35
0.25
0.15
0.05
Total Foraging Frequency
14
12
10
8
6
4
2
0
Week
0
1
2
3
4
5
6

Fig. 3.3: a) Swim frequency and b) time spent motionless of redfish larvae (*Sebastes* spp.) over time. Each week is comprised of three days of observations taken every other day. Values are means for each treatment per observational period ± 1 SE; n = 30 larvae per week.



0 Prey L-1
500 Prey L-1
1500 Prey L-1
4500 Prey L-1
A
Swim Frequency
36
32
28
24
20
16
12
8
4
0
B
Motionless Duration (sec)
58
56
54
52
50
48
46
44
42
40
0
1
2
3
4
5
6
Week

Table 3.3: Tukey's pairwise comparison of (a) orient frequency and (b) bite frequency in larval redfish. Significant differences occur where 0 lies outside the range of listed confidence intervals of each test. *Significant difference ($p <$ family error rate of 0.05).

[a]Weeks 1-5 are comprised of three observational periods taken over a six day period. Week 6 is comprised of two observations taken over a five day period.

A

Week[a]	0-500		0-1500		0-4500		500-1500		500-4500		1500-4500	
1	-2.428	0.161	-2.928	-0.339*	-2.761	-0.172*	-1.557	0.557	-1.390	0.724	-0.890	1.224
2	-3.540	2.122	-4.807	0.855	-5.407	0.255	-3.341	0.807	-3.941	0.207	-2.674	1.474
3	-4.847	0.113	-5.247	-0.287*	-4.913	0.047	-1.832	1.032	-1.499	1.365	-1.099	1.765
4	------	------	------	------	------	------	-2.454	1.021	-2.287	1.187	-1.387	1.721
5	------	------	------	------	------	------	-2.826	0.559	-3.554	-0.076*	-2.420	1.057
6	------	------	------	------	------	------	-4.802	-1.264*	-3.888	-0.305*	-0.808	2.681

B

Week[a]	500-1500		500-4500		1500-4500	
1	-0.3948	0.3282	-0.4282	0.2948	-0.3948	0.3282
2	-1.459	0.259	-1.459	0.259	-0.859	0.859
3	-1.0132	0.0798	-0.8798	0.2132	0.4132	0.6798
4	-2.453	0.087	-0.987	1.553	0.331	2.603*
5	-2.787	-0.813*	-0.710	1.317	1.090	3.117*
6	-3.196	-1.426*	-0.875	0.916	1.459	3.204*

Table 3.4. Tukey's pairwise comparisons of bite frequency in larval redfish. Significant differences occur where 0 lies outside the range of listed confidence intervals of each test. *Significant difference ($p <$ family error rate of 0.05)

[a]Weeks 1-5 are comprised of three observational periods taken over a six day period. Week 6 is comprised of two observations taken over a five day period.

Week[a]	0-500		0-1500		0-4500		500-1500		500-4500		1500-4500	
1	-5.570	-0.644*	-8.746	-3.850*	-6.834	-1.929*	-4.595	-1.787*	-2.687	0.137	0.530	3.302*
2	-6.648	3.078	-9.115	0.612	-10.215	-0.488*	-6.029	1.096	-7.129	-0.004*	-4.662	2.462
3	-7.926	0.860	-9.260	-0.474*	-8.426	0.360	-3.870	1.203	-3.036	2.036	-1.703	3.370
4	------	------	------	------	------	------	-6.766	1.032	-3.766	4.032	-0.487	6.487
5	------	------	------	------	------	------	-8.026	-1.241*	-4.078	2.893	0.555	7.526*
6	------	------	------	------	------	------	-11.978	-5.389*	-5.274	1.396	3.496	9.993*

Table 3.5. Tukey's pairwise comparisons of time spent motionless in larval redfish. Significant differences occur where 0 lies outside the range of listed confidence intervals of each test. *Significant difference ($p <$ family error rate of 0.05)

[a]Weeks 1-5 are comprised of three observational periods taken over a six day period. Week 6 is comprised of two observations taken over a five day period.

Week[a]	0-500		0-1500		0-4500		500-1500		500-4500		1500-4500	
1	-3.197	5.684	-0.847	8.034	-0.167	8.714	-1.276	5.976	-0.596	6.656	-2.946	4.306
2	-4.297	10.263	-3.244	11.317	-0.280	14.280	-4.280	6.386	-1.316	9.350	-2.370	8.296
3	-2.452	10.785	-1.452	11.785	-1.852	11.385	-2.821	4.821	-3.221	4.421	-4.221	3.421
4	------	------	------	------	------	------	-2.314	5.821	-5.917	2.217	-7.241	0.035
5	------	------	------	------	------	------	-1.250	5.317	-5.199	1.548	-7.232	-0.485*
6	------	------	------	------	------	------	4.608	12.075*	-4.061	3.499	-12.304	-4.941*

Growth and condition of larvae was dependent on prey concentration. Larval SL varied between treatments over time (Table 3.6), and larvae in the 1500 prey L^{-1} treatment were significantly larger than larvae in 500 prey L^{-1} and 4500 prey L^{-1} by the end of the experiment (Table 3.7a; Fig. 3.4a). Myotome height (MH) was also significantly different between treatments (Table 3.6), but differences occurred earlier than SL for larvae in the 1500 prey L^{-1} treatment (Table 3.7b; Fig. 3.4b). The larvae in 1500 prey L^{-1} showed highest overall mean growth at 0.074 mm SL d^{-1} compared to 4500 prey L^{-1} and 500 prey L^{-1} at 0.055 and 0.054 mm SL d^{-1} respectively (Fig. 3.5a). Larval condition increased as a function of age in all treatments (Table 3.6), but larvae had best overall condition at the end of the experiment in 1500 prey L^{-1} (Table 3.8; Fig. 3.5b).

Because growth rates varied between treatments, behavioural measurements were plotted as a function of size (MH) independent of age. Myotome height was used in place of standard length (SL) because it appeared to be less affected by morphological changes associated with metamorphosis e.g. posterior notochord flexion. Mean orient frequency as a function of size (Fig. 3.6a) indicates that prey encounter rates in the middle and high prey treatments are higher than the low prey treatment. However, significant differences did not occur until the largest size class (0.60-0.65 mm) (Tukey's pairwise comparison < 0.0193). Influences of size on mean total foraging (Fig. 3.6b) shows a similar trend as plots against age (Fig. 3.2b). Similar sized larvae appear to be foraging at different rates between treatments as they approach 0.6 mm. This is shown statistically between the 1500 and 500 prey L^{-1} treatment (Tukey's pairwise comparison < 0.0193) but not between 1500 and 4500 prey L^{-1} (Tukey's pairwise comparison > 0.0193) at the 0.60-0.65 mm size

Fig. 3.4: a) Standard length (SL: mm) and b) myotome height (MH: mm) of larval redfish (*Sebastes* spp.) subjected to prey concentrations of 0, 500, 1500 and 4500 prey L^{-1}. Values are means (n = 10) + 1 SE.

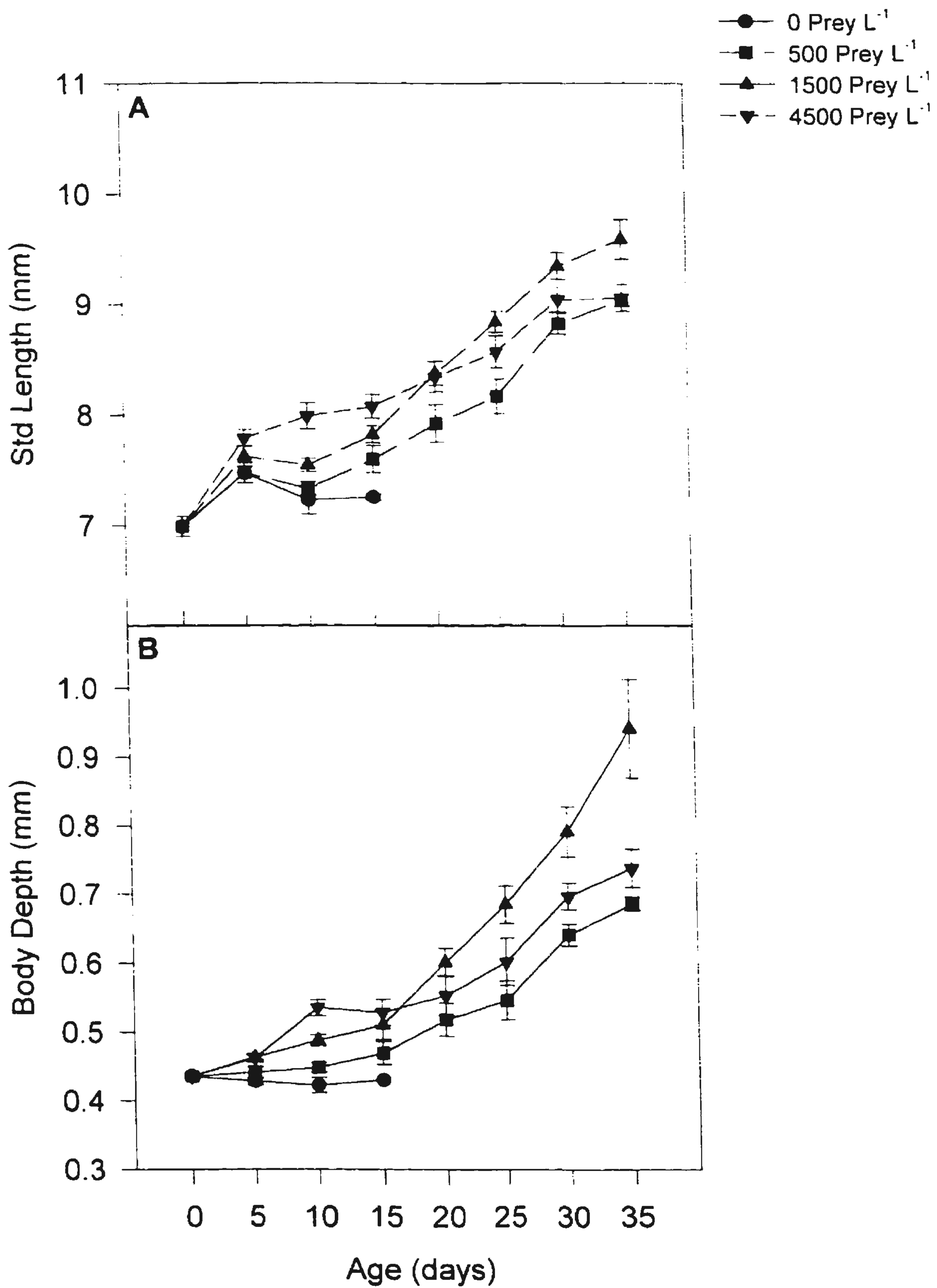

0 Prey L^{-1}
500 Prey L^{-1}
1500 Prey L^{-1}
4500 Prey L^{-1}
A
Std Length (mm)
11
10
9
8
7
B
Body Depth (mm)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
5
10
15
20
25
30
35
Age (days)

Fig. 3.5: a) Growth rates (mm SL d^{-1}) and b) condition index ± 1 SE (MH/SL) of larval redfish (*Sebastes* spp.) subjected to prey concentrations of 0, 500, 1500 and 4500 prey L^{-1}. Values are means (n=10).

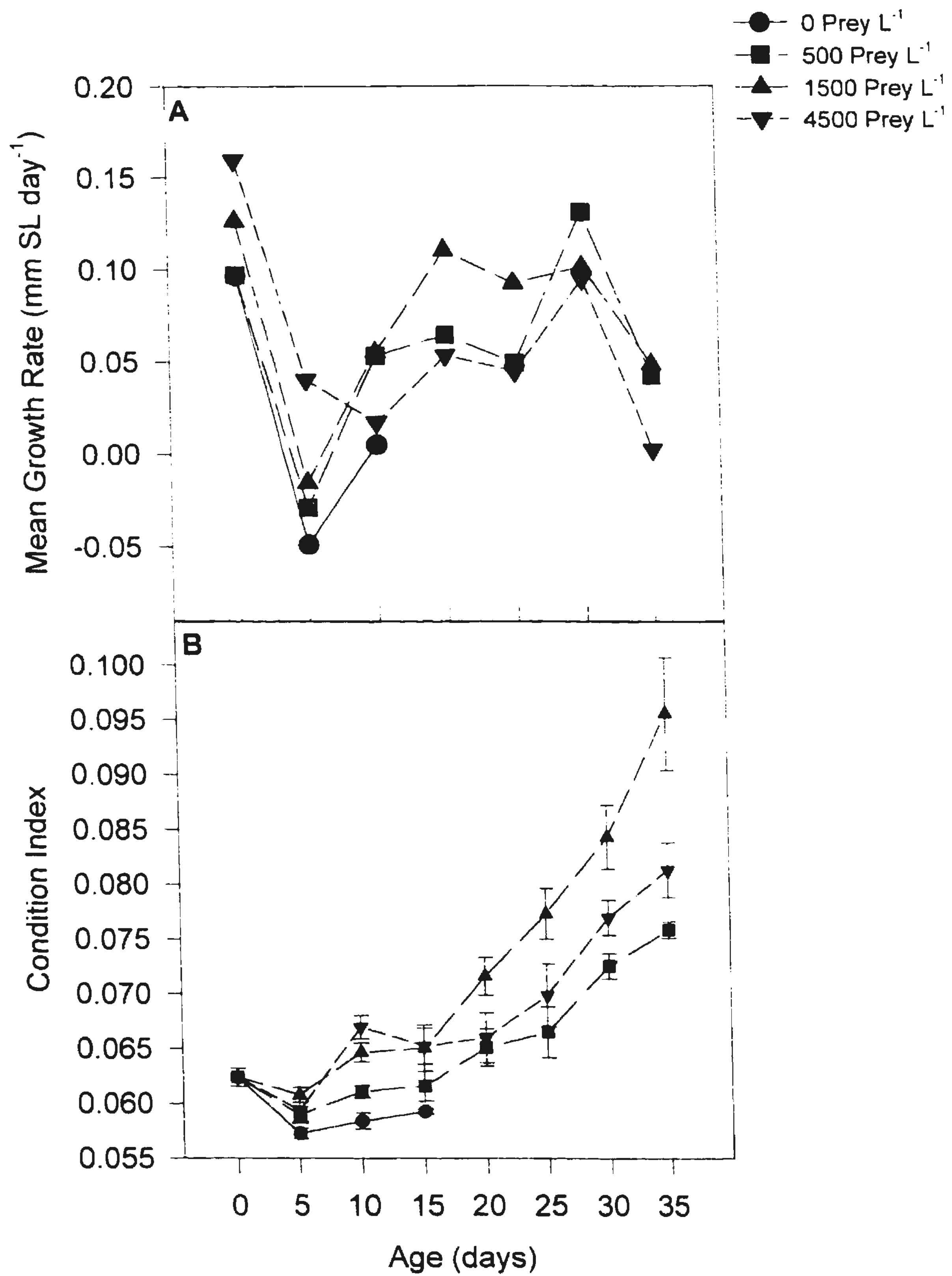

0 Prey L-1
500 Prey L-1
1500 Prey L-1
4500 Prey L-1
A
Mean Growth Rate (mm SL day-1)
0.20
0.15
0.10
0.05
0.00
-0.05
B
Condition Index
0.100
0.095
0.090
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0
5
10
15
20
25
30
35
Age (days)

Fig 3.6: a) Orient frequency and b) total foraging frequency of redfish larvae (*Sebastes* spp.) in prey concentrations of 0, 500, 1500 and 4500 prey L^{-1} as a function of larval size (myotome height). Myotome height values are means (n=10) taken every five days and behavioural values are means taken ± 2 days of size values (n=20-30).

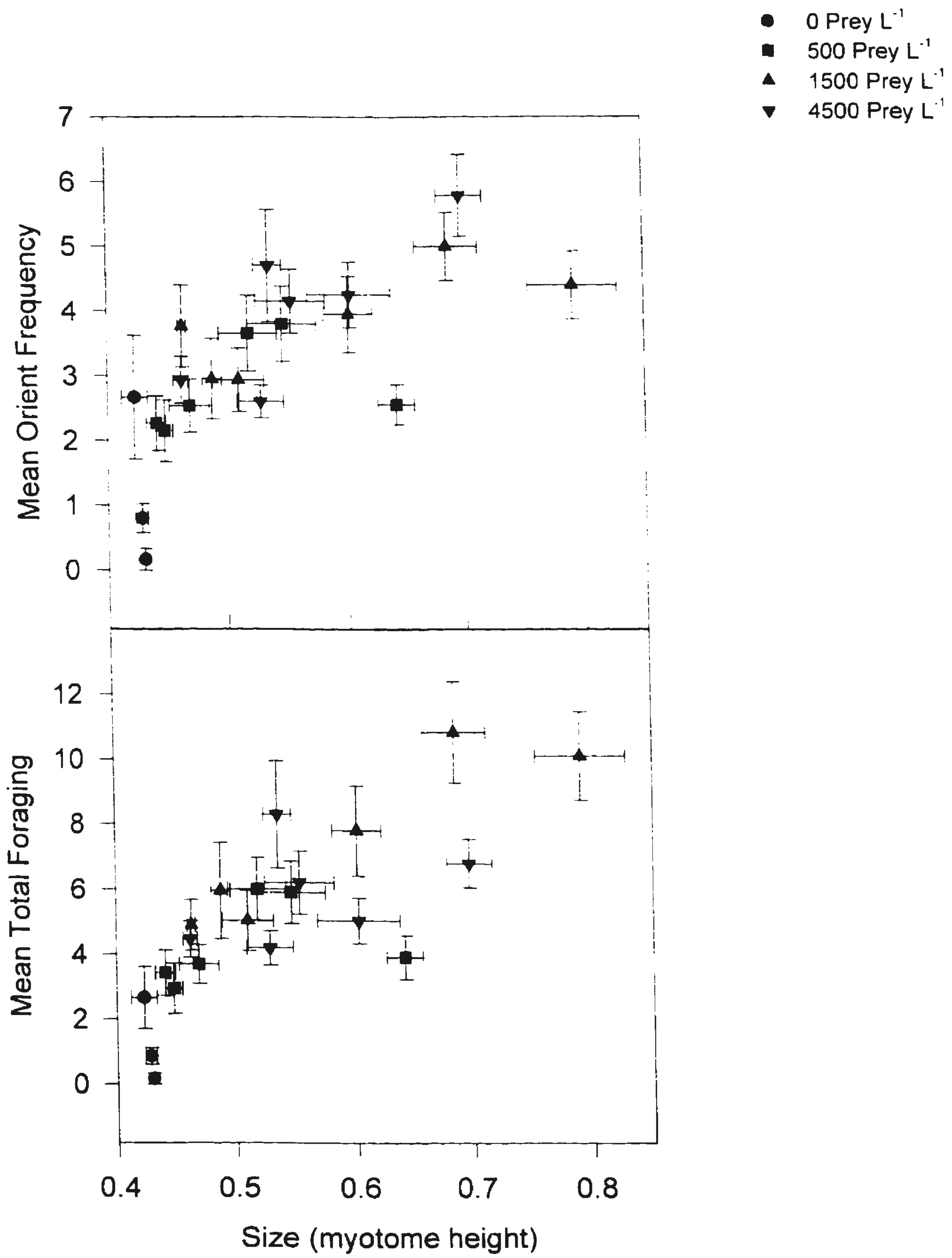

0 Prey L-1
500 Prey L-1
1500 Prey L-1
4500 Prey L-1
Mean Orient Frequency
0
1
2
3
4
5
6
7
Mean Total Foraging
0
2
4
6
8
10
12
0.4
0.5
0.6
0.7
0.8
Size (myotome height)

Table 3.6: Summary of ANCOVA results for morphometric and condition changes for larvae at varying prey concentrations. (α=0.05)

Morphometric	Source	df	F	P
Standard Length	Covariates (day)	1	1094.37	<0.0001
(SL)	Treatment	3	13.08	<0.0001
	Error	293		
Myotome Height	Covariates (day)	1	540.53	<0.0001
(MH)	Treatment	3	15.08	<0.0001
	Error	293		
Condition	Covariates (day)	1	281.88	<0.0001
(MH/SL)	Treatment	3	13.66	<0.0001
	Error	293		

Table 3.7: Tukey's pairwise comparisons of (a) standard length (SL) and (b) myotome height (MH) measured at five day intervals. Significant differences occur where 0 lies outside the range of listed confidence intervals of each test. *Significant difference ($p <$ family error rate of 0.05)

A

Day	0-500		0-1500		0-4500		500-1500		500-4500		1500-4500	
5	-0.399	0.395	-0.546	0.248	-0.712	0.082	-0.472	0.178	-0.638	0.012	-0.491	0.159
10	-0.529	0.325	-0.743	0.111	-1.188	-0.335*	-0.536	0.108	-0.981	-0.337*	-0.767	-0.123
15	-0.911	0.226	-1.135	0.002	-1.390	-0.253*	-0.610	0.162	-0.865	-0.093*	-0.641	0.131
20	-----	-----	-----	-----	-----	-----	-0.947	0.041	-0.917	0.071	-0.464	0.524
25	-----	-----	-----	-----	-----	-----	-1.144	-0.194*	-0.876	0.074	-0.207	0.743
30	-----	-----	-----	-----	-----	-----	-0.905	-0.137*	-0.603	0.165	-0.082	0.686
35	-----	-----	-----	-----	-----	-----	-1.002	-0.104*	-0.481	0.441	0.072	0.994*

B

Day	0-500		0-1500		0-4500		500-1500		500-4500		1500-4500	
5	-0.041	0.015	-0.063	-0.007*	-0.062	-0.006*	-0.045	0.001	-0.044	0.002	-0.022	0.024
10	-0.071	0.020	-0.111	-0.021*	-0.158	-0.068*	-0.074	-0.006*	-0.121	-0.053*	-0.081	-0.013*
15	-0.143	0.065	-0.184	0.024	-0.202	0.006	-0.111	0.029	-0.129	0.011	-0.088	0.052
20	-----	-----	-----	-----	-----	-----	-0.169	0.003	-0.121	0.051	-0.038	0.134
25	-----	-----	-----	-----	-----	-----	-0.245	-0.033*	-0.162	0.050	-0.023	0.189
30	-----	-----	-----	-----	-----	-----	-0.240	-0.060*	-0.146	0.034	0.004	0.184*
35	-----	-----	-----	-----	-----	-----	-0.385	-0.089*	-0.204	0.101	0.033	0.338*

Table 3.8: Tukey's pairwise comparisons of larval condition measured at five day intervals. Significant differences occur where 0 lies outside the range of listed confidence intervals of each test. *Significant difference ($p <$ family error rate of 0.05)

Day	0-500		0-1500		0-4500		500-1500		500-4500		1500-4500	
5	-0.0054	0.0020	-0.0072	0.0002	-0.0058	0.0016	-0.0048	0.0012	-0.0034	0.0026	-0.0016	0.0044
10	-0.0069	0.0015	-0.0104	-0.0021*	-0.0127	-0.0044*	-0.0067*	-0.0004*	-0.0090	-0.0027*	-0.0055	0.0008
15	-0.0117	0.0071	-0.0152	0.0036	-0.0153	0.0035	-0.0099	0.0029	-0.0100	0.0028	-0.0066	0.0062
20	-----	-----	-----	-----	-----	-----	-0.0133	0.0002	-0.0076	0.0059	-0.0011	0.0124
25	-----	-----	-----	-----	-----	-----	-0.0200	-0.0020*	-0.0122	0.0055	-0.0014	0.0163
30	-----	-----	-----	-----	-----	-----	-0.0189	-0.0046*	-0.0116	0.0027	0.0002	0.0145*
35	-----	-----	-----	-----	-----	-----	-0.0314	-0.0080*	-0.0175	0.0066	0.0022	0.0263*

range. Bite:orient frequencies between larvae in the 500 and 1500 prey L^{-1} treatments were not significantly different at the largest size class (0.60-0.65 mm) (Tukey's pairwise comparison > 0.0193) (Fig. 3.7) but were significantly lower for larvae in 4500 prey L^{-1} than 1500 prey L^{-1} in this size class (Tukey's pairwise comparison > 0.0193).

Increased growth rates in the 1500 prey L^{-1} treatment corresponded with increased larval survival and developmental rates. Although some larvae lived to day 18 in the 0 prey L^{-1} treatment, 50 % mortality occurred by day 5. Survival of larvae in food treatments was otherwise dependent upon prey concentration. By the end of the experiment, larvae in the 1500 prey L^{-1} treatment had the highest survival at 26 % whereas both the 500 prey L^{-1} and 4500 prey L^{-1} treatment had lower survival at ~7 % (Fig. 3.8a). Of these survivors, ~90 % of the larvae sampled showed signs of flexion in 1500 prey L^{-1} compared to ~50 % of larvae in both the 500 and 4500 prey L^{-1} treatments (Fig. 3.8b). While no larvae in the 500 and 4500 prey L^{-1} treatments were classified past a pre-flexion state of metamorphosis, 78 % of the larvae in the 1500 prey L^{-1} treatment showed signs of full flexion.

3.4 Discussion:

With the exception of the 0 prey L^{-1} treatment, larvae fed, grew, survived and initiated metamorphosis in all the prey treatments in the study. Larvae fed at 1500 prey L^{-1}, however, had the highest overall survival, condition, and growth at the end of the experiment. Because optimal foraging conditions can be influenced by a number of factors such as stocking density (Houde 1975, 1977), light levels (Puvanendran and Brown 1998), turbidity (Grecay and Targett 1996), and turbulence (Sundby and Fossum

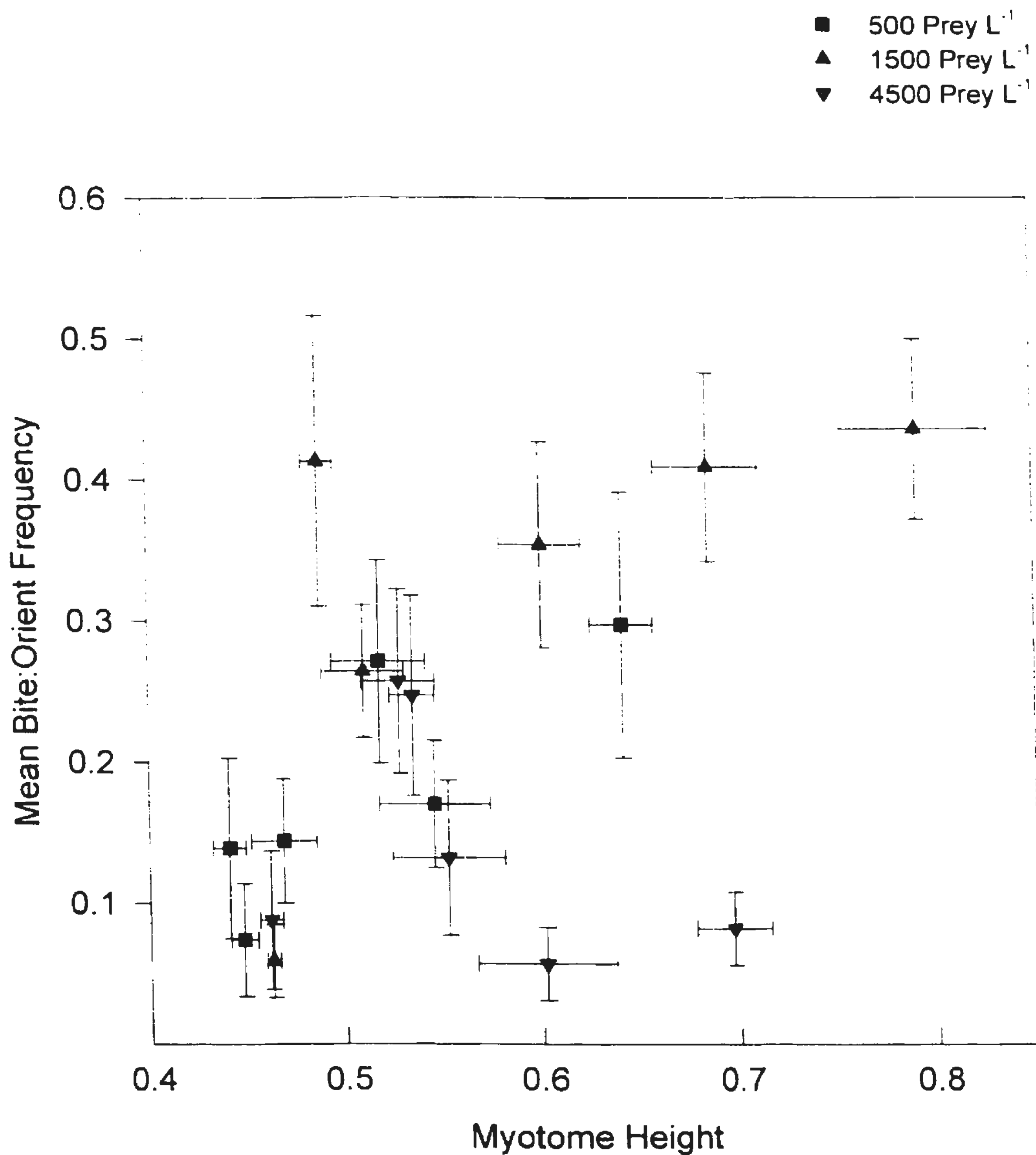
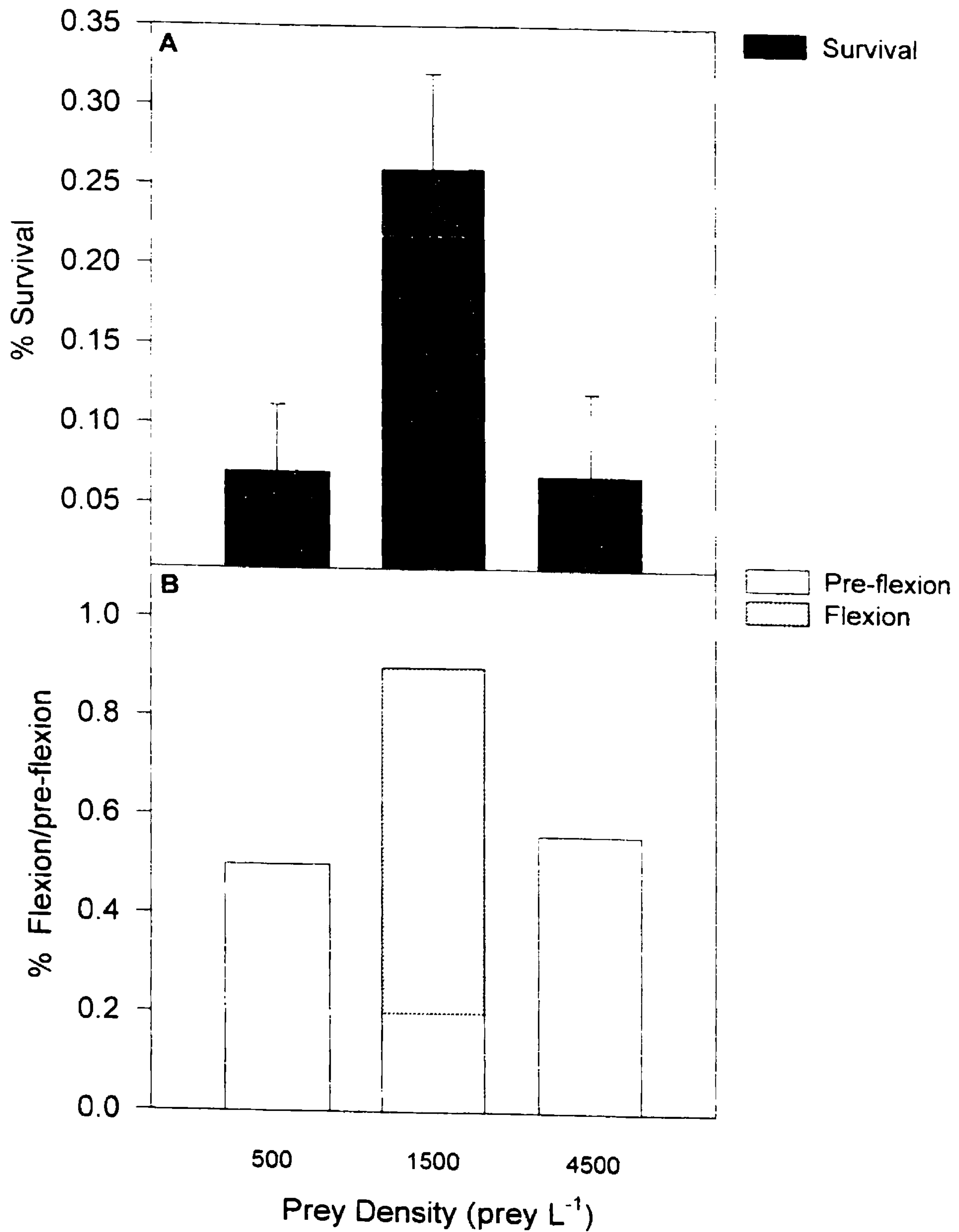


Fig. 3.7: Mean bite:orient frequency of larval redfish subjected to prey concentrations of 500, 1500 and 4500 prey L-1 as a function of mean larval size (myotome height). Myotome height values are means (n=10) taken every 5 days and behavioural values are means taken within 2 days of size values (n=20-30).

Fig 3.8: a) Percent survival and b) metamorphosis of redfish larvae (*Sebastes* spp.) in prey concentrations of 500, 1500 and 4500 prey L^{-1} at day 35. Metamorphosis values are expressed as a percentage of larvae showing signs of either pre-flexion or flexion. Survival is the average of two replicates + 1 SE.



0.35
0.30
0.25
0.20
0.15
0.10
0.05
% Survival
A
Survival
B
Pre-flexion
Flexion
1.0
0.8
0.6
0.4
0.2
0.0
% Flexion/pre-flexion
500
1500
4500
Prey Density (prey L-1)

1990), this result should be considered as a 'starting point' towards understanding the early foraging ecology of redfish larvae.

Attack and consumption rates of fish larvae are typically asymptotic when using a range of prey densities in the laboratory (Houde and Schekter 1980). Subsequent survival and growth also follow a similar curve at higher prey densities until an asymptote is reached (Werner and Blaxter 1980). The significant reduction in growth and survival at 4500 prey L^{-1} in this experiment, therefore, was unexpected. Two separate hypotheses may explain this result for the high prey treatment: 1) water quality or 2) a confusion effect.

Increased release of metabolites by *Artemia* nauplii at high densities in the lab (> 8000 prey L^{-1}) has been suggested as being detrimental for fish larvae (Houde 1975). Atlantic cod have been shown to have reduced survival at high densities (>8000 prey L^{-1}) (Puvanendran and Brown submitted) as have other species of marine fish larvae (see Léger et al. 1986 for review). *Brachionus* sp., however, have not been reported to reduce water quality in the same manner, and these prey at densities > 4500 prey L^{-1} in our lab with other fish species have not been shown to be detrimental (Gotceitas et al. 1996). Lastly, flow rates set at 1 L min^{-1} exchanged ~ 5 tank volumes of water per day, so it is unlikely that poor water quality contributed to reduced larval growth and survival in the high prey treatment.

The second hypothesis (i.e. a confusion effect) is evidenced by patterns in the foraging behaviour data. Mean total foraging frequency was significantly higher in the 1500 prey L^{-1} treatment than the 4500 prey L^{-1} treatment by week 5. Equal frequencies of

orienting behaviour, however, existed for larvae in the 1500 and 4500 prey L^{-1} treatment throughout the entire experiment. Accounting for growth differences, larvae in the high prey treatment are actually encountering more prey at a given size than larvae in lower prey densities. The bite:orient data, however, show that larval foraging efficiencies in the high prey treatment were markedly lower than larvae at 1500 prey L^{-1} as well as 500 prey L^{-1} when accounting for size differences. The low ratio for larvae in the 4500 prey L^{-1} treatment indicates that these larvae were orienting ~ 10 times for every bite within the 0.60-0.65 mm size class. In contrast, larvae in the low and high densities are ~ 3 times more efficient at this size i.e. orienting ~ 3 times per bite. It would appear that larvae were re-orienting as more prey items entered their field of vision. Redfish foraging routines (orient, fixate and bite) sometimes lasted more than 6 sec in smaller larvae, and it was often noted in the high prey treatment that larvae restarted their foraging routine when another prey item swam past their field of vision.

It is interesting to note that this confusion effect in the high prey treatment appears to become more pronounced with larval maturity. This may be explained by developmental changes occurring within the eye resulting in greater perceptual fields for fish larvae. In general, perceptual distance and angle increase both with increasing size of fish and prey (Hunter 1981). As the larval fish grows it has to discriminate among more stimuli within its perceptual range. Hairston et al. (1982) suggested that higher amounts of prey detected from increased visual fields should lead to higher feeding rates in planktivorous fish. Under initially high prey densities, however, redfish larvae would appear to contradict this theory; increased encounter rates from larger visual fields

resulted in fewer attacks toward prey and proved detrimental in terms of larval growth and survival.

Three-spined sticklebacks have been shown to be confused when offered *Daphnia* sp. at high densities in the laboratory (Milinski 1977; Heller and Milinski 1979). In these studies fish preferred to forage only at higher densities when at hungrier states because they had higher 'attack readiness'. With increased satiation, fish were able to forage much more efficiently (had higher capture success) in lower densities than higher densities. Heller and Milinski (1979) suggest that there is an increased predation risk when feeding in higher prey densities; more concentration is needed to overcome confusion and less time can be devoted towards predator vigilance.

Redfish larvae forage in their environment by repositioning themselves through abrupt swimming movements and then pausing to search for prey. Termed 'saltatory search' (O'Brien et al. 1989), this foraging strategy has been described in a number of larvae fish species including white crappie (*Pomoxis annularis*; Browman and O'Brien 1992a), golden shiner (*Notemigonus crysoleucas*; Browman and O'Brien 1992b) and Atlantic cod (*Gadus morhua*; Puvanendran et al. submitted). Swimming frequency in the 0 prey L^{-1} treatment would be predicted to be highest assuming search time increases with lower prey availability. This has been demonstrated in larval cod (Munk 1995) and Atlantic herring (Munk and Kiorboe 1985). Although swimming frequency in the present study was higher in the 0 prey L^{-1} treatment during week 1 and 2, it was not significant. By week 3, however, larvae at 0 prey L^{-1} swam significantly less than other prey treatments. A critical period or 'point-of-no-return' (PNR) has been described for marine

larvae shortly after yolk reserves are depleted and exogenous feeding must ensue (Blaxter and Hempel 1963). This period is typified by high mortality, sluggish swimming and general inactivity so it is possible that redfish underwent such a critical period shortly after day 10. Although highest mortality in this experiment was observed in the first few days, it is probably not an accurate reflection of food availability as yolk-sacs were not observed to be exhausted until day five. Initial mortality in this experiment is more likely due to handling stress in the transfer from the research vessel to lab aquaria.

Further evidence for a critical period is provided in the growth rate data. Estimates of growth rates for larval redfish can vary both intra- and interannually, depending upon availability of preferred prey types (Anderson 1994), changes in seasonal heating cycles (Anderson 1984) and cohort strength (Penny and Evans 1985). The highest average growth rate achieved in our experiment was 0.074 mm SL d^{-1} by larvae in the 1500 prey L^{-1} treatment. Anderson (1984) reports mean growth rates of larval redfish averaging 0.136 mm SL d^{-1} in 1981 using a length frequency method. Penny and Evans (1985) report lower estimates of 0.109 mm d^{-1} for the same larval samples by correlating daily otolith increment with total length. These studies, however, have consistently shown larval redfish to have relatively low or declining growth rates within the first two weeks of development. Mean growth rates of larvae in all treatments in our experiment also declined initially with the lowest growth rates being found in the 0 prey L^{-1} treatment. Anderson (1984) reports increased mortality during this same period of reduced growth, strongly suggesting a critical period during this time if mortalities are indeed growth related. Unfortunately, day to day mortality data is not available for the prey treatments

to compare with the mean growth rates, but it would be reasonable to expect higher mortalities between days 5 and 20 when growth rates were lowest.

The noticeable drop in foraging activity during week 3 may be the result of weak larvae at the end of their critical period. Orient, fixate and bite frequency increased in all treatments from week 1 to week 2, followed by a noticeable decline during week 3, and then rebounded during week 4. The decline could have been due to reduced foraging activity from weak larvae during this period of time. The similar response across treatments for all foraging behaviour suggests that treatment effects were not responsible for the trend.

Comparisons of these results to those found in the field with redfish larvae, however, should be exercised with caution. The enriched rotifers used in this experiment likely have a different nutritional value compared with preferred wild zooplankton, namely *Calanus finmarchicus*. Rearing marine larvae in the laboratory also often requires using prey densities higher than those typically reported in the field. To date, no experiments have successfully taken larvae from first-feeding to metamorphosis using prey levels reported from field studies. Either field estimates are not sensitive to patchy prey distributions or laboratory rearing conditions do not closely simulate field conditions (MacKenzie et al. 1990). Increased turbulence has been shown to increase encounter rates of larval cod over calm water conditions in the lab (MacKenzie and Kiørboe 1995) and field studies have shown feeding rates to be higher for larval fish in more turbulent environments (Sundby and Fossum 1990). Turbulence is theorized to function by increasing predator-prey encounter rates (Rothschild and Osborn 1988; for other views

see Browman 1996), but we were able to accomplish this in our experiment by increasing prey densities.

The ecological significance of this study can best be understood by comparing redfish larvae with other marine fish larvae observed under similar conditions. Atlantic cod larvae are ideal candidates as they overlap temporally and spatially with redfish larvae during spring spawning off the coast of Greenland (Bainbridge and McKay 1968), Flemish Cap (Anderson and Akenhead 1981; Anderson 1982) and to a smaller extent on Georges Bank (Sherman et al. 1981). Both larvae show a preference for *Calanus* copepods during early development, but cod larvae have been shown to switch to a more diverse diet earlier than redfish larvae (Bainbridge and McKay 1968). This diet switch is likely due to the much higher growth rates reported for cod than redfish. Cod growth rates in the laboratory are reported to be 0.20 mm SL d^{-1} for the first 30 days of development (Puvanendran and Brown submitted), nearly three times faster than maximum redfish growth rates achieved in our experiment. As a consequence, laboratory reared cod larvae require high prey densities (~4000 prey L^{-1}) and do not survive below 1000 prey L^{-1} when reared with *Brachionus* sp. (Puvanendran and Brown submitted). Older and larger cod larvae seem more tolerant of lower prey densities (Munk 1995), but this is a typical phenomenon for fish larvae as they grow and develop better vision and digestive capabilities (Noakes and Godin 1988) while swimming ability increases (e.g. Rosenthal and Hempel 1971; Laurence 1972).

It is therefore possible that low prey availability is less limiting for redfish than it is for cod in the field. Anderson (1994) found that growth and survival of redfish larvae

was better in a year when overall prey abundance was lower than the following year. He concluded that qualitative aspects of zooplankton such as size and type may be more limiting to survival than prey quantity. Cod larvae on the other hand are likely more sensitive to a mismatch in prey abundance than redfish larvae. Evidence that cod are susceptible to mismatches in prey availability exists from both studies in the laboratory (Gotceitas et al. 1996) and the field (Cushing 1990). The role of prey quality on growth and survival of this species remains poorly understood, but it is reasonable to speculate that the more diverse diet of cod larvae increases tolerance to years when prey types are more varied. The different prey requirements of redfish and cod may be a means by which these two species avoid resource competition during the spring spawning season when they often co-occur in high numbers.

CHAPTER 4

SUMMARY AND FUTURE RESEARCH

Variability in endogenous resources as well as behaviour, growth and survival in accordance with exogenous resources suggests that recruitment processes in redfish potentially start at an early stage. Recruitment processes may include either direct starvation or increased predation at vulnerable sizes as a results of decreased growth rates. Atlantic redfish requirements for endogenous and exogenous resources during early larval development, however, have only been partially described in this thesis. Several areas in this early life history should be explored to both gain further insight into the resiliency of matches and mismatches in prey as well as understand the evolutionary success of these species.

Lipid and fatty acid profiles of eggs prior to the 1st developmental stage described would be useful for comparisons with other oviparous teleosts. Biochemical information on non-oviparous marine teleosts is limited making it difficult to compare lipid/fatty acid changes of *Sebastes* spp. with other fish species because developing sequences are not analogous. Developing egg sequences of *Sebastes* spp., despite being fertilized, would be more comparable to developing oviparous eggs from other marine teleosts. Detailed stage descriptions of *Sebastes* spp. egg sequences would have to be constructed *a priori*, however, since egg age cannot reliably be used in internal developers.

The metabolism of yolk and oil globule after parturition would also help determine critical periods for first-feeding in redfish larvae. Larvae began feeding prior

to day 5, shortly after yolk sacs were exhausted. However, some larvae were able to live well past this point to day 18 despite having no prey available. While this may be the result of metabolising alternative energy reserves (e.g. muscle or phospholipid), a more likely source of energy was the oil globule. Typically oil globules are rich in neutral lipids such as TAG, and these lipid reserves are often consumed later in development than yolk reserves (Wiegand 1996). Therefore, it would be interesting to investigate both the metabolism of lipid reserves after extrusion as well as the partitioning of these resources between yolk and oil globule.

Qualitative aspects of prey (e.g. size or nutritional content) would be an important area for further study. As shown in the feeding experiment, prey density requirements for redfish larvae are considerably lower than they are for Atlantic cod larvae. This may imply that larval redfish are less sensitive to mismatches in prey abundance than cod larvae. Qualitative mismatches in prey, as evidenced by Anderson (1994), may be more limiting to redfish survival than quantitative mismatches. Laboratory experiments would thus be useful in determining if 1) larval redfish show biased preference for prey of different quality offered at equal concentrations and 2) these choices subsequently affect growth and survival.

Lastly, our understanding of recruitment variability of redfish would greatly be improved with feeding experiments conducted during the juvenile stage. Weight changes after metamorphosis within the first year of life in marine fish can exceed 10^5 –fold for some species, suggesting that growth rate variability during this stage could significantly influence recruitment (Houde 1987). Like the early larval stage of redfish, information

on juvenile feeding dynamics is restricted to gut content analysis from a few field studies (Bainbridge and McKay 1968; Anderson 1994). How the early life foraging strategies of juvenile redfish change with changing prey availability would therefore be useful in our understanding of post-larval matches and mismatches in food availability.

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