LIPID BIOMARKERS AND ESSENTIAL FATTY ACIDS IN TROPHIC ECOLOGY AND NUTRITION OF AGE-0 GADIDS

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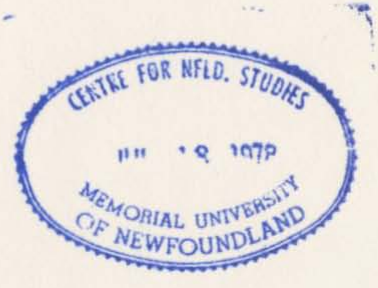
CENTRE FOR NFLD. STUDIES
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Lipid Biomarkers and Essential Fatty Acids in Trophic Ecology and Nutrition of Age-0 Gadids.

by

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

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ABSTRACT:

Over the last 30 years, lipids have been used to study patterns of energy flow and food web dynamics in cold and temperate marine ecosystems. I used fatty acids (FAs) and lipid classes to better understand the early survival, nutrition, habitat use and growth of age-0 Atlantic and Pacific gadids, both through laboratory and field approaches. Firstly, I used lipids to examine condition of age-0 juvenile Atlantic cod (*Gadus morhua*) as they settled in eelgrass (*Zostera marina*) nursery habitat. Cod reduced lipids per wet weight at the time of settlement, indicating that energy was directed towards rapid growth rather than storage for overwintering. Secondly, I used both FA biomarkers (FABMs) and compound specific carbon isotopes of FAs to show that increased terrestrial carbon entered the diet of juvenile cod during settlement. Higher dietary short chain polyunsaturated FAs (PUFA), coupled with low proportions of dietary essential FAs (EFAs) in the nearshore foodweb, indicated that the functional significance of eelgrass was refuge, and not elevated nutritional food quality. Thirdly, I conducted a laboratory experiment on the nutritional requirements of Pacific cod (*Gadus macrocephalus*) larvae to compare with literature values for Atlantic species. This comparative approach indicated that trends in larval nutrition could not be generalized across the family Gadidae. Pacific cod larvae grew fastest with diets containing high levels of n-3 PUFA, similarly to Atlantic species. Unlike Atlantic cod, however, Pacific cod larvae did not show elevated growth and survival with higher dietary proportions of DHA (22:6n-3) relative to EPA (20:5n-3). Fourthly, I investigated the rate of uptake of two C_{18} PUFAs in tissues of two species of Pacific juvenile gadids (Pacific cod, *Gadus macrocephalus* and

walleye Pollock, *Theragra chalcogramma)*. I examined how both biotic and abiotic factors influenced the rate of uptake in liver, flesh and heart tissues. C_{18} PUFAs showed high temporal sensitivity, and were evident in fish tissues after only one week of feeding. The differential uptake of these FABMs among tissues could represent a new tool to disentangle timing of offshore-inshore nursery migrations in juvenile fish. Throughout, this thesis I have shown that lipid classes, FABMs and EFAs can be successfully used to indicate critical trophic events during the early life history of gadid fish.

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My parents, Margaret and Douglas Copeman and my sister, Laura Copeman, gave me inspiration to continue. Their great work ethic and no nonsense advice were a driving force behind this thesis. The love, support, and childcare that they provided throughout this process were essential to a successful outcome.

I cannot thank my husband, Ben Laurel, enough! He provided endless support on a personal level, while on an academic level, his successful grant writing and academic collaboration were essential to the last two chapters of this thesis.

Finally thanks to my wonderful son, Samuel Laurel.

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid (20:4n-6)
ai-	*anteiso-*
AMPL	Acetone Mobile Polar Lipids
DHA	Docosahexaenoic Acid
EFAs	Essential Fatty Acids
EPA	Eicosapentaenoic acid (20:5n-3)
FABM	Fatty Acid Biomarkers
FAME	Fatty Acid Methyl Esters
FAs	Fatty Acids
FFA	Free Fatty Acids
GC	Gas Chromatography
GC-IRMS	Gas Chromatography Isotope Ratio Mass Spectrometry
HC	Aliphatic Hydrocarbons
i-	*iso-*
ME	Methyl Esters
NMID	Non-Methylene Interrupted Dienes
PL	Phospholipids
PUFA	Polyunsaturated Fatty Acids
ST	Sterols
TAG	Triacylglycerols

LIST OF ABBREVIATIONS

TLC	Thin Layer Chromatography
TLC-FID	Thin Layer Chromatography with Flame Ionization Detection
WE	Wax Esters

CHAPTER 1: General Introduction

For over eighty years scientists have searched for rules to explain patterns of energy flow in food webs (Elton 1927), reflecting the importance of trophic relationships in explaining patterns of ecosystem structure and function (Odum 1969). In marine fisheries, trophic ecology has played a central role in illuminating variable recruitment of commercially important species of fish. This was first stated as Hjort's "critical period hypothesis", in which he proposed that the survival of a year class of marine fish was determined in the early larval stage, shortly after yolk absorption (Hjort 1926). During this period, larvae must quickly find suitable nutrition or else face starvation or size-dependent mortality. Since then, the trophic links to recruitment success have expanded across multiple critical periods in development, including flexion, metamorphosis, settlement, and juvenile overwintering. All of these critical periods can occur within the first year of life, yet may account for more than 90% of the recruitment variability in a fish population (Houde 2008).

I have studied the trophic ecology of fish from the family Gadidae during two critical periods in their first year of life: first-feeding and settlement in the nearshore. First-feeding refers to the period during which larval fish transition from endogenous yolk reserves to exogenous feeding on zooplankton, while settlement refers to the transition of juvenile fish from a planktonic existence to a close association with benthic habitats (Levin 1994, Methvan & Bajdik 1994) The Family Gadidae, includes commercially important groups such as cod, haddock, whiting and pollock, with most species found in temperate waters of the northern hemisphere (Cohen 1998). There is a

long history of commercial exploitation of fish from this family, with the best known example resulting in the tragic collapse of Atlantic cod (*Gadus morhua)* stocks in the western North Atlantic (Rose 2007). Global annual capture rates for Atlantic cod climaxed in the late 1960s at 4,000,000 tonnes and collapsed in the early 1990s with global values today remaining under ~900,000 tonnes (FAO 2010). Pacific fisheries have shown the opposite trends in landings of walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*), which rank 1st and 2nd in groundfish biomass and product value annually (http://www.afsc.noaa.gov/species). Global landings in the 1960s were less than 2,000 tonnes for both species, whereas currently walleye pollock and Pacific cod account for 7,000,000 and 600,000 tonnes, respectively, on an annual basis (FAO 2010).

Atlantic cod is a well-studied species, with thousands of publications on aspects of their biology, ecology, and physiology in refereed scientific literature. However, there is still much uncertainty about their trophic ecology during the first year of life. Further, it is uncertain to what degree the physiology and biochemical processes in Atlantic cod can be compared to their closely related Pacific congeners. Like Atlantic cod, walleye pollock and Pacific cod spawn in the winter-spring and their pelagic larvae remain in the upper depths of the water column (Brodeur & Rugen 1994, Bradbury et al. 2008). However, the egg characteristics differ among all species (Atlantic cod=pelagic egg, pollock = bathypelagic, and Pacific cod = demersal; Laurel et al. 2010) and it is often the egg stage that defines early lipid and fatty acid (FA) nutritional requirements of the first-feeding larvae (Sargent 1989). Previous studies have shown that poor broodstock

nutrition can affect the fatty acid composition of eggs, hatching success and the nutritional status and survival of first-feeding larvae (Rainuzzo et al. 1997). Like Atlantic cod, Pacific cod larvae are thought to be transported shoreward to nursery areas (Rugen & Matarese 1988, Hurst et al. 2009), whereas the bulk of the walleye pollock juvenile populations likely remain pelagic in the offshore (Brodeur & Wilson 1996). Pelagic juvenile walleye pollock, as in Atlantic cod, occur in coastal regions in the summer and fall, where they prefer structural habitats such as kelps (*Laminaria* spp.) and seagrass (*Zostera marina*) beds (Laurel et al. 2007, Stoner et al. 2008). The subsequent behavior and distribution of older Pacific juvenile cod is unknown, including their use of coastal waters during the fall and subsequent year.

Lipids and fatty acids (FAs) play a vital role in tracing trophic links and understanding critical periods in fishes (Sargent et al. 1989, St. John & Lund 1996, Koussoroplis et al. 2010). They have a vast diversity in structure (Figures 1 & 2) and serve as catabolic substrates, structural apparatus in cellular membranes, and in the production of 'localized hormones' during development of marine fish (Sargent et al. 1999, Copeman et al. 2002, Tocher 2008). Lipids are considered to be a limiting aspect of prey quality for cold-water marine fish (Arts et al. 2001, Dalsgaard et al. 2003, Litzow et al. 2006) and are particularly limiting to growth and survival at the onset of first-feeding (Izquierdo 2006).

The diversity of lipid classes in marine food webs was reviewed in detail by Parrish (1988). However, marine gadids generally contain only three major lipid classes (Figure 1): triacylglycerols (TAG), sterols (ST) and phospholipids (PL, Copeman & Parrish

2004). TAG is generally the major storage lipid class in larval and juvenile gadids whereas PL and ST are important components of cellular membranes (Garcia et al. 2008). Recent studies showed that PL is also important as an energy source in eggs and larval fish (Evans et al. 1998, Laurel et al. 2010), although further research is required to understand lipid class metabolism in low-lipid juveniles such as Atlantic cod. Relative improvements in larval condition in species such as herring (*Clupea harengus*) and Atlantic cod have previously been attributed to elevated total lipid (TL), TAG per dry weight and TAG/ST ratios (Fraser 1989, Lochman et al. 1995).

During the early life history of many marine fish, storage of TAG is quite limited because most energy reserves are invested in growth in order to avoid early size-selective predation (Sogard 1997). However, around the time of settlement many marine species enter estuaries and other nearshore habitats and start to accumulate neutral lipid reserves, presumably in order to increase overwinter survival (Hurst 2007). Settlement in Atlantic cod represents a critical period, because they must successfully switch dietary carbon sources (i.e. marine pelagic to nearshore epibenthic) as well as foraging behaviors (Grant & Brown 1998). Juvenile Pacific and Atlantic gadids settle in nearshore regions where summer temperatures are warm and they can maintain growth, avoid predation, and have access to an abundant food supply (Grant & Brown 1998, Laurel et al. 2003, Laurel et al. 2007). Coastal nursery areas with eelgrass are essential to many juvenile fish and invertebrates, and also maintain bay-scale population structure in Atlantic cod juveniles (Orth et al. 1984, Bradbury et al. 2008). Despite the importance of this life history stage to gadid recruitment (Gregory et al. 2004), little is known about their trophic ecology and

bioenergetics during settlement into these nearshore essential habitats. Further, although eelgrass habitat is known to play an important role as a refuge from predation (Laurel et al. 2003), studies on the functional significance of biogenic habitat to the trophic ecology of juvenile gadids are lacking.

FAs are the major 'building blocks' of both storage (TAG) and structural (PL) lipids and they play an important role in the early nutrition of marine fishes (Figure 1 & 2). However, fishes and higher vertebrates have limited capacity to synthesize several essential FAs *de novo*, which has inspired both nutritional and biomarker studies (Dalsgaard et al. 2003, Budge et al. 2006). Specifically, the importance of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are widely studied in aquaculture in relation to growth, survival, pigmentation and metamorphosis in many marine species (Sargent et al. 1999, Copeman et al. 2002). However, much less is known about the nutrition and trophic relationships of gadids in the wild or from regions outside the Atlantic.

Budge et al. (2006) indicated three major uses for FA biomarkers (FABMs) in ecological trophic studies. Firstly, FABMs within a predator species can indicate changes in diet on a variety of spatial or temporal scales. Secondly, unique FABM can be used to demonstrate predator feeding upon a specific prey type (as indicated by the presence of a unique FABM). Thirdly, FABMs are useful in quantitative models of predator diet composition based on the FA composition of both predator and prey (Budge et al. 2006, Iverson 2009). Dalsgaard et al. (2003) stated that despite over 30 years of research on marine lipids, general application of the FABM approach to food web

relationships was still mostly qualitative and echoed Sargent's (1976) description of FA analyses as a 'blunt tool' for describing food web relationships. However, Iverson (2009) pointed to the utility of FAs as quantitative determinants of diet; with the caveat that detailed information is needed on both prey FA composition as well as the effects of predator metabolism on tissue FA deposition. In order to understand the observed patterns of FA deposition in predator tissues, detail knowledge of predator selective retention of specific FAs, *de novo* FA synthesis, as well as an understanding of tissue lipid/FA deposition patterns is required (Iverson 2009, Copeman et al. 2002). This information is most easily collected through controlled laboratory feeding experiments. Within this thesis, I have taken both laboratory and field approaches to further the understanding of juvenile gadid critical periods and trophic ecology, using both qualitative and quantitative FABM approaches.

I outline below, the chapter approach that I took to exploring the trophic ecology of age-0 Atlantic and Pacific gadids. I combined field and laboratory studies to look at the lipid requirements of gadids during two critical periods during the first year of life. I combined both lipid class and FA applications to investigate fish condition, trophic relationships, changes in sources of organic carbon, and to determine the rate of uptake of FABM in relation to both biotic and abiotic factors. These chapters provide the first investigations of Pacific gadid early lipid nutrition as well as the first use of FABM to determine trophic relationships during settlement in the nearshore.

In Chapter 2, I examined lipid class metabolism in age-0 Atlantic cod during their initial months in eelgrass habitat prior to the onset of winter. To do this, I measured

changes in storage of specific lipid classes and FAs while simultaneously characterizing the changes in the surrounding prey field of eelgrass habitat. Based on limited knowledge of cod physiology and juvenile cod-eelgrass relationships from behavioural studies (e.g., high sensitivity to predator-risk), I predicted that juvenile cod would preferentially metabolize lipids to maximize growth in a manner reflective of the available resources in their surrounding habitat. I discuss these results in the context of freshwater/estuarine systems where comparable data are available.

In Chapter 3, I examined lipid biomarkers (FABM and select compound specific isotopes) in the food web of juvenile cod settling in eelgrass beds during 2002. I used multiple lipid biomarker techniques to clarify the utility of Σ18:3n-3 &18:2n-2 as an indicator of terrestrial sourced carbon input into the diet of juvenile cod. I also investigated changes in essential lipids in zooplankton and correlated these proportions with levels of polyunsaturated fatty acids (PUFA) in cod flesh. These techniques allowed me to investigate the dietary sources of organic carbon utilized by juvenile Atlantic cod during settlement in eelgrass habitat.

In Chapter 4, I designed a laboratory experiment to examine the first-feeding EFA nutrition of Pacific cod larvae. Specifically, I investigated how changes in DHA to EPA dietary proportions affected Pacific cod growth and survival. Pacific cod were chosen because 1) they are highly abundant and play an important functional role in predator-prey dynamics in the North Pacific (Hunt et al. 2002) and 2) they offer an interesting comparison with their well-studied congener, Atlantic cod. I tested two hypotheses: 1) whether energetically similar diets comprised of varying levels of EPA and DHA

impacted size-at-age and survival in Pacific cod larvae, and 2) whether the highest levels of DHA:EPA (e.g., 2:1) are optimal for marine fish larvae in the Pacific as has been shown for Atlantic species. I discuss these experimental results in relation to natural variation in the lipid/FA composition of prey in the North Pacific.

In Chapter 5, I examined how and whether the uptake rate of nearshore FABMs (Σ18:3n-3 & 18:2n-6) in Pacific juvenile gadids changed as a function of biotic and abiotic factors. To parameterize the effect of species and temperature on the uptake of FABMs in gadids I fed two different gel food diets to walleye pollock and Pacific cod juveniles over an 8-wk feeding trail. Diets were identical except that one was enriched with marine oil (cod liver) whereas the other was enriched with terrestrial plant oil (flax seed). I tested the following hypotheses: 1) the proportion of FABM in the tissues of juvenile gadids is species-specific, 2) temperature mediates the rate of FABM uptake in gadid tissues, 3) the proportion of FABM uptake is specific to tissue type (liver, flesh, heart) and 4) the ratios of nearshore markers in different tissue types (i.e. % FABM in liver : % FABM in flesh) show temporal trends that could be applied to field data in order to show residency times in the nearshore. Quantification of the rate of uptake of these FAs in laboratory studies on juvenile gadids is a necessary prerequisite to the application of the FABM approach to field studies that aim to assign residency times within the nearshore.

In Chapter 6, I summarize the results of this work and provide implications for juvenile gadid feeding ecology and the utility of the FABM approach in elucidating food web linkages.

THESIS OBJECTIVES

The specific thesis objectives were to:

1. Examine the importance of lipid classes and FAs to the growth and survival of age-0 gadids during two critical periods: settlement and first-feeding
2. Examine changes in lipid class metabolism in relation to condition of age-0 Atlantic cod during settlement in the nearshore.
3. Use lipid classes, FAs, and compound specific isotopes to examine the functional significance of eelgrass habitat to juvenile Atlantic cod during settlement.
4. Investigate the importance of dietary EFAs to the size-at-age, survival, and condition of first-feeding Pacific cod larvae
5. Compare the EFA requirements of Pacific cod larvae to better-studied Atlantic cod larvae
6. Investigate the abiotic and biotic factors that affect the rate of uptake of nearshore FABM in two juvenile Pacific gadids
7. Explore the use of differential rates of FABM uptake in fish tissues to questions of residency times within the nearshore.

CO-AUTHORSHIP STATEMENT

I am the first author on all of the manuscripts produced from this thesis. I identified the research questions and designed and performed all experimental and field sampling protocols. I performed the laboratory experiments (Chapters 4 and 5) as well as the field collections (Chapters 2 to 3). I completed all data analyses and manuscript preparations.

Dr. Chris Parrish is a co-author on the manuscripts that were produced from all chapters except Chapter 4. On co-authored papers, Dr. Parrish helped with identification of research ideas, manuscript preparation, and financial support. Dr. Parrish performed the GC-IRMS analysis for compound specific isotopes in Chapter 3.

Dr. Ben Laurel is a co-author on the manuscripts that were produced from Chapters 4 and 5. He provided full financial support for the completion of these chapters. He also helped to develop research questions and aided in the review of manuscripts.

Jeanette Wells is a co-author on the manuscripts that were produced from Chapters 2 and 3. She helped significantly with the lipid class and FA laboratory analysis and also performed select extractions for these chapters during my absence for maternity leave.

Dr. Gregory is a co-author on the manuscripts that were produced from Chapters 2 and 3. He helped with identification of these research ideas and manuscript preparation. Dr. Gregory also provided financial support of the field programs described in these chapters.

Dr. Robyn Jamieson is a co-author on the manuscript that was produced from Chapters 3. She aided significantly with the development of ideas and completion of the field work.

Dr. Michael Whiticar is a co-author on the manuscript that was produced from Chapter 3. He provided laboratory access for the completion of the GC-IRMS analysis at University of Victoria, BC, Canada.

The publications produced from this thesis include:

Chapter 2: Copeman LA, Parrish CC, Gregory RS, Wells J (2008) Decreased lipid storage in juvenile Atlantic cod (*Gadus morhua*) during settlement in cold-water eelgrass habitat. Marine Biology 154(5): 823-832

Chapter 3: Copeman LA, Parrish CC, Gregory RS, Jamieson RE, Wells J, Whiticar MJ (2009) Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*. Marine Ecology Progress Series 386: 237-251

Chapter 4: Copeman LA, Laurel BJ (2010) Experimental evidence of fatty acid limited growth and survival in Pacific cod (*Gadus macrocephalus*) larvae. Accepted in Marine Ecology Progress Series

Chapter 5: Copeman LA, Parrish CC, Laurel BJ. Rate of uptake of terrestrial fatty acid biomarkers in juvenile gadids as a function of temperature and species. Target Journal is Canadian Journal of Fisheries and Aquatic Sciences.

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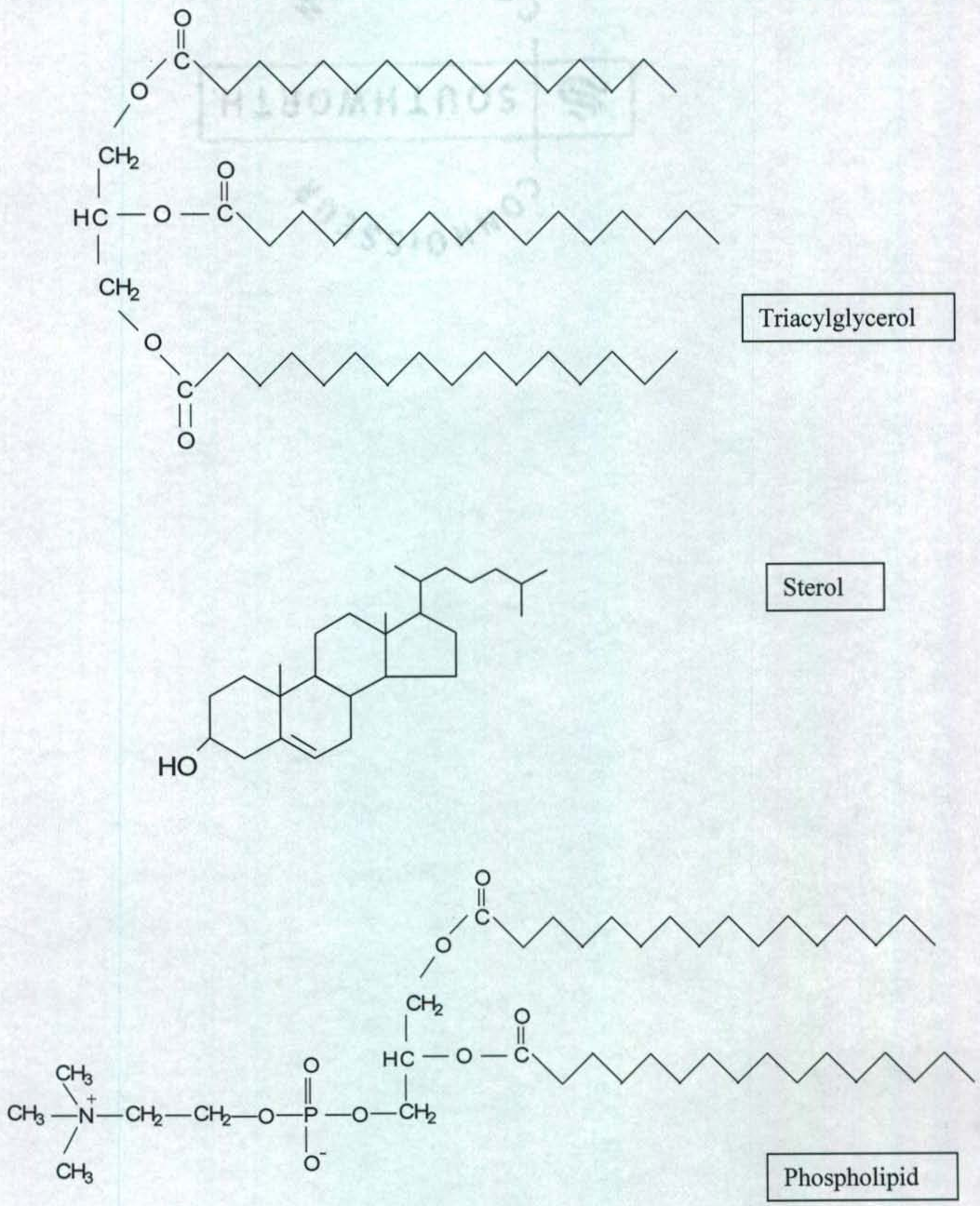

Figure 1: Structure of the three major lipid classes found in marine gadids.

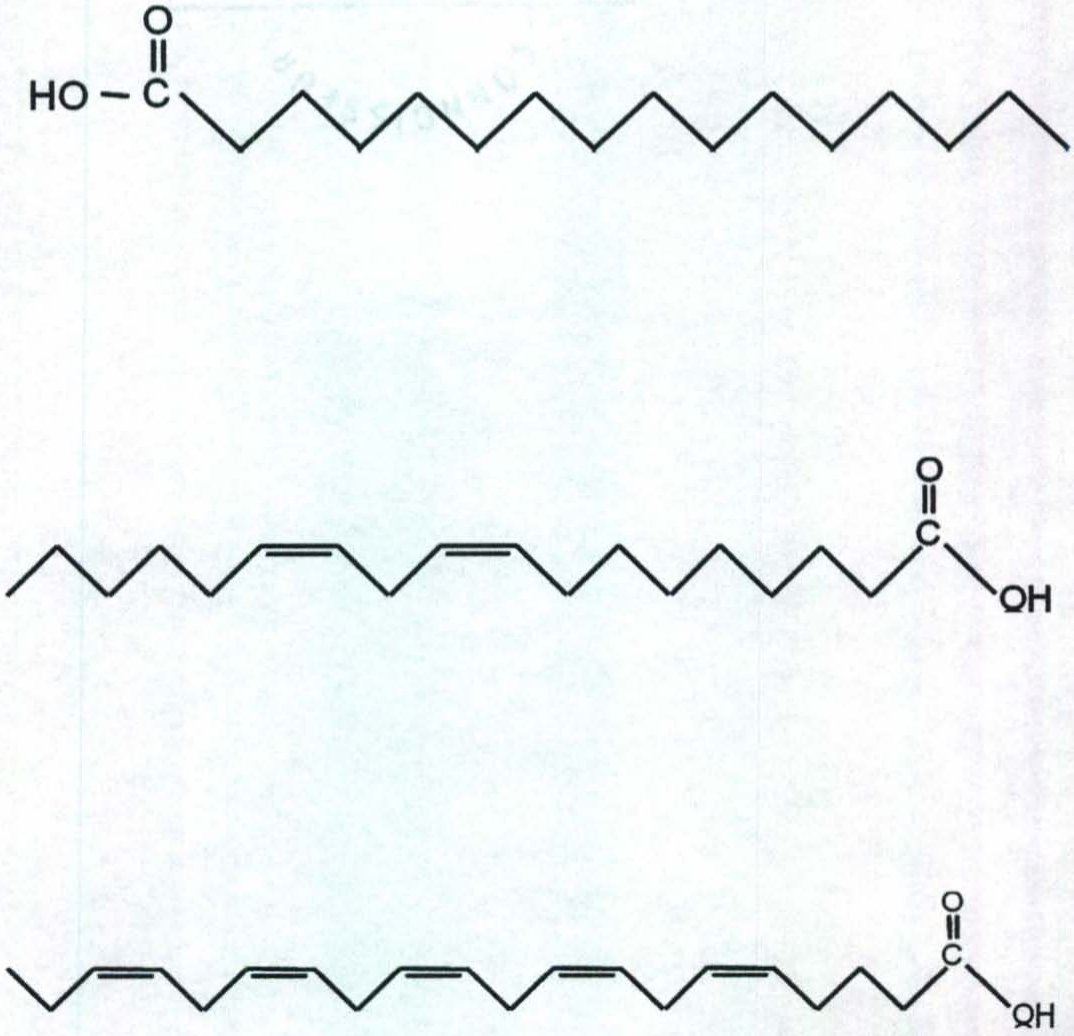

Figure 2: Representative structure of a saturated (16:0) and polyunsaturated (18:2n-6, 20:5n-3) fatty acids. FAs are named using shorthand notation of A:Bn-X, where A indicates the number of carbon atoms, B is the number of double bonds and X indicates the position of the first double bond relative to the terminal methyl group.

CHAPTER 2: Decreased Lipid Storage in Juvenile Atlantic Cod (*Gadus morhua*) During Settlement in Cold-Water Eelgrass Habitat.

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ABSTRACT:

I characterized the prey field and the lipid classes/fatty acids (FAs) in the flesh of age-0 juvenile cod (*Gadus morhua*) during their late-summer/fall arrival and settlement into eelgrass (*Zostera marina*) in coastal Newfoundland. Examination of available prey demonstrated a high abundance of small zooplankton (*Acartia, Microsetella* and *Oithona* sp.) with no larger *Calanus* sp. prey. Breakpoint analysis showed significant changes in the accumulation of relative (mg. g^{-1} wet weight) and absolute (μg. $fish^{-1}$) amounts of lipid with standard length at the time of settlement (~60 mm standard length). Settling juvenile cod showed an alternate lipid utilization strategy where they catabolized phospholipids (PL) to a greater extent than triacylgylcerols (TAG). Polyunsaturated FAs (PUFA) content in cod flesh decreased as fish grew indicating that nearshore zooplankton quality was not optimal for PL formation. The dramatic reduction in cod PL was likely due to both catabolism of muscle and a lack of dietary PUFA suitable for PL synthesis. However, juvenile cod continued to grow, leading to decreased lipid stores and suggesting that cod settling into eelgrass are under intense selection pressure for growth prior to the onset of winter. These data contrast better-studied freshwater and estuarine systems in which lipid storage is critical for successful overwintering.

INTRODUCTION:

Stocks of Atlantic cod (*Gadus morhua*) collapsed off the coast of Canada in the early 1990s and despite a commercial fishing moratorium (Myers et al. 1996) there has been no significant population recovery (Lilly et al. 2003). In 2003, the Newfoundland and Labrador stock was termed endangered by the Committee on the Status of Endangered Wildlife in Canada, which emphasized the need for further understanding of factors affecting recruitment (COSEWIC, 2003). Currently, inshore aggregations represent the bulk of the remaining cod biomass (Hutchings 1996, Lawson & Rose 2000) and there has been increased interest in the nursery role of eelgrass habitat (*Zostera marina*) for this species (Gregory & Anderson 1997, Laurel et al. 2003). Recently, the abundance of juveniles in nearshore eelgrass habitat has been used as a predictor of the relative strength of adjacent year classes (Gregory et al. 2004). Eelgrass areas have also been deemed necessary for the persistence of bay-scale populations of cod in Newfoundland (Bradbury et al. 2008). Therefore, factors affecting the vital rates of juvenile cod in these nearshore habitats are critical to an understanding of recruitment dynamics in this species.

For juvenile fish living in temperate climates, patterns of energy storage are driven by the interaction between the threat of over-wintering starvation and predation (Post & Parkinson 2001, Hurst & Conover 2003). Most of these studies have been based in freshwater or estuarine systems and have shown a significant increase in lipid prior to the onset of winter (Griffiths & Kirkwood 1995, Hurst & Conover 2003). This increase is generally viewed as an adaptive response to prevent winter starvation. However, there

is some evidence that juvenile cod begin to reduce their condition and catabolize lipid prior to winter, in the fall just following settlement (Grant & Brown 1999). Settlement in flatfish has been shown to correlate with a decrease in condition (Christensen & Korsgaard 1999). However, cod continue to increase in body length at this time (Grant & Brown 1999), suggesting that predation pressure may be a more important influence on mortality than the risk of starvation following settlement. Previous work on juvenile cod in coastal eelgrass habitats has indicated significant levels of predation by both larger conspecifics and other fish species (Linehan et al. 2001, Laurel et al. 2006).

Adult cod store energy as lipid in the liver (~24% of wet weight) and have low fat flesh (~0.5% of wet weight). Specifically, the majority of lipid is stored as triacylglycerols (TAG) in the liver, while lipid in the flesh is mostly phospholipids, PL (Copeman & Parrish 2004). During times of reduced food availability, adult cod utilize TAG from the liver first and then protein reserves from muscle (Black & Love 1986). In contrast, wild juvenile cod are generally low in lipid (1.5-2.2 % of wet mass), and approximately 50% of the lipid present is PL, which is usually considered to be structural. PL along with sterols (ST) form the vital lipid component of cell membranes (Ackman 1989). However, PL have been shown to be used as metabolic fuel, particularly in lipid-limiting situations such as during egg or early larval development (Tocher et al. 1985, Laurel et al. 2010). No detailed lipid class analyses have been conducted during the time of settlement in juvenile cod. Further, given that juvenile cod have an intermediate lipid profile, that does not mirror the adult stage, it is likely that they use an alternate energy and resource allocation strategy. Juvenile fish likely utilize PL as

a source of energy which more closely resembles the pattern found in larval/egg stages of development.

Marine fish generally cannot synthesize long-chain polyunsaturated FAs (PUFAs) from shorter chain precursors and therefore must rely on dietary input for normal physiological function especially in cold temperatures (Sargent 1995). PUFAs are formed in primary producers and are transferred and concentrated as they move throughout the food web (Copeman & Parrish 2003, Budge et al. 2001). Specifically the long chain PUFA, 22:6n-3 (DHA) has been found to be essential for marine fish larvae (Copeman et al. 2002, Bell et al. 2003) and is found in high levels in the PLs of Atlantic cod (Jangaard et al. 1967). Conversely, elevated dietary levels of shorter chain PUFAs such as 18:3n-3 and 18:2n-6 have been correlated with negative effects on growth and survival in marine larvae (Sargent et al. 1999). These shorter chain PUFAs are characteristic of terrestrial plant oils and can compete with 22:6n-3 for the transacylases and acylases that esterify FAs onto PL backbones. High relative levels of short chain PUFA have previously been reported in the near shore (Budge et al. 2001) and they may have a negative impact on food quality available to juvenile cod at settlement.

The purpose of this study was to qualitatively examine lipid metabolism in age-0 Atlantic cod during their initial months in eelgrass habitat prior to the onset of winter. To do this, I measured changes in storage of specific lipid classes/FAs while simultaneously characterizing the changes in the surrounding prey field of eelgrass habitat. Based on the limited understanding of cod physiology and juvenile cod-eelgrass relationships from behavioural studies (i.e., high sensitivity to predator-risk), I predicted that juvenile cod

would preferentially metabolize lipids to maximize growth in a manner reflective of the available resources in their surrounding habitat. Results are discussed in the context of freshwater/estuarine systems where comparable data are available.

MATERIALS & METHODS:

Field collections

Samples of fish and zooplankton were collected at two eelgrass sites during four sampling periods in the late summer and fall of 2002 (Table 1, Figure 1). In November, more extensive sampling included animals from both inner and outer sound locations (Table 1). This extensive sampling of fish in November was done opportunistically as part of a larger study on the effects of diet quality on juvenile cod condition.

Sampling for this study was conducted in Newman Sound, a small protected fjord located in Bonavista Bay off the NE coast of Newfoundland. Age-0 pelagic juvenile cod enter eelgrass areas in Newman Sound as a series of recruitment pulses, starting in late July and ending in November (Gregory et al. 2004, Grant & Brown, 1998b). Juvenile cod were collected during this period using a 25 m demersal seine net deployed 55 m from shore using a small boat (Table 1). Net hauls were retrieved by two people standing along the shoreline 16 m apart. Fish were unloaded into plastic tubs filled with seawater and were identified, enumerated and measured. Juvenile cod were considered 'pre-settled' at less than 60 mm and 'post-settled' at greater than 60 mm as established by Methven & Bajdik (1994) and Laurel et al. (2003). Fish were placed on ice immediately and frozen within 3 hr of sampling.

Zooplankton samples were collected monthly at a depth of 1 m above the eelgrass canopy using a Jabsco Vane Puppy Reversible 12 Volt Pump (Model 18680) to examine zooplankton species composition. Samples were collected over a 2.0 minute period and an average of 27.2 L of seawater was collected and directly filtered onto a 222 μm mesh sieve and backwashed into collection jars. Duplicate samples were collected at Dockside and Mistaken Cove sites in Newman Sound during the four sampling periods. Samples were preserved in 5% formalin in seawater, buffered with borax. Identification of whole samples was made using dichotomous keys developed for the purpose at the Ocean Sciences Centre, Memorial University of Newfoundland.

Lipid analyses

Prior to lipid sampling, standard length (± 0.1 mm), body depth (± 0.1 mm), and wet weight (±0.0001g) were recorded. Fish intestinal tracts were removed and fish were washed with filtered seawater, blotted dry, weighed and stored in chloroform under nitrogen until extraction. A condition index – Fulton's K – was calculated using, $K= 100W/L^3$, where K was the condition factor, W was the mass (g), and L was the length (mmSL) (Ricker 1975, Grant & Brown 1999). A comparison of condition indices based on hepatosomatic index, dry eviscerated, and ash free dry eviscerated body weight have been shown to be more reliable that those based on wet weight (Grant & Brown, 1999). These condition indices respond rapidly (1 to 2 weeks) to changes in food intake. Condition based on wet weight of pre-frozen fish is susceptible to water loss during the sampling procedure and are less sensitive. However, this index has been found to be

biologically meaningful for both juvenile (Grant & Brown, 1999) and adult cod (Lambert & Dutil, 1997). Here I was required to use a wet weight condition factor because of my lipid extraction protocol.

Lipids were extracted in chloroform/methanol according to Parrish (1987) using a modified Folch procedure (Folch et al. 1957). Lipid classes were determined using thin layer chromatography with flame ionisation detection (TLC/FID) with a MARK V Iatroscan (Iatron Labratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three stage development system was used to separate lipid classes. The first separation consisted of 20-min developments in 99:1:0.05 hexane:diethyl ether:formic acid. The second separation consisted of a 40-min development in 80:20:1 hexane:diethyl ether:formic acid. The last separation consisted of 15-min developments in 100 % acetone followed by 10-min developments in 5:4:1 chloroform:methanol:water. After each separation, the rods were scanned and the 3 chromatograms were combined using T-data scan software (RSS Inc., Bemis, TN, USA). The signal detected in millivolts was quantified using lipid standards (Sigma, St. Louis, MO, USA). Lipid classes were expressed both in relative ($mg.g^{-1}$ wet weight) and absolute amounts ($\mu g.animal^{-1}$).

TL was analysed for FA composition. FA methyl esters (FAME) were prepared by transesterification with 10% BF_3 in methanol at 85°C for one hour (Morrison & Smith 1964, Budge 1999). A Varian model 3400 GC equipped with a Varian 8100 autosampler was used for FA analysis (Varian, CA, USA). The column was an Omegawax 320 column, 30 m, 0.32 mm i.d., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA).

Hydrogen was used as the carrier gas and the flow rate was set at 2 ml min^{-1}. The column temperature profile was: 65°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C min^{-1}, and hold at 220°C for 0.75 min after ramping at 2°C min^{-1}. The injector temperature increased from 150 to 250°C at 200°C min^{-1}. Peaks were detected by flame ionisation with the detector held at 260°C. FA peaks were integrated using Varian Star Chromatography Software (version 4.02) and identification was made with reference to known standards (PUFA 1 and 37 Component FAME Mix, Supelco Canada, ON).

Data Analysis

I used linear regression approaches to describe the relationships between fish length and lipid composition. In addition to linear relationships, I examined the data for evidence of an ontogenetic shift in lipid characteristics by fitting bi-phasic regressions. I used a piece-wise nonlinear fitting algorithm to test the hypothesis that the relationship between lipid composition and length was better explained by two linear segments rather than a single linear relationship (Statistica™ non-linear regression; Post & Parkinson 2001). This analysis provided best fit estimates of the slopes and intercept plus the value of the inflection point in the biphasic relationship (Breakpoint).

The following model was used to test the null hypothesis that $b_2 = 0$:

Lipid parameter = $b_0 + (b_1 * \text{length}) + ((b_2 * (\text{length}-\text{break})) * (\text{length}>\text{break}))$ where (b_0) is the intercept, (b_1) is the slope before break, and (b_2) is the change in slope after break. This hypothesis tests whether a single linear relationship best represents the data and if the hypothesis is rejected ($p<0.05$) there is a significant variable break. 'Break' is the

point at which there is a change between the scaling of lipid composition with length; with segment one representing small fish and segment two larger fish. When the break-point model did not fit the data I used a simple linear regression in Minitab version 10.1 to determine the relationship between other lipid parameters and standard length.

RESULTS:

Figure 2 shows the percentage abundance of zooplankton sampled at two eelgrass sites in the inner Newman Sound (Dockside and Mistaken Cove) during the late summer and fall of 2002. *Acartia* sp., bivalve veligers, *Microsetella* sp. and *Oithona* sp. made up at least ~80% of the zooplankton identified in pumped samples at both eelgrass sites, during August, September, and October. In November, a greater proportion of the sample (23-37%) consisted of *Temora* sp. and no calanoid copepods were identified in any of the samples.

The length frequency distribution of juvenile cod captured in Newman Sound throughout 2002 indicated that three different age classes were present in shallow water – age-0, 1, and 2 year old fish based on established length-at-age data (Gregory et al. 2004). The fish collected for lipid analysis were restricted to 40 -84 mmSL during the late summer and fall, ensuring all fish analyzed were the age-0 cod. However, the continued arrival of age-0 fish throughout the season did not restrict the sampling of small fish to one sampling period (Figure 3). Pre-settled juveniles were present on all sampling dates throughout the period, but settled individuals greater than 60 mmSL were only present later in October and November. I did not observe any increase in the size of juveniles

that were sampled for lipid analyses between August and September indicating that the first recruitment pulse of juvenile fish in 2002 was only sampled in August, and probably failed to contribute substantively to subsequent age groups, compared to subsequent recruitment pulses that year (Figure 3).

An analysis of the change in Condition (Fulton's K) with standard length (mm) of juvenile fish was described by the following linear regression: Condition (Fulton's K) = 0.814 + 0.00238*SL (mm), $p<0.05$, $r^2=0.07$, n=80. Although a significant relationship existed between condition factor and length, a very low value of the coefficient of determination was found ($r^2=0.07$). Therefore, this relationship was not considered to be biologically meaningful (Lloret & Ratz 2000). The assumption of isometric growth associated with the use of Fulton's K was respected, as the slope of the regression between log weight (g) and log length (mm) was approximately three (3.15; log weight = - 5.29 + 3.15 log length, $p<0.0001$, $r^2=0.97$, n=80).

The relationship between absolute lipids per animal ($\mu g.animal^{-1}$) and length (mmSL) was variable, and depended on lipid class (Figure 4). Results from breakpoint analysis indicated that STs (61.9, $p<0.01$), PLs (60.4, $p<0.001$) and TLs (66.2, $p<0.001$) showed significant breakpoints (Figure 4, Table 2). However, TAG was better described by the following linear regression:

$\mu g\ TAG.individual^{-1} = - 17197 + 439*$ length mm ($p<0.0001$, $r^2=0.60$)

The change in mass of lipids per wet weight with standard length showed a variable pattern depending on lipid class (Figure 5). Both TL and TAG showed a significant biphasic relationship with length (Table 3). A significant breakpoint was

found at 59 mm for TL while a significant breakpoint of 64 mm was found for TAG. After the breakpoint, a significant decrease in the slope (-.30 for TAG and -.53 for TL) was observed indicating that in larger fish, the relative amount of TL and TAG decreased with size. This biphasic relationship for TL was likely driven by the observed relationship for TAG as a biphasic relationship was not observed for ST or PL. Rather the decrease in the concentration of these lipid classes with size was better described using a simple linear regression (Figure 5, Table 4).

I examined the relationship between C_{18} PUFA and longer chain 22:6n-3 with length. These FA were chosen as they are known to compete for transacylases that are used in esterification of FA onto PL backbones. PL are essential to normal membrane structure and function. The regression of short chain C_{18} PUFA (18:2n-6+18:3n-3+18:4n-3) and the long chain PUFA (22:6n-3, DHA) and length showed opposite relationships. Proportions of C_{18} PUFA increased with length (r^2 =0.40, $p<0.01$) while levels of 22:6n-3 showed a decrease with length (r^2 =0.22, $p<0.01$, Figure 6).

DISCUSSION:

Previous studies on lipid dynamics in juvenile fish have mostly investigated freshwater and estuarine species (e.g., Fullerton et al. 2000), which are generally higher in lipid and may face different constraints than low-fat marine species. The post-settlement juvenile period in cod, like other marine fishes, represents a period of high growth and changing predator fields (Sogard 1997). Ontogenetic changes impose constraints on where and what food resources fish will have access to and can

subsequently feed upon. In this study, I interpret the changes in lipid composition of juvenile cod to be reflective of the trade-offs in growth (i.e., predation avoidance), prey availability and overwintering success.

Small juvenile fish in north temperate climates have competing demands for energy usage prior to the onset of winter (Hurst 2007). In both freshwater and marine systems, juvenile fish generally have a higher metabolic rate than larger conspecifics and are also faced with high rates of piscivory. In freshwater fish and estuarine systems, studies have shown that accumulating energy stores in the form of lipids is also necessary to reduce susceptibility to over-winter starvation mortality (Thompson et al. 1991, Pangle et al. 2004). However, in my study, I found that juvenile cod did not increase in condition or relative amounts of lipid during the late summer and early fall as would be predicted from these freshwater/estuarine studies. Instead juvenile cod: 1) grew after settlement with no concomitant increase in absolute amounts of lipid; 2) decreased in the relative amounts of both neutral and polar lipid reserves during this growth period; and 3) decreased in relative amounts of long chain PUFAs while shorter chain PUFAs increased. I interpret these patterns as the result of decreased late-summer food quality (discussed below) coupled with high selection pressure for growth.

The zooplankton community at my sites in Newman Sound was typical of shallow coastal areas elsewhere in Newfoundland. Zooplankton in Newman Sound was dominated by *Acartia* sp. bivalve veligers, *Microsetella* and *Oithona* sp. which comprised at least ~80% of the zooplankton identified in pumped samples. In November, a greater proportion of the samples (23-37%) were *Temora* sp., which is in agreement with

previous work in coastal embayments in Newfoundland (Grant & Brown 1998a). Grant and Brown (1998a) found a significant increase in the relative importance of *Temora* sp. in the diet of cod during the late fall with levels increasing to 48% of identified prey items in December. Interestingly, no *Calanus* sp. were identified in any of the samples taken from Newman Sound in this study. The importance of *Calanus* copepods to the diet of juvenile cod have previously been noted by Grant & Brown (1998a), although, the *Calanus* copepods were only present in the diet of juvenile cod in October (21.5% relative importance) and November (5.8%) in a September-December survey. *Calanus* copepods form large lipid globules comprised of neutral wax-esters that are easily digested and provide high amounts of energy for juvenile fish (Sargent 1999). Evjemo et al. (2004) described the lipid composition of nearshore marine copepods used for feeding larval fish. In that study, the relative lipid composition (% dry mass) of *Calanus* (~10% in April to a high to > 20% in June and July) was higher than both *Eurytemora* sp. (~10%) and *Temora* (~10%). Net tows in Newman Sound showed that small zooplankton (80-220μm) had a lipid composition of only 1.8-3.8% dry mass while larger zooplankton contained 2.9-4.5% (Chapter 3, Copeman et al. 2009). Grant and Brown (1999) also noted a decrease in condition when larger *Calanus* sp. disappeared from the diet. Together, these data suggest that food quality in Newman Sound during 2002 could have been sub-optimal.

Generally, body condition in cod (i.e., Fulton's K in this case) reflects the storage of energy under "surplus food conditions" and the reduction in energy storage under "replete food conditions" (Love 1970, Lambert & Dutil 1997). Previous studies indicate

adult Atlantic cod exhibit a seasonal pattern of growth and condition (Jangaard et al. 1966). Here I found that juvenile age-0 cod had a Fulton's Condition Index that ranged from a low of ~0.7 to a high of ~1.2 with an average of 0.95 ± 0.1. These results are within the range previously reported for Trinity Bay juveniles (~0.95 to 1.05) during the late summer and fall and are well above the predetermined lethal limit (~0.63, Grant & Brown 1999).

At ~60 mm (i.e., 'settlement'), there was a significant shift in the deposition of PL, ST and TL. Juveniles <60 mm increased with length in the absolute amount ($\mu g.fish^{-1}$) of all lipid classes examined, but the rate of lipid accumulation either stopped or decreased after this size. However, absolute TAG deposition continued to trend upward and did not show a significant change in slope after 60 mm SL (Figure 4, Table 2). Still, despite the analysis's inability to detect a significant breakpoint between TAG ($\mu g.animal^{-1}$) and fish size, the same analysis on a log-log scale indicated that the rate of TAG deposition was less than isometric. Further, when this relationship was expressed per wet weight the relative amounts of TAG also decreased after settlement albeit to a much lesser extent than PL.

Although liver and flesh were not analyzed separately, it is probable that the bulk of the decrease in PL, ST, and TL after 60 mm was due to a reduction in lipid in the muscle, while the continued increase in absolute amounts of TAG was likely in the liver. Prior examination of lipid classes in adult cod muscle and liver have shown that the majority of the lipid in the liver is TAG (69% of TLs) while the bulk of the lipid in the muscle is PL (55%); ST account for 13% and 3% of the lipids in the muscle and liver,

respectively (Copeman & Parrish, 2004). Therefore, ST and PL are generally considered to be structural components of the muscle with TAG viewed as a storage component in the liver. Based on this understanding and the findings from this study, it appears that PL was being utilized more quickly than TAG and this catabolism was likely due to a reduction in membrane density during protein and PL utilization in the muscle.

This low absolute increase in TAG with a decrease in absolute amounts of PL can likely be explained by previously noted changes in food quality late in the fall. The relationship between fish size and FAs in the flesh of juvenile cod were negative with DHA but positive with shorter chain C_{18} PUFA (18:2n-6 + 18:3n-3 + 18:4n-3). Previous studies on organisms living at a range of latitudes have found that fish and bivalves living in cold-water environments have increased levels of highly unsaturated FAs in their flesh (Dunstan et al. 1999, Hall and Parrish 2000). This level of membrane specificity for DHA in lean fish is necessary so that they can maintain cell membrane fluidity and function at cold temperatures. Further, the neutral and polar lipids in larval and juvenile fish have been shown to maintain high levels of DHA in the polar lipids even when fed a diet of lower nutritional quality (Copeman et al. 2002). Therefore, the counter-intuitive pattern of TAG storage and PL reduction after settlement likely reflects both food quality which is unsuitable for PL formation, and a reduction in absolute amounts of polar lipid associated with catabolism of muscle.

CONCLUSIONS:

The examination of lipid classes and selected FAs in age-0 juvenile Atlantic cod revealed a reduction in energy storage that was not evident in the condition factor based on wet weight. The accumulation and catabolism of lipids were lipid class- and fish size-dependent. The absolute amount of TAG in juvenile cod did not show a significant breakpoint at settlement (i.e., 60 mm SL) like other lipid classes, but, reduced deposition of TAG was evident on a relative scale following this period. Further, the log-log relationship of absolute amounts of all lipid classes with length revealed that storage was less than isometric after settlement, indicating that the water content of the muscle was increasing. This increase led to a reduction in the relative amounts of all lipid classes in juvenile cod after settlement. The preferential storage of TAG and decreased proportions of DHA indicate that the nutritional quality in eelgrass was likely not optimal. Increased size with decreased energetic reserves after settlement, suggest that piscivory could be a factor driving energy allocation in juvenile cod. Further, decreased nutritional condition following settlement may indicate that the functional significance of eelgrass habitat is not elevated food quality but rather increased refuge.

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Table 1: Sampling location and dates for zooplankton and cod collections within Terra Nova National Park, Bonavista Bay, Newfoundland Canada, 2002.

Date	Location	# Zooplankton ID samples	# Fish Samples
August 29 to 31, 2002	Dockside (DS)	2	7
	Mistaken (MC)	2	5
September 10 to 12, 2002	Dockside (DS)	2	8
	Mistaken (MC)	2	6
October 03 to 04, 2002	Dockside (DS)	2	10
	Mistaken (MC)	2	10
November 01 to 08, 2002	Dockside (DS)	2	2
	Mistaken (MC)	2	13
	South Broad Cove (SB)	0	2
	Newbridge (NB)	0	5
	Little south Broad Cove (LSB)	0	7
	Minchin's Cove (MI)	0	4
	Heffern's Cove (HC)	0	3
	Mt Stanford (MS)	0	1

Table 2: Results of piecewise regression for the relationship between absolute amounts (μg /fish) of (a) ST, (b) PL and (c) TL on length of age-0 Atlantic cod from Newman Sound during the late summer and fall of 2002. A breakpoint model could not be fit to the relationship between TAG and length.

Model: Lipid parameter = $b_0 + (b_1 * \text{length}) + ((b_2 * (\text{length}-\text{break})) * (\text{length}>\text{break}))$

	ST		PL		TL	
	Estimate	P-value	Estimate	p-value	Estimate	p-value
Intercept (b_0)	-2966.5	<0.01	-14322.6	0.1	-44762.2	<0.0001
Slope before break (b_1)	96.70	<0.0001	476.3	0.004	1297.8	<0.0001
Change in slope after break (b_2)	-98.30	<0.0001	-657.32	<0.001	-1359.0	0.0002
Segment 2 slope (Calculated change)	-1.6	-	-181.02	-	-61.2	-
Break	61.87	<0.0001	60.37	<0.001	66.2	<0.0001
Variance Explained by the model r^2.	69%		54%		55%	

Table 3: Results of piecewise regression for the relationship between relative amounts (mg/g wet weight) of (a) TAGs, (b) TLs on length of age-0 Atlantic cod from Newman Sound during the late summer and fall of 2002. A breakpoint model could not be fit to the relationship between PLs and STs with length.

Model: Lipid parameter = $b_0 + (b_1 * \text{length}) + ((b_2 * (\text{length}-\text{break})) * (\text{length}>\text{break}))$

	TAGs		TL	
	Estimate	p-value	Estimate	p-value
Intercept (b_0)	-4.1	0.09	14.7	0.009
Slope before break (b_1)	0.16	0.05	0.08	0.45
Change in slope after break (b_2)	-0.30	<0.09	-0.60	<0.001
Segment 2 slope (Calculated change)	-0.14	-	-0.52	-
Break	62.38	<0.001	59.00	<0.001
Variance Explained by the model r^2,	0.22		0.44	

Table 4: Results from linear regression of ST and PL (mg/g wet weight) with length in age-0 Atlantic cod from Newman Sound during the late summer and fall of 2002.

	Intercept	Slope	R^2	p-value
STs	3.5	-0.03	0.54	<0.001
PLs	19.2	-0.20	0.54	<0.001

FIGURE CAPTIONS:

Figure 1: Map of Newman Sound in Terra Nova National Park showing sampling sites for collection of fish and zooplankton in 2002.

Figure 2: Percentage abundance of zooplankton at two eelgrass sites in Terra Nova National Park during the late Summer and fall of 2002

Figure 3: Standard length (mmSL) of age-0 Atlantic Cod captured by beach seine in Newman Sound, Bonavista Bay in Newfoundland. Shown are both size ranges of total animals captured (n=530) as well as those used for lipid analysis in this study (n=83). P1, P2, P3 indicate the categorical periods of the sequential recruitment pulses arriving to Newman Sound over the course of the season. The fine dotted line indicates the size at which juvenile cod are considered settled i.e., >60 mm SL.

Figure 4: Relationship between the absolute amount (ug/g fish) of (a) TAG (b) ST (c) PL and (d) TL with standard length in age-0 Atlantic cod (*Gadus morhua*) during late summer and fall of 2002.

Figure 5: Relationship between the relative amounts (mg/g wet weight) of (a) TAG (b) ST (c) PL and (d) TL with standard length in age-0 Atlantic cod (*Gadus morhua*) during late summer and fall of 2002. Statistics in Table 3.

Figure 6: Relationship between the proportion of 22:6n-3 and C_{18} PUFA (18:2n-3 + 18:3n-3 +18:4n-3) in the whole juvenile cod and standard length (mm) during the summer and fall of 2002.

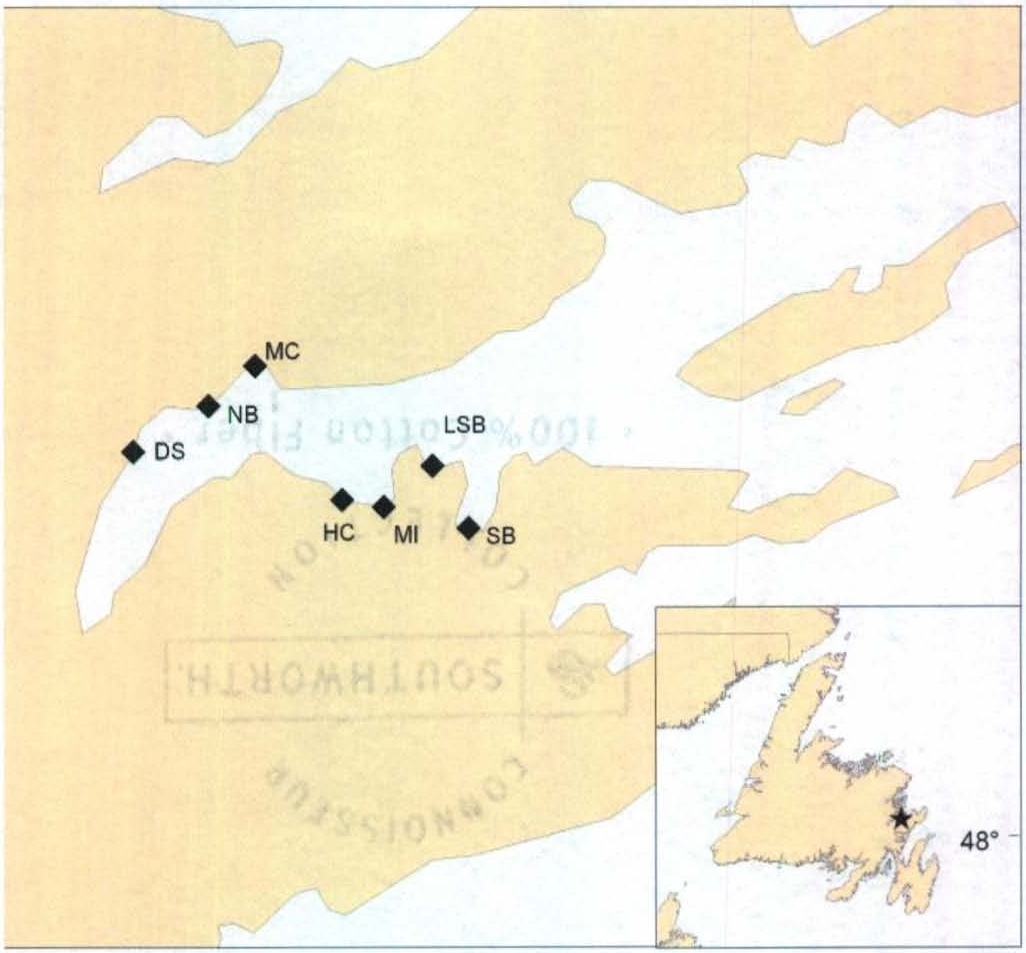

Figure 1

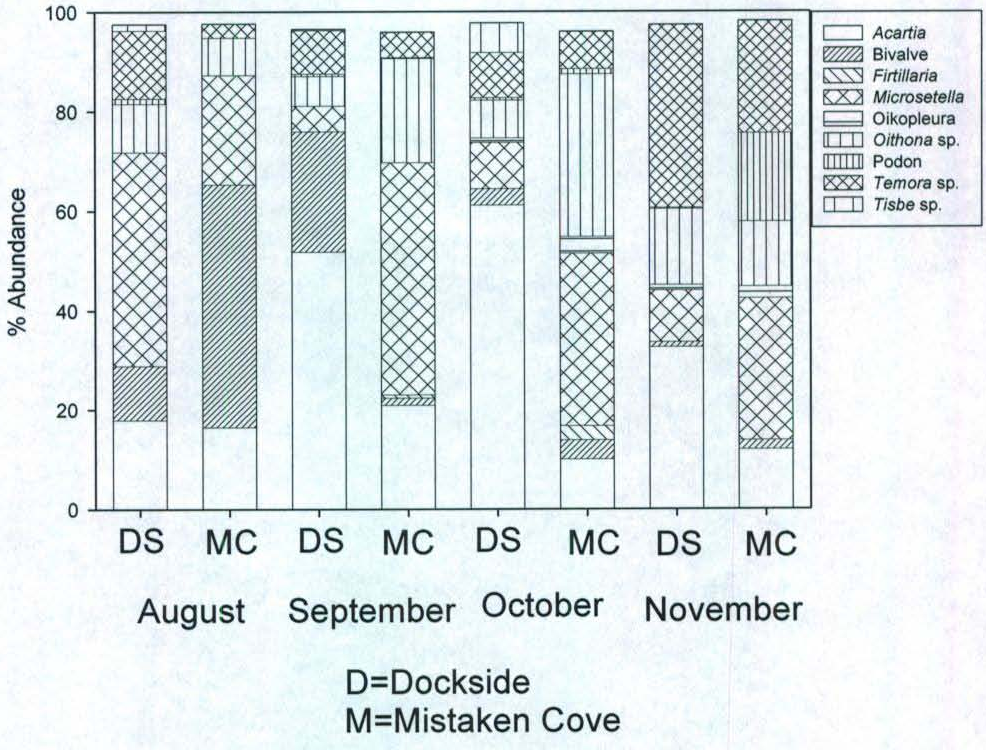
100
80
60
40
20
0
% Abundance
DS
MC
DS
MC
DS
MC
DS
MC
August
September
October
November
D=Dockside
M=Mistaken Cove
Acartia
Bivalve
Firtillaria
Microsetella
Oikopleura
Oithona sp.
Podon
Temora sp.
Tisbe sp.

Figure 2

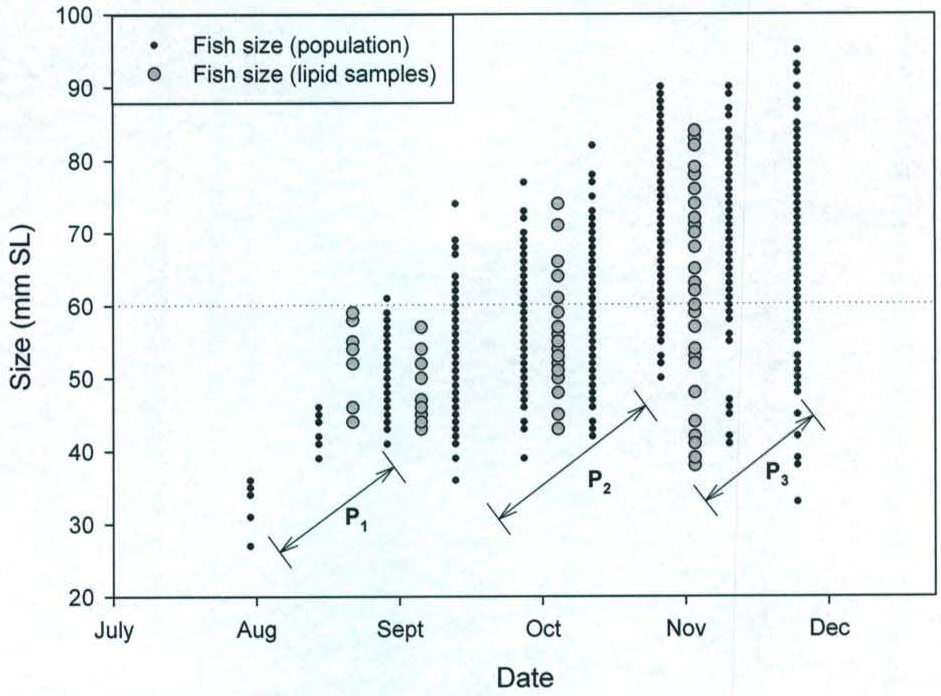
Fish size (population)
Fish size (lipid samples)
Size (mm SL)
100
90
80
70
60
50
40
30
20
July
Aug
Sept
Oct
Nov
Dec
Date
P1
P2
P3

Figure 3

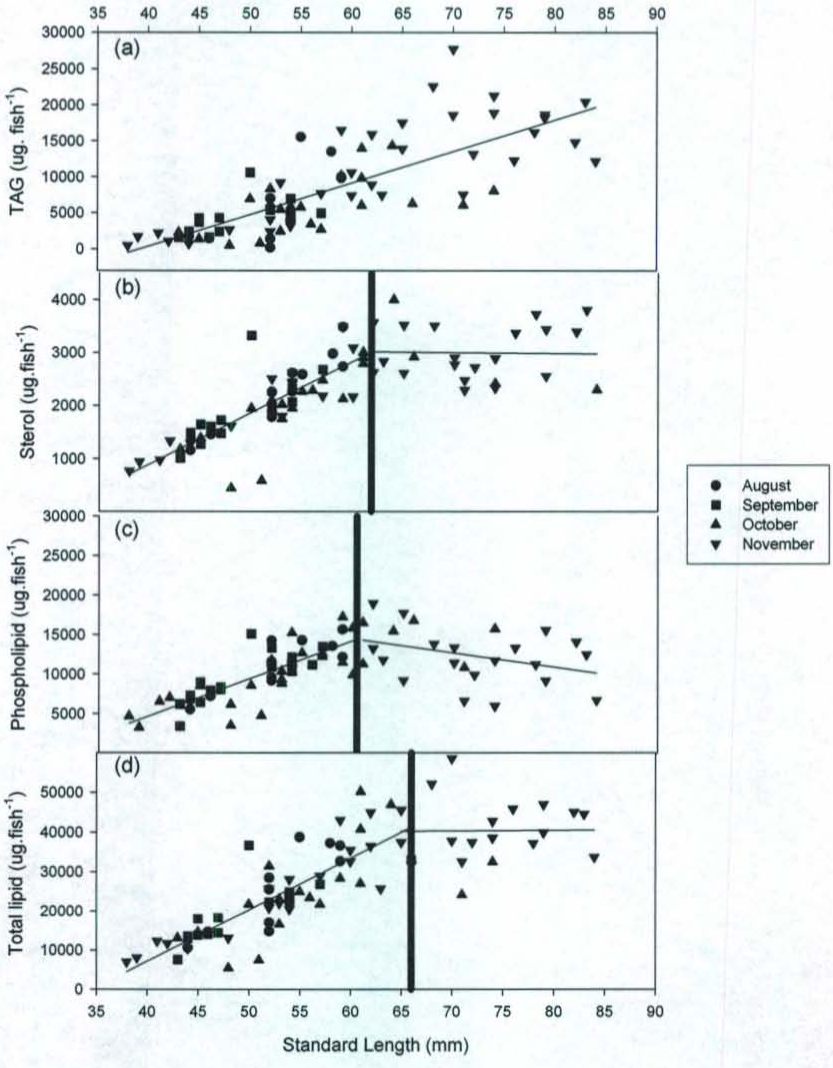
(a)
(b)
(c)
(d)
TAG (ug. fish-1)
Sterol (ug.fish-1)
Phospholipid (ug.fish-1)
Total lipid (ug.fish-1)
Standard Length (mm)
August
September
October
November

Figure 4

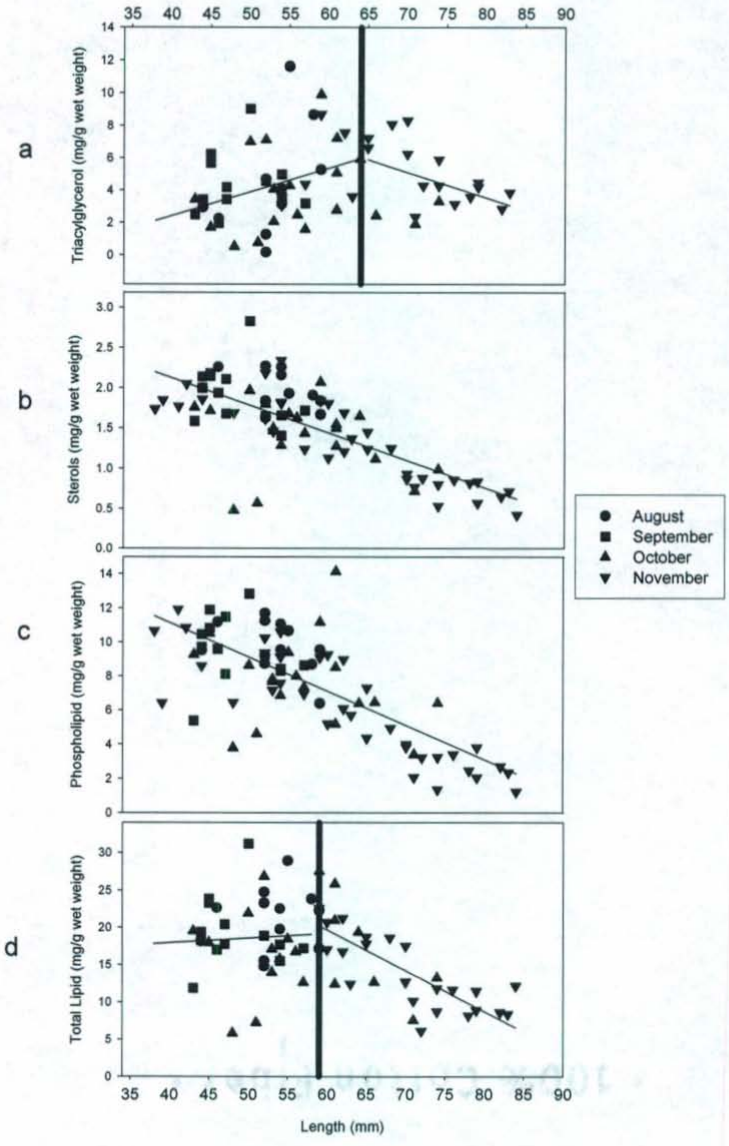
a
b
c
d
Triacylglycerol (mg/g wet weight)
Sterols (mg/g wet weight)
Phospholipid (mg/g wet weight)
Total Lipid (mg/g wet weight)
Length (mm)
August
September
October
November

Figure 5

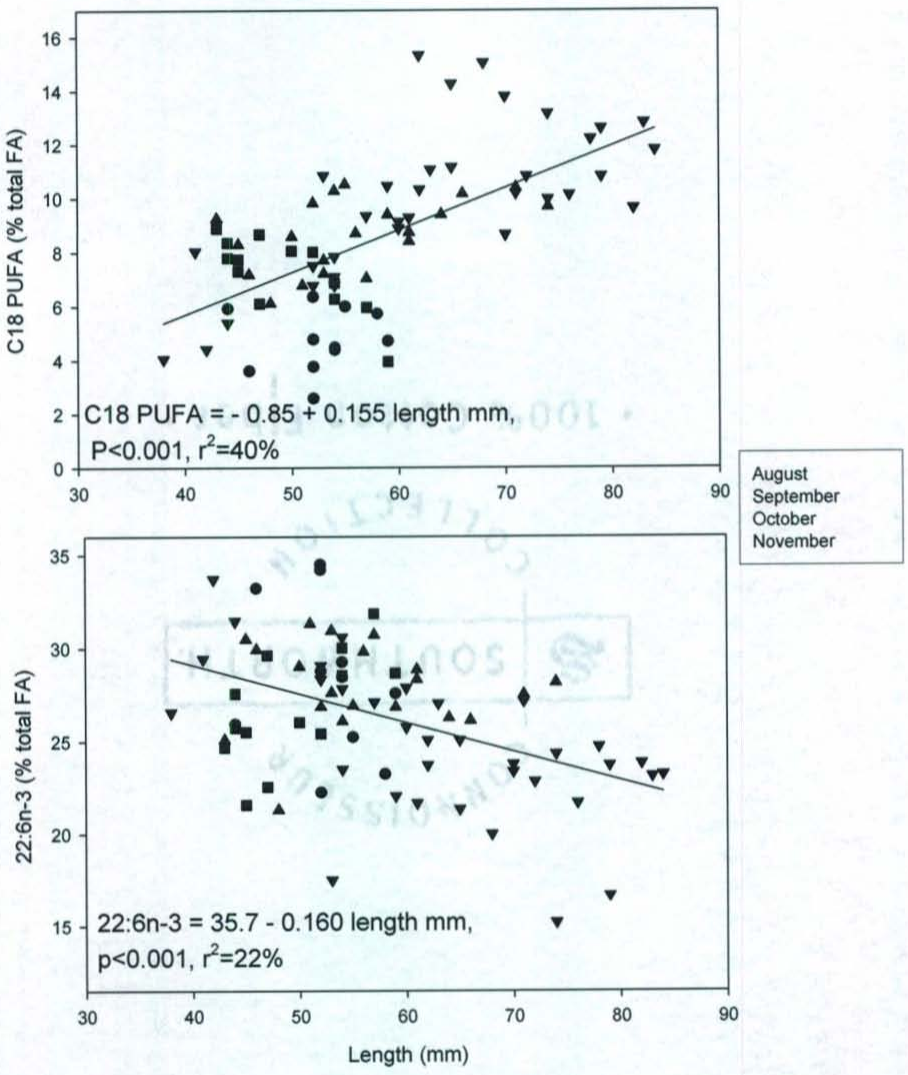
C18 PUFA (% total FA)
C18 PUFA = - 0.85 + 0.155 length mm,
P<0.001, r²=40%
22:6n-3 (% total FA)
22:6n-3 = 35.7 - 0.160 length mm,
p<0.001, r²=22%
Length (mm)
August
September
October
November

Figure 6

CHAPTER 3: Fatty Acid Biomarkers in Cold Water Eelgrass Meadows: Elevated Terrestrial Input to the Food Web of Age-0 Atlantic cod (*Gadus morhua*).

*** A version of this chapter was previously published and formatted for the journal Marine Ecology Progress Series (Copeman et al. 2009).**

ABSTRACT:

Lipid classes, fatty acids (FAs), and stable carbon isotopes of FAs were used to investigate dietary sources of organic carbon for juvenile Atlantic cod (*Gadus morhua)* during settlement into eelgrass, *Zostera marina*. Primary producers, epibenthic prey, zooplankton, and fish were collected from August to November 2002 in shallow (<10 m) eelgrass in Bonavista Bay, Newfoundland, Canada. Lipid data indicated that zooplankton (>80 μm) were associated with fresh organic material, while seston (5–80 μm) had high levels of bacterial FA and non-acyl lipids, typical of sedimentary material. Zooplankton, mysids, and amphipods showed a seasonal decrease in 22:6n-3 with a concomitant increase in the ubiquitous terrestrial indicators 18:2n-3 and 18:3n-3. Based on essential FA composition of prey, there was a decrease in the quality of food available to juvenile fish from August until November. Earlier (August) pelagic juveniles had higher levels of marine-sourced FA (22:6n-3) than late (November) arrivers. Further, in October and November settled juveniles had higher proportions of terrestrial FA biomarkers than pelagic cod, indicating an increased dietary terrestrial input at settlement. Isotopic evidence demonstrated that eelgrass was the most enriched (–14‰) source of organic carbon and supported multivariate FA analysis, confirming that eelgrass was not incorporated into the food web of juvenile cod. Increased terrestrial input of organic carbon, coupled with low proportions of dietary essential FAs indicate that the functional significance of this habitat is refuge and not nutrition.

INTRODUCTION:

Eelgrass (*Zostera marina*) is a colonial marine flowering plant that occurs in shallow soft sediments. It is globally distributed in coastal waters and is among some of the most productive marine habitat, forming vital nursery areas for both juvenile fish and invertebrates (Duarte 1989, Sogard & Able 1991, Mattila et al. 1999). Newfoundland eelgrass meadows, found in sheltered coastal bays, represent an important nursery area for many species of juvenile fish, including Atlantic cod (*Gadus morhua,* Laurel et al. 2003b). Juvenile fish choose to settle in eelgrass beds due to a combination of factors including increased refuge, high food levels, and reduced physical exposure (Orth et al. 1984, Gotceitas et al. 1995, 1997, Parker et al. 2001).

Stocks of Atlantic cod collapsed in the early 1990s and despite a commercial fishing moratorium (Myers et al. 1996) there has been no population recovery (Lilly et al. 2003). Little is known about the relative input of different primary producers to the diet of juvenile Atlantic cod, or if these contributions change following cod settlement. Examination of dietary carbon sources could result in expansion of critical habitat for juvenile Atlantic cod to include terrestrial buffer zones draining into eelgrass beds.

A number of constraints to the traditional gut analysis methodology has lead investigators to use alternative methods to resolve nearshore foodweb linkages; these include lipid biomarker, bulk stable isotope and compound specific isotope analyses (Canuel et al. 1997, Kharlamenko et al. 2001, Jaschinski et al. 2008). The small size of consumers and the vast number of primary producers in eelgrass systems make the use of biomarkers compelling to investigators (Kharlamenko et al. 2001).

Lipids play a fundamental role in fish as a source of energy, as important structural components for cell membranes and as precursors for biologically active compounds (Sargent et al. 1989, Arts et al. 2001). Fatty acids (FAs) provide information on dietary intake and food constituents leading to sequestration of lipid reserves over a long period of time (St. John & Lund 1996, Auel et al. 2002). C_{20} and C_{22} polyunsaturated FAs (PUFA) are particularly important in cold water marine systems allowing animals to maintain cell membrane fluidity (Cossin & Lee 1985, Cossin et al. 1997). Marine fish generally cannot synthesize adequate quantities of long chain PUFA from shorter chain precursors to satisfy their metabolic requirements and therefore rely on dietary input for normal physiological function. PUFA are formed in primary production and are transferred and concentrated in consumers throughout the food web (Arts et al. 2001, Budge et al. 2001, Copeman & Parrish 2003). Other sources of PUFA in the nearshore marine environment include shorter chain C_{18} PUFA from terrestrial sources, however, these are of lower nutritional value than longer chain PUFA (Sargent 1987, Sargent et al. 1989).

Lipid biomarkers have been defined as compounds that can be used as signatures of a species, groups of organisms, or environmental processes (Parrish et al. 2000). FA biomarkers are normally synthesized at low trophic levels and ideally remain unchanged when transferred through food webs (Reuss & Poulsen 2002, Dalsgaard et al. 2003). They have been used for determining sources of terrestrial and anthropogenic carbon as well as to assess health of ecosystems (Colombo et al. 1997, Budge & Parrish 1998). Specific FAs have been correlated with various sources of primary production such as

diatoms, bacteria, dinoflagellates and terrestrial run-off both in plankton and sediments (Budge & Parrish 1999, Fraser & Sargent 1989, Mayzaud et al. 1989, Maziane & Tsuchiya 2000, Parrish et al. 2000).

Bulk stable carbon isotopes have previously been widely used in food web studies to determine sources of dietary primary production in different trophic levels (Fry & Sherr 1984). Here I used compound specific isotopes of FAs in select primary producers, secondary consumers, and juvenile Atlantic cod to elucidate food web linkages. Previous analyses of eelgrass for bulk carbon-isotope composition has shown that eelgrass has much higher values (-15‰ to -7‰) than other sources of primary production in the nearshore (Kharlamenko et al. 2001, Cloern et al. 2002, Jaschinski et al. 2008). Further, little change in the $^{13}C/^{12}C$ ratio occurs with each successive trophic level, making it possible to estimate the importance of various sources of organic carbon to secondary consumers (DeNiro & Epstein 1978). The use of either FA or bulk $^{13}C/^{12}C$ ratios alone can have limited power for deducing linkages, but the combination of multiple biomarkers with multivariate statistics is a powerful approach to clarifying trophic relationships (Canuel et al. 1997, Parrish et al. 2000, Ramos et al. 2003). Using this approach, I investigated temporal changes in the dietary composition of juvenile Atlantic cod during settlement into nearshore eelgrass. These data will be important in expanding the definition of critical habitat for juvenile Atlantic cod.

MATERIALS AND METHODS:

Study Site

All samples were collected in Newman Sound, Terra Nova National Park, Newfoundland during late summer and fall, 2002. Newman Sound is a sheltered fjord (45 km^2) located on the northeast coast of Newfoundland (53.93°W, 48.58°N) and surrounded by Terra Nova National Park (Fig. 1). Within the shallow littoral zone (3-10 m) there is an abundance of sand substrate interspersed with eelgrass (*Zostera marina*) and brown macroalgae (*Laminaria* sp.). Eelgrass predominates on southern and western facing shores while north facing shores are characterized by unvegitated gravel habitat (Cote et al. 2003, Laurel et al. 2003b).

Field Collections

Thirty six horizontal surface net tows were taken over shallow sites (10 m) in Newman Sound (Table 1). Sampling was concentrated over two eelgrass sites (Dockside and Mistaken Cove), except in late November when additional eelgrass locations were added. Triplicate tows were taken at each station using a10 μm mesh plankton net over a 640 m distance. Plankton was collected in a cod end (meshed collection pouch) and samples were backwashed into clean plastic vials and kept on ice until filtration (within 7 hr). Prior to filtration, three size fractions were separated, >220 μm, 220-80 μm, and 10-80 μm, and samples were collected for lipid and weight determinations. Identification sub-samples from each size class of plankton (100 ml) were preserved by adding 1 ml of Lugol's iodine and 1 ml of 10% buffered formaldehyde. Plankton samples used for weight and lipid determination were collected on pre-combusted pre-weighed glass fiber

filters (Whatman GF/C). Filters were washed with 5 ml of 3% ammonium formate to remove salt, dried at 75°C for 24 hr, weighed and then ashed at 450°C 24 hr and re-weighed. Lipid samples were placed in lipid clean glass vials and immersed in chloroform in the lab and stored under nitrogen at -20°C until extraction.

During plankton surveys, samples of eelgrass (n=9) and macroalgae (n=4) were also collected below 2 m depth along the waterline. Samples were placed in clean bags of seawater and kept on ice until returning to the lab. Samples of macroalgae were washed with filtered seawater. Eelgrass samples were scraped clean with a blunt metal spatula and their epiphytes (n=9) filtered onto clean GF/C filters for lipid analysis. Macroalgae and clean eelgrass blades were blotted dry, weighed and stored in chloroform under nitrogen until extraction.

Epibenthic invertebrates, amphipods (*Gammarus* sp.) and mysids (*Mysis stenolepis*), were taken at beach seine sites during fish sampling (Table 1). Amphipods were collected using dip nets by dragging the nets along the bottom at a depth of < 2 m while mysids were by-catch from seine hauls. Animals were placed in clean plastic bags filled with filtered seawater and stored at 4°C for 24 hr to depurate. Animals were then washed in filtered seawater and placed in lipid clean tubes under chloroform and nitrogen at -20°C until extraction. The number of individuals per sample decreased as the season progressed: fewer individuals of a larger size were required for lipid analysis. These numbers ranged from a high of 25 amphipods per sample in August (0.5-1.5 mg wet wt.ind^{-1}) to a low of 12 in November (0.8-22.9 mg wet wt.ind^{-1}). Similarly, numbers of

mysids per sample decrease from 20 in August (5.2-12.3 mg wet wt.ind^{-1}) to 6 in November (16.5 to 27.2 mg wet wt.ind^{-1}).

Juvenile Atlantic cod were collected in Newman Sound using a 25 m demersal seine net which was deployed 50 m from shore using a small boat (Table 1). Hauls were retrieved by people standing along the shoreline 16 m apart. Fish were unloaded into plastic tubs filled with seawater and identified, enumerated and measured. Juvenile cod were considered pre-settled at <60 mm and post-settled at >60 mm (Methvan & Bajvik 1994, Laurel et al. 2003b). Fish were placed on ice immediately and frozen within 3 hr of sampling. During lipid sampling, standard length, body depth, and wet weight were recorded. Fish stomachs were removed and animals were washed with filtered seawater, blotted dry, weighed and stored in chloroform under nitrogen until extraction.

Lipid & Isotope Analysis

Lipids were extracted in chloroform/methanol according to Parrish (1988) using a modified Folch procedure (Folch et al. 1957). Lipid classes were determined using thin layer chromatography with flame ionisation detection (TLC/FID) with a Mark V Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three stage development system was used to separate lipid classes. After each separation, rods were scanned and the 3 chromatograms were combined using T-data scan software (RSS Inc., Bemis, TN, USA). The signal was quantified using lipid standards (Sigma, St. Louis, MO, USA).

FA methyl esters (FAME) were prepared from total lipid extracts by transesterification with 10% BF_3 in methanol at 85°C for one hour (Morrison & Smith

1964, Budge 1999). FAME were injected in a Varian 3400 GC equipped with a 8100 autosampler (Varian, CA, USA) and an Omegawax 320 column, 30 m, 0.32 mm i.d., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA). Hydrogen was the carrier gas and the flow rate was 2 ml min^{-1}. The column temperature profile was: 65°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C min^{-1}, and hold at 220°C for 0.75 min after ramping at 2°C min^{-1}. The injector temperature increased from 150 to 250°C at 200°C min^{-1}. Peaks were detected by flame ionisation with the detector held at 260°. FA peaks were integrated using Varian Star Chromatography Software (version 4.02) and identification was made with reference to standards (PUFA 1 and 37 Component FAME Mix, Supelco Canada, ON).

The FAME carbon isotope ratios (‰) were determined, relative to the Vienna PDB standard, after combustion at 850EC, in a continuous flow isotope ratio mass spectrometer (Finnigan MAT 252: Veefkind 2003). FAME were separated on a Supelco SPB-PUFA column (30 m X 0.25 mm i.d. X 0.2 :m film) in a Varian 3400 GC with the outlet connected to the combustion chamber. The measured natural carbon isotope composition for esters is:

$$\delta^{13}C_{sample} = 1000\ H\{(^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{PDB}) - 1\}$$

All FA $\delta^{13}C$ data were corrected for the contribution made by the derivatizing agent, BF_3/CH_3OH, whose ratio, determined by bulk isotope ratio mass spectrometry (Finnigan Delta Plus XL Thermo Quest) was, on average, -38.23‰.

Statistical Analysis

Select lipid classes and FAs in zooplankton, epibenthic prey, and fish, were compared between months or (for fish) pelagic *versus* settled using a one-way analysis of variance (ANOVA) with Tukey's pairwise comparisons. Residuals *versus* fitted values were examined for normality and heteroscedasticity and certain percentage data were arcsine square-root transformed in order to meet these assumptions.

Principal components analysis (PCA) was used to simplify multivariate FA and lipid class data by transforming correlated variables into a set of uncorrelated principal components (Minitab, version 10.5, Meglen 1992). This technique was employed using 10 to 12 highly discriminatory lipid variables (based on previously run PCA analysis) from net tows, epibenthic prey, and cod samples. The first two principal components accounted for 54 to 72% of the variance among samples, which allowed a display of the major trends within the data set without significant loss of the total original variation. PCA lipid loading coefficients are defined as the correlation coefficients between the original lipid variables and the PCA axis. PCA scores are defined as the position of the original variables along the new PCA axes (Meglen1992). Lipid variables were chosen based on biological significance and the degree of variance explained by a given lipid class or FA. Addition of other lipid classes did not change the clustering of observations, indicating that the models were robust.

RESULTS & DISCUSSION:

Net Tows

The net tow sampling locations and dates are shown in Table 1 & Figure 1. At each sampling, two net tows from each size class were examined microscopically in order to determine zooplankton availability for settling juvenile cod. The smallest plankton (10-80 μm) had over ~80% of the field-of-view covered with an amorphous matrix and no recognizable phytoplankton cells. Fecal pellets and detritus were noted and microscopically these samples resembled re-suspended sedimentary "fluff". Therefore, the smallest fraction is referred to as seston rather than plankton.

Enumeration of the two larger size fractions of net tow collected zooplankton showed a variable species abundance, but *Acartia* sp., *Oithona* sp., *Temora* sp., *Microsetella* sp., and copepod nauplii made up >85% of the zooplankton abundance in all samples (Figure 2). Averaged over the whole sampling period, the largest size fraction (>220 μm) had high levels of *Microsetella* (56±22%), copepod nauplii (25±22%) and *Oithona* (12±12%). The medium size fraction (80-220 μm) had increasing numbers of *Temora* later in the fall with an average of 36±22% present over the whole sampling period. Seasonally averaged levels of *Microsetella* and *Oithona* were 24±21% and 19±18% respectively (Fig. 2).

This distribution of zooplankton is characteristic of other studies on zooplankton abundance during the fall in nearshore Newfoundland (Davis 1982, Grant & Brown 1998). Grant and Brown found a shift in prey abundance in juvenile cod diets from high lipid *Calanus* sp. in summer to smaller low lipid prey items such as *Temora* sp. in late

autumn. Davis also observed a reduction in the size of copepods and a shift in species abundance to higher levels of smaller copepods.

No *Calanus* sp. were identified in any of the samples taken from Newman Sound during 2002. Grant and Brown (1998a) previously found *Calanus* to be important to the diet of juvenile cod. This prey item was only present in their diet in October (21.5% relative importance) and November (5.8%) and was not found in either September or December. *Calanus* form large globules of wax ester that are easily digested by juvenile fish and provide high amounts of energy (Sargent 1989). Previously, a decrease in juvenile cod condition has been noted when larger *Calanus* sp. disappeared from the diet (Grant & Brown 1999). The absence of *Calanus* sp. in my samples may indicate that during 2002 food quality was not optimal.

Evjemo et al. (2003) described the lipid composition of nearshore marine copepods used for feeding larval fish. They examined the lipid composition as a percent of dry mass from April to July and found *Calanus* (~10% in April to a high > 20% in June and July) had higher levels than *Eurytemora* (~10%), or *Temora* (~10%). In Newman Sound I report lipid values for the 80-220 μm zooplankton which averaged only 2.6% of dry mass while large zooplankton contained 3.5%. These levels are low compared to whole net tows sampled during the height of the spring bloom in Conception Bay, Newfoundland ~ 6% (Parrish et al. 2005) but equivalent to values of 4.2% for net tows collected in late summer in Gilbert Bay, Labrador (Copeman & Parrish 2003); suggesting that our reported levels are within the late summer/ fall expected range.

Both sizes of zooplankton, discussed above, had significantly higher levels of lipid per dry weight than found in seston ($p<0.001$, Table 2). The major lipid classes in both sizes of zooplankton were triacylglycerols (TAG, 20-27%) and phospholipids (PL, 34-42%), while seston had significantly higher levels ($p<0.001$) of hydrocarbons (HC) and acetone mobile polar lipids (AMPL) than zooplankton (~2.5 and 8.5% respectively). Saturated FAs (SFA), monounsaturated FAs (MUFA) and PUFA comprised 29-31%, 19-25%, and 44-52%, respectively of total FAs in both sizes of zooplankton. Seston had lower levels of PUFA (32%) and elevated levels of SFA (39%) and MUFA (29%). Terrestrial FAs (18:3n-3+18:2n-6) accounted for ~6% of total FAs in all size fractions. High levels of 18:4n-3 were found in net tows, with large zooplankton having the most elevated proportions (5.2±1.4%). Levels of bacterial FAs in the two sizes of zooplankton were ~4.7%, with seston (10-80 μm) having the highest proportions, 7.9±2.6% (Table 2).

This study was designed to look at the food web of eelgrass beds in relation to diet of settling juvenile Atlantic cod. Therefore, net tow data presented here, from late summer and fall, are probably not representative of plankton at other times of the year. The typical seasonal pattern of phytoplankton abundance in cold North Atlantic nearshore waters shows diatom lipid markers dominating in spring, followed by terrestrial plant material, small flagellates, zooplankton, and bacterial sources becoming more important in late summer and fall (Mayzaud et al. 1989, Parrish et al. 1995, 2000). Seasonal changes in lipid composition of particulate matter can be caused by changes in taxonomic source or physiological changes within a taxonomic group (Mayzaud et al.

1989). Algal lipid composition may be influenced by a number of physical factors, including irradiance, temperature, and nutrients (Thompson et al. 1990, 1992).

The lack of intact phytoplankton in my net tow samples indicates that the food-web of Newman Sound during the autumn of 2002 was not dominated by large phytoplankton cells. Rather, smaller flagellates, bacteria, and terrestrial material likely accounted for most of the seston. Parrish et al. (1995) looked at seasonal seston microplankton species composition in South Broad Cove, also in Newman Sound. They also noted high levels of microzooplankton in the water column during summer, particularly after a storm mixing event. Further, nanoflagellates (2-20 μm) which are likely inefficiently caught in my 10 μm mesh net, made up a significant proportion of the late fall plankton community. However, they noted a distinct fall diatom bloom which I did not observe in 2002.

Lipid classes and FAs in seston were more similar to "fluff" material than phytoplankton, in agreement with microscopic evaluation. High levels of terrestrial FAs (18:2n-6+18:3n-3) were found in all sizes of plankton (5.5 to 6.1%, Table 2). Previously, Budge et al. (2001) used a level of >2% in marine samples to indicate significant terrestrial input. My levels are much higher, indicating Newman Sound has a high level of terrestrial carbon in the water column. Copeman & Parrish (2003) previously reported similarly high levels of terrestrial markers (~6%) in the fall in a shallow sheltered Bay with significant terrestrial input (Gilbert Bay, Southern Labrador).

Bacterial markers in Newman Sound seston were elevated averaging 7.9% of identified FAs (Table 2). These levels were similar to those found in other shallow

sheltered embayments such as Gilbert Bay (~5%, Copeman & Parrish 2003) and Notre Dame Bay (~5.5%, Budge et al. 2001). Values of bacterial FA markers for net tows taken at the same latitudes but in deep fjord-like systems, such as Trinity Bay (2%, Budge & Parrish 1998) and Conception Bay (2-4%, Parrish et al. 2005) Newfoundland were much lower. Elevated organic matter in eelgrass sediments provide a substrate for bacterial production. Likely sources of sedimentary carbon include seagrass detritus (Holmer & Nielsen 1997), root organic contributions (Moriarty et al 1986), and benthic microalgae (Boschker et al. 2000). In Newman Sound, eelgrass sites were very shallow (<10 m) and high levels of re-suspension of degraded material from sediments likely contributed to elevated levels of bacteria observed in net tow samples (Table 2).

Principal components analysis (PCA) allows the simplification of a large number of correlated FA variables into a few uncorrelated axes, which explain most of the original variance among the samples (Manly 1986). PCA of eleven FA and lipid class variables allowed us to view the lipid composition of net tows in terms of seasonal trends and size differences (Figures 3a & 3b). PC1 explained 38% of the variance and examination of the lipid loading coefficients indicated that this axis represents a freshness axis with high levels of PUFA, organic matter, 22:6n-3 and PL positively loaded (Figure 3a). Lipid classes typical of degradation (Parrish 1998) and bacterial FAs loaded negatively (Figure 3a). Scores for net tows showed larger zooplankton were associated with fresh material while seston was negatively loaded (Figure 3b).

PC2 explained 26% of the variance and represented a seasonal axis showing lipid parameters associated with marine lipids such as 22:6n-3 positively loaded while those

associated with terrestrial sources (18:2n-6 and 18:3n-3) were negatively loaded (Figure 3a). PL and TAG are not diagnostic for marine or terrestrial sources, however, in this study it is clear that PL was associated with marine derived lipids while TAG was associated with terrestrially derived lipids. The distribution of the sample scores along PC2 shows a definite seasonal trend with samples taken in August and September positively located and those from the later in the fall negatively loaded (Figure 3b).

TAG and PL clustered with PUFA and were elevated in zooplankton compared to seston. TAG are important for storage and during the spring bloom in Newfoundland, high levels of TAG sink through the water column (Parrish 2000). PL are essential components of cell membranes and marine organisms generally have high levels of PUFA. Previously, this lipid class has been used to indicate freshly biosynthesized carbon (Derieux et al. 1998). HC and alcohols (ALC) occur at elevated levels in sediments compared to phytoplankton (Copeman & Parrish 2003, Parrish 1998)

Higher proportions of 18:4n-3 were found in large zooplankton (>200 μm) compared to seston (Table 2). The origin of this FA has previously been a source of some debate (Ramos et al. 2003), however, formerly it has been linked to bacterial (Murphy & Abrajano 1994) and dinoflagellate sources (Dalsgaard et al. 2003). Further, this FA is likely an intermediate step in the chain elongation and desaturation pathway that copepods such as the harpacticoid, *Tisbe holothuriae*, use to form 22:6n-3 and 20:5n-3 from 18:3n-3 (Norsker & Stottrup 1994). The position of 18:4n-3 close to terrestrial markers in PCA analysis and away from other bacterial markers is evident in Figure 3a. This indicates that elevated 18:4n-3 in copepods is associated with chain elongation of

terrestrial material within zooplankton and not due to an increase in bacterial or dinoflagellate sources. Compound specific isotope data, presented below, supports the origin of significant amounts of 18:4n-3 as being from a terrestrial carbon source.

Primary Producers

The lipid composition of three photoautotrophs (eelgrass blades, macroalgae and eelgrass epiphytes) is shown in Table 2. The two major lipid classes in eelgrass blades were PL (32%) and AMPL (34%), while the major FAs were 16:0 (22%), 18:2n-6 (13%) and 18:3n-3(27%). Levels of these FAs agree with previous studies on FA signature of eelgrass blades and roots (Kharlamenko et al. 2001). Epiphytic algae had increased proportions of 16:1n-7 (diatom marker) and high levels of bacterial markers (8.3%), while macroalgae were characterized by elevated levels of 20:4n-6 relative to other sources of primary production (Table 2).

High levels of 18:2n-6 and 18:3n-3 in eelgrass makes the use of these FAs as a terrestrial marker in eelgrass habitat problematic. However, different values of $\delta^{13}C$ in eelgrass and terrestrial plants allow us to rule out any significant input of eelgrass into the diet of juvenile cod (see below). Further, other studies have used FAs and $\delta^{13}C$ to validate the terrestrial marker, 18:2n-6+18:3n-3, in both shallow (Budge et al. 2001) and fjord-like systems (Ramos et al. 2003). Additionally, seasonal rainfall data collected by Environment Canada in Newman Sound, showed much higher levels of rainfall in November (130 mm monthly) than compared to earlier in August (23 mm monthly).

Increased rainfall would contribute to elevated levels of terrestrial carbon in run-off to surrounding eelgrass habitat.

Epibenthos

When averaged over the late summer and fall, mysids (*Mysis stenolepis*) and amphipods (*Gammarus* sp.) had similar lipid class and FA profiles (Table 3). TAG (~34%) and PL (~33%) were the major lipid classes; however, amphipods contained a high level of free FAs (20%). The major SFA in amphipods and mysids was 16:0 (~17%), while 16:1n-7 (~6%) and 18:1n-9 (~12%) were the most abundant MUFA, and 20:5n-3 (~18%) and 22:6n-3 (14%) the major PUFA. The 22:6n-3/20:5n-3 ratio was lower in mysids and amphipods (0.8:1) than in zooplankton (1.4:1) while higher proportions of 20:4n-6 (4.2%) were found in both types of epibenthic prey compared to that in zooplankton (2%).

Figure 4 shows the seasonal change in a marine (22:6n-3) and selected terrestrial (18:3n-3 & 18:2n-6) PUFA for epibenthic prey and zooplankton. Significantly higher levels of 22:6n-3 and lower levels of terrestrial markers occurred in August. Conversely, lower levels of 22:6n-3 and elevated levels of terrestrial markers were observed in November. The only non-significant trend in these FA was the change in 18:2n-6 from August to November in epibenthic prey.

Using twelve lipid class and FA variables I was able to explain 54% of the variation in zooplankton and epibenthic prey collected from August to November in Newman Sound (Fig. 5) in the first two PCA axes. The lipid coefficient distribution showed FAs associated with marine sources were loaded positively on PC1 (32% of the

variance, Fig. 5a) while those associated with terrestrial material were negatively loaded. PC2 explained 22% of the variation and FAs associated with the benthos, including benthic diatoms (16:1n-7) and macroalgae (20:4n-6), were negatively positioned. Conversely, FAs and lipid classes associated with water column terrestrial (18:2n-6 & 18:3n-3, HC and AMPL) and marine sources (DHA and PL) were positively loaded.

The scores for epibenthic prey and zooplankton show both seasonal trends and trophic differences. Prey items were divided into those collected in August and September and those collected in October and November. On PC1 zooplankton and mysids collected in summer were positively located, indicating a significant marine input to their diet. Zooplankton collected later in October and November shifted to the negative side of PC1, indicating enrichment in terrestrial material later in the season. However, zooplankton samples showed more variation along this axis indicating a greater shift in carbon source throughout the season compared to mysids and amphipods.

Along the pelagic to benthic axis (PC2, Fig. 5b) zooplankton were located positively, indicating they had dietary input from pelagic terrestrial and marine sources and not from macroalgae and benthic diatoms. Oppositely, mysids had a negative position along PC2 which indicates that they were not preying on zooplankton and that the major dietary carbon sources were benthic diatoms and macroalgae. Amphipods showed an intermediate position along PC2, and this may demonstrate consumption of a mixture of organic material.

Juvenile Cod

Grant and Brown (1998a) compared the diet of age-0 and age-1 cod foraging over eelgrass in Trinity Bay, Newfoundland. They found that after settlement into eelgrass, juvenile cod developed a diel feeding strategy. During the day they actively forage on pelagic prey over eelgrass and at night they reduce feeding and disperse into bottom cover. Benthic and epibenthic prey were of minor importance to the diet of age-0 cod. Conversely, mysids and amphipods were found to be the major prey item for age-1 juvenile cod foraging at night over eelgrass. Thus, an ontogenetic shift in diet and lipid markers is expected after settlement, with benthic food sources increasing in importance.

Table 4 summarizes lipid class and FA compositions of juvenile Atlantic cod during the four periods. Detailed analysis of lipid classes in relation to juvenile cod condition and timing of settlement are found in Chapter 2 (Copeman et al. 2008). Significant differences between pelagic and settled juvenile fish during both October and November were found with settled fish having lower relative amounts of lipids ($mg.g^{-1}$ wet weight) in November and lower proportions of phospholipids and STs in both October and November. The FA composition of settled fish in both months showed a significantly greater input of terrestrial FAs in settlers compared to pelagic juveniles (Table 4). When pelagic juveniles were compared across four months, fish collected in August showed significantly lower levels of terrestrial FAs than pelagic fish collected later in the season (Table 4).

PCA of specific FAs and lipid classes in both settled and pelagic juvenile cod from August until November is given in Figure 6. PC1 explained 51% of the variance and

examination of the lipid loading coefficients indicates that the marine FAs found in cod phospholipids are positively loaded on PC1 while terrestrial FAs that are normally stored in neutral lipids are negatively loaded. When the scores for fish are examined, the August pelagic juveniles are located positively on PC1 with pelagic juveniles from later in the season moving to the negative side of the axis. Most negatively loaded onto this axis are the settled juveniles from November that have the highest relative amounts of terrestrial FAs and the lowest level of lipid as a proportion of wet weight. This confirms that fish mimic both pelagic and benthic prey items with increased terrestrial makers in November compared to August. Higher levels of C_{18} PUFA in both zooplankton and epibenthic prey likely also decreased food quality for fish in November.

Lipid classes and FAs from juvenile cod are shown as a function of month and settlement status (Table 4). Total lipids were 1.3-2.1% of wet weight, while previous reports on Newfoundland adult cod lipid composition showed that the flesh had 0.6% lipids while the liver contained 24% (Copeman & Parrish 2003). Settlement has previously been defined as 60 mm in standard length based on behavioral and pigmentation characteristics (Methvan & Bajvik 1994, Laurel et al. 2003a) and more recently based on biochemical characteristics (Chapter 2, Copeman et al. 2008). Table 4 shows that pelagic juvenile cod had low levels of total lipid and high proportions of PL relative to TAG, indicating few lipid reserves were present in the liver during settlement in the nearshore. Further, Copeman et al. (2008, Chapter 2) found a significant break point in the regression of lipid class parameters with standard length that occurred at the time of settlement. Cod also increased utilization of PL and STs and decreased relative

amounts of lipid in the flesh at ~60 mm standard length. Continued growth, with reduced energy storage, suggests predation pressure could be a significant factor driving lipid utilization during settlement. This theory is supported by high predation rates measured over eelgrass habitat in Newman Sound (Linehan et al. 2001; Laurel et al. 2003a).

A comparison of lipid class composition between settled and pelagic fish in November showed unexpectedly that settled fish had significantly lower relative proportions of total lipid and lower proportions of PL per wet weight than pelagic fish (Table 4). Most studies on juvenile fish have been based in freshwater or estuarine systems and have shown a significant increase in lipid prior to the onset of winter (Griffiths & Kirkwood 1995, Hurst & Conover 2003). Lipid class utilization at settlement in juvenile cod has been shown to be driven in large part by utilization of PL (Copeman et al. 2008, Chapter 2). This pattern is also unexpected given the classic adult cod scenario of utilizing TAG in the liver first followed by protein in the muscle (Black & Love 1986, Hemre et al. 1993). However, PL utilization does occur in lipid-limiting situations such as during egg or early larval development (Tocher et al. 1985), indicating juvenile fish may be more similar to larval stages in terms of lipid metabolism.

The main SFA, MUFA, and PUFA in the whole bodies of cod were 16:0, 18:1n-9, and 20:5n-3 + 22:6n-3, respectively (Table 4). Levels of these FAs are in agreement with amounts previously found in whole animal analysis of cod (Kirsech et al. 1998). Like most organisms, fish can carry out *de novo* biosynthesize of SFA and MUFA with chain lengths up to C_{18}, however, synthesis of longer chain $C_{20} + C_{22}$ PUFA is inadequate to

meet physiological demand (Henderson & Sargent 1985, Sargent 1989). Therefore, high levels of long chain PUFA are required pre-formed in the diet.

Increased proportions of 22:6n-3 are found in neural PL of marine fish (Mourente et al. 1992, Sargent 1999). Earlier work in fish nutrition has shown that inclusion of high levels of C_{18} PUFA negatively affect growth and survival. The mechanism of this effect is via competition between 18:3n-3 with longer chain 22:6n-3 and 20:5n-3 for transacylases and acylases that esterify FAs onto PL backbones. Given the specificity of cod membranes for 22:6n-3, a dietary 22:6n-3/20:5n-3 ratio of 2:1 has been recommended for marine fish (Sargent et al. 1999, 1995). This ratio was higher in zooplankton than epibenthic prey but decreased in all potential prey from August to November, further indicating a decrease in food quality.

Compound Specific Isotope Analysis

Figure 7a shows the stable carbon isotope values for specific FAs found in various primary producers in eelgrass habitat. Unfortunately, samples of epibenthic algae from eelgrass were too small to be analysed using this method. For other primary producers, an early August sample and a November sample were analysed. Early values were more enriched in ^{13}C compared to later values. When the $\delta^{13}C$ weighted mean was calculated for all identified FAs, the following ranges were found: eelgrass (early: -13.50‰, late: -14.6‰), macroalgae (early: -20.7‰, late: -22.9‰) and seston (early: -23.1‰, late: -24.7‰). Values for $\delta^{13}C$ in the smallest net tow fraction were in agreement with literature values for plankton sampled in late October in Notre Dame Bay

in Newfoundland (Budge et al. 2001). 18:3n-3 and 18:2n-6 had lower $\delta^{13}C$ values in seston and zooplankton (Fig. 7).

The stable carbon isotope values for specific FAs found in fish (n=6), zooplankton (n=2), epibenthic prey (n=4), and literature values for Newfoundland marine plankton and a terrestrial plant are given in Figure 7b (Budge et al. 2001). Mysids and amphipods had more $\delta^{13}C$ enriched values than zooplankton and fish. While equisetum, a terrestrial plant, showed the most depleted values for the terrestrial markers (18:2n-2 & 18:3n-3). Similar values for $\delta^{13}C$ in 18:4n-3 and 18:3n-3 found in copepods in Figure 7b, further support the assertion they have similar origins.

August samples of zooplankton and fish were more enriched in $\delta^{13}C$ compared to later values. When the isotopic weighted average was calculated for consumers the following ranges were found: mysid (early: -22.8‰. late: -21.9‰), amphipod (early: -22.9‰, late: -22.2‰), zooplankton (early: -26.4‰, late: -26.8‰), and fish (early: -25.9‰, late: -27.4‰).

Much higher values of $\delta^{13}C$ in both 18:2n-6 and 18:3n-3 from eelgrass (-14‰, Fig. 7a) compared to plankton, epibenthic prey, and fish (-22 to -29‰, Fig. 7b) indicate that there is very little contribution from eelgrass to these two FAs which are elevated throughout the food web during the fall. The most likely sources of large amounts of depleted 18:2n-6 and 18:3n-3 are from C_3 terrestrial plants (-28‰, bulk carbon) or certain freshwater algae. Levels of $\delta^{13}C$ in freshwater algae vary considerably from -20‰ to -45‰ in bulk carbon depending on their source of dissolved CO_2 (Fry 2006).

Recently, Boschker et al. (2005) examined the phospholipid-derived FAs in the plankton community along a salinity gradient in a North Sea estuary. Their results varied with salinity (1-32 ppt), but at salinities similar to ours (31 ppt) they found levels of $\delta^{13}C$ in their green algae marker (18:3n-3) to be -26‰. I report values of 18:3n-3 in the fall as depleted as -29‰. Further, Boschker et al. (2005) reported very low concentrations of freshwater algae derived FAs (18:3n-3, 18:2n-6, 18:4n-3) at salinities > 5 compared to samples collected at < 5 ppt. Also, my identification of net tow samples showed no fresh phytoplankton cells, with my smallest size fraction dominated by fecal pellets and detritus. Therefore, freshwater algae are not a likely source of the depleted FAs (18:3n-3, 18:2n-6, 18:4n-3) that I observed in increasing proportions during fall.

In this study, the most likely source of elevated levels of depleted 18:3n-3 and 18:2n-6 is terrestrial C_3 plants. Budge and Parrish (1998) analysed a number of terrestrial sources of carbon surrounding Trinity Bay, Newfoundland for the sum of the proportions of 18:2n-6 and 18:3n-3. They found high levels in *Equiseteum* (~50%) *Carex* (~30%) and Pine pollen (~16%). Further, parallel samples taken in Newman Sound during 2002, give bulk carbon isotope levels of -30.4‰ in spruce tree (*Picea mariana*) needles and -28.6‰ in alder (*Alnus incana*) leaves (Jameison et al. unpublished data). When I adjust these values with the consideration that lipid synthesis imparts an additional fractionation of approximately 3 to 5‰ relative to total biomass, this puts the lipid composition of these two terrestrial plants between -31.5 and -35.5‰. Contributions of terrestrial run-off containing high proportions of terrestrial FAs that are depleted in carbon would explain the lighter value of these FAs seen throughout the food web.

Isotope evidence indicates a more ^{13}C enriched food source for amphipods and mysids than for zooplankton and fish. Other possible sources of primary production for mysids and amphipods are macroalgae and benthic diatoms (high levels of 20:4n-6 and 16:1n-7). The isotopic signature agrees with trends from PCA analysis of epibenthic prey and zooplankton, where epibenthic prey were closer to a benthic food source (Figure 5). Isotopically, fish do not resemble epibenthic prey, but rather are very similar to zooplankton.

CONCLUSIONS:

The examination of lipid biomarkers and compound specific isotopes in eelgrass beds during 2002 provided complimentary data indicating increased terrestrial carbon input into the diet of juvenile cod during the fall. The use of these two techniques clarified the utility of 18:3n-3+18:2n-2 as an indicator of terrestrial carbon and isotopic data distinguished terrestrial sources from eelgrass carbon. I found no evidence that eelgrass was utilized either by juvenile cod or their prey as a source of carbon. Decreased food quality and a reduction in PUFA content and total lipids in cod flesh during the fall indicate the functional significance of eelgrass is not increased nutrition but potentially refuge. Given the indicated input of terrestrial carbon into the diet of juvenile cod at settlement, terrestrial buffer zones surrounding eelgrass habitat should also be considered in the evaluation of essential fish habitat for this species.

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Table 1: Sampling locations and dates within Newman Sound, 2002.

Date	Location	# Plankton tows	# Epibenthic prey samples	# Cod Samples
28/08/02 to 29/08/02	Dockside	3	6	7
	Mistaken Cove	3	2	5
10/09/02 to 12/09/02	Dockside	3	4	8
	Mistaken Cove	3	0	6
03/10/02 to 04/10/02	Dockside	3	6	10
	Mistaken Cove	3	6	10
27/10/02 to 08/11/02	Dockside	3	8	2
	Mistaken Cove	3	6	13
	South Broad Cove	3	4	2
	Minchin's Cove	3	4	4
	Heffern's Cove	3	3	3
	Mt Stanford	3	3	1

Table 2: Major lipid classes (>4% in at least one group) and FAs (>1.5%) in net tows and primary producers collected from August to November (2002) over *Zostera marina* beds in Newman Sound.

(n=25-30 for net tows, n=7-12 for primary producers, mean ± SD, Bacterial FAs were: ∑15:0, *ai*15:0, *i*15:0, *i*16:0, *ai*16:0, 15:1, 17:0, 17:1).

	Large Zooplankton (>220 µm),	Medium Zooplankton (220-80 µm)	Seston (10-80 µm)	Epiphytes	Eelgrass	Macroalgae
Total lipid (mg.g^{-1} dry weight)	34.5±12.7	25.79±12.95	14.1 ± 12.8			
Total lipid (µg.g^{-1} organic weight)	173±116	103±84	29.5±27.4			
% of total lipid						
Hydrocarbons	1.65±1.68	3.38±2.31	8.40±4.58		1.48±0.84	
Steryl/Wax Esters	8.39±7.97	3.69±4.68	4.98±7.83		0.31±0.55	
Triacylgylcerols	19.90±11.59	26.98±16.37	8.85±6.43		8.34±2.36	
Free FAs	11.54±4.32	11.42±3.40	6.68±3.38		8.44±5.43	
Sterols	6.33±2.13	6.27±2.72	7.72±4.72		8.95±2.17	
Acetone Mobile Polar Lipids	6.59±2.75	10.44±3.89	21.35±6.96		34.20±3.61	
Phospholipids	42.19±11.48	34.19±16.39	33.54±11.56		32.22±4.40	
FAs (%)						
14:0	4.0±1.5	3.7±0.9	4.5±1.3	4.6±1.3	2.0±0.8	10.3±1.2
16:0	17.4±1.4	19.1±2.1	20.0±3.6	13.9±2.4	21.8±2.8	9.9±1.5
18:0	2.3±0.8	3.1±2.8	5.5±6.4	3.3±1.9	2.1±0.3	0.7±0.2
22:0	0.3±0.2	0.3±0.3	0.8±0.8	0.6±0.7	4.8±0.7	0.1±0.0
24:0	0.7±3.3	0.1±0.2	0.6±0.6	0.8±1.0	1.9±0.5	0.1±0.0
ΣSFA	29.0±4.0	30.5±2.9	38.6±8.4	30.7±5.1	39.3±2.7	22.2±2.9
16:1n-7	3.1±0.8	4.4±1.8	6.4±2.8	11.6±3.4	1.4±1.3	1.3±0.2
17:1	0.7±0.8	0.5±0.4	0.9±0.8	1.0±0.8	3.4±0.8	0.2±0.1
18:1n-9	6.4±3.3	10.1±4.8	10.8±5.0	13.2±4.2	3.4±0.7	29.5±19.9
18:1n-7	2.7±0.8	4.7±2.5	3.7±1.3	3.9±0.7	1.7±1.0	8.8±17.2
ΣMUFA	18.6±5.1	25.4±9.4	29.4±6.1	39.4±5.2	13.0±2.5	41.7±3.2
18:2n-6	3.7±1.3	3.3±1.0	3.8±1.0	3.6±1.1	12.6±3.0	9.3±2.0
18:3n-3	2.4±0.8	2.2±0.5	2.1±0.6	2.0±0.6	26.5±5.7	3.2±1.1
18:4n-3	5.2±1.4	3.5±1.1	3.2±1.8	2.0±1.2	0.4±0.2	2.3±0.4
20:4n-6	0.7±0.2	0.6±0.2	0.6±0.4	1.3±0.3	0.3±0.3	11.0±1.8
20:5n-3	13.6±2.3	11.0±3.4	6.5±2.7	6.1±4.3	4.6±2.9	5.4±0.5
22:6n-3	19.5±6.8	15.2±8.0	6.1±4.1	4.6±4.6	0.7±0.6	1.1±0.3
ΣPUFA	52.4±6.5	44.2±8.9	32.0±8.0	29.9±8.7	47.6±4.9	36.2±3.2
DHA:EPA	1.4±0.4	1.3±0.3	0.9±0.3	0.8±0.4	0.3±0.5	0.2±0.0
Bacterial	4.9±1.6	4.4±1.1	7.9±2.6	8.3±3.0	7.2±1.3	1.0±0.1

Table 3: Major lipid classes (>4% in at least one group) and FAs (>2%) in mysids and amphipods collected from August to November (2002) over *Zostera marina* beds in Newman Sound (mean ± SD).

	Mysids (n=26)	Amphipods (n=32)
Total lipid (mg.animal^{-1})	1.2±0.7	0.3±0.3
% Lipid classes		
Triacylglycerols	35.3±18.4	33.7±14.5
Free FAs	8.3±4.6	19.5±7.0
Sterols	8.1±2.1	7.7±3.0
Diacylglycerols	3.5±1.7	1.9±2.0
Acetone Mobile Polar Lipids	4.3±1.3	5.4±1.9
Phospholipids	38.4±17.6	27.8±12.1
% FAs		
14:0	3.2±1.07	1.8±0.6
16:0	17.3±1.6	14.9±1.3
ΣSFA	25.5±1.7	21.4±2.0
16:1n-7	8.5±2.8	3.2±1.1
18:1n-9	8.1±0.7	16:8±4.5
18:1n-7	3.0±0.2	2.7±0.4
ΣMUFA	24.2±2.2	28.4±5.4
18:2n-6	1.8±0.5	4.8±1.2
18:3n-3	1.5±0.5	2.0±0.4
18:4n-3	3.1±1.5	2.1±1.0
20:4n-6	4.1±1.4	4.2±2.5
20:5n-3	17.5±1.5	18.2±3.3
22:6n-3	14.4±3.5	13.7±3.1
ΣPUFA	50.3±2.3	50.2±4.9
DHA:EPA	0.8±0.2	0.8±0.1
Bacteria	4.4±0.9	3.8±0.6

Table 4: Major lipid classes (>4%) and FAs (>2%) in settled (>60 mm) and pelagic (<60 mm) cod collected from August to November (2002) in Newman Sound *Zostera marina* beds (mean ± SD).

	August	Sept	October		November	
	Pelagic (n=12)	Pelagic (n=13)	Pelagic (n=13)	Settled (n=7)	Pelagic (n=14)	Settled (n=23)
Total lipid ($mg.g^{-1}$ wet wt)	21.2± 4.2	19.5±4.7	17.1±6.4	16.0±6.2	18.0±3.0[a]	12.5±5.1[b]
Total lipid ($mg.animal^{-1}$)	25.2±9.7	19.0±7.7	19.7±7.8[a]	36.4±9.9[b]	19.5±10.0[a]	40.4±8.8[b]
% of total lipid						
TAG	20.9±11.7	21.6±5.0	19.6±8.8	25.1±5.6	18.8±10.6[a]	35.7±11.0[b]
FFA	13.5±3.2	9.9±3.0	8.8±2.0	9.6±3.3	10.6±3.4	10.3±3.9
ST	9.6±2.4	10.1±1.3	8.9±1.3	8.3±1.5	10.2±1.7[a]	7.7±1.6[b]
AMPL	5.2±1.8	5.7±1.6	7.1±2.2	7.2±1.6	6.8±3.2	8.2±3.3
PL	47.1±7.9	49.8±4.6	50.6±8.7	44.86±7.2	49.8±8.4[a]	31.5±9.9[b]
Major FAs (% total)						
14:0	2.1±0.5	2.4±0.4	1.7±0.3	1.7±0.4	1.8±0.4	1.9±0.6
16:0	16.1±1.6*	17.5±0.8	17.7±2.6	16.5±0.7	18.5±2.6[a]	14.8±3.4[b]
18:0	4.8±0.6*	4.3±0.5	4.5±0.5	4.5±0.4	4.4±0.5	4.0±0.6
16:1n-7	1.9±0.4	1.9±0.4	1.6±0.2	1.9±0.5	2.1±0.4[a]	2.4±0.5[b]
18:1n-9	9.2±1.0	9.3±0.7	9.2±0.8	9.3±0.5	9.8±1.5	9.8±1.0
18:1n-7	2.1±0.2	2.1±0.4	2.1±0.2[a]	2.3±0.2[b]	2.6±0.3	2.7±0.4
20:1n-9	3.0±1.2	2.5±0.9	1.9±0.9	2.1±0.8	2.3±1.0	2.9±1.9
22:1n-11&13	3.1±2.0	1.8±0.8	0.9±0.9	1.1±0.9	1.1±0.8	1.8±1.6
18:2n-6	1.3±0.4*	2.2±0.2	2.7±0.3[a]	3.1±0.4[b]	2.7±0.7[a]	3.9±0.9[b]
18:3n-3	1.0±0.2*	1.7±0.2	1.9±0.3	2.1±0.1	1.5±0.4[a]	2.3±0.4[b]
18:4n-3	2.1±0.7*	3.3±0.5	3.5±0.8	3.9±0.3	3.0±0.9[a]	4.7±1.2[b]
20:5n-3	11.5±0.8	11.5±0.7	12.2±1.3	12.8±1.0	12.6±1.6	13.4±1.2
22:6n-3	28.5±4.0	26.9±3.1	27.9±2.9	26.7±2.5	25.3±6.5	23.7±3.7
Bacterial	3.2±0.2*	3.0±0.6	2.6±0.3	2.5±0.3	2.5±1.0	2.2±0.5
Terrestrial	2.3±0.2*	3.9±0.4	4.6±0.6[a]	5.2±0.4[b]	4.2±1.1[a]	6.2±1.2[b]

*Significant effect of month on lipid composition between pelagic fish only (ANOVA, $p<0.05$). [a,b]Significant difference between pelagic and settled fish within a month (ANOVA $p<0.05$)

FIGURE CAPTIONS:

Figure 1: Sample Collection sites in Newman Sound, Bonivista Bay (MC = Minchin's Cove, DS = Dockside, HC = Heffern's Cove, MI = Mistaken Cove, and SB = South Broad Cove).

Figure 2: Major zooplankton species abundance (>5%) in two size classes of plankton collected during the late summer and fall of 2002 (n=2).

Figure 3: Principal components analysis of lipid class and FA proportions in plankton tows collected in Newman Sound during summer and fall 2002. (a) Lipid loading coefficients for PC1 and PC2 (b) Different sized net tows scores for PC1 and PC2. Acetone Mobile Polar Lipids (AMPL), Alcohols (ALC), Hydrocarbons (HC), % Organic matter (ORG), Polyunsaturated to Saturated FA ratio (P/S).

Figure 4: Seasonal changes in PUFA levels in two types of epibenthic and two sizes of zooplankton during the late summer and fall of 2002.

Figure 5: Principal components analysis of epibenthic prey and zooplankton available from August to November (2002) in Newman Sound. (a) Lipid loading coefficients for PC1 and PC2 (b) Scores of summer and fall zooplankton (ZP), mysids (M) and amphipods (A) for PC1 and PC2.

Figure 6: Principal component analysis of lipid class and FA proportions in juvenile cod collected in Newman Sound during summer and fall 2002. (a) Lipid loading coefficients for PC1 and PC2 (b) Scores for pelagic and settled fish for PC1 and PC2. Wax esters & steryl esters (WE/SE).

Figure 7: Compound specific FA isotope data for (a) primary producers and (b) secondary consumers in August (A) and November (N) during 2002. Lines represent the weighted averages for all FAs in a given sample. *Literature data for net tow and terrestrial plants (*Equisetum*) are from Budge et al. (2001)

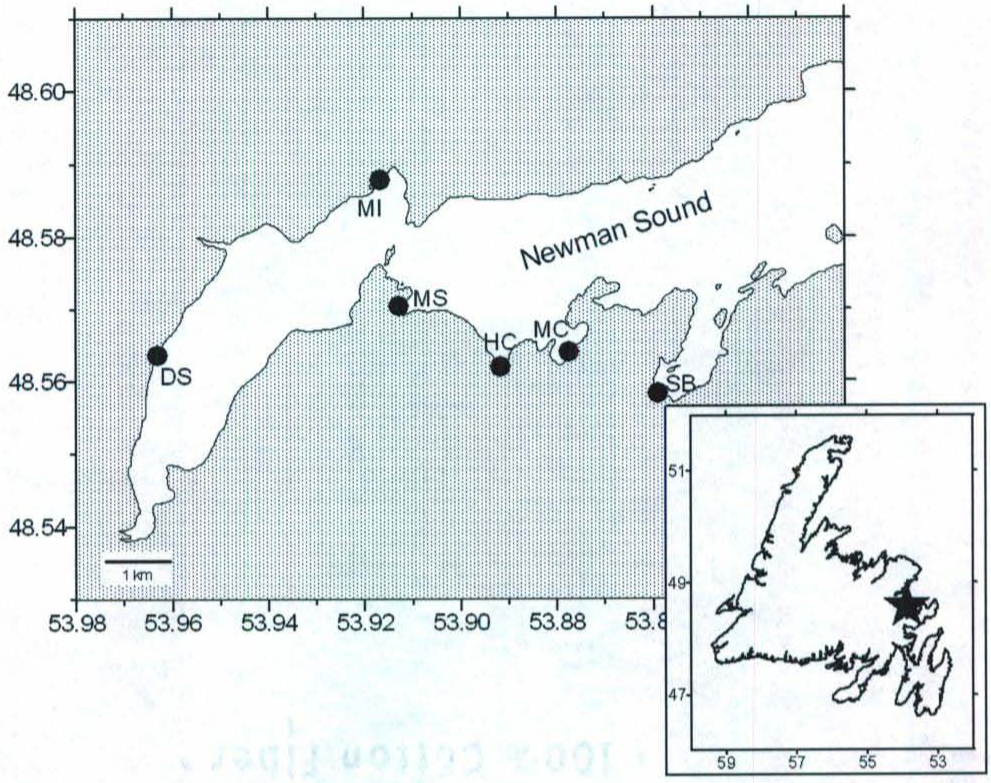

48.60
48.58
48.56
48.54
MI
Newman Sound
MS
HC
MC
DS
SB
1 km
53.98
53.96
53.94
53.92
53.90
53.88
51
49
47
59
57
55
53

Figure 1

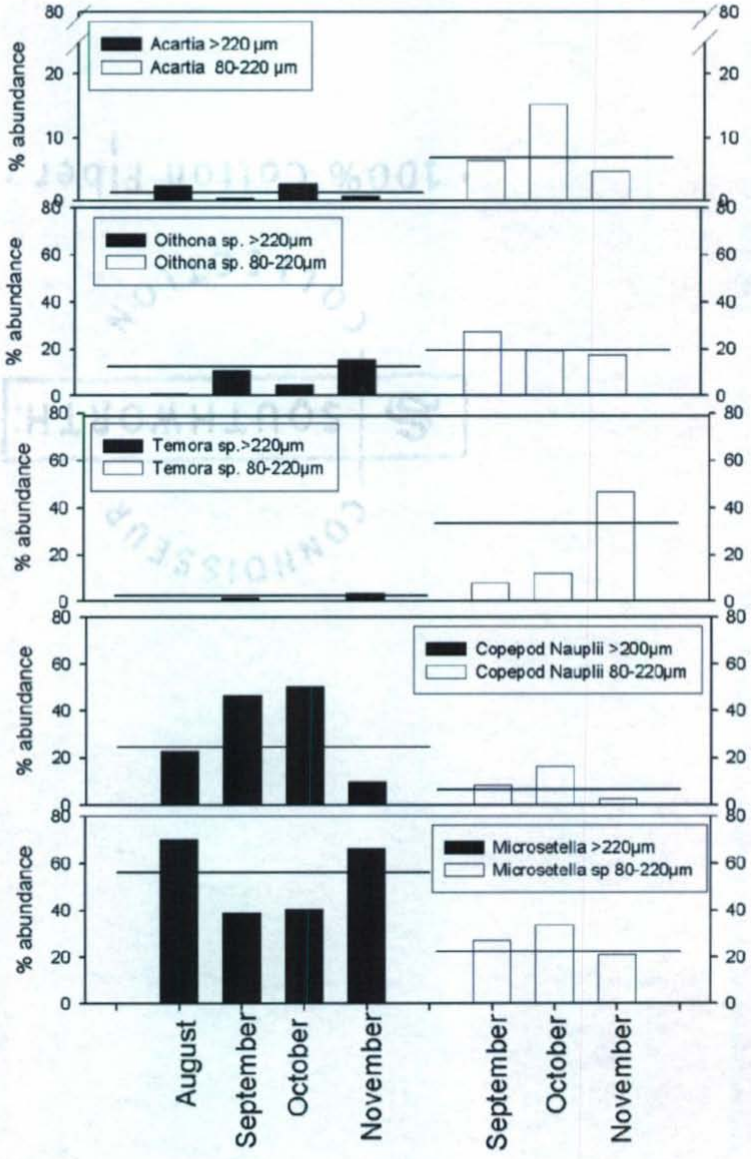

Acartia >220 µm
Acartia 80-220 µm
Oithona sp. >220µm
Oithona sp. 80-220µm
Temora sp.>220µm
Temora sp. 80-220µm
Copepod Nauplii >200µm
Copepod Nauplii 80-220µm
Microsetella >220µm
Microsetella sp 80-220µm
% abundance
August
September
October
November
September
October
November

Figure 2

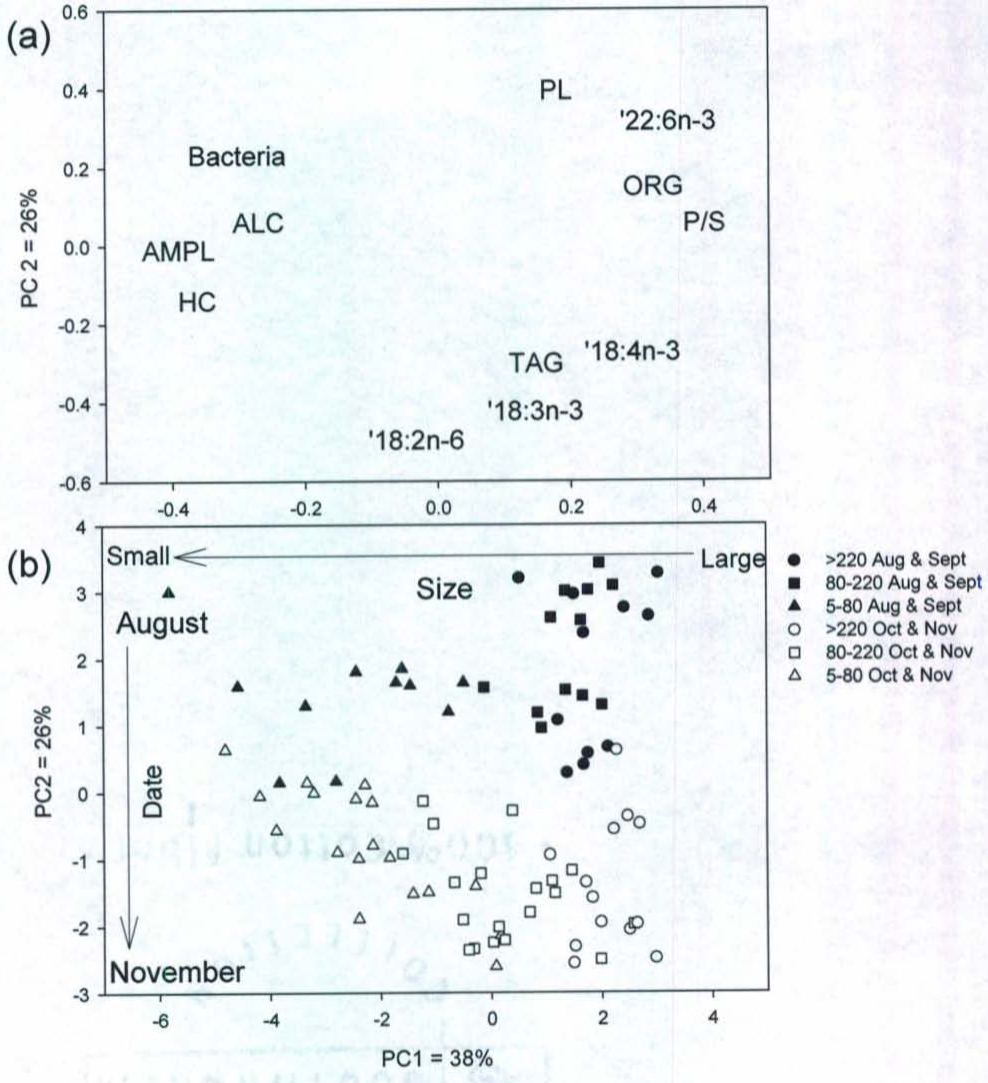
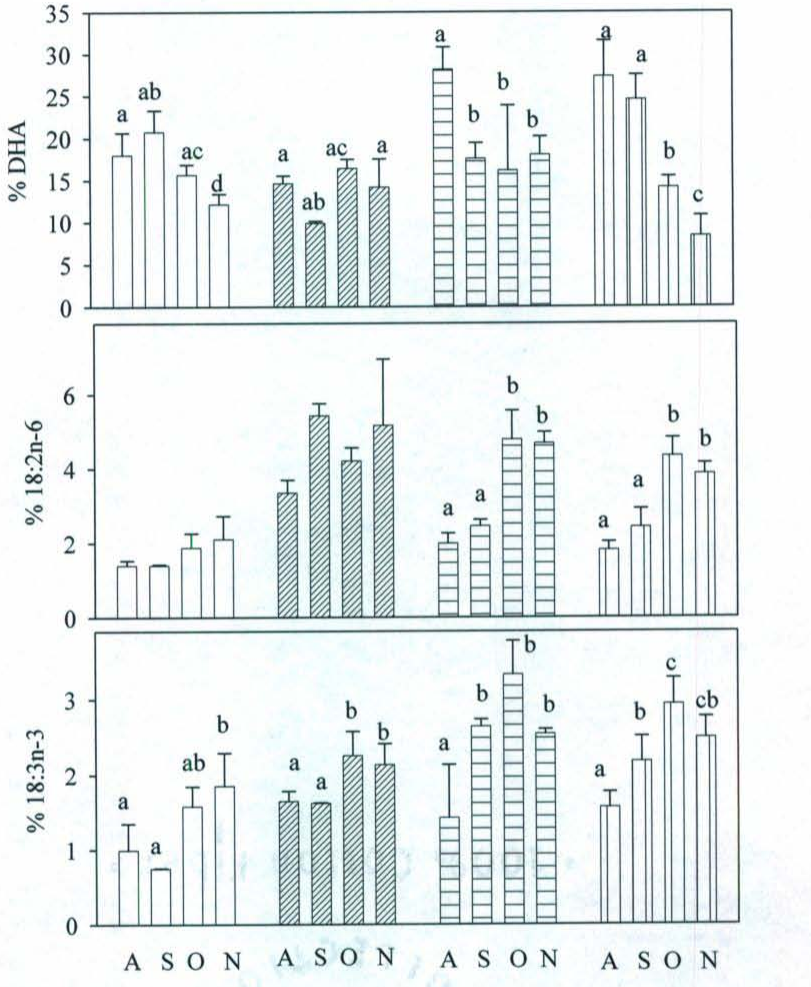

(a)
PL
'22:6n-3
Bacteria
ORG
ALC
P/S
AMPL
HC
TAG
'18:4n-3
'18:3n-3
'18:2n-6
PC 2 = 26%
(b)
Small
Large
Size
August
Date
November
PC2 = 26%
PC1 = 38%
>220 Aug & Sept
80-220 Aug & Sept
5-80 Aug & Sept
>220 Oct & Nov
80-220 Oct & Nov
5-80 Oct & Nov

Figure 3



(A=August, S=September, O=October, N=November, One-Way ANOVA, P=0.05)

Figure 4

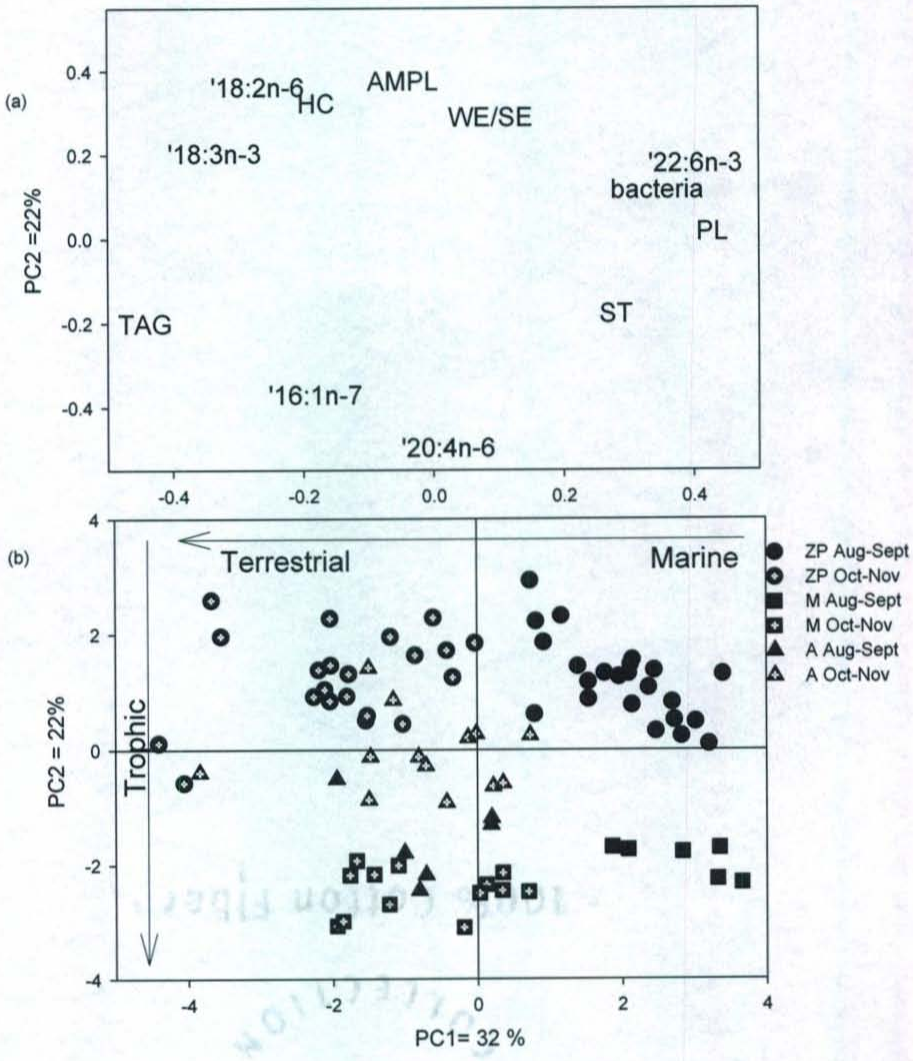

(a)
'18:2n-6
HC
AMPL
WE/SE
'18:3n-3
'22:6n-3
bacteria
PL
ST
TAG
'16:1n-7
'20:4n-6
PC2 =22%
0.4
0.2
0.0
-0.2
-0.4
-0.4
-0.2
0.0
0.2
0.4
(b)
Terrestrial
Marine
Trophic
PC2 = 22%
PC1= 32 %
4
2
0
-2
-4
-4
-2
0
2
4
ZP Aug-Sept
ZP Oct-Nov
M Aug-Sept
M Oct-Nov
A Aug-Sept
A Oct-Nov

Figure 5

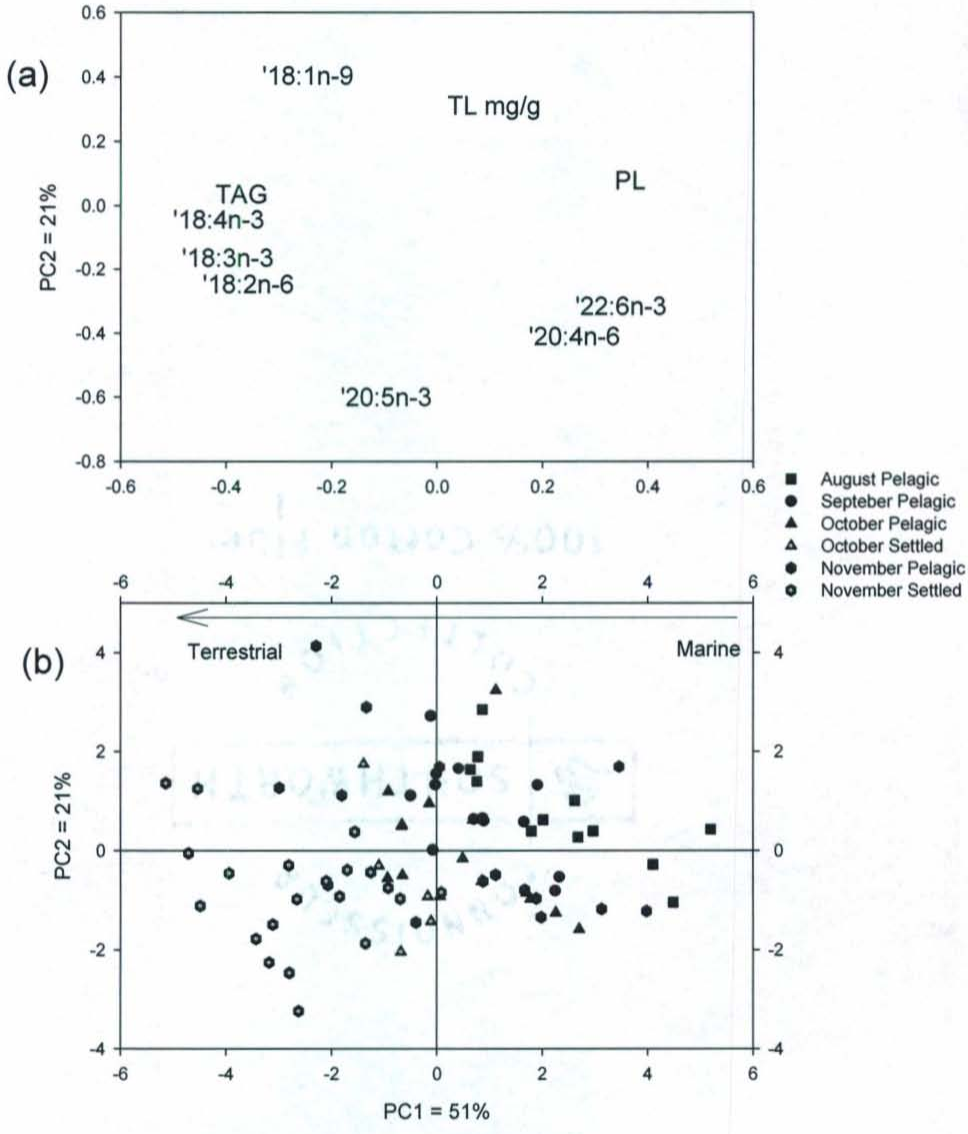

(a)
'18:1n-9
TL mg/g
PL
TAG
'18:4n-3
'18:3n-3
'18:2n-6
'22:6n-3
'20:4n-6
'20:5n-3
PC2 = 21%
August Pelagic
Septeber Pelagic
October Pelagic
October Settled
November Pelagic
November Settled
(b)
Terrestrial
Marine
PC2 = 21%
PC1 = 51%

Figure 6

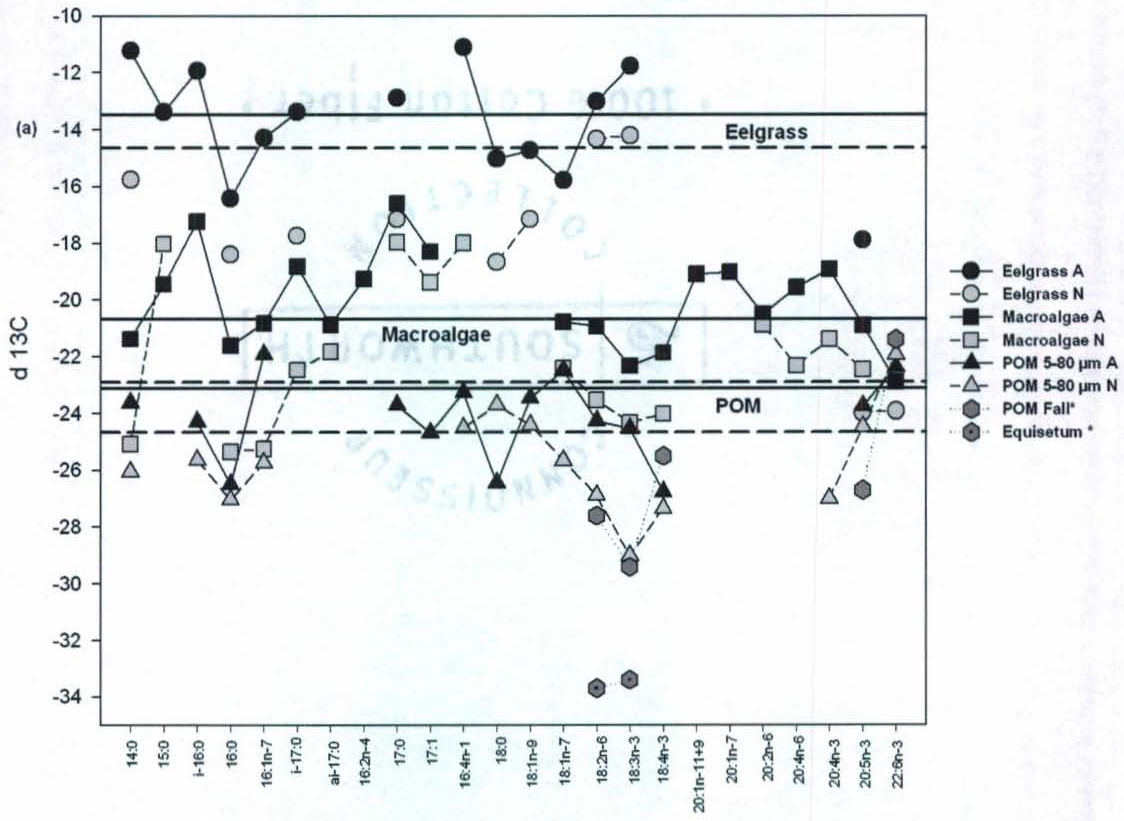

(a)
d 13C
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
-34
Eelgrass
Macroalgae
POM
14:0
15:0
i-16:0
16:0
16:1n-7
i-17:0
ai-17:0
16:2n-4
17:0
17:1
16:4n-1
18:0
18:1n-9
18:1n-7
18:2n-6
18:3n-3
18:4n-3
20:1n-11+9
20:1n-7
20:2n-6
20:4n-6
20:4n-3
20:5n-3
22:6n-3
Eelgrass A
Eelgrass N
Macroalgae A
Macroalgae N
POM 5-80 µm A
POM 5-80 µm N
POM Fall*
Equisetum *

Figure 7A

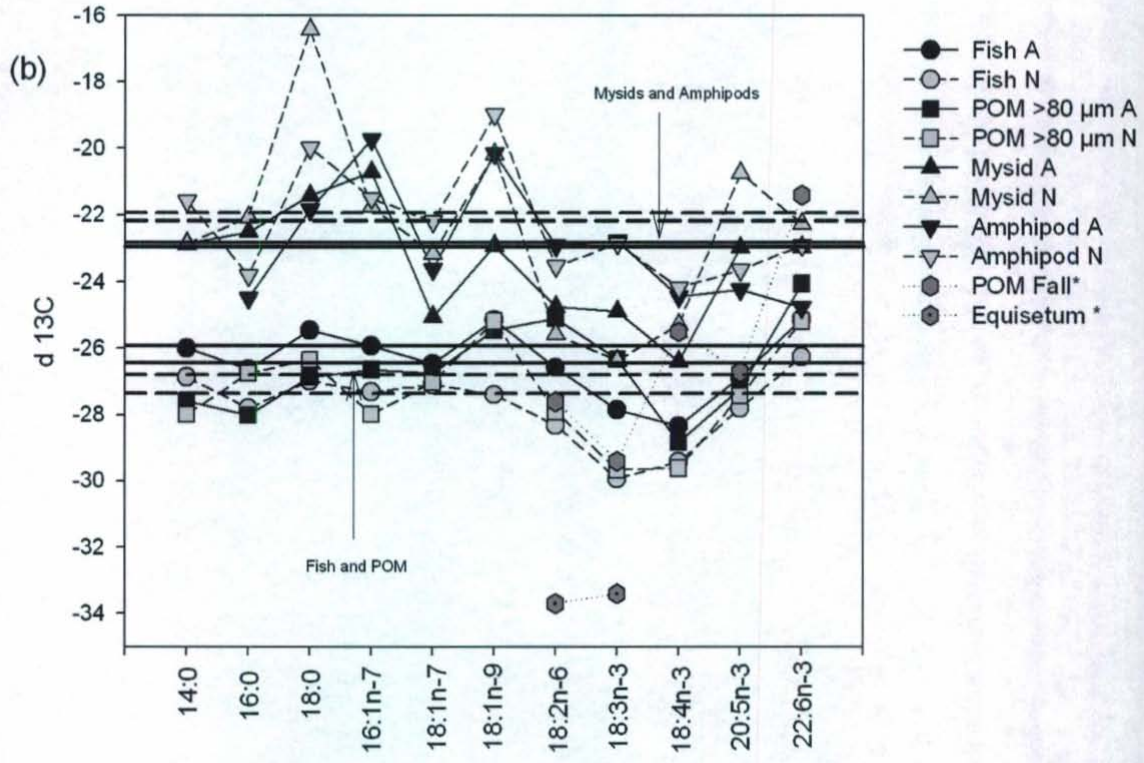

(b)
d 13C
-16
-18
-20
-22
-24
-26
-28
-30
-32
-34
Mysids and Amphipods
Fish and POM
14:0
16:0
18:0
16:1n-7
18:1n-7
18:1n-9
18:2n-6
18:3n-3
18:4n-3
20:5n-3
22:6n-3
Fish A
Fish N
POM >80 µm A
POM >80 µm N
Mysid A
Mysid N
Amphipod A
Amphipod N
POM Fall*
Equisetum *

Figure 7B

Chapter 4: Experimental Evidence of Fatty Acid Limited Growth and Survival in Pacific Cod (*Gadus macrocephalus*) Larvae

*** A version of this chapter was previously published and formatted for the journal Marine Ecology Progress Series (Copeman & Laurel 2010).**

ABSTRACT:

Changing environmental conditions in the North Pacific are altering the lipid/fatty acid (FA) composition of zooplankton assemblages, but the consequences to resident fish larvae are unknown. In the laboratory, I reared Pacific cod (*Gadus macrocephalus*) larvae over a 4-wk period on prey enriched with varying levels of two essential FAs, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), to determine how this species might respond to such changes in prey quality. Ratios of DHA:EPA were chosen to represent the natural variation observed in zooplankton of the North Pacific. I tested two hypotheses: 1) whether energetically similar diets comprised of varying levels of EPA and DHA impact growth and survival in Pacific cod larvae, and 2) whether the highest levels of DHA:EPA (2:1) are optimal for Pacific cod larvae, as it has been shown for Atlantic species. Results indicated that Pacific cod larvae grew fastest with diets containing high levels of n-3 PUFA (>22%). Diets with the same total lipid content but different ratios of DHA:EPA (<0.1:1 to 2:1) also mediated growth and lipid composition of the larvae. Interestingly, unlike Atlantic cod, Pacific cod larvae did not show as high a requirement for DHA relative to EPA but rather achieved largest size-at-age with intermediate DHA:EPA ratios ranging from of 0.8:1 to 1.1:1. This range most closely resembled average levels of DHA:EPA reported from copepods of the North Pacific, suggesting anomalous years with an over- or under-abundance of DHA-rich

dinoflagellates or EPA-rich diatoms may be detrimental to survival and growth of Pacific cod larvae in the field.

INTRODUCTION:

Prey quality is an important but poorly understood factor regulating growth and survival in larval fish (Cushing 1990, Munk 1997, Beaugrand et al. 2003). Changing climate and emerging concerns of ocean acidification have the potential to impact fish communities through large-scale changes in planktonic communities. Changes in dietary quality for larval fish can be manifested both through changes in available copepod and zooplankton populations or through compositional change in specific nutritional components (i.e. essential FAs) within a given zooplankton prey species. In the North Pacific, zooplankton species composition changes dramatically between warm and cold years (Batten & Welsh 2004), with cold-years typified by large boreal copepods and warm years characterized by higher numbers of small, southern species (Mackas, 2007). Further, biochemical changes in a major North Pacific copepod species, *Neocalanus plumchrus,* have been correlated with changes in phytoplankton species abundance and have resulted in shifts in zooplankton FA composition (El-Sabaawi et al. 2009a). However, while qualitative changes in the planktonic community such as these are hypothesized to impact growth and survival of marine fish larvae, they have rarely been examined explicitly. This is largely due to the difficulty in determining *a priori* which qualitative components of prey are limiting (e.g., prey size, energy, proximate composition, etc.) and the difficulty in manipulating those conditions experimentally for marine fish larvae (Rainuzzo et al. 1997).

From aquaculture studies, lipids and FAs are considered to be a limiting factor in determining prey quality for cold-water marine fish, as they play a vital role both as a source of energy and as important structural components of cell membranes (Sargent et al. 1989; Arts et al. 2001). In particular, polyunsaturated FAs (PUFAs) have been shown to be a critical component of larval fish nutrition and have been found to affect growth, survival, metamorphosis and pigmentation in many species (Wantanabe 1993, Sargent et al. 1999, Copeman et al. 2002).

Two PUFAs, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) are abundant in organisms found in cold-water marine ecosystems, but are considered essential FAs (EFAs) to marine fish as they cannot be synthesized in adequate amounts from shorter chain precursors. Marine larvae must therefore rely on dietary input of DHA and EPA stemming from primary production (Sargent 1995, Arts et al. 2001, Budge et al. 2001, Copeman & Parrish 2003). Levels of EPA in plankton have been correlated with diatom production whereas DHA is found at higher proportions in dinoflagellates (Dunstan et al. 1993, Parrish et al. 2000, Stevens et al. 2004). Although these FAs have both been found to be essential to marine fish larvae, many species have shown a higher level of membrane specificity for DHA than EPA (Copeman et al. 2002, Rodriguez et al. 1997). Given that DHA is naturally found at high levels in neural tissue, it is thought to play a specialized role in neural membrane structure and function (Bell and Dick, 1991). Therefore, higher dietary EPA in comparison to DHA is postulated to have a negative impact on larval neural function and, consequently, impact growth and survival (Bell et al 1995, Rodriguez et al 1997).

Together with arachidonic acid (20:4 n-6, AA), EPA is also found to be an important substrate for the formation of biologically active localized hormones such as prostaglandins (Sargent et al. 1999). Localized hormones have been indicated to be important in a wide range of fish physiological processes such as stress responses, ionic regulation and pigmentation development (Sargent 1995). EPA and AA are both substrates for the formation of eicosanoids, with AA being the preferred substrate and producing eicosanoids of higher biological activity (Bell et al. 1994). EPA produces eicosanoids of lower biological activity and therefore modulates the efficiency of AA. Therefore, it is often important to consider the ratio of DHA:EPA:AA as has been indicated in recent nutritional larval fish studies on Atlantic species (Copeman et al. 2002, Garcia et al. 2008a).

Generally, a DHA:EPA ratio of 2:1 in larval fish diets is cited as being optimal for growth and survival (Sargent 1995), largely based on egg composition of Atlantic species. However, Saito & Kotani (2000) found FA profiles of wax esters from four species of North Pacific copepods had low DHA:EPA ratios, 0.2:1 to 0.4:1. A more recent study from the North Pacific also indicated that DHA:EPA ratios were lower than the Atlantic, but varied considerably depending on the relative abundance of EPA-rich diatoms and DHA-rich dinoflagellates (0.3:1 to 1.1:1, El-Sabaawi et al. 2009a). Interestingly, Laurel et al. (2010) found levels of DHA:EPA in Pacific cod eggs were 1.4:1, lower than the 2:1 reported in their Atlantic congeners. The degree to which lower DHA:EPA ratios impact Pacific marine fish larvae remains uncertain, largely because the majority of cold-water larval fish nutrition is based on Atlantic aquaculture species

(Sargent et al. 1999) with little comparison to wild zooplankton assemblages (St. John et al. 2001).

Here I designed a laboratory experiment to examine how changes in DHA and EPA affected the growth and survival in the larvae of a cold-water Pacific marine fish species, Pacific cod (*Gadus macrocephalus*). Pacific cod were chosen because 1) they are highly abundant and play an important functional role in predator-prey dynamics in the North Pacific (Hunt et al. 2002) and 2) they make an interesting comparison with their well-studied Atlantic congener, *Gadus morhua*. I tested two hypotheses: 1) whether energetically similar diets comprised of varying levels of EPA and DHA impacted size-at-age and survival in Pacific cod larvae, and 2) whether the highest levels of DHA:EPA (e.g., 2:1) are optimal for marine fish larvae in the Pacific as it has been shown for Atlantic species. I discuss these experimental results in relation to natural variation in the lipid/FA composition of prey in the North Pacific.

MATERIALS AND METHODS:

Experimental design:

Four rotifer enrichments were formulated with varying levels of the essential FAs DHA and EPA and thus, variable DHA:EPA ratios (Dr. Moti Harel, Advanced BioNutrition, Columbia, MD, USA). Enriched rotifers were harvested twice daily throughout the experiment and fed to larvae for four wks. During this time I measured changes in lipid composition of the larvae while simultaneously monitoring size-at-age and survival.

Rotifer emulsions

The four experimental emulsions included one control emulsion that was high in monounsaturated FAs (MUFA, Diet 1) and three emulsions that were high in PUFA (Diets 2, 3 & 4, Table 2). The target ratio of DHA:EPA in enriched rotifers was from < 0.1:1 in control rotifers to a high of 2:1 in diet 4. Three of the PUFA emulsions were formulated by blending different ratios of algae oil (DHASCO-S) and a marine oil (Cod Liver Oil). The DHA -rich algal oil was extracted from the heterotrophically grown *Schizochitrium* sp. (Martek BioSci., Columbia, MD, Harel et al. 2002). The FA composition of DHASCO-S was 27% DHA and no EPA while cod liver oil contained approximately 20% DHA and 30% EPA (Information provided by manufacturer). The control emulsion was prepared using only olive oil, which was low in PUFA. A mixture of 5% lecithin, 1% vitamin E, 1% ascorbic acid and 1% Tween-80(w/oil weight) was added to the oils. Oil mixtures were emulsified with equal amounts of distilled water by first homogenizing at low speed (Ultra-turrax T8, IKA Labortechnik, Staufen, Germany) for 15 s and then sonicating for an additional 15 s at one third of the maximum sonication energy level (Sonifier 450: Branson Sonic Power: Danbury, CT.). Emulsions were stored under nitrogen at 4°C for daily use.

Rotifer culture and enrichment

Rotifers were cultured in a high-density rotifer culture system (150L) and maintained on *Nannochloropsis* Premium 3600 (Suantika et al. 2000, Aquatic Ecosystems, Inc). Twice daily rotifers were harvested from the continuous high-density rotifer culture system and were placed in smaller enrichment vessels in order to produce 4 AM and a 4 PM batches of enriched rotifers for daily larval fish feedings. Rotifer

enrichments were carried out in small 50L vessels at a density of 500 rotifers L^{-1}. During enrichment rotifers were gently aerated using an air stone placed in the bottom of the conical 50L vessel. Unenriched rotifer batches were enriched for 8 hr (8am to 4pm) and 16 hrs (4pm to 8am) each 24 hr period by adding 0.1g of oil per 500,000 rotifers at the beginning of each enrichment (Dhert et al. 2001, Copeman et al 2002). Emulsion oils were blended for approximately 30 sec in 2L of distilled water and added to enrichment cylinders. Enriched rotifers were sampled from each enrichment vessel in triplicate for lipid analysis two times in the AM and two times in the PM during the experiment.

Larviculture

Fish for larval experiments were reared in the laboratory from eggs collected from spawning adults. In April 2008, 2 female and 3 male Pacific cod were caught by commercial jigging gear from spawning grounds in Chiniak Bay, Kodiak Island, Alaska. The gametes were mixed and placed into 4 L incubation trays at 4°C. At 24 h post-fertilization, fertilized eggs were shipped in insulated containers filled with 4°C chilled seawater to Alaska Fisheries Science Center (AFSC) laboratory facilities in Newport, Oregon. Eggs were transferred to 4 L plastic flow-through trays and incubated at 4°C until hatching, following procedures described by Laurel et al. (2008). Hatching occurred 19-22 days post-fertilization, after which larvae were transferred into 100 L cylindrical upwelling tanks. Larval tanks were stocked with larvae from multiple hatching trays in order to avoid differential effects due to egg-incubation environment. Three larval tanks were assigned to each of four dietary treatments for a total of 12 larval first-feeding culture tanks.

The light regime during larval rearing was maintained at 12:12 h light:dark, with light provided by overhead fluorescent bulbs at a level of 6.7 μmol photons•$m^{-2}s^{-1}$ at the water surface. The larval feeding experiment was carried out in 12 conical upwelling 100 L fiberglass tanks with dark green interiors. Larvae were randomly sorted into tanks with 3 replicate tanks assigned to each of 4 dietary treatments. Tanks were kept at 12:12 photoperiod to approximate day length conditions experienced by cod larvae in the Gulf of Alaska in March-April. Water was supplied at a rate of 250 ml min^{-1} through central-bottom intake to minimize disturbance to the larvae. Gentle aeration was provided by an airstone to provide additional circulation in the tanks. Differentially enriched rotifers were added to tanks twice per day at a density of 4000 prey L^{-1}, from day 2 until the end of the experiment. Prey densities of 4000 prey L^{-1} are considered optimal, saturating food conditions for cod larvae (Brown et al. 2003). Tanks were "greened" with *Nannochloropsis* Premium 3600 (Aquatic Ecosystems, Inc) twice daily at a density of $1.06*10^{9}$ cells L^{-1}. Greening larval tanks with the addition of microalgae provides larviculture benefits that are not fully understood, but are related to beneficial effects on foraging activity and increased nutritional condition in larvae through direct ingestion of microalgae (van der Meeren et al. 2007a)

Size-at-age and survival

Larvae were sacrificed from experimental tanks at wks 1, 2, 3 & 4 for morphometric measurements. Ten larvae $tank^{-1}$ wk^{-1} were taken from three replicate tanks per treatment (30 larvae $treatment^{-1}$) for measurements of standard length (SL), length in millimeters from the tip of the snout to the end of the notochord, and body depth (BD), width of the larvae just posterior to the anus not including the fin fold, using an

image analysis system connected to a dissecting microscope. Dry weight (DWT) measurements were made with a microbalance (Sartorius R16OP) to the nearest µg. Ten larvae were pooled to give DWT estimates resulting in 1 measure $tank^{-1}$ and 3 measures $treatment^{-1}$ wk^{-1}. Larvae for DWT measure were first rinsed collectively in 3% ammonium formate solution to rid excess salt and inorganic material before being transferred to 1.5-cm^2 preweighed aluminum foils and an oven set at 68°C for 48 hrs. Average individual DWTs were calculated by subtracting the known foil weight and dividing by the number of individuals on the foil. Foils were then stored in a desiccator and reweighed within 1 hr. Survival was determined at the end of the study by counting all remaining larvae left in experimental tanks.

Lipid analysis:

Total lipids and lipid classes were measured on both rotifers and larval fish to determine differences in dietary quality and their effects on larval condition. It was also important to analyze rotifers to verify differences in EFA proportions, while other lipid parameters remained constant. The major lipid classes in fish include triacylglycerols (TAG), sterols (ST) and phospholipids (PL). TAG is generally considered as the major storage lipid class in larval fish while PL and ST are important components of cellular membranes. However, recent studies have shown that PL is also important as an energy source in eggs and larval fish as well as in low-lipid juveniles (Evans et al. 1998, Copeman et al. 2008, Laurel et al. 2010). Relative improvements in larval condition in other species, such as herring and Atlantic cod have been attributed to elevated total lipid, TAG per dry weight and TAG/ST ratios (Fraser 1989, Lochman et al. 1995).

Lipid samples of larvae were collected at time zero and at the end of wk 2 and wk 4. Not enough larvae remained in diet 1 to sample at wk 4 so only larvae from the three high PUFA diets were sampled for lipids at this end point. One sample of 50 pooled larvae was collected per tank for a total of three samples per diet at wk 2 and wk 4. Individuals were pooled in order to obtain sufficient material for lipid class and FA analysis. Lipids were extracted in chloroform/methanol according to Parrish (1987) using a modified Folch procedure (Folch et al. 1957). Lipid classes were determined using thin layer chromatography with flame ionisation detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three stage development system was used to separate lipid classes. The first separation consisted of 20-min developments in 98.95:1:0.05 hexane:diethyl ether:formic acid. The second separation consisted of a 40-min development in 79:20:1 hexane:diethyl ether:formic acid. The last separation consisted of 15-min developments in 100 % acetone followed by 10-min developments in 5:4:1 chloroform:methanol:water. After each separation, the rods were scanned and the 3 chromatograms were combined using T-data scan software (RSS Inc., Bemis, TN, USA). The signal detected in millivolts was quantified using lipid standards (Sigma, St. Louis, MO, USA). Lipid classes were expressed both in relative (mg g^{-1} wet weight) and absolute amounts (μg $animal^{-1}$).

Total lipid was analysed for FA composition. FA methyl esters (FAME) were prepared by transesterification with 14% BF_3 in methanol at 85°C for one and a half hours (Morrison & Smith 1964, Budge 1999). The FAMEs were analyzed on a HP 6890 GC FID equipped with a 7683 autosampler and a ZB wax+ GC column (Phenomenex,

U.S.A.). The column was 30 cm in length, with an internal diameter of 0.25 μm. The column temperature began at 65°C and held this temperature for 0.5 minutes. The temperature ramped to 195°C at a rate of 40°C min^{-1}, held for 15 minutes then ramped to a final temperature of 220°C at a rate of 2°C min^{-1}. This final temperature was held for 3.25 minutes. The carrier gas was hydrogen and flowed at a rate of 2 $ml \cdot min^{-1}$. The injector temperature started at 150°C and ramped to a final temperature of 250°C at a rate of 200°C·min^{-1}. The detector temperature stayed constant at 260°C. Peaks were identified using retention times from standards purchased from Supelco (37 component FAME, BAME , PUFA 1, PUFA 3). Chromatograms were integrated using the HP ChemStation Chromatograghy Software (Version B00.00).

Data analysis

Differences in size-at-age and lipid profiles were performed on tank means. Examination of differences between treatments in size-at-age measurements were analyzed using a repeated measures ANOVA with the explanatory variables being 'dietary treatment', 'wk', and the interaction between 'diet' and 'wk', (Statistix 7, Hicks 1982). There was a significant interaction between the effect of 'wk' and 'diet' on larval size-at-age and therefore, I examined weekly effects of diet on morphometrics and lipid composition using one-way ANOVAs with Tukey's multiple comparison tests. Data were examined for normality, homogeneity and independence to satisfy the assumption of the ANOVA. Significance for all tests was set at $\alpha=0.05$. FA percentage data were arcsine-square root transformed to meet the assumptions of the model. Differences in the lipid

classes and FAs of enriched rotifers were compared across diets using a one-way ANOVA with Tukey's multiple comparison tests.

Principal component analysis (PCA) was used to simplify multivariate FA and lipid class data by transforming correlated variables into a set of uncorrelated principal components (Minitab, version 15; Meglen 1992). This technique was employed using eight highly discriminatory lipid variables from first-feeding Pacific cod larvae, and larvae analyzed at wks 2 & 4 from all four diets. The first two principal components (PC1, PC2) accounted for 78% of the variance among samples, which allowed a display of the major trends within the data set without significant loss of the total original variation. PCA lipid loading coefficients are defined as the correlation coefficients between the original lipid variables and the PCA axis. PCA scores are defined as the position of the original variables along the new PCA axis (Meglen 1992). Lipid variables were chosen based on biological significance (found at >1.5% in all samples) and the degree of variance explained by a given lipid class or FA. A process of elimination was used in which all lipid variables of biological significance were used and then only those with the greatest explain variability were kept in the finial PCA analyses. A correlation matrix was used and scores were grouped by cluster analysis using single linkage.

RESULTS:

Enriched rotifers

Following rotifer enrichment there was no significant difference in the total lipid or the proportion of different lipid classes among differently enriched rotifers ($F_{3,12}$=1.35,

$p>0.5$, Table 1). On average, all groups had 11.3% of their DWT as lipid, 38% as triacylglycerols (TAG) and 29% as phospholipids (PL).

Feeding different emulsions to rotifers resulted in four diets with significantly different FA profiles (Table 2, $p<0.05$). The three high PUFA diets (Diets 2, 3, 4) did not vary significantly in their levels of total ∑SFA (24%), total ∑MUFA (33%), or total ∑PUFA (42%). In contrast, the control diet (diet 1), had significantly lower levels of ∑SFA (22%), higher ∑MUFA (51%), and lower ∑PUFA (27%) than the other enriched rotifer treatments. Elevated levels of 18:1n-9 in diet 1 reflected the use of olive oil for enrichment while varying levels of DHA, EPA, AA, and n-6 DPA were found in the three other diets. Diet 2 had a significantly lower DHA:EPA ratio (0.8:1) than diet 4 (2:1), while diet 3 had an intermediate value (1:1). Levels of total n-3 were also significantly different, ranging from 9% in diet 1 to 27% in diet 2, while total ω-6 PUFA ranged from 19% in diet 4 to 14% in diet 2 (Table 2)

Growth and survival

There was a significant effect of week of sampling on all three measurements of size-at-age (standard length, body depth and DWT $F_{3,23}$ $F>75$, $p<0.001$). Repeated measures also indicated a significant interaction between the effect of dietary treatment and the week of sampling on all three morphometric measurements ($F_{9,23}>4.11$, $p<0.003$, Figure 1a). Weekly differences in size-at-age were examined using one-way ANOVA with Tukey's pairwise comparisons. At wk 4 larvae from Diet 1 were significant shorter in standard length than larvae from all other dietary treatments. Further, body depth of larvae in diet 1 were significantly different than all other treatments at the end of wk 4 ($F_{3,7}=8.3$, $p<0.011$, Figure 1b). Dry weight of larvae were also significantly affected by

dietary treatment and examination of differences at the end of the experiment showed that larvae in diet 1 weighed significantly less than all of the PUFA treatments. Further, larvae in diets 2 and 3 weighed more than larvae from diet 4 ($F_{3,10}$=42.3, $p<0.001$, Figure 1c).

Larvae which were removed for lipid analysis and morphometric measurements were not considered in the calculation of survival. At the end of wk 4, the lowest survival was found in Diet 1, where there were not enough larval left to collect lipid data at wk 4 (average survival was 0.2±0.2%). Survival in the high PUFA treatments was similar, with diet 2 having 6±3%, diet 3 showing 6±3%, and diet 4 having 7±3%.

Lipid class composition of larvae

On a dry weight basis there was no significant increase in the amount of total lipid over the course of the entire feeding trial ($F_{2,19}$=0.07, p=0.933, Figure 2). Larvae had an average of 110 μg mg^{-1} of lipid per dry weight per individual throughout all treatments during the 3 sampling periods. There was a trend towards higher levels of lipid per dry weight in Diet 2 compared to the other dietary treatments, but this was not significant (wk 2, $F_{2,5}$=2.4, p=0.08). Absolute amounts of lipid per animal did increase from an average of 15.6 ± 5.1μg in all dietary treatments at wk 2 to 31.9 ± 10.0 μg per animal at the end of the experiment (data not shown).

The effect of dietary treatment on the proportion of TAG present in the larvae was not significant at wks 2 or 4. However, by pooling all dietary treatments there was a significant effect of sampling wk on the proportion of TAG in the larvae ($F_{2,19}$=22.9, $p<0.001$). Larvae at the beginning the experiment had significantly higher levels of TAG than larvae at wk 2, while larvae at wk 4 had the highest levels of TAG (Figure 2b).

The TAG/ST ratio followed the same trend as TAG, with an initial decrease to wk 2 and then an increase to wk 4. However, at wk 4 there was a significant difference in this ratio among dietary treatments ($F_{2,5}$=6.5, p=0.04). At wk 4, larvae in Diet 2 had a significantly higher TAG/ST condition index than larvae in Diet 4 (Figure 2c). There was no significant effect of diet on the proportion of phospholipids (PL) in the larvae at either wk 2 or wk 4 (figure 2d).

Fatty Acid Composition of Larvae

Total FAs per dry wt ($\mu g.mg^{-1}$) varied from 59 $\mu g\ mg^{-1}$ in Diet 1 at wk 2 to 87 $\mu g\ mg^{-1}$ in Diet 2 at the end of the experiment. When both weeks were pooled, Diet 2 had significantly more FAs per dry weight than Diet 4 ($F_{3,15}$=5.23, p=0.01).

After just two weeks of feeding, larvae showed significant differences in the levels of individual FAs (Table 3). Larvae in Diet 1 had higher levels of 18:1n-9 (~17%) and lower levels of many of the longer chain PUFAs than the other three larval groups. Survival was not measured at wk 2, however, larvae from Diet 1 were noted to have higher mortality at wk 2 than seen in the other PUFA diets. The DHA:EPA ratio in larvae at wk 2 varied from 1.4:1 in diet 1 to 4.3 :1 in Diet 4.

After four weeks of feeding, larvae in the three high PUFA diets showed variable levels of individual PUFA but no significant differences (p>0.05) in the ΣSFA, ΣMUFA or ΣPUFA. DHA levels reached a high of 24% in Diet 4, while EPA was highest in Diet 2 (12%). The DHA:EPA ratio in the larvae was significantly higher in Diet 4 (4.2:1) than in either Diet 2 or Diet 3 (~2:1). 22:5n-6 also varied significantly among larval groups with the highest levels found in Diet 4 (6.5%) and significantly lower levels found in Diets 1 and 2 (1.7% and 3.6%, respectively).

Levels of dietary FAs affected PUFA retention in larval tissue after only two weeks of feeding on enriched rotifers (Figure 3a). Larvae in the control diet had much higher levels of all PUFA in their tissues compared to dietary levels (1.7x more ∑PUFA, 60x more DHA, Figure 3a) and higher levels of 18:0, while levels ∑MUFA and 18:1n-9 were lower in all larvae than in their diets.

At 4 wks, only enough larvae remained in the PUFA enriched treatments (Diets 2, 3 and 4) to perform lipid analysis. Larvae from these diets showed conservation of DHA, 22:5n-3, 22:5n-6 (ω6DPA), 20:4n-6 (AA), and 18:0 at the rate of 1.5-2x more in their flesh than in the rotifers. EPA was present at levels approximately equal to that found in the rotifers while MUFA and the short chain PUFA 18:2n-6 were present at ~50% the proportions found in the diet (Figure 3b).

PCA of nine FA and lipid class variables simplified the lipid composition of larvae in terms of weekly and dietary effects (Figure 4). Figure 4a shows the first two principal components and separates larvae in terms of dietary differences. PC1 explained 44% of the variance, and shows a separation of the PUFA with high levels of DHA and AA on the positive side of the axis and higher levels of total lipids, and EPA on the negative side of the axis. Examination of the lipid loading coefficients for PC 2 (33%, Figure 4a) indicated that this axis represented an unsaturation axis, with MUFA loaded on the positive side and high levels of PUFA and increased condition (TAG/ST) on the negative side of the axis.

Examination of sample scores showed that larvae in Diet 4 from wks 2 and 4 were associated with high levels of DHA, AA, and ω6DPA. These larvae clustered together along the positive side of the axis. On the negative side of the axis, first-feeding larvae

clustered together with the four-week old larvae from Diet 2, indicating that these larvae had the most similar lipid composition, typified by higher levels of EPA relative to DHA. Larvae from Diet 1 had the lowest condition and low levels of PUFA, with the highest levels of MUFA. They clustered separately at the top of PC2 with an outlying larva from Diet 2. Larvae from Diet 3 showed an intermediate lipid composition.

Figure 5 shows the relative proportions of the essential FAs DHA, EPA and the ratio of DHA:EPA in differently enriched rotifers compared to that reported in wild copepods. I calculated the average and standard error of six years of data on *Neocalanus plumchrus* from the Straits of Georgia (El-Sabaawi et al. 2009a). DHA, EPA and DHA:EPA ranged from 3.9 to 8.6%, 6.6 to 17%, and 0.3 to 1.1, respectively (El-Sabaawi et al. 2009a). Proportions of DHA in the rotifers ranged from a low of 0.2% in Diet 1 to a high of 11.4% in Diet 4, while EPA was also lowest in Diet 1 (5.2%) and highest in Diet 2 (11.1%). The ratio of DHA:EPA ranged from a low of <0.1:1 in Diet 1 to a high of 2:1 in Diet 4. A comparison of *N. plumchrus* with my rotifer diets indicates that Diet 2 was the most well-matched diet compared to wild copepods in terms of all three PUFA measures (Figure 5).

DISCUSSION:

The results of this experiment support my predictions that larval Pacific cod do require high levels of n-3 PUFA for normal growth and development. Further, I found that energetically similar diets with different ratios of DHA:EPA did affect size-at-age and lipid composition. However, Pacific cod larvae did not show a high requirement for DHA relative to EPA but, rather, demonstrated highest growth at ratios ranging from

1.1:1 to 0.8:1. Diets 2 and 3 produced elevated growth and increased lipid condition indices. After four wks of feeding, larvae from diet 2 most closely resembled the levels of PUFA found in wild-spawned first-feeding Pacific cod larvae (Laurel et al. 2010). Further, a DHA:EPA ratio of 0.8:1 in diet 2 rotifers resembled the ratio reported for four species of cold-water Pacific copepods (*Neocalanus plumchrus*, *Calanus marshallae*, *Euchaeta elongate* and *Eucalanus bungii*, El-Sabaawi et al 2009b).

Rotifers (*Brachionus plicatilis*) are not a natural prey item for Pacific cod, but are commonly used in both ecological and aquaculture studies on small marine fish larvae (Puvanendran & Brown 1998, Jordaan & Brown 2003, Imsland et al. 2006). This is largely because rotifers are more easily mass-cultured than wild zooplankton and can still be enriched with lipids/FAs to resemble specific prey types. Further, rotifers do not show significant retro-conversion of long chain PUFA into shorter chain PUFA like other live-cultured prey e.g., *Artemia* (Navarro et al. 1999). Still, fish larvae often grow and survive better on natural prey in the laboratory (e.g., Imsland et al. 2006), likely because lipid-enriched rotifers lack some of the amino acids, vitamins, minerals and digestibility characteristics of wild zooplankton (Sargent 1999, Evjemo et al. 2003). Although it would ideal to culture Pacific cod larvae on marine copeopods with variable ratios of EFAs, the techniques for controlled lipid enrichment of marine copepods have not been fully developed (Olivotto et al. 2008). For these reasons rotifers still represent the best vehicle to experimentally examine the effects of prey quality on fish larvae.

Pacific cod and Atlantic cod larvae have been observed to feed opportunistically on protozoans, copepod nauplii and copepodites in the wild (Takatsu et al. 2002, van der Meeren & Naess 1993). Takatsu et al. (2002) showed that Pacific cod larvae off Japan

consumed a variety of copepod nauplii and copepodites. Over a three-year study, smaller larvae (3.6-7.0 mm TL) had higher levels of nauplii (41-83% of prey items in the gut) while larger larvae (7.1-15.5 mm TL) had mostly copepodites (80.4-99.5%). Here I examined just the effect of FA composition of one live-food on larval size-at-age and condition. Although Pacific cod larvae are capable of growing on rotifers up to six weeks at similar temperatures (Laurel et al. In Press), I cannot rule out the possibility that prey size constrained growth in these larvae during the last week of the experiment. However, this factor would likely have been more significant in larger larvae from diets 2 and 3 than for smaller larvae in diet 1. Therefore, any potential effect of prey size constraints in my experiment would have made these conclusions about dietary EFA on size-at-age somewhat conservative.

I intentionally formulated diets ranging in DHA:EPA ratios to represent the natural annual variation in plankton FAs associated with food webs based on diatoms and dinoflagellates (Budge & Parrish 1998, El-Sabaawi et al. 2009a). Previous studies on lipid nutrition in gadids have largely been based on commercial live-food enrichments, which often have simultaneous variability in a number of factors such as total lipids per dry wt, protein, lipid classes as well as multiple FAs (Park et al. 2006, Garcia et al. 2008a). Controlled studies using formulated experimental enrichment oils have been conducted to examine the importance of the EFAs; DHA (22:6n-3), EPA (20:5n-3) and AA (20:4n-6). However, most of these studies have been based on commercially important Atlantic species (Copeman et al. 2002, Villalta et al. 2005a, Lund et al. 2007).

The functional significance of dietary DHA:EPA can be observed in terms of competitive interactions between FAs for incorporation into phospholipids; specifically, competition for the enzymes that esterify FAs onto the glycerophospho-based backbone (Sargent et al. 1999). The functional significance of the ratio of DHA:EPA has now been investigated in both primary consumers (Arendt et al. 2005, El-Sabaawi et al. 2009a) and fish larvae (Sargent et al. 1999, Izquierdo et al. 2000) as well as at the ecosystem level (Litzow et al. 2006). Specifically, this ratio has been well studied in relation to the dietary requirements of many marine fish that are candidates for aquaculture development. Nutritional requirements for DHA and EPA have been found to be both species- and developmentally-specific (Villalta et al. 2005b, Copeman 2001) and therefore cannot always be generalized across species and life-history stages.

From a population perspective, reproductive and early life history differences between Pacific and Atlantic cod suggest that Pacific cod larvae may be more susceptible to changes in prey quality in the field. Although both species are highly fecund, and likely susceptible to high variation in survival during the first few wks of life (May 1967, McCain 2003), Pacific cod eggs are semi-adhesive and are released in one batch during the spring spawning season (Mecklenburg et al. 2002). This contrasts to Atlantic cod eggs which are pelagic and are released in batch spawning events across multiple months in the spring and fall (Kjesbu 2006). These differences in spawning characteristics of Pacific cod, both temporally (i.e. single batch-spawing) and spatially (i.e., reduced dispersal potential of eggs), have been hypothesized to make Pacific cod more vulnerable to changes in their prey field than Atlantic cod (Laurel et al. 2008).

Pacific cod also differ from Atlantic cod in their ability to synthesize and convert lipids and FAs during egg development. Pacific cod eggs synthesize large amounts of lipid (presumably from protein) just prior to hatch, and demonstrate extreme conservation and possible synthesis of DHA as yolk-sac larvae (Laurel et al. 2010). This conservation of DHA by Pacific cod may reflect a unique ability to produce DHA from shorter chain precursors, however, the mechanism for this synthesis needs further investigation. Levels of DHA:EPA in wild Atlantic cod eggs have been reported to be 2:1 (Finn et al. 1995) while levels in pre-feeding larvae have been found to be 1.8:1 (Garcia et al. 2008a) and 2.6:1 (Finn et al. 1995). Levels of DHA:EPA in wild Pacific cod have been analyzed from 2006 and 2008 and have shown slightly lower DHA:EPA ratios in both eggs and newly hatched larvae, 1.5:1 and 1.8:1 respectively (Laurel et al. 2010). Therefore, some of the differences in the natural history of these two gadid species, coupled with lower levels of DHA:EPA in wild caught samples may indicate a lower dietary requirement for DHA and a higher requirement for EPA in Pacific cod than in their Atlantic congener. However, these differences may just represent ecosystem differences rather than egg or larval requirements.

EPA is an important FA both for inclusion in membranes and for the production of biologically active compounds called eicosanoids or 'localized hormones'. Eicosanoids play a wide variety of physiological roles in fish that can range from ionic regulation to stress responses (Sargent 1995, Logue et al. 2000). AA (20:4n-6) is also used in the production of these localized hormones that include prostaglandins, thromboxanes, and leukotrienes. In fish, AA is the preferred substrate for the formation of eicosanoids and has been found to produce eicosanoids of higher biological activity

than EPA (Bell et al. 1994). Previously, flatfish fed high levels of AA relative to EPA have been found to develop high rates of malpigmentations (Estevez et al. 1999, Copeman et al. 2002). Elevated levels of AA relative to EPA maybe stressful to fish in culture conditions. AA is always found in low levels in wild zooplankton and Pacific cod embryos (<2.5%, Laurel et al. 2010, El-Sabaawi personal communication, Budge & Parrish 1998). I had a range of EPA:AA ratios in larval diets with Diet 4 showing the lowest levels of 3:1 while Diet 2 showed the highest levels at 6.9:1. van der Meeren et al. (2007b) measured levels of EPA:AA in a number of copepods and found that the levels of EPA:AA were never less than 7.5:1 and could be as high as 49.5:1 due to very low levels of AA in the wild. The importance of this FA has led fish nutritionists to discuss optimum ratios in terms of a three part index of DHA:EPA:AA. Previously this has been hypothesized to be 10:1:1 (Park et al 2006) and 11:1.5:1 (Garcia et al. 2008a) in Atlantic cod. Based on the higher weight and lipid condition indices for larvae in Diet 2, I would suggest a preliminary dietary ratio of DHA:EPA:AA of 5:7:1 for first-feeding Pacific cod.

Rotifers enriched with all four experimental emulsions had the same total lipids per dry weight and the same proportions of different lipid classes. This ensured that the proximate lipid composition of the rotifers did not differ between dietary treatments. I enriched the rotifers only once every 12hrs. However, for maximal lipid retention and growth potential these experimental emulsions should ideally be added to rotifer cultures more repeatedly e.g., every 4 hrs. This is likely why the levels of total lipid per dry wt in these data were ~11% while Copeman et al. (2002) reported levels of ~16% using similar experimental emulsions. For my purposes, enrichment every 12 hrs provided the

variation in DHA:EPA ratios that I required to mimic natural variation reported in zooplankton of the North Pacific, despite possible reductions in growth and survival potential.

Temperature and food availability are often emphasized as the most limiting factors regulating the vital rates of fish larvae (Buckley et al. 2004). However, this experiment indicates that prey quality can explain similar variance in growth and survival. This experiment was conducted at 8°C, yet Pacific cod larvae exposed to Diet 1 grew at rates similar to Pacific cod larvae reared at 3°C (2%.day^{-1} ; Laurel et al in Press). In Diets 2 and 3, the observed growth rates (i.e., ~ 5% day^{-1}) are on the lower range of those reported for Pacific cod larvae reared at similar temperatures (5-12% day^{-1}), but this is likely attributable to using experimental enrichment emulsions as opposed to commercially formulated rotifer enrichments. While Diet 1 was an extreme and unlikely scenario for Pacific cod to face in the field, the growth variation among the 3 PUFA diets was measurable and would likely have significant survival consequences for Pacific cod larvae in the field when faced with size-dependent predation.

Survival at the end of the experiment was low in all treatments, with an average of 6% in the three PUFA diets and only ~1% in diet 1. While natural mortality of marine fish larvae is extremely high in the field, mortality in larviculture laboratory experiments can be driven by numerous uncontrolled environmental factors and tank effects. Interestingly, survival was much lower after wk 2 than observed after wk 1, most notably in Diet 1. Further, a reduction in the TAG/ST ratio and proportion of TAG was observed at wk 2. This was followed by a dramatic increase in condition and lipids at wk 4. Week 2 samples therefore likely contained larvae that had not successfully started feeding or

were starving due to inadequate nutrition. Day 13-18 post-hatch at 8°C is the period at which 100% mortality occurs in non-feeding Pacific cod larvae (Laurel et al. 2008). Lipid class analysis showed that larvae fed low PUFA had a significantly lower TAG/ST ratio than larvae in all other treatments. Relative improvements in larval condition in other species, such as herring and Atlantic cod, have been attributed to elevated total lipid, TAG per dry weight, and TAG/ST ratios (Fraser 1989; Lochman et al., 1995).

Although levels of DHA, EPA, and AA have been well investigated for their effects on the early survival, growth, and development in fish and marine invertebrates (Sargent 1999, Arts 2001), more recently ω6DPA (22:5n-6) has received attention as an EFA. Parrish et al. (2007) used stable isotope data and FA proportions to show that this FA was conserved at very high levels in larval tissue. Further isotopic evidence showed that these high levels were due to conservation of this long chain ω-6 PUFA rather than increases due to chain elongation of shorter chain precursors. These results confirm that this FA was also conserved at high levels in Pacific cod larvae, at a rate of 1.5x what was found in the diet. This is similar to the level of conservation of ω3DPA and lower than the levels of 2.5x seen for DHA in all larvae at wk 4. Despite the conservation of this FA in larval tissues, inclusion in the diet of Pacific cod did not result in increased growth or survival. This is contrary to reports for both larval Atlantic cod and larval scallops (*Argopecten irradians*), where ω6DPA has been associated with increased growth (Garcia et al. 2008b, Milke et al 2006). However, the addition of this FA to the diet of larval haddock did not increase growth or survival despite retention of this FA at high levels within larval tissues (Garcia et al. 2008b). Future work is required to test the importance of this FA to the growth and survival of marine species without simultaneous

variation in other highly essential FAs such as DHA and EPA. It has been suggested that this FA can be used as a C_{22} PUFA substitute in larval tissues when inadequate levels of DHA are present in the diet (Garcia et al. 2008a).

CONCLUSIONS:

In the North Pacific, shifts between EPA-rich diatoms and DHA-rich dinoflagellates have resulted in variability in the DHA:EPA ratios in zooplankton (El-Sabaawi et al. 2009a) but the impacts on Pacific marine fish larvae remain poorly studied. I have shown that Pacific cod larvae are sensitive to changes in the ratios of EFAs in their diet and that optimum dietary levels are comparable to those seen (on average) in the wild. Therefore, given the sensitivity of marine fish larvae to the nutritional composition of zooplankton, further efforts should be made to determine not only the effect of changes in zooplankton abundance and species composition (Beaugrand et al. 2003, Batten 2004, Mackas et al. 2007) but also the effect of changes in zooplankton nutritional quality for fish larvae. Furthermore, it will be important to determine when such prey quality is most critical in developing fish larvae. Given the sensitivity of zooplankton to phytoplankton species composition and FA proportions (El-Sabaawi et al. 2009a) it is likely that food quality will help explain a portion of variability in year class strength observed in Pacific cod throughout the North Pacific.

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Table 1: Lipid class composition of rotifers enriched for 8-16 hrs using four different oil emulsions (mean ± SEM, n=4)

	Diet 1	Diet 2	Diet 3	Diet 4
Ingredients (g)				
DHAsco-S	0	3.5	9.5	21.8
Cod Liver Oil	0	37.7	25.5	25.5
Olive Oil	45	3.8	10	10
Water	50	50	50	50
Total n-3	0	8.7	8.7	8.7
Total Lipids* ($\mu g.mg^{-1}$)	132.8 ± 42.8	133.7 ± 65.1	80.1 ± 38.9	107.2 ± 7.3
Lipid class (% total lipid)				
Steryl/wax esters	2.6 ± 0.2	1.8 ± 0.4	1.6 ± 1.0	2.2 ± 0.6
Triacylglycerols	35.0 ± 4.3	44.2 ± 3.5	35.4 ± 5.8	38.0 ±3.8
Free FAs	7.7 ± 1.1	6.0 ±1.5	6.7 ± 1.2	7.4 ± 0.9
Alcohols	2.7 ± 1.3	5.6 ± 0.5	6.7 ± 2.5	3.7 ± 2.7
Sterols	3.7 ± 0.5	3.3 ± 0.5	8.8 ± 4.9	6.2 ± 1.0
Acetone Mobile Polar Lipids	6.1 ± 0.7	8.7 ± 1.3	16.2 ± 6.5	11.0 ± 2.1
Phospholipids	38.5 ± 6.0	27.7 ± 0.8	22.0 ± 7.5	29.4 ± 2.6

*Also contained <2.5% Hydrocarbons, ethyl esters, methyl esters, ethyl ketones, methyl ketones, alcohols, and diacylglycerols.

*All emulsions contained 5% lecithin, 1% vitamin E, 1% ascorbic acid and 1% Tween-80(w/oil weight)

Table 2: FA composition of rotifer enriched for 8-16 hours using four different oil emulsions (mean ± SEM, n=4)

FA (% total FAs)	Diet 1	Diet 2	Diet 3	Diet 4
14:0	1.1 ± 0.1^{a}	3.1 ± 0.2^{b}	2.8 ± 0.2^{bc}	2.3 ± 0.2^{c}
16:0	16.7 ± 0.1^{a}	16.4 ± 0.4^{a}	16.9 ± 0.4^{ab}	19.0 ± 0.9^{b}
18:0	3.0 ± 0.2	3.0 ± 0.2	2.8 ± 0.2	2.8 ± 0.2
ΣSFA	21.7 ± 0.4^{a}	23.5 ± 0.4^{ab}	23.7 ± 0.4^{ab}	25.4 ± 1.2^{b}
16:1n-7	4.4 ± 0.5^{a}	6.4 ± 0.6^{b}	5.8 ± 0.5^{ab}	4.0 ± 0.3^{a}
18:1 n-9	41.2 ± 2.2^{a}	18.0 ± 0.5^{b}	18.9 ± 0.3^{b}	23.1 ± 0.9^{b}
18:1 n-7	2.5 ± 0.1^{a}	2.8 ± 0.1^{a}	2.5 ± 0.1^{a}	2.0 ± 0.1^{b}
20:1 n-9	0.9 ± 0.1^{a}	2.0 ± 0.2^{b}	1.8 ± 0.2^{ab}	0.6 ± 0.2^{a}
ΣMUFA	51.2 ± 1.6^{a}	33.0 ± 0.5^{b}	33.0 ± 0.5^{b}	33.0 ± 0.4^{b}
18:2 n-6	15.0 ± 0.7^{a}	10.2 ± 0.8^{b}	10.4 ± 0.7^{b}	10.9 ± 0.7^{b}
18:3n-3	0.7 ± 0.1^{a}	1.4 ± 0.2^{b}	1.1 ± 0.2^{ab}	0.6 ± 0.1^{a}
20:4 n-6 (AA)	0.9 ± 0.3^{a}	1.6 ± 0.0^{ab}	1.8 ± 0.1^{b}	2.1 ± 0.2^{b}
20:5n-3 (EPA)	5.2 ± 0.8^{a}	11.1 ± 0.6^{b}	9.3 ± 0.7^{b}	6.0 ± 0.5^{a}
22:5 n-6 (n6DPA)	$<0.01^{a}$	1.1 ± 0.2^{ab}	2.4 ± 0.3^{b}	4.7 ± 0.7^{c}
22:5n-3	2.5 ± 0.2^{a}	3.8 ± 0.1^{b}	3.6 ± 0.1^{abc}	3.0 ± 0.2^{ac}
22:6n-3 DHA	0.2 ± 0.1^{a}	8.3 ± 0.7^{b}	9.8 ± 0.5^{bc}	11.4 ± 0.8^{c}
ΣPUFA	26.6 ± 1.7^{a}	42.6 ± 0.9^{b}	42.7 ± 0.9^{b}	41.2 ± 1.4^{b}
Σn-3	9.1 ± 1.1^{a}	26.8 ± 0.5^{b}	25.6 ± 0.5^{b}	21.7 ± 0.7^{c}
Σn-6	16.9 ± 0.9^{a}	14.0 ± 0.6^{b}	15.9 ± 0.3^{ab}	18.8 ± 0.6^{a}
DHA/EPA	$\mathbf{<0.1^{a}}$	$\mathbf{0.8 \pm 0.1^{b}}$	$\mathbf{1.1 \pm 0.1^{b}}$	$\mathbf{2.0 \pm 0.3^{c}}$
EPA/AA	4.7 ± 0.4^{a}	6.9 ± 0.2^{b}	5.1 ± 0.5^{a}	3.0 ± 0.4^{c}

[a,b,c] Different letters represent a significant difference among groups; $P < 0.05$, $F_{3,12}$, one-way ANOVA with Tukey's multiple comparison test.

Table 3: FA composition of Pacific cod larvae fed differentially enriched rotifers for the first four weeks post-hatch (mean ± SEM, n=3).

	First Feeding	Diet 1 (WK 2)	Diet 2 (WK 2)	Diet 3 (WK 2)	Diet 4 (WK 2)	Diet 2 (WK 4)	Diet 3 (WK 4)	Diet 4 (WK 4)
Total FAs ($\mu g.mg^{-1}$dry wt)	86.7 ± 6.0	58.8 ± 4.9	75.5 ± 2.1	65.5 ± 6.4	54.4 ± 4.7	86.7 ± 9.0	61.5 ± 11.7	50.6 ± 10.3
16:0	20.9 ± 0.3	19.9 ± 3.0	16.9 ± 0.7	19.1 ± 1.8	16.6 ± 1.0	13.8 ± 0.5	14.7 ± 0.6	16.8 ± 1.2
18:0	4.8 ± 0.1	7.4 ± 0.7	6.6 ± 0.3	7.7 ± 1.0	6.4 ± 0.3	5.0 ± 0.1	5.3 ± 0.3	6.1 ± 0.3
ΣSFA[1]	**28.3 ± 0.3**	**30.9 ± 4.7**	**26.7 ± 1.0**	**29.9 ± 2.8**	**25.0 ± 1.5**	**21.5 ± 0.5**	**22.6 ± 1.0**	**24.9 ± 1.6**
16:1 n-7	2.7 ± 0.1	2.4 ± 0.1	4.0 ± 0.4	3.3 ± 0.3	2.4 ± 0.1	4.2 ± 0.1	3.7 ± 0.1	2.7 ± 0.2
18:1 n-9	8.5 ± 0.2	16.6 ± 1.2	11.9 ± 1.0	12.3 ± 0.7	13.6 ± 0.2	11.7 ± 0.2	12.9 ± 0.1	14.6 ± 1.0
18:1 n-7	5.0 ± 0.1	3.2 ± 0.1	3.9 ± 0.1	3.6 ± 0.3	3.1 ± 0.1	3.2 ± 0.0	3.1 ± 0.1	2.8 ± 0.2
ΣMUFA[2]	**20.2 ± 0.3**	**26.3 ± 1.5**	**23.8 ± 1.8**	**22.9 ± 1.0**	**22.2 ± 0.3**	**22.6 ± 0.3**	**22.7 ± 0.4**	**22.7 ± 1.42**
18:2 n-6	0.5 ± 0.0	7.4 ± 0.8	5.4 ± 0.6	4.9 ± 0.5	5.2 ± 0.3	6.5 ± 0.2	6.4 ± 0.2	5.8 ± 0.3
20:4 n-6	2.9 ± 0.0	3.4 ± 0.3^{a}	3.1 ± 0.2^{a}	3.2 ± 0.1^{a}	4.3 ± 0.1^{b}	3.3 ± 0.0^{a}	3.8 ± 0.1^{a}	4.5 ± 0.2^{b}
20:5n-3	15.2 ± 0.0	9.1 ± 0.8^{a}	9.6 ± 0.3^{a}	7.8 ± 0.3^{ab}	5.9 ± 0.4^{b}	12.3 ± 0.2^{a}	9.9 ± 0.4^{b}	5.7 ± 0.1^{c}
22:5 n-6	0.3 ± 0.0	0.3 ± 0.0^{a}	1.4 ± 0.1^{b}	2.8 ± 0.1^{c}	5.5 ± 0.2^{d}	1.7 ± 0.0^{a}	3.6 ± 0.1^{b}	6.5 ± 0.6^{c}
22:5n-3	1.6 ± 0.0	5.9 ± 0.6^{a}	4.9 ± 0.1^{ab}	3.7 ± 0.2^{bc}	3.1 ± 0.1^{c}	6.2 ± 0.1^{a}	4.9 ± 0.2^{b}	3.2 ± 0.1^{c}
22:6n-3	27.8 ± 0.1	12.5 ± 1.0^{a}	20.2 ± 2.6^{ab}	20.4 ± 1.6^{ab}	25.1 ± 0.7^{b}	20.4 ± 0.3	21.9 ± 0.5	24.1 ± 2.3
ΣPUFA[3]	**51.3 ± 0.1**	**42.8 ± 3.4**	**49.5 ± 2.2**	**47.2 ± 2.2**	**52.8 ± 1.8**	**55.7 ± 0.2**	**54.7 ± 0.7**	**52.4 ± 3.0**
DHA:EPA	**1.8 ± 0.0**	**1.4 ± 0.1^{a}**	**2.1 ± 0.3^{ab}**	**2.6 ± 0.1^{b}**	**4.3 ± 0.2^{c}**	**1.7 ± 0.1^{a}**	**2.2 ± 0.1^{a}**	**4.2 ± 0.3^{b}**

[1]14:0, i15:0, ai15:0, 15:0, i16:0, ai16:0, i17:0, ai17:0, 17:0, 20:0, 22:0, 23:0, 24:0
[2]14:1, 15:1, 16:1 n-11, 16:1 n-9, 16:1 n-5, 17:1, 18:1 n-11, 18:1n-9, 18:1 n-6, 18:1 n-5, 20:1 n-11, 20:1 n-7, 22:1 n-11(13), 22:1 n-9, 22:1 n-7, 24:1
[3]16:2 n-4, 16:3 n-4, 16:4n-3, 16:4 n-1, 18:2 n-4, 18:3 n-6, 18:3 n-4, 18:3n-3, 18:4n-3, 18:4 n-1, 18:5n-3, 20:2a, 20:2b, 20:2 n-6, 20:3 n-6, 20:3n-3, 20:4n-3, 22:2NIMDa, 22:2NIMDb, 22:4 n-6, 22:4 n-3.
[a,b,c] Different letters represent a significant difference among groups; $P < 0.05$, $F_{3,7}$ at WK 2, $F_{2,5}$ at WK 4, one-way ANOVA with Tukey's multiple comparison test.

FIGURE CAPTIONS:

Figure 1: Morphometric measurements and mass of Pacific cod larvae reared on four types of differently enriched rotifers for the first four WKs post-hatch. Data are mean ± SEM, each symbol represents 30 individuals. Standard length, body depth and dry weight. Different letters (^{a,b,c}) represent significant differences among dietary groups (ANOVA, Tukey's multiple comparison).

Figure 2: Lipids in Pacific cod reared on four types of differently enriched rotifers for the first four WKs post-hatch. Data are ±SEM of Lipid (dry weight $^{-1}$), TAG (%), TAG/ST and PL.. * Represents a significant difference among dietary groups, ^{a,b,c}Different letters represent significant differences among dietary groups (ANOVA, Tukey's multiple comparison).

Figure 3: Relative proportions of specific FAs in larvae compared to dietary levels after (a) 2 WKs and (b) 4 WKs of feeding on differently enriched rotifers. Data are mean ± SEM, n = 3. The solid bar indicates that the proportion of FAs in the larvae equals that of those in the diet.

Figure 4: Analysis of the first two principal components of lipid data from first-feeding larvae and larvae after two and four WKs of feeding on differentially enriched rotifers. The FA and lipid class parameters used were: AA (20:4n-6), DHA (22:6n-3), n-6DPA (22:5 n-6), EPA (20:5n-3), DHA:EPA, ∑MUFA (Monounsaturated FAs), total per dry (total FAs per dry weight $\mu g.mg^{-1}$), TAG:ST (Triacylglycerols: sterols), ∑PUFA (Polyunsaturated FAs). Groups were determined by cluster analysis. Lipid parameter coefficients, symbols represent orientation along the third principal component axis and larval fish scores for the first two principal components.

Figure 5: Relative proportions of DHA, EPA, and DHA:EPA in four type of differently enriched rotifers and *Neocalanus plumchrus*. *Neocalanus plumchrus* data is the average of six years of zooplankton collected in the Strait of Georgia, British Columbia Canada, El-Sabaawi et al. 2009a.

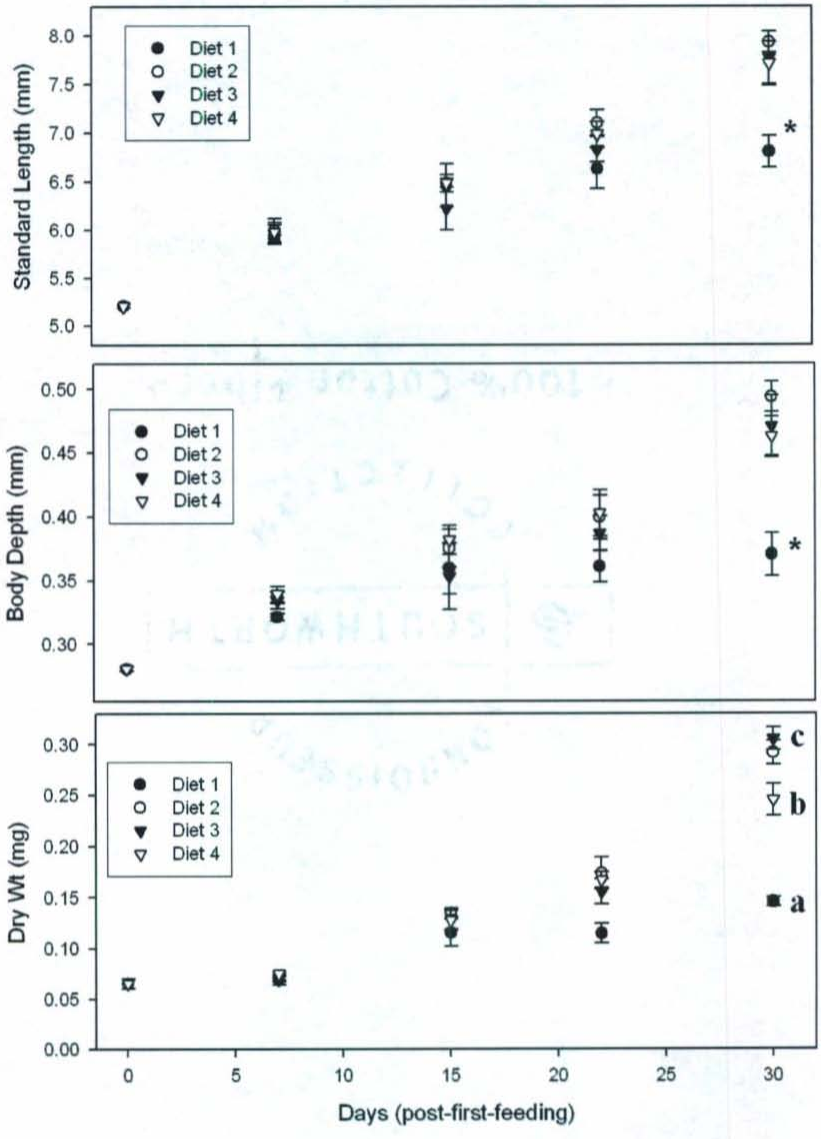
Standard Length (mm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
Diet 1
Diet 2
Diet 3
Diet 4
*
Body Depth (mm)
0.50
0.45
0.40
0.35
0.30
Diet 1
Diet 2
Diet 3
Diet 4
*
Dry Wt (mg)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Diet 1
Diet 2
Diet 3
Diet 4
c
b
a
0
5
10
15
20
25
30
Days (post-first-feeding)

Figure 1

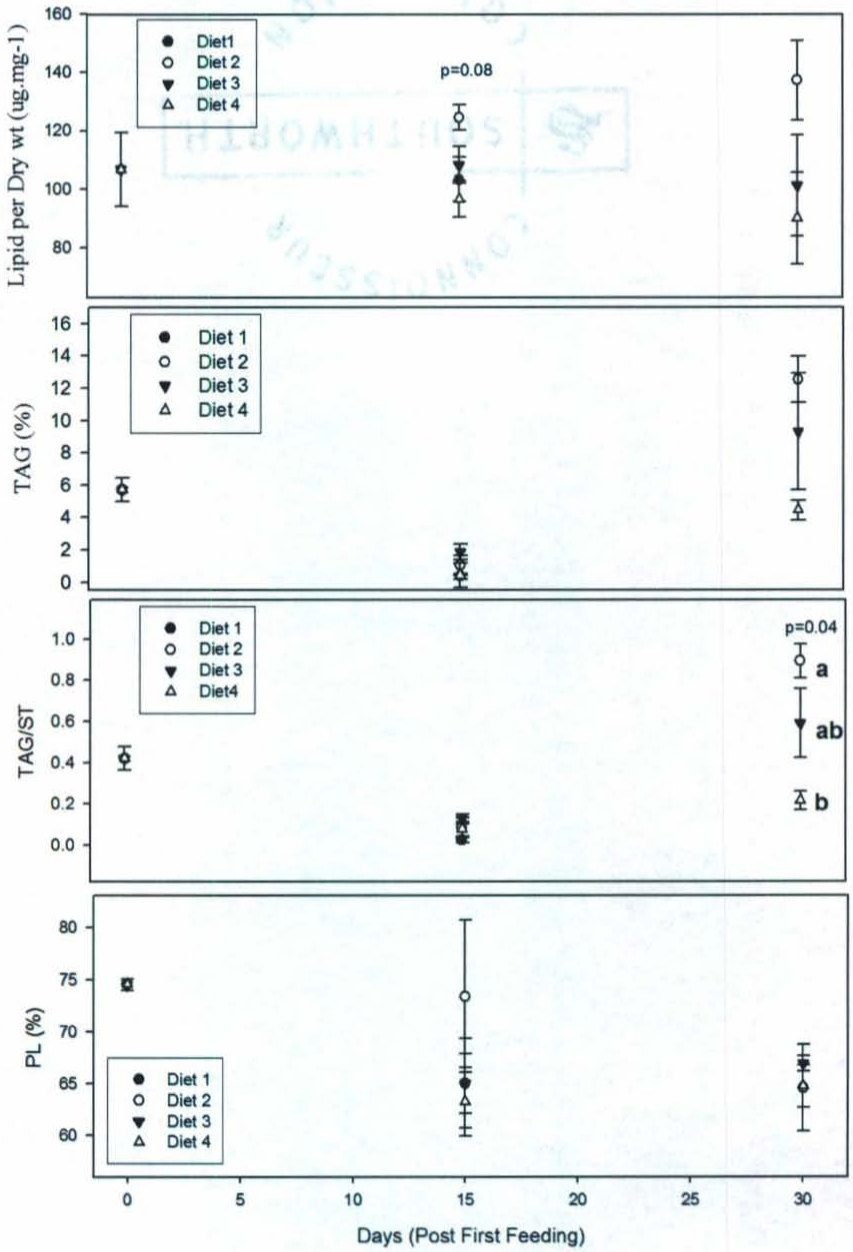
Lipid per Dry wt (ug.mg-1)
Diet1
Diet 2
Diet 3
Diet 4
p=0.08
TAG (%)
Diet 1
Diet 2
Diet 3
Diet 4
TAG/ST
Diet 1
Diet 2
Diet 3
Diet4
p=0.04
a
ab
b
PL (%)
Diet 1
Diet 2
Diet 3
Diet 4
Days (Post First Feeding)

Figure 2

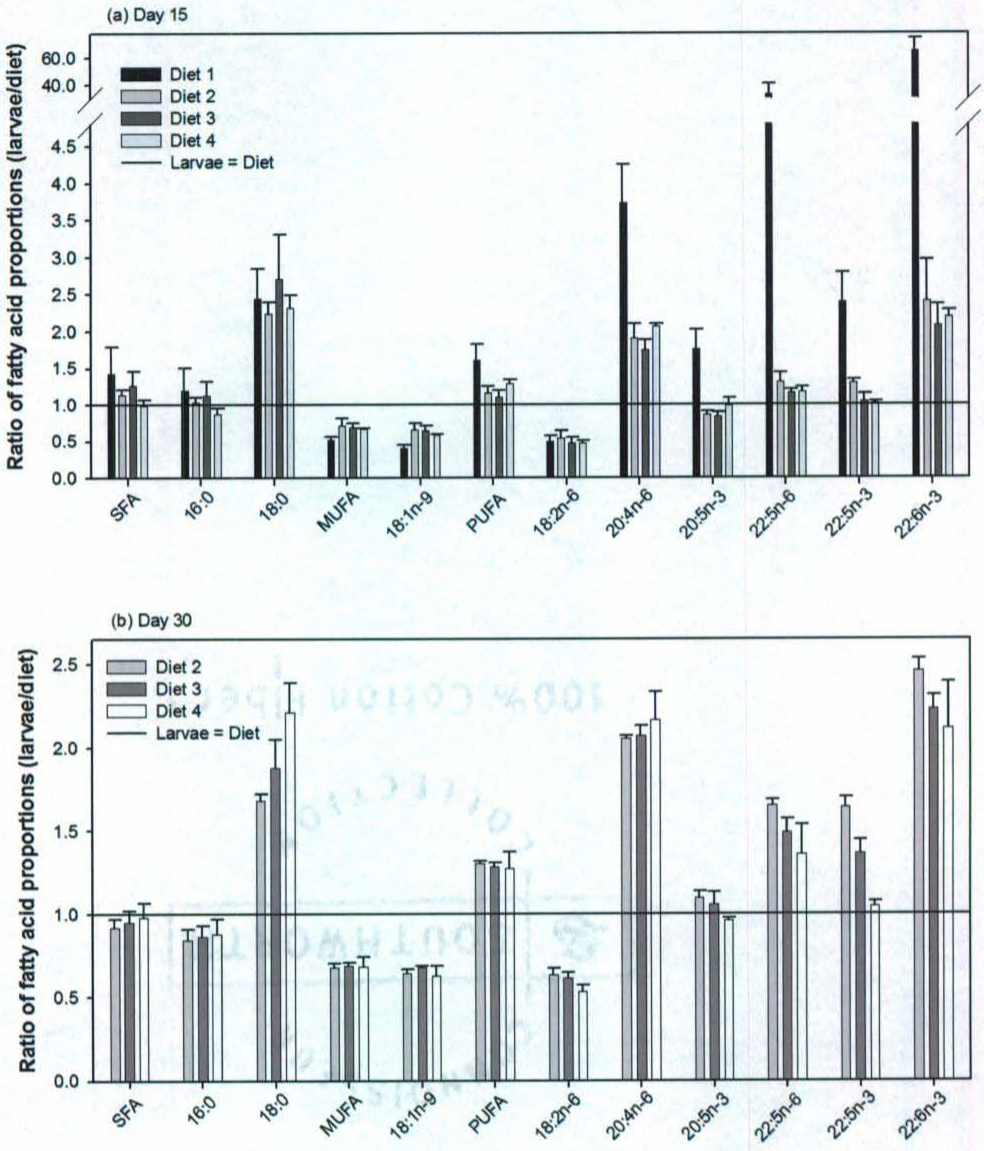
(a) Day 15
Ratio of fatty acid proportions (larvae/diet)
Diet 1
Diet 2
Diet 3
Diet 4
Larvae = Diet
SFA
16:0
18:0
MUFA
18:1n-9
PUFA
18:2n-6
20:4n-6
20:5n-3
22:5n-6
22:5n-3
22:6n-3
(b) Day 30
Ratio of fatty acid proportions (larvae/diet)
Diet 2
Diet 3
Diet 4
Larvae = Diet
SFA
16:0
18:0
MUFA
18:1n-9
PUFA
18:2n-6
20:4n-6
20:5n-3
22:5n-6
22:5n-3
22:6n-3

Figure 3

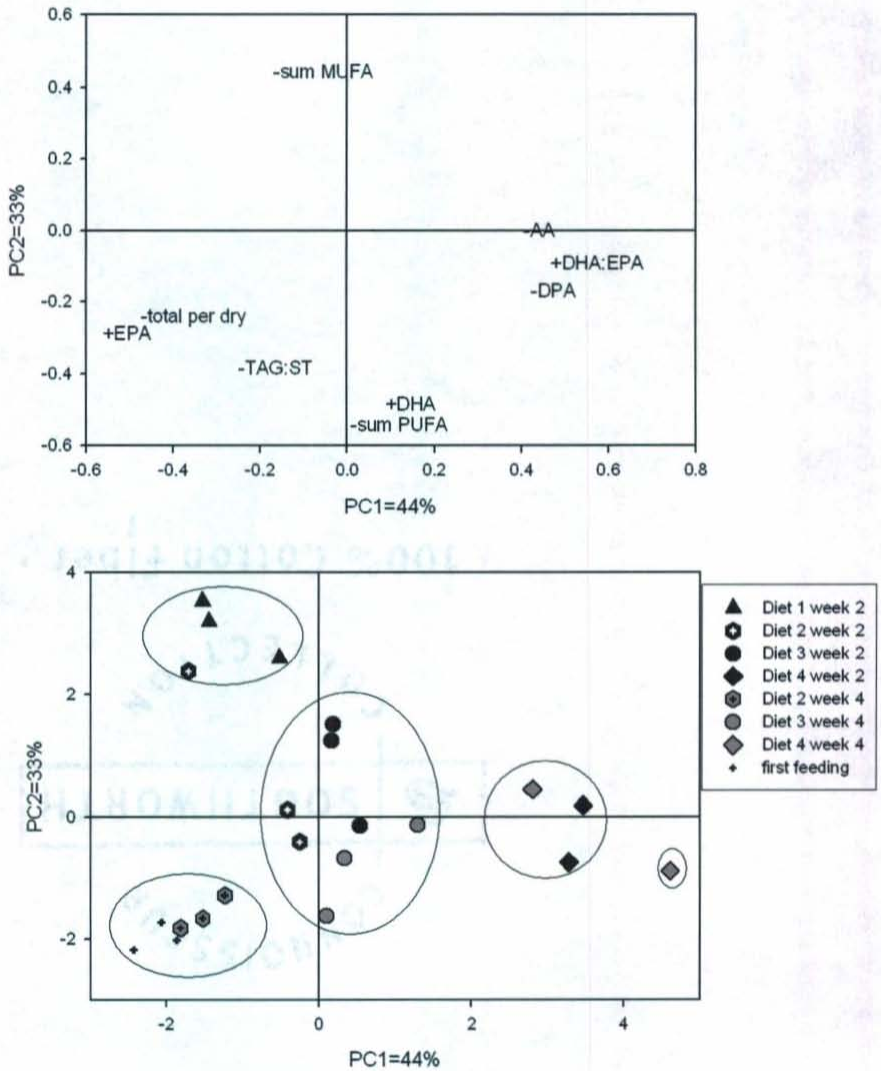
-sum MUFA
-AA
+DHA:EPA
-DPA
-total per dry
+EPA
-TAG:ST
+DHA
-sum PUFA
PC2=33%
PC1=44%
Diet 1 week 2
Diet 2 week 2
Diet 3 week 2
Diet 4 week 2
Diet 2 week 4
Diet 3 week 4
Diet 4 week 4
first feeding
PC2=33%
PC1=44%

Figure 4

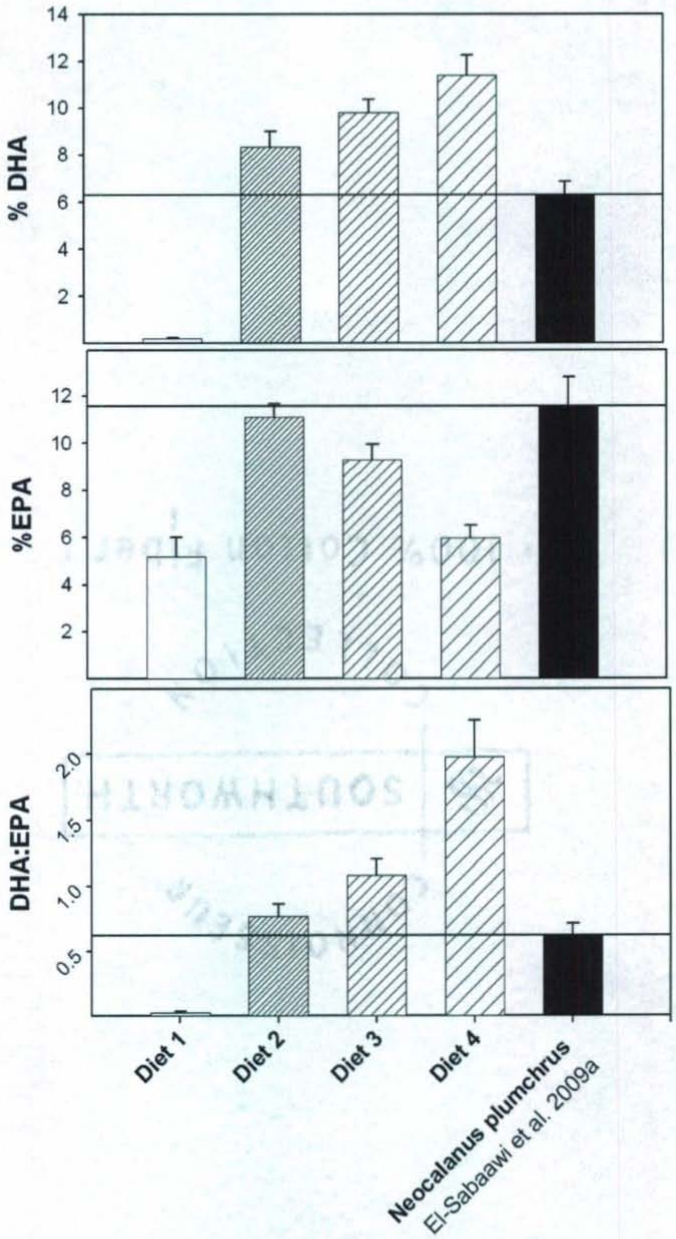
% DHA
%EPA
DHA:EPA
Diet 1
Diet 2
Diet 3
Diet 4
Neocalanus plumchrus
El-Sabaawi et al. 2009a

Figure 5

Chapter 5: Effect of Temperature on the Rate of C_{18} PUFA Uptake in Two Juvenile Gadids: Pacific Cod (*Gadus macrocephalus*) and Walleye Pollock (*Theragra chalcogramma*).

***This chapter has not yet been submitted to a peer-reviewed journal.**

ABSTRACT

Fatty acid biomarkers (FABMs) can be used to understand both dietary history and the timing of habitat shifts in larval and juvenile stages of marine fish. However, the utility of this approach is still limited by a lack of knowledge about how biotic and abiotic factors determine the rate of biomarker uptake in fish tissues. An 8-wk laboratory feeding experiment was conducted in which two species of gadids were reared at 3°C or 9°C and fed either a diet based on marine oil (cod liver) or terrestrial oil (flax seed). Non-linear models were fitted to investigate how tissue type (liver & flesh) and temperature mediated rates of 18:2n-6 and 18:3n-3 (nearshore indicator FAs) uptake in both species. At each temperature, uptake rates were similar for both species, indicating that uptake trends may be generalized in closely related taxa. Dietary biomarkers also showed high temporal sensitivity across temperatures, and were evident in all fish tissues after only one wk of feeding. Cod held at 9 °C had a significantly higher rate of uptake of the C_{18} polyunsaturated fatty acids (PUFAs) in their liver, but this effect diminished from wk 1 to wk 8, as fish tissue reached fatty acid (FA) saturation. The proportion of C_{18} PUFAs was also significantly higher in liver than in heart tissue or flesh. The differential uptake of C_{18} PUFAs among tissues (e.g., FA in liver:FA in heart) may provide temporal patterns that could help disentangle timing of offshore-inshore nursery migrations in juvenile fish.

INTRODUCTION

For over 30 years, FA biomarkers (FABMs) have been used to define food chain interrelationships in both marine (Sargent 1976, Dalsgaard et al. 2003, Budge et al. 2006) and freshwater ecosystems (Arts et al. 1999). The FABM approach is based on the distinctive FA compositions of primary producers (Budge et al. 2006, Iverson et al 2009) whose signatures are incorporated conservatively into secondary consumers such as zooplankton (Sargent et al. 1989, Stevens et al. 2004). However, application of the biomarker approach is increasingly difficult at higher trophic levels where omnivory and carnivory play a more significant role.

In fish, FABMs have helped to identify both dietary and habitat shifts in larval and juvenile stages. For example, FABMs linked juvenile cod (*Gadus morhua*) to either a diatom-based frontal mixing zone or to a dinoflagellate-based stratified region of the North Sea (St. John & Lund 1996). FABMs have also been used to assess the sources of dietary organic matter available to juvenile Atlantic cod in the nearshore. Copeman et al. (2009, Chapter 3) demonstrated a seasonal change in diet for juvenile cod settling into cold-water eelgrass meadows (Newfoundland, Canada) where elevated nearshore indicator FAs (Σ18:2n-6 + 18:3n-3) in settled individuals showed increased utilization of terrestrial organic matter. To date, most studies on juvenile fish have utilized a qualitative approach to FABM analysis, with few quantitative examples. Dalsgaard & St. John (2004) provide a rare example of a quantitative approach to FABM accumulation in juvenile sand eel (*Ammondytes tobianus*), where they found that ration had no significant effect on growth rate based models of overall uptake of two different ^{13}C labeled FAs.

However, significant knowledge gaps still prevent the quantitative application of the FABM approach to ecological questions. These gaps include how the differential rate of FABM uptake is affected by a range of abiotic and biotic factors (temperature, ration, tissue type, condition, species-specific effects, ontogenetic state and degree of omnivory).

I conducted laboratory studies to examine the rate of 18:3n-3 and 18:2n-6 uptake in the tissues of two juvenile gadids that use nearshore nursery areas in the Northeast Pacific. Walleye pollock (*Theragra chalcogramma*, semi-pelagic) and Pacific cod (*Gadus macrocephalus*, demersal) are the two most commercially important species in the Alaskan finfish fishery. Both species have pelagic larvae (4-30 mm) that settle (30-40 mm) into complex biogenic habitat (eelgrass, kelp) in shallow, coastal areas (2-4 m; Laurel et al. 2007). These nursery areas can be within meters of shore and are prone to terrestrial run-off through annual late-spring freshets when juvenile gadids begin to settle.

In this study, I examined how and whether the uptake rate of two C_{18} PUFAs changed as a function of biotic and abiotic factors. To examine the effect of species and temperature on the uptake of these nearshore biomarkers in gadids, two different gel food diets were fed to walleye pollock and Pacific cod juveniles over an 8-wk feeding trail. Diets were identical except that one was enriched with marine oil (cod liver) whereas the second was enriched with terrestrial plant oil (flax seed). I tested the following hypotheses: 1) the proportion of 18:3n-3 + 18:2n-6 in the tissues of juvenile gadids is species-specific, 2) temperature mediates the rate of C_{18} PUFA uptake in gadid tissues, 3) the proportion of 18:3n-3 + 18:2n-6 is specific to tissue type (liver, flesh, heart) and 4) the ratios of C_{18} PUFA between different tissue types (i.e. FABM in liver:FABM in flesh)

show temporal trends that could be applied to field data in order to show residency times in the nearshore environment. Quantification of the rate of uptake of these FAs in laboratory studies on juvenile gadids is a necessary prerequisite to the application of the FABM approach to field studies that aim to assign residency times within the nearshore. Further, these lab rates will lay the foundation to connect juveniles of ecologically and commercially important fish species to different trophic regimes or habitat types.

MATERIALS AND METHODS:

Fish Husbandry and Sampling

Juvenile walleye pollock were collected on June 4, 2008 with lights and lift nets suspended from a dock in Pt. Townsend Bay, Washington, USA (48°6'N 122°48'W). Fish were held for 24 hrs in ambient seawater prior to shipment to the National Marine Fisheries Service laboratory in Newport, Oregon. Once at the laboratory, Pollock (20-40 mm initial length) were placed in flow-through seawater 450 L tanks at 9 °C. For the first two wks, I fed fish a mixture of krill, *Euphausia superba*, and gel food (Table 1). After August 21, 2008 fish were moved into 3140 L tanks and fed krill and gel food 3 X per wk.

Pacific cod were collected in Kodiak, Alaska (USA) by beach seine on July 15, 2008. Fish were allowed to depurate for 24 hrs and were then shipped overnight at low densities in containers with an overlying saturated O_2 layer. Upon arrival in Newport, Oregon, cod were quarantined for 6-wks in 3104 L tanks. Cod (20-50 mm initial size)

were maintained for over 6 wks at 9 °C on the same feeding schedule as Pollock, with 3X per wk krill and gel food, until the beginning of the feeding trail in early October.

Dietary trials were run in twenty-four cylindrical upwelling 100 L green tanks with water through-flow set at 1.5 $L.min^{-1}$. Due to availability, twelve juvenile pollock or ten juvenile cod were stocked per tank five days prior to the start of the experiment, and over the next three days, temperatures in half of the tanks were gradually lowered to 3 °C while the other tanks remained at 9 °C.

I initiated the feeding trial on October 7, 2008 where fish were hand-fed either the marine oil based diet or the terrestrial oil based diet to satiation (all feeding behavior stopped) 5X per wk. Table 2 shows the allocation of the 24 tanks between two species, two temperatures and two diets. Diets were formulated to contain the same level of lipid per wet weight and the same lipid class composition (Table 1). Gelatinized diets previously shown to provide adequate nutrition for growth in Pacific marine juveniles (Hurst et al. In press), were modified by addition of either a marine oil (cod liver oil) or a terrestrial oil (flax seed oil). Diets contained a combination of squid, krill, pacific cod fillets, commercial food, amino acid supplements, and vitamins. Ingredients were blended together and then bound by the addition of a warm gelatin to the mixture. Diets were frozen immediately after the addition of the gelatin. Small (~1 cm wide) strands of gel food, suitable for juvenile fish gape size, were produced by grating frozen blocks of gel food using a kitchen cheese grater. The marine diet had higher levels of the marine PUFAs, DHA and EPA, whereas the terrestrial diet had elevated proportions of C_{18} PUFAs, 18:2n-6 and 18:3n-3 (Table 3).

Three pollock and three cod were sampled prior to differential feeding, at the beginning of the experiment. For lipid samples, fish were first euthanized and then blotted dry with paper towels. Then their total length (mm) and wet weight (g) were recorded prior to removal of tissues for lipid extractions. During tissue sampling, both liver and flesh were sampled separately from each animal. Whole fish livers (0.02 to 0.2 g) were removed for extraction while a representative flesh sample (~0.25-0.35 g) was removed from each fish and weighed. Lipid sampling was conducted in this manner on three fish per species at the beginning of the experiment and on one fish per tank at the end of weeks 1, 2, 4 and 8. This resulted in a total of 3 samples of liver and flesh per treatment at each time period. Whole fresh hearts were sampled from 3 cod and 3 pollock at time zero and from one fish per tank at the end of the 8-week experimental period. All lipid samples were placed in chloroform and under nitrogen immediately after sampling and were stored at -80°C for less than 4 months prior to extraction.

Lipid Analysis

Lipids were extracted in chloroform/methanol according to Parrish (1987) using a modified Folch procedure (Folch et al. 1957). Lipid classes were determined using thin layer chromatography with flame ionisation detection (TLC/FID) with a MARK VI Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three stage development system was used to separate lipid classes. The first separation consisted of 20-min developments in 98.95:1:0.05 hexane:diethyl ether:formic acid. The second separation consisted of a 40-min development in 79:20:1 hexane:diethyl ether:formic acid. The last separation

consisted of 15-min developments in 100% acetone followed by 10-min developments in 5:4:1 chloroform:methanol:water. Data peaks were integrated using Peak Simple software (ver. 3.67, SRI Inc) and the signal detected in millivolts was quantified using lipid standards (Sigma, St. Louis, MO, USA). Lipid classes are expressed per wet weight (mg g^{-1} wet weight) and as proportions (% total lipid).

Total lipid was analysed for FA composition. FA methyl esters (FAME) were prepared by transesterification with 14% BF_3 in methanol at 85°C for 90 min (Morrison & Smith 1964, Budge 1999). FAMEs were analyzed on an HP 6890 GC FID equipped with a 7683 autosampler and a ZB wax+ GC column (Phenomenex, U.S.A.). The column was 30 m in length, with an internal diameter of 0.25 μm. The column temperature began at 65°C where it was held for 0.5 minutes. Temperature was increased to 195°C (40°C min^{-1}), held for 15 minutes then increased again (2°C min^{-1}) to a final temperature of 220°C. The final temperature was held for 3.25 min. The carrier gas was hydrogen, flowing at 2 ml·min^{-1}. The injector temperature started at 150°C and increased (200°C·min^{-1}) to a final temperature of 250°C. The detector temperature was constant at 260°C. Peaks were identified using retention times based on standards purchased from Supelco (37 component FAME, BAME, PUFA 1, PUFA 3). Chromatograms were integrated using Galaxie Chromatography Data System (Ver. 1.9.3.2, Varian). Individual FAs are expressed as a percentage of total FAs.

In order to express FAs as μg per mg wet wt conversion factors were used based on the average FA molecular weight for each sample and its lipid class composition. These calculations involve subtracting the glycerol, phosphate, and other functional

groups from the acyl lipid class mass in order to obtain the mass of FAs per lipid class. Conversion factors for the major lipid classes used here were ~0.47 steryl/wax esters, ~0.95 triacyglycerols (TAG), 1.0 free FAs (FFA), ~0.90 diacylglycerols (DAG), ~0.37 acetone mobile polar lipids (AMPL), and ~0.72 for phospholipids (PL). These conversion factors are in agreement with previous reviews on the nutritional composition of fats in seafoods (Ackman 1989).

Statistical Analysis

The effect of diet, wk, tissue type (flesh or liver), species, temperature and tanks within treatments on the proportion of C_{18} PUFA in juvenile gadids was performed using a General Linear Model (SYSTAT 12 for windows). Because the nested effect of tanks within treatments was not significant, I dropped it from further models. When interaction terms were significant, separate 3-way ANOVAs were performed for different tissue types and different diets to investigate the effect of species, temperature, and wk on FA proportions in tissues. Differences in the proportion of FAs in all three different tissue types (liver, flesh and heart) at the end of the 8-wk feeding trial were investigated using a two-way ANOVA, to look at the effect of species and tissue type on the proportion of FA. All proportional FA data were arcsine square root transformed whereas wk data was log transformed in order to meet the assumptions of normality. Significance was set at α=0.05.

The rate of uptake of 18:3n-3 and 18:2n-6 was fitted to a 3-parameter exponential rise to a maximum model ($\mathbf{Y_x = Y_0 + a[1-exp(-bX)]}$) (Cober et al. 2006, Sigma Plot version 10.0). In the model, $\boldsymbol{Y_0}$ refers to the proportion of FA (%) in the tissue at time-0, $\boldsymbol{a}$ is the

maximum proportion found in the tissue (%), ***b*** relates to the initial slope of the FA uptake curve (%.day^{-1}), and ***X*** refers to the days of feeding.

The ratio between the level of 18:2n-6 or 18:3n-3 in the liver and that in the flesh over the 8-wk feeding trial was fitted to a dome shaped curve explained by the Gaussian 3 parameter peak model: $\mathbf{Y_1=a*exp(-.5*((X_1-X_0)/b)^2}$. Y_1 refers to the ratio of FABM in the liver relative to the flesh at time 1, ***a*** is the maximum proportion in the liver:flesh, X_1 is the days of feeding, X_0 is the days until a slope was zero or at the max peak, ***b*** is the width of the peak in days.

Growth rates were calculated as the change in standard length per day (mm per day) = (mm wk 8 – mm wk 0)/days), or as standard growth rate (SGR=((ln(wwt wk 8)-ln(wwt wk 0))/day)*100). Individual growth rates were not measured throughout the experiment, so treatment averaged growth rates from wk 0 to wk 8 were presented. Differences in average growth rate between treatments were analyzed using a one-way ANOVA with Tukey's multiple comparison tests, α=0.05.

RESULTS:

Summary Lipid Data

Summary tables of the major lipid classes and FAs in the tissues of cod and pollock fed two different diets are shown for time 0 and at the end of the eight wk feeding trial (Tables 4 to 6, See Appendices for detailed lipid class and FA data). Cod and pollock flesh contained 5 to 7 mg.g^{-1} FAs per wet weight throughout the experiment with phospholipids (PL) as the major lipid class. PL ranged from 74 to 76% at time 0 to 84 to 86% at the end of the experiment in both species. The major saturated FAs (SFA) in both

cod and pollock were 16:0 and 18:0, which declined during the experimental trial for marine and terrestrial diet treatments, resulting in a ΣSFA at wk 8 of ~21% compared to 31 to 39 % at time 0. The major monounsaturated FAs (MUFAs) in cod and Pollock, 18:1n-9 and 18:1n-7, varied little throughout the experiment and remained between 23 to 27% through the 8-wk feeding trial.

Total PUFAs were ~40% at time 0 in cod and pollock, but increased in both marine fed and terrestrial fed fish to a high of ~53%. However, in fish fed the marine diet this increase was caused by a rise in 20:5n-3 and 22:6n-3 (marine based PUFA), whereas in terrestrial diet fish there were large increases in 18:2n-6 and 18:3n-3. Levels of nearshore indicators (18:2n-6 +18:3n-3) rose to a high of ~8% of the total FAs in the flesh of terrestrial diet fish at the end of wk 8.

The summary lipid data for the livers of cod and pollock (Table 5, Detailed data in Appendices), show 115 $mg.g^{-1}$ of lipid per wet weight in cod livers at time 0 compared to 386 $mg.g^{-1}$ in pollock livers. At the end of the feeding trial, liver lipid ranged from 292 $mg.g^{-1}$ in cod fed the marine diet to 542 $mg.g^{-1}$ in pollock fed the terrestrial diet. Triacylglycerols (TAG), the major lipid class in gadid livers, ranged from a low of 50% in cod at time 0 to a high of 83% in pollock at time 0.

Although livers had similar major FAs to flesh, liver MUFA levels were higher than those in flesh, with a low of 37% in cod livers at time 0 and a high of 47% in pollock livers at wk-8. Livers contained proportionally less PUFA than the flesh with a high of 40% compared to a high of 54% in flesh (Tables 4 and 5).

PL was their major lipid class in hearts, ranging from 37% in pollock at time 0 to a high of 84% in pollock at the end of the feeding experiment. Proportions of FA in the heart and flesh tissue were similar. Uptake of C_{18} PUFAs in the heart during the 8-wk feeding trail reflected dietary FA proportions, in a similar uptake pattern as found in the flesh tissue (Table 6)

Survival

Pollock survival (90-100%) was higher than cod (47-75%) throughout the 8-wk feeding trial. In cod, the low of 47% survival in the cold-water terrestrial diet contrasted sharply with the high of 75% in the warm-water terrestrially diet. Survival differences between the two species may have been related to lower condition (total lipids in the liver) in cod at the beginning of the experiment (Table 1 & 5).

FA uptake: 18:3n-3

GLM analysis of the effect of week, tissue type, species, temperature, and diet on the proportion of 18:3n-3 in juvenile gadid tissues is shown in Table 7. Tanks nested within treatments did not have a significant effect on the level of FAs in fish tissue (Table 7), and the nested term was dropped from all subsequent analysis (tanks within treatment, $p=0.68$). There were several significant 3-way interactions (i.e. Tissue*Wk*Diet, $p<0.001$, Table 7) with tissue type in the multi-factorial GLM. Therefore, analysis of species, temperature and wk of sampling effects on FA proportions were separately analyzed in smaller 3-way ANOVAs for each tissue type and each diet (Table 8, 9 & 10).

The proportion of 18:3n-3 in the liver of gadids fed the marine diet was not affected by either species or temperature; however, there was a significant difference in

the proportion of this FA from wk 0 to wk 8, reflecting the sensitivity of flesh levels of this FA to slight changes in diet (Table 8). The proportion of 18:3n-3 in flesh of juveniles fed the marine diet did not differ between species, temperature or wk (Table 8)

In gadids fed the terrestrially enriched diet, 18:3n-3 increased significantly in flesh tissue from wk 0 to wk 8 ($p<0.001$, Table 8), but there was no significant effect of either species or temperature on the proportion of this marker. In juveniles fed the terrestrial diet, there was a significant interaction between the effect of species and temperature on the proportion of 18:3n-3 in the liver. Therefore, I ran separate 2-way ANOVAs to look at the effect of temperature and wk on the proportion of 18:3n-3 in the liver for cod and pollock. In both species, wk was significant ($p<0.001$), however, temperature had a significant effect on the proportion of 18:3n-3 in cod livers ($F_{1,16}=7.32$, $p=0.016$) but not in pollock ($F_{1,16}=0.545$, $p=0.471$, Figure 1).

FA uptake: 18:2n-6

The liver of gadids fed the marine diet showed an effect of species on the percentage of 18:2n-6 (Table 9), with cod having higher percentages averaged over the 8-wks (2.19±0.06, n=30) than pollock (1.9±0.06, n=30). Uptake of 18:2n-6 in the liver of gadids fed the terrestrial diet showed a significant effect of species but not temperature (Table 9). Cod consistently had higher proportions of 18:2n-6 in their livers than pollock (Figure 2). The percentage of 18:2n-6 in the flesh tissue of terrestrial fed gadids increased significantly from wk 0 to wk 8 but did not differ between species or temperature treatments (Table 9).

Uptake of Σ18:2n-6+18:3n-3

The liver of gadids fed the marine diet showed no effect of temperature on Σ18:2n-6 + 18:3n-3 uptake but did show a significant effect of species ($p<0.001$) on the proportion of these C_{18} PUFA, with lower levels in Pollock (~2.7%, n=30) averaged over all sampling periods than cod (~3%, n=30) (Table 10). Proportions of Σ18:2n-6 + 18:3n-3 in the flesh tissue of marine diet gadids were significantly different from wk 0 to wk 8, showing the sensitivity of gadid flesh tissue to even slight changes in their diet (from the herring-based maintenance-diet to the marine-diet enriched with cod-liver oil).

Uptake of Σ18:2n-6 + 18:3n-3 in the liver of gadids fed the terrestrial diet showed a significant effect of species but not temperature (Table 10). Averaged over all sampling periods, cod consistently had higher proportions of C_{18} PUFAs in their livers (~11.9%) than pollock (~9.8%) (Figure 3). The percentage of Σ18:2n-6 + 18:3n-3 in the flesh of terrestrial fed gadids increased significantly from wk 0 to wk 8 but was not significantly affected by either species or temperature treatments ($p>0.05$, Table 10).

Rate of uptake

In Figures 1, 2 and 3 I used a "3-parameter exponential rise to a maximum" model to describe the relationship between the proportion of 18:3n-3, 18:2n-6 and the sum of these two FAs, respectively, in gadid tissue throughout 8-wks of feeding. This model describes a rapid initial rate of uptake followed by a decreased rate and a saturation maximum (Cober et al. 2006). Graphs were based on the 3-way ANOVAs, with data pooled for variables with no significant differences. Table 11 summarizes the model

parameters and shows that the rate of initial uptake was elevated in liver (0.1 to 0.04% per day) compared to flesh (0.02% per day). Uptake rates were similar for 18:3n-3 (0.02% per day) and 18:2n-6 (0.02 % per day) in flesh tissue, despite very different proportions of these FAs present in the terrestrial diet (Table 3). Pollock had lower proportions of both 18:3n-3 and 18:2n-6 than cod in their tissues at time 0 (Y_o) and maxima (a) were also slightly lower at the end of the 8-wk feeding period (Table 11). Trends in Σ 18:2n-6 & 18:3n-3 were similar to their individual component FAs, with higher initial slopes in liver (0.06 to 0.09%.day^{-1}) than in flesh (0.02%.day^{-1}) tissue. Cod liver (15.3%) trended towards higher maxima than pollock liver (13.6%, Figure 3) tissue.

Proportion in the liver and flesh relative to the diet

Figure 4 shows ratios of proportions of 18:3n-3 in the liver:diet (Figure 4a) and flesh:diet (Figure 4b) for juveniles fed either a marine or a terrestrial diet. Despite very different levels of 18:3n-3 in the diet, the tissue:diet ratios were similar at the end of the experiment for marine and terrestrially fed fish. In Figure 4a, fish fed both diets approached a 1:1 ratio of 18:3n-3 in the liver:18:3n-3 in the diet. Fish fed the cod-liver oil enriched marine diet remained near this 1:1 ratio throughout the 8-wk feeding trial, indicating similar signals from the marine diet and the maintenance diet that all fish received prior to the 8-wk feeding trial. In contrast, the ratio in those fish switched to the terrestrial diet at wk 0 increased from ~0.1:1 at wk 0 to 0.5:1 at wk 2, and to 1:1 (liver:diet) at wk 8.

The proportion of 18:3n-3 in flesh tissue relative to the diet reached a maximum of approximately 0.5:1, which is lower than for the liver (Figure 4a,b). Gadids fed a

terrestrial diet showed a low ratio of ~0.025:1 at week 0; however, this ratio increased to a high of ~0.5:1 after eight wks of feeding, approaching the ratio in fish fed a marine diet. The same pattern was observed for 18:2n-6 and the sum of Σ18:2n-6 + 18:3n-3 (data not shown).

Proportion in the liver relative to the flesh

Proportions of 18:3n-3 and 18:2n-6 in the liver relative to those in the flesh tissue of marine diet fish did not change significantly over the 8-week feeding trial (Figure 5). Therefore, the approximately 2:1 ratio in the liver relative to the flesh represents a saturation level in fish with no large recent dietary change. However, in fish fed the terrestrial diet, the relationship between the proportions in liver relative to flesh tissue was best described by a dome-shaped Gaussian 3 parameter peak model, indicating a rise in the ratio to a maximum at 1 month followed by a decline to original saturation levels at 2-months. The relationship between liver to flesh was similarly dome shaped for 18:2n-6, even though this FA represented only 5.4% of the total FAs in the diet compared to 13.9% for 18:3n-3 (Figure 5b, Table 12).

Growth rates, size and biomarker uptake

Individual growth rates were not collected throughout the experiment because of limited numbers of juvenile gadids per tank and lethal lipid sampling. However, growth rates of cod and pollock remaining at the end of the 8-wk growth period differed significantly with temperature (one-way ANOVA, $F_{7,151}=9.92$, $p<0.001$, Figure 6). There were no differences between species reared at the same temperature, or between fish at the same temperature but fed different diets (Figure 6).

The relationship between size-at-age and the proportion of 18:3n-3 in the fish was not linear (Figure 7). Experimental fish did not increase significantly in length or weight until after the first 4 wks of feeding. However, during this same period the proportion of Σ18:2n-6 + 18:3n-3 increased significantly in flesh tissue and especially in liver tissue. This duration of maximum slope for the liver ranged from 7.7 to 23.8 days (from the ***1/b*** term in Figures 2 & 3, Table 11), but was less than 50 days for flesh (Table 9). Therefore, C_{18} PUFA uptake is apparently uncoupled from growth during the first four wks of feeding, particularly in liver tissue.

Biomarker Uptake in Heart Tissue

Samples of heart tissue were taken at time 0 and after 8 wks of feeding. In Figure 8 I show the proportion of 18:3n-3 and 18:2n-6 in the hearts, flesh and liver of juvenile gadids at time 0 and after 8 wks of feeding on the terrestrially enriched diet. Liver had higher levels of these two FAs after 8-wks than either the flesh or the heart tissue in both cod (18:3n-3, one-way ANOVA $F_{2,15}=42.66$, $p<0.001$; 18:2n-6 one-way ANOVA $F_{2,15}=48.34$, $p<0.001$) and pollock (18:3n-3, one-way ANOVA $F_{2,15}=38.86$, $p<0.001$; 18:2n-6 one-way ANOVA $F_{2,15}=29.88$, $p<0.001$). However, pairwise comparisons showed that by the end of the 8-wk period there were no significant differences in levels of 18:2n-6 +/or 18:3n-3 between the flesh and the heart tissue in either species (Figure 8).

DISCUSSION:

Quantification of the rates of uptake and the determination of factors that may affect these rates are vital to the interpretation of the FABM technique. This is especially the case when FABM are used to address questions of residency time within a specific

habitat, or when they are used to address the timing of a dietary switch in a wild collected marine organism. In this study, the rate of uptake of 18:2n-6 & 18:3n-3 was quantified as a function of species, temperature, and tissue type. Definition of the factors that drive the rate of uptake will allow a more useful application of the FABM approach and coupled with field validation studies, may allow an assignment of residency times to juvenile gadids within the nearshore.

In this experiment, terrestrial short chain PUFA was rapidly assimilated into juvenile gadid tissues, with maximal rates of uptake in the first four wks of feeding (Figures 1 to 3). The livers of both cod and pollock had the shortest duration of maximal slope (**1/b**, Table 11) for the uptake of Σ18:2n-6 + 18:3n-3 with durations of 16 and 11 days, respectively. Gadid flesh showed a longer duration of maximal slope (**1/b**, Table 11) at approximately 50 days for Σ18:2n-6 + 18:3n-3. This rapid and significant adjustment of FA composition of the flesh and liver is consistent with other laboratory (Copeman et al. 2002, Dalsgaard et al. 2004) and field (Copeman et al. 2009- Chapter 3, Koussoroplis et al. 2010) studies on age-o fish. Kirsch et al. (1998) examined dietary effects on the FA signature of whole adult Atlantic cod and found significant differences after only 3 wks of feeding. Thus, juvenile and adult fish flesh is sensitive to changes in dietary FA composition and nearshore FAs are likely to be rapidly evident in fish tissues in the field. Further, juvenile fish are likely to show increased proportions of FABM more rapidly than seen for adult fish.

Surprisingly, there was no effect of temperature or species on the rate of uptake in juvenile gadid flesh, suggesting that the rates reported here can potentially be applied 1)

to juveniles of other low-fat marine species and 2) throughout the range of temperatures during gadid settlement in temperate nearshore habitats.

Temperature determines fish growth by increasing food consumption and driving the metabolic processes that can change growth efficiency (Brett 1979). Previously it has been shown that there is a significant impact of temperature on growth rate, both in juvenile walleye pollock (Kooka et al. 2007) and Pacific cod (Hurst et al. in review). Within, I report similar rates in an 8-wk averaged growth period (Figure 6). Despite this similarity, there were not significant differences in the proportion of C_{18} PUFAs in the flesh of gadids reared at either 3°C or 9°C even after 8-wks of feeding. These patterns suggest a decoupling of growth rate and FABM uptake in juvenile gadids. It was not possible to accurately determine weekly growth rates, as individual growth was not tracked and only 1 fish was sampled per tank on a weekly basis. However, temperature-mediated growth rates based on larger numbers of fish at time-0 and at time-"8-wks" are in agreement with previous reports in both species; showing on average 0.4 mm length.day^{-1} at 9°C and 0.2 mm length.day^{-1} at 3°C (Figure 6, Kooka et al. 2007, Hurst et al. In Press).

Previous studies calculated the rate of FA uptake from the relative change in mass or growth rate (Dalsgaard & St. John 2004) or from FA dilution (Jobling 2003) following a change in fish diet. Jobling (2003) investigated the change in Atlantic salmon (*Salmo salar*) flesh when switched from a terrestrial to marine oil enriched diet and then modeled dilution of the terrestrial signal based on the proportional increase in the amount of fillet fat over time. They also stated that relative changes in body mass could be used as a

surrogate provided that both fillet yield and fillet fat percentage changed little over time. I found there was little relationship between weekly measures of size-at-age (wet wt or total length) and the proportion of the C_{18} PUFA in the flesh of juvenile gadids during the first 4-wks of feeding (Figure 7), perhaps because of small sampling sizes or reduced growth during the first four wks of feeding. Increased stress associated with changes in diet or the development of feeding hierarchies in experimental tanks may have delayed growth. Despite this reduced growth, C_{18} PUFA was highest in the liver and significantly increased in flesh tissue. For reasons discussed above, growth may decouple from FABM uptake, suggesting feeding duration (not growth) is a more sensitive indicator of the proportion of FABM accumulating in juvenile gadid flesh (Figure 7).

Species differences in the uptake of FABM in the flesh of juvenile gadids were not evident (Tables 8 to 10). Generally, Pacific cod juveniles are considered to be more tightly connected to coastal nursery areas than walleye pollock (Hurst et al. 2009, Brodeur & Wilson 1996). In a recent study of nearshore habitat use in juvenile gadids, Laurel et al. (2007) showed higher abundance of age-0 juvenile Pacific cod and saffron cod (*Eleginus gracilis*) than walleye pollock during settlement (July and/or August) in the Kodiak, Alaska nearshore. However, all gadids preferentially used macrophytes over bare substratum during settlement; Pacific cod closely associated with *Laminaria*, saffron cod with eelgrass, and walleye pollock equally with both habitats.

Reduced uptake of dietary C_{18} PUFAs in walleye Pollock livers compared to cod may reflect differential utilization of this FA. The majority of offshore pollock juveniles probably never encounter elevated levels of C_{18} PUFA in the wild, whereas cod routinely

settle in the nearshore where 18:2n-6 and 18:3n-3 are abundant. Alternatively, lower proportions of 18:2n-6 and/or 18:3n-3 in the livers of pollock may have resulted from a higher relative hepatosomatic index (HI) in walleye pollock at time-0 than in Pacific cod (data not shown). Elevated HI in pollock may also have related to relatively better condition and survival in pollock (~90%) compared to cod (~70%) throughout the experiment. Pollock had more liver lipid than Pacific cod, and thus greater storage of marine FA at the beginning of the experiment. Therefore, during the 8-wks of feeding, pollock may have retained their originally elevated marine based FAs, reducing the relative proportion of C_{18} PUFA stored in the liver.

In both the North Atlantic and Pacific, many large-scale studies on FA signatures in fish have added to large prey databases that provide a platform on which to base trophic investigations of larger predatory fish, sea birds and marine mammals (Budge et al. 2002, Iverson et al. 2002). Given that fish are generally eaten whole, these analyses mostly encompass whole body lipids. However, from the fish feeding ecology perspective, temporal trends can be inferred from separate analysis of storage lipids (liver) and membrane lipids (flesh). I found that proportions of FAs in the tissues of juvenile gadids differed as a result of elevated rates of liver uptake compared to flesh and heart tissues. Liver uptake was more sensitive than other tissues to species or temperature, with higher proportions of nearshore indicator FAs in Pacific cod than walleye Pollock. Further, initial liver uptake was higher in fish reared at 9°C. Interestingly, both 18:2n-6 and 18:3n-3 approached saturation levels before the end of the 8-wk experiment even though 18:3n-3 comprised a much higher proportion of the diet

than 18:2n-6. This similarity may indicate that the saturation time for a FA in the flesh and liver of juvenile fish may not depend on the proportion available in the diet.

Proportions of 18:3n-3 in the liver of fish fed a terrestrially enriched diet approached a maximum of ratio of 1:1 (liver:diet), whereas their flesh levels approached a lower ratio of 0.5:1. At the end of the two-month experiment, maximal ratios in terrestrial-fed fish were similar to those of marine-fed fish, indicating saturation (Figure 4). Different ratios in the liver and flesh relative to the diet likely reflected the higher proportion of TAG in liver (~70 to 80% at wk 8) compared to PL in flesh tissue (~85% at wk 8). Tocher et al. (2008) recently reviewed the physiological roles of phospholipids (PLs) in the nutrition and metabolism of teleost fish; specifically their importance in membrane function, formation of eicosanoids, and as an energy source. PLs are a lipid class characterized by a common backbone of phosphatidic acid, formed from L-glycerol 3-phosphate with two FAs esterified on positions 1 and 2. Normally a SFA or MUFA is esterified to the sn-1 position while a PUFA is preferentially esterified to the sn-2 position (Bell & Tocher 1989). Bell & Dick's (1991) study of PLs in cod fed on a natural marine diet reported major molecular species such as 16:0/22:6 and 16:0/20:5 in both the liver and flesh, but not 18:2 or 18:3. The significant increase in the proportion of C_{18} PUFA here indicates that there is considerable flexibility in the sn-2 position of gadid PLs.

Conversely, triacylglycerols (TAGs) are the main storage lipid class in fish. Functionally, TAGs are esters composed of a glycerol backbone bound to three FAs, but in early stages of marine fish the FA specificity in TAGs is less than in PLs (Copeman et

al. 2002). These functional differences in the lipid classes that typify the liver and flesh of fish likely explain part of the differences in saturation levels of 18:3n-3 in the flesh (0.5:1) and liver (1:1) tissues relative to those provided in the diet.

The differential rate of C_{18} PUFA uptake in the two tissues of juvenile gadids resulted in the dome-shaped relationship between the proportions of C_{18} PUFAs in liver relative to flesh tissue (Figure 5). Maximal differences in the liver:flesh ratio were observed after 1-month of feeding on the terrestrial diet. These differences seemed to have reached saturation levels after two months of feeding, as they were at a level similar to that found in the marine fed fish (2:1 liver:flesh). This relationship holds promise for estimating residency time in the nearshore, especially when coupled with size-at-age data that is routinely collected in field studies. Based on the dome-shape relationship, the ratio of C_{18} PUFA in the liver:flesh are similar after two wks or six wks on a new diet (Figure 5). However, when this information is coupled with basic size-at-age population structure it may be possible to assign a 6-wk residency to a ~100 mm fish and a 2-wk residency to a ~50 mm fish, despite a similar liver to flesh ratio of 18:3n-3 & 18:2n-6. A general increase in the proportion of C_{18} PUFAs (% total FAs) that occurs with residency in the nearshore (2 wks versus 6 wks) could help validate the liver:flesh ratio, as could select $\delta^{13}C$ isotopic FA analysis.

Recently, Koussoroplis et al. (2010) analyzed neutral and polar lipids for FA $\delta^{13}C$ in juvenile leaping grey mullet (*Liza saliens*) during settlement and demonstrated that the neutral lipids took on the dietary FA signal and isotopic values of the new lagoon habitat faster (at a smaller size) than the polar lipids from the same animals. Further, within a

relatively short settlement growth period (20 mm to 50 mm, August to September) the isotopic values of the FAs in the neutral and polar lipids equilibrated. Koussoroplis et al (2010) showed a similar time-scale to that observed here for juvenile gadids: maximal differences in liver (neutral) to flesh (polar) of one month followed by equilibration at 2 months.

The rate of $\delta^{13}C$ labeled 18:3n-3 uptake in juvenile sand eel (*Ammodytes tobianus*) corresponded to an exponential model based on growth and FA metabolism (Dalsgaard & St. John 2004) which predicted a FA uptake rate of $0.035.day^{-1}$ for 18:3n-3 and $0.0086.day^{-1}$ for 16:0, in whole bodies of sandeel. I estimated rates of uptake on a 3-parameter growth to a maximum model for flesh and liver separately. Results for 18:3n-3 are within the range of Dalsgaard & St. John (2004), at $0.021\%.day^{-1}$ in the flesh and 0.6 to $0.13\%.day^{-1}$ in the liver, depending on temperature and species. Similar values were reported for 18:2n-6 and the sum of the total FAs (Table 11). This indicates that similar rates of uptake may be valid for a number of juvenile fish species.

Copeman et al. (2009- Chapter 3) reported levels of terrestrial FAs in both pelagic and settled juvenile Atlantic cod from cold-water eelgrass beds in Bonavista Bay Newfoundland, Canada. Levels of Σ18:2n-6 &18:3n-3 in pelagic juveniles in September (3.9%) increased in settled juveniles to a high in November (6.2%), indicating a 2.3% proportional uptake. A simple calculation of the proportional change in Σ18:3n-3 +18:2n-6 over the number of days in the nearshore (~56), yields an uptake rate of $0.041\%.day^{-1}$. This calculation for transition from pelagic-marine to nearshore-enriched food webs does not account for concurrent changes in FA composition of zooplankton in

the nearshore, however, it is consistent with a laboratory study on juvenile sand eel by Dalsgaard & St. John's (2004) and laboratory rates that I report for juvenile Pacific gadids (Chapter 5). Interestingly, the calculation for whole-bodied field-captured Atlantic cod is intermediate ($0.041\%.day^{-1}$) between the flesh ($0.020\%.day^{-1}$) and liver ($0.075\%.day^{-1}$) tissue derived rates. This intermediate rate likely reflects the elevated uptake in TAG from the liver and the reduced uptake in PL from the flesh, noting that whole juvenile Atlantic cod contained 35% TAG and 70% PL (Copeman et al. 2008-Chapter 2).

CONCLUSIONS:

I found that neither species nor temperature affected FABM uptake rate in juvenile Pacific gadid flesh over a 2-month study period. Although temperature affected liver uptake rate during the first few wks of feeding, there were no significant temperature effects on FABM proportions in flesh or liver tissue at the end of the 8-wk feeding trial. Both tissues were saturated by the end of the 8-wk study period, during which C_{18} PUFA increase was described by growth-to-a-maximum curves. Tissue specificity led to different proportions of the biomarker in the flesh, liver and hearts of juvenile gadids. This differential retention produced liver and flesh tissue ratios for juvenile gadids that show promise for determining nearshore residency time. I found maximal differences in liver:flesh ratio after 1-month of feeding which equilibration after 2-months of feeding, as found in previous field studies using $\delta^{13}C$ labeled FAs (Koussoroplis et al. 2010). The laboratory developed rates for juvenile Pacific gadids

match those in previous studies using $\delta^{13}C$ labeled FAs (Dalsgaard et al. 2004) as well as previous field reports for Atlantic juvenile cod (Copeman et al. 2009- Chapter 3).

Application of these quantitative lab determined rates of C_{18} PUFA uptake will aid in future field studies that aim to determine the dietary carbon source of juvenile fish. Additionally, the proportions of C_{18} PUFA in the tissues of juvenile gadids could help to determine the duration of residence within nearshore essential fish habitats. Coupling these results with selected $\delta^{13}C$ labeled FABM analysis in field studies will provide a more complete understanding of dietary requirements of juvenile fish during settlement within nearshore habitats. Significant input of dietary terrestrial carbon could expand the definition of critical habitat for juvenile Pacific cod and saffron cod to include nearshore biogenic structure (eelgrass and kelp) as well as surrounding terrestrial buffer zones that supply carbon to the food web during summer and fall settlement.

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Table 1: Ingredients used in the formulation of a maintenance gel-food diet (herring), marine gel-food diet (Pacific cod fillets and cod liver oil) and terrestrially enriched gel-food diet (Pacific cod fillets and flax seed oil).

Ingredient	Maintenance Diet*	Marine Diet	Terrestrial Diet
Herring	568 g	-	-
Pacific Cod	-	568 g	568 g
Cod Liver Oil	-	45.5 g	-
Flaxseed Oil	-	-	45.5 g
Freeze dried krill	28.4 g	28.4 g	28.4 g
Squid	284 g	284 g	284 g
Othohime EP4	864 g	864 g	864 g
Phoenix Amino Solution	50 ml	50 ml	50 ml
Powdered gelatin	800 ml	800 ml	800 ml
Water	1700 ml	1700 ml	1700 ml
Vitamins	2 tablets	2 tablets	2 tablets

*fed for >6-wks prior to onset of feeding experiment

Table 2: Tank assignment and percentage survival (expressed relative to number of fish remaining after lipid sampling at the end of the experiment) for the 8-wk dietary study on the effects of species and temperature on C_{18} PUFA uptakes.

Species	Temperature	Diet Type	# tanks	% survival (tanks n=3 ± SEM)
Cod	3°C	Marine	3	63± 3
Cod	9°C	Marine	3	61±11
Cod	3°C	Terrestrial	3	47±18
Cod	9°C	Terrestrial	3	75±9
Pollock	3°C	Marine	3	95±4
Pollock	9°C	Marine	3	90±9
Pollock	3°C	Terrestrial	3	100±0
Pollock	9°C	Terrestrial	3	96±4

Table 3: FA composition of marine based and terrestrial based experimental gel-food diets fed to juvenile gadids for 8-wks feeding trail (n=6, average ± SEM).

	Cod Liver Oil Diet	Flaxseed Oil Diet
Total Lipid per Wet Wt ($mg.g^{-1}$)	40.3 ± 1.4	37.0 ± 5.9
Triacylglycerols	52.6 ± 2.8	55.9 ± 1.2
Free FAs	9.8 ± 0.8	8.5 ± 0.8
Sterols	2.3 ± 0.5	1.7 ± 0.0
Acetone Mobile Polar Lipids	4.5 ± 1.3	3.5 ± 0.9
Phospholipids	29.2 ± 1.7	29.1 ± 1.8
FAs		
14:0	5.9 ± 0.1	4.4 ± 0.1
16:0	17.1 ± 0.5	15.2 ± 0.6
18:0	3.3 ± 0.1	3.5 ± 0.1
Σ SFA	**27.6 ± 0.5**	**24.2 ± 0.1**
16:1 n-7	6.6 ± 0.2	4.7 ± 0.1
18:1n-7	3.6 ± 0.1	3.4 ± 0.1
18:1n-9	11.2 ± 0.3	11.6 ± 0.4
20:1n-9	2.4 ± 0.2	2.2 ± 0.3
20:1n-11	1.8 ± 0.2	1.1 ± 0.5
22:1n-11+13	3.1 ± 0.3	2.2 ± 0.5
Σ MUFA	**31.6 ± 1.1**	**27.7 ± 1.1**
16:2n-4	1.2 ± 0.1	0.9 ± 0.1
18:2n-6	**2.6 ± 0.0**	**5.4 ± 0.1**
18:3n-3	**0.8 ± 0.2**	**13.9 ± 0.7**
18:4n-3	2.2 ± 0.0	1.7 ± 0.0
20:4n-6	0.9 ± 0.0	0.7 ± 0.0
20:5n-3	13.1 ± 0.2	10.1 ± 0.4
22:5n-3	1.7 ± 0.0	1.2 ± 0.0
22:6n-3	13.0 ± 0.4	11.0 ± 0.6
Σ PUFA	**39.9 ± 0.9**	**47.4 ± 1.0**
Σ 18:2n-6 +18:3n-3	**3.5 ± 0.2**	**19.3 ± 0.8**

Table 4: Total lipid and FA concentrations and summary lipid proportions (>2% in at least one sample type) for flesh of juvenile gadids fed either a marine or terrestrial diet for 8-wks (temperatures are pooled). Data are mean ± SDM, n=3 fish at time-0, n=6 fish at wk 8.

	Cod Time 0	Cod Marine Wk 8	Cod Terrestrial Wk 8	Pollock Time 0	Pollock Marine Wk 8	Pollock Terrestrial Wk 8
Total Lipids per wet wt $mg.g^{-1}$	8.4±0.6	25.4±16.5	10.4±1.2	11.3±0.9	9.6±1.7	8.7±1.6
% TAG	1.4±0.2	1.1±1.0	1.6±2.0	2.2±1.8	1.2±0.8	1.2±1.2
% PL	73.9±6.0	85.3±5.0	85.6±4.3	75.9±3.0	83.7±1.9	84.8±7.0
Total FAs $mg.g^{-1}$ wet weight	5.2±0.5	5.9±1.4	6.8±0.8	6.6±1.4	6.3±1.3	5.6±1.1
16:0	26.7±7.3	14.4±0.6	13.8±0.1	21.25±3.6	15.2±0.1	14.3±0.3
18:0	9.4±2.8	4.6±0.2	4.8±0.3	7.1±1.3	4.2±0.1	4.5±0.1
ΣSFA	39.2±10.8	21.5±0.2	20.7±0.2	31.4±5.5	21.7±0.2	20.6±0.4
18:1n-9	11.9±0.2	13.0±0.2	13.0±0.4	12.6±0.6	12.4±0.3	13.2±0.2
18:1n-7	5.3±0.5	4.3±0.2	3.9±0.2	4.2±0.1	4.4±0.1	4.0±0.1
ΣMUFA	23.3±0.3	26.8±0.6	24.7±0.5	26.0±0.9	25.3±0.5	24.5±0.8
18:2n-6	0.7±0.2	1.5±0.1	3.0±0.2	0.9±0.1	1.5±0.0	2.5±0.1
18:3n-3	0.4±0.0	0.4±0.1	5.8±0.4	0.3±0.0	0.6±0.1	5.3±0.3
20:5n-3	10.6±3.3	16.6±0.4	14.8±0.3	12.5±1.8	16.1±0.3	14.3±0.3
22:6n-3	20.5±6.0	24.3±0.8	23.2±0.8	22.6±2.9	25.9±0.6	25.2±1.1
ΣPUFA	37.0±10.7	51.3±0.6	54.1±0.7	42.2±5.5	52.4±0.6	54.5±0.9
Σ 18:2n-6 +18:3n-3	1.0±0.2	1.9±0.1	8.7±0.6	1.2±0.2	2.1±0.1	7.9±0.4

* Detailed lipid class and fatty acid data found in Appendices.

Table 5: Total lipid and FA concentrations and summary lipid proportions (>5% in at least one sample type) for liver of juvenile gadids fed either a marine or terrestrial diet for 8-wks (pooled temperatures). Data are mean ± SEM, n=3 time-0, n=6 wk 8.

	Cod Time 0	Cod Marine Wk 8	Cod Terrestrial Wk 8	Pollock Time 0	Pollock Marine Wk 8	Pollock Terrestrial Wk 8
Total Lipids per wet wt $mg.g^{-1}$	115.3±15.2	292.0±57.7	364.6±78.8	386.2±108.7	364.2±46.1	542.2±60.2
% TAG	49.9±12	69.6±5	69.9±6	82.9±1.6	77.1±0.7	80.3±1.6
% PL	21.4±7.7	13.2±3.5	17.7±4.5	5.7±1.1	7.9±1.3	8.0±0.9
Total FAs $mg.g^{-1}$ wet weight	80.0±13.0	260.0±52.5	329.3±75.5	264.2±77.6	336.8±42.3	499.5±57.4
16:0	14.5±0.3	11.9±0.7	10.7±0.5	15.7±0.8	9.3±1.9	11.2±0.5
ΣSFA	22.5±0.4	18.0±1.2	16.3±0.7	23.5±1.1	15.3±1.7	16.0±0.9
18:1n-9	17.1±0.6	20.1±1.0	21.8±0.8	20.3±0.8	21.3±0.8	22.0±1.1
18:1n-7	5.9±0.1	4.8±0.3	4.4±0.3	5.0±0.1	5.2±0.4	5.0±0.2
ΣMUFA	36.5±0.8	42.8±1.5	40.1±1.0	45.3±0.8	47.5±2.5	43.0±2.1
18:2n-6	1.7±0.1	2.5±0.1	5.4±0.2	1.6±0.0	2.2±0.1	4.6±0.2
18:3n-3	0.7±0.0	1.0±0.0	13.6±0.5	0.7±0.0	0.9±0.0	11.3±0.6
20:5n-3	12.5±0.5	12.9±0.4	8.6±0.3	10.2±0.3	12.6±0.3	9.1±0.4
22:6n-3	16.6±0.4	12.2±1.0	8.1±0.4	9.6±0.2	11.2±1.1	8.2±1.6
ΣPUFA	40.0±1.0	38.4±1.9	43.0±1.3	30.3±0.3	36.4±1.4	40.4±2.4
Σ 18:2n-6 +18:3n-3	2.5±0.1	3.5±0.1	19.1±0.7	2.4±0.1	3.1±0.1	15.9±0.8

* Detailed lipid class and fatty acid data found in Appendices.

Table 6: Total lipid and FA concentrations and summary lipid proportions (>2.5% in at least one sample type) for hearts of juvenile gadids fed either a marine or terrestrial diet for 8-wks (pooled temperatures). Data are mean ± SEM, n=3 time-0, n=6 wk 8.

	Cod Time 0	Cod Marine Wk 8	Cod Terrestrial Wk 8	Pollock Time 0	Pollock Marine Wk 8	Pollock Terrestrial Wk 8
% PL	52.7±0.5	77.0±4.6	82.5±3.3	37.3±2.5	84.1±3.1	84.2±2.5
14:0	4.1±2.4	1.9±0.5	1.2±0.2	1.2±0.2	1.4±0.3	0.7±0.2
16:0	33.9±10.8	18.3±0.7	15.0±0.6	21.9±6.0	21.8±1.5	14.7±2.7
18:0	11.4±0.1	7.2±0.6	7.1±0.4	9.6±3.2	6.5±1.4	7.4±1.2
ΣSFA	51.2±14.1	28.7±1.2	24.5±1.2	33.8±9.5	31.1±1.9	23.6±3.9
18:1n-9	10.4±5.7	15.9±0.7	12.9±0.7	16.5±2.1	14.6±3.2	12.5±3.0
18:1n-7	5.5±3.1	6.0±0.3	4.4±0.4	8.8±2.5	7.4±0.4	4.4±1.2
ΣMUFA	22.4±9.3	33.0±1.0	25.6±1.5	32.5±4.7	33.2±3.4	35.3±6.8
18:2n-6	1.0±0.6	2.3±0.4	2.7±0.3	1.1±0.2	1.9±0.2	2.4±0.3
18:3n-3	0.2±0.1	2.2±1.2	5.3±1.1	0.4±0.4	1.5±0.9	4.4±0.8
20:5n-3	5.2±1.5	8.7±0.8	10.0±0.7	7.8±2.7	8.6±1.1	9.5±1.4
22:6n-3	15.2±1.3	20.5±2.2	23.4±2.3	20.4±9.5	15.2±3.1	17.8±3.8
ΣPUFA	25.4±5.2	37.1±2.0	49.3±2.2	33.4±14	34.8±4.5	40.5±6.6
Σ 18:2n-6 +18:3n-3	1.2±0.7	4.5±1.6	8.0±1.3	1.6±0.5	3.4±1.1	6.8±1.1

Table 7: Result of GLM analysis of variance for the complete model of the effect of wk, tissue type, species, temperature and diet on the proportion of FA in juvenile gadids (Systat 12).

Analysis of Variance					
Source	Type III SS	df	Mean Squares	F-ratio	p-value
Natural log week	503.51	4	125.88	61.29	<0.001
Tissue	352.30	1	352.30	171.59	<0.001
Species	9.06	1	9.06	4.41	0.037
Temperature	8.42	1	8.42	4.101	0.044
Diet	1139.12	1	1139.12	554.71	<0.001
Species*Tissue	15.19	1	15.19	7.40	0.007
Temperature*Tissue	1.38	1	1.38	0.67	0.413
Temperature*Species	7.72	1	7.72	3.76	0.054
Tissue*Diet	251.62	1	251.62	122.53	<0.001
Species*Diet	9.23	1	9.23	4.49	0.035
Temperature*Diet	7.58	1	7.58	3.69	0.056
Wk*Diet	469.82	4	117.46	57.20	<0.001
Temperature*Species*Tissue	1.28	1	1.28	0.62	0.431
Tissue*Species*Diet	11.21	1	11.21	5.46	0.021
Tissue*Temperature*Diet	0.86	1	0.86	0.42	0.519
Tissue*Wk*Diet	86.03	4	21.51	10.47	<0.001
Species*Temperature*Diet	8.72	1	8.72	4.25	0.041
Species*Wk*Diet	5.21	4	1.30	0.64	0.638
Temperature*Wk*Diet	2.36	4	0.59	0.29	0.886
Tanks(Treatment)	26.09	8	3.26	1.59	0.131
Tissue*Species*Temp*Diet	1.42	1	1.42	0.69	0.407
Tissue*Species*Wk*Diet	5.00	4	1.25	0.61	0.657
Tissue*Temperature*Wk*Diet	1.68	4	0.42	0.21	0.936
Species*Temperature*Wk*Diet	6.79	4	1.70	0.83	0.510
Tissue*Species*Temperature*Wk*Diet	12.32	4	3.08	1.50	0.204
Error	367.58	179	2.05		

Table 8: Results of multiple 3-way ANOVA examining the effect of wk, species, and temperature on the proportion of 18:3n-3 in either flesh or liver of juvenile gadids fed either a marine or terrestrial based diet during an 8-wks feeding trial.

Marine Diet Liver			
Source	DF	F-ratio	P-value
Wk	4	6.634	**0.001**
Species	1	2.686	0.111
Temp	1	2.654	0.113
Wk*Species	4	1.706	0.186
Wk*temp	4	1.522	0.228
Species*temp	1	0.503	0.484
Wk*species*temp	4	0.234	0.872
Error	40		
Terrestrial Diet Liver			
Wk	**4**	**15.113**	**<0.001**
Species	**1**	**8.249**	**0.007**
Temp	1	0.898	0.351
Wk*Species	4	1.090	0.367
Wk*temp	4	0.515	0.675
Species*temp	**1**	**4.652**	**0.039**
Wk*species*temp	4	2.627	0.067
Error	40		
Marine Flesh			
Wk	4	0.178	0.911
Species	1	3.985	0.054
Temp	1	0.148	0.703
Wk*Species	4	0.480	0.699
Wk*temp	4	0.509	0.679
Species*temp	1	0.120	0.732
Wk*species*temp	4	1.105	0.361
Error	40		
Terrestrial Flesh			
Wk	**4**	**12.659**	**<0.001**
Species	1	0.454	0.505
Temp	1	2.025	0.165
Wk*Species	4	0.375	0.772
Wk*temp	4	0.276	0.842
Species*temp	1	2.688	0.111
Wk*species*temp	4	1.359	0.273
Error	40		

Table 9: Results of multiple 3-way ANOVA examining the effect of wk , species, and temperature on the proportion of 18:2n-6 in either flesh or liver of juvenile gadids fed either a marine or terrestrial based diet during an 8-wks feeding trial.

Marine Diet Liver			
Source	DF	F-ratio	P-value
Wk	4	10.130	**<0.001**
Species	1	17.450	**<0.001**
Temp	1	1.633	0.210
Wk*Species	4	0.839	0.483
Wk*temp	4	2.421	0.084
Species*temp	1	0.307	0.584
Wk*species*temp	4	0.648	0.590
Error	40		
Terrestrial Diet Liver			
Wk	**4**	**7.533**	**0.001**
Species	**1**	**10.767**	**0.003**
Temp	1	1.537	0.224
Wk*Species	4	0.074	0.973
Wk*temp	4	0.330	0.804
Species*temp	1	1.167	0.288
Wk*species*temp	4	0.816	0.495
Error	40		
Marine Flesh			
Wk	**4**	**2.963**	**0.047**
Species	1	2.140	0.153
Temp	1	3.473	0.072
Wk*Species	4	0.852	0.476
Wk*temp	4	0.084	0.968
Species*temp	1	2.954	0.095
Wk*species*temp	4	1.437	0.250
Error	40		
Terrestrial Flesh			
Wk	**4**	**10.421**	**<0.001**
Species	1	0.032	0.858
Temp	1	1.523	0.227
Wk*Species	4	0.655	0.586
Wk*temp	4	0.243	0.866
Species*temp	1	2.220	0.146
Wk*species*temp	4	1.339	0.280
Error	40		

Table 10: Results of multiple 3-way ANOVA examining the effect of wk, species, and temperature on the proportion of Σ18:2n-6 + 18:3n-3 in either flesh or liver of juvenile gadids fed either a marine or terrestrial based diet during an 8-wks feeding trial.

Marine Diet Liver			
Source	DF	F-ratio	P-value
Wk	4	18.173	**<0.001**
Species	1	15.189	**<0.001**
Temp	1	1.427	0.239
Wk*Species	4	1.337	0.273
Wk*temp	4	2.177	0.089
Species*temp	1	0.651	0.425
Wk*species*temp	4	0.401	0.807
Error	40		
Terrestrial Diet Liver			
Wk	**4**	**42.158**	**<0.001**
Species	**1**	**9.366**	**0.004**
Temp	1	2.649	0.111
Wk*Species	4	0.707	0.592
Wk*temp	4	0.310	0.870
Species*temp	1	2.591	0.115
Wk*species*temp	4	1.346	0.270
Error	40		
Marine Flesh			
Wk	**4**	**7.272**	**<0.001**
Species	1	3.480	0.069
Temp	1	0.276	0.602
Wk*Species	4	0.702	0.595
Wk*temp	4	0.869	0.491
Species*temp	1	0.054	0.817
Wk*species*temp	4	1.688	0.172
Error	40		
Terrestrial Flesh			
Wk	**4**	**34.245**	**<0.001**
Species	1	0.180	0.674
Temp	1	2.528	0.120
Wk*Species	4	0.502	0.735
Wk*temp	4	0.437	0.781
Species*temp	1	3.402	0.073
Wk*species*temp	4	0.992	0.424
Error	40		

Table 11: Parameters for the rise to a maximum shaped uptake of 18:3n-3, 18:2n-6 and Σ18:2n-6 + 18:3n-3 into the liver and flesh of juvenile gadids.

The equation $\mathbf{Y_x = Y_0 + a[1-exp(-bX)]}$, exponential rise to a maximum, was used with the following parameter: $\mathbf{Y_0}$ refers to the proportion of FA (%) in the tissue at time 0, **a** is the maximum proportion found in the tissue (%), **b** relates to the initial slope of the FA uptake curve (%.day^{-1}), **X** refers to the days of feeding, while **1/b** refers to the duration of the maximum slope (days) (Data are means ± standard errors of the mean).

Treatment	Y_0 (%)	a (max % FA)	b (% FA.day^{-1})	1/ b (days)	R^2
18:3n-3					
Gadid flesh	0.64 ± 0.32	6.90 ± 2.15	0.02 ± 0.01	47.6	0.85
Pollock Liver	0.25 ± 0.90	11.09 ± 1.32	0.06 ± 0.02	15.9	0.90
Cod Liver 9°C	0.92 ± 1.15	12.00 ± 1.42	0.13 ± 0.04	7.69	0.95
Cod Liver 3°C	1.10 ± 1.50	13.13 ± 3.09	0.04 ± 0.02	23.8	0.94
18:2n-6					
Gadid Flesh	0.94 ± 0.15	2.55 ± 1.08	0.02 ± 0.02	50.0	0.77
Pollock Liver	1.66 ± 0.22	2.79 ± 0.30	0.08 ± 0.02	12.7	0.91
Cod Liver	1.75 ± 0.36	3.37 ± 0.44	0.12 ± 0.04	8.20	0.86
Σ18:2n-6+18:3n-3					
Gadid Flesh	1.58 ± 0.46	9.40 ± 3.18	0.02 ± 0.01	50.0	0.83
Pollock Liver	2.27 ± 1.09	13.60 ± 1.61	0.06 ± 0.02	16.1	0.90
Cod Liver	2.72 ± 1.64	15.27 ± 2.14	0.09 ± 0.03	11.4	0.85

Table 12: Parameters for the peaked shaped ratio of the proportion of 18:3n-3 and 18:2n-6 in the liver relative to the flesh for juvenile gadids.

Gaussian 3 parameter peak model: $Y=a*exp(-.5*((X_1-X_0)/b)^2)$: **Y** refers to the ratio of 18:3n-3 in the liver relative to the flesh, **a** is the maximum proportion in the liver:flesh, $\mathbf{X_1}$ is the days of feeding, $\mathbf{X_0}$ is the days until a slope was zero or max peak, **b** is the width of the peak in days (data are means ± standard errors of the mean).

FA ratio in liver to flesh	a (max proportion unite less)	X_0 (days)	b (days)	R^2
18:3n-3	4.34 ± 0.26	28.99 ± 1.59	24.23 ± 2.00	0.90
18:2n-6	3.10 ± 0.23	28.09 ± 2.39	28.19 ± 3.62	0.76

FIGURE CAPTIONS

Figure 1: Proportion of 18:3n-3 in fish reared on either a marine or terrestrial diet for 8 weeks: (a) flesh with species and temperature combined, (symbols represents mean ± SEM, n=3 per symbol), (b) liver of pollock with temperatures combined (symbols represents mean ± SEM n=3 per symbol), (c) liver of cod with temperatures separate (symbols represents mean ± SEM n=3 per symbol). * graphs are pooled/separate for species and temperature based on ANOVA results in table 8.

Figure 2: Proportion of 182n-6 in fish reared on either a marine or terrestrial diet for 8 weeks: (a) flesh with species and temperature combined, (symbols represents mean ± SEM, n=3 per symbol), (b) liver of pollock with temperatures combined (symbols represents mean ± SEM n=3 per symbol), (c) liver of cod with temperatures combined (symbols represents mean ± SEM n=3 per symbol). * graphs are pooled/separate for species and temperature based on ANOVA results in table 9.

Figure 3: Proportion of 18:3n-3 + 182n-6 in fish reared on either a marine or terrestrial diet for 8 weeks: (a) flesh with species and temperature combined, (symbols represents mean ± SEM, n=3 per symbol), (b) liver of pollock with temperatures combined (symbols represents mean ± SEM, n=3 per symbol), (c) liver of cod with temperatures combined (symbols represents mean ± SEM, n=3 per symbol). * graphs are pooled/separate for species and temperature based on ANOVA results in table 10.

Figure 4: Proportion of 18:3n-3 in the (a) liver (pooled temperatures) and (b) flesh (pooled temperatures and species) of juvenile gadids relative to the proportion in the diet. ((a) symbols are n=6, mean ± SEM, (b)symbols are n=12, mean ± SEM).

Figure 5: Ratio of (a) 18:3n-3 and (b) 18:2n-6 in the liver relative to the proportion in the flesh for juvenile gadids switched to a terrestrially enriched diet. Symbols are n=12 mean ± SEM.

Figure 6: Growth rates of juvenile cod (n=10 to 14) and Pollock (n=24 to 26) reared at two temperatures and fed either a marine diet or a terrestrially enriched diet for 8 wks.

Figure 7: Relationship between wet weight (g) and proportion of 18:3n-3 (%) in the flesh tissue of juvenile gadids over a 8-wk feeding trial (n=12, mean ± SD).

Figure 8: The proportion of (a) 18:2n-6 and (b) 18:3n-3 in the flesh, liver and heart of two species of juvenile gadids switched from a marine diet to 8 wks of feeding on a terrestrially enriched diet (n=6, mean ± SEM).

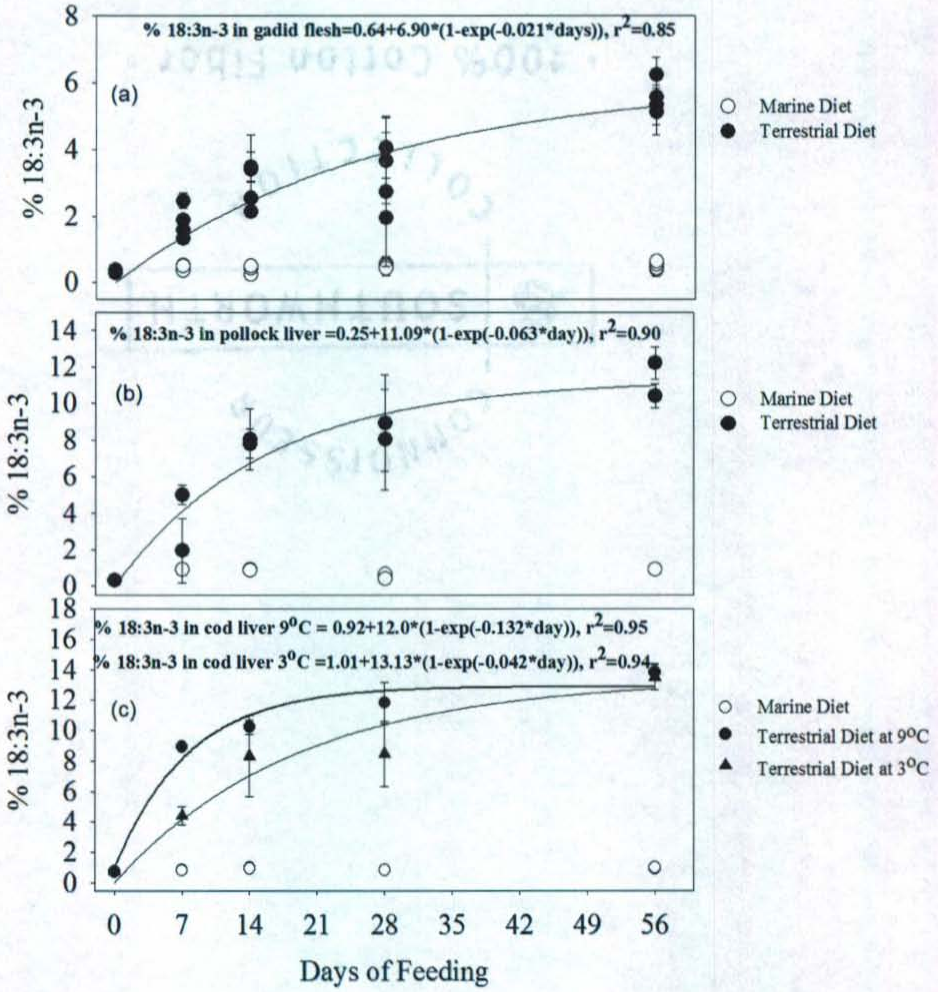
% 18:3n-3 in gadid flesh=0.64+6.90*(1-exp(-0.021*days)), r2=0.85
(a)
% 18:3n-3
Marine Diet
Terrestrial Diet
% 18:3n-3 in pollock liver =0.25+11.09*(1-exp(-0.063*day)), r2=0.90
(b)
% 18:3n-3
Marine Diet
Terrestrial Diet
% 18:3n-3 in cod liver 9°C = 0.92+12.0*(1-exp(-0.132*day)), r2=0.95
% 18:3n-3 in cod liver 3°C =1.01+13.13*(1-exp(-0.042*day)), r2=0.94
(c)
% 18:3n-3
Marine Diet
Terrestrial Diet at 9°C
Terrestrial Diet at 3°C
0 7 14 21 28 35 42 49 56
Days of Feeding

Figure 1

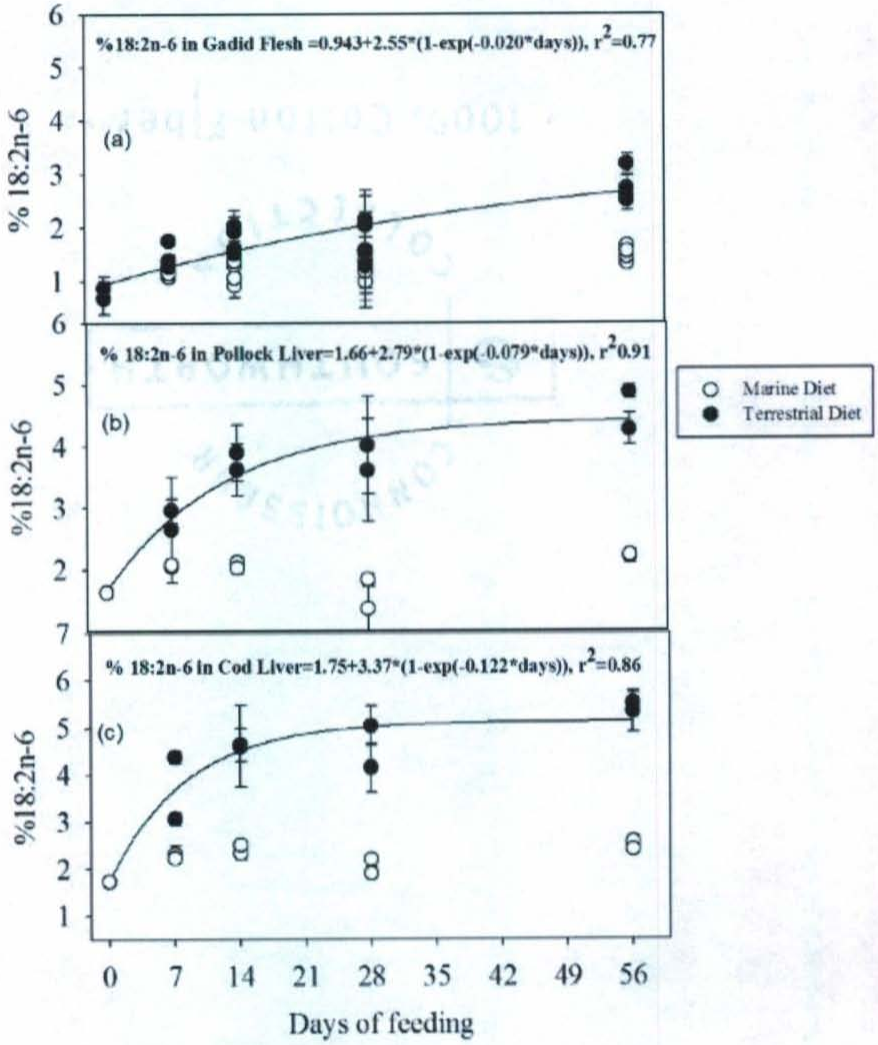
%18:2n-6 in Gadid Flesh =0.943+2.55*(1-exp(-0.020*days)), r2=0.77
(a)
% 18:2n-6
% 18:2n-6 in Pollock Liver=1.66+2.79*(1-exp(-0.079*days)), r20.91
(b)
%18:2n-6
% 18:2n-6 in Cod Liver=1.75+3.37*(1-exp(-0.122*days)), r2=0.86
(c)
%18:2n-6
Marine Diet
Terrestrial Diet
0 7 14 21 28 35 42 49 56
Days of feeding

Figure 2

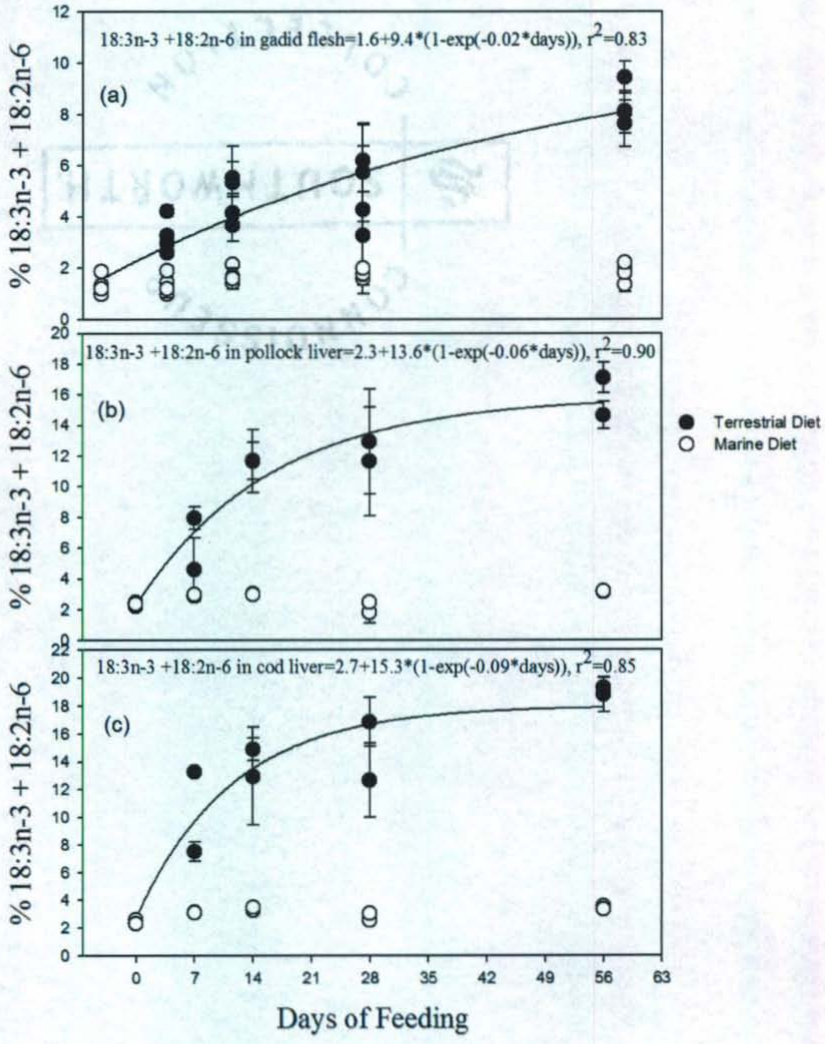
18:3n-3 +18:2n-6 in gadid flesh=1.6+9.4*(1-exp(-0.02*days)), r2=0.83
(a)
18:3n-3 +18:2n-6 in pollock liver=2.3+13.6*(1-exp(-0.06*days)), r2=0.90
(b)
18:3n-3 +18:2n-6 in cod liver=2.7+15.3*(1-exp(-0.09*days)), r2=0.85
(c)
% 18:3n-3 + 18:2n-6
Days of Feeding
Terrestrial Diet
Marine Diet

Figure 3

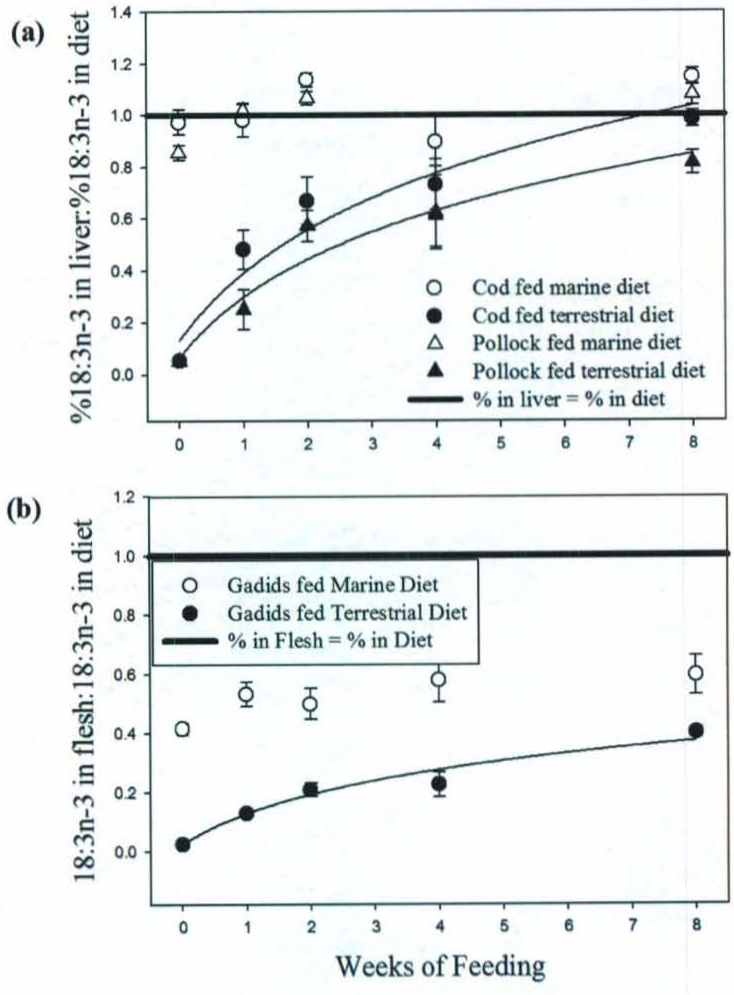
(a)
%18:3n-3 in liver:%18:3n-3 in diet
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
7
8
Cod fed marine diet
Cod fed terrestrial diet
Pollock fed marine diet
Pollock fed terrestrial diet
% in liver = % in diet
(b)
18:3n-3 in flesh:18:3n-3 in diet
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Gadids fed Marine Diet
Gadids fed Terrestrial Diet
% in Flesh = % in Diet
Weeks of Feeding

Figure 4

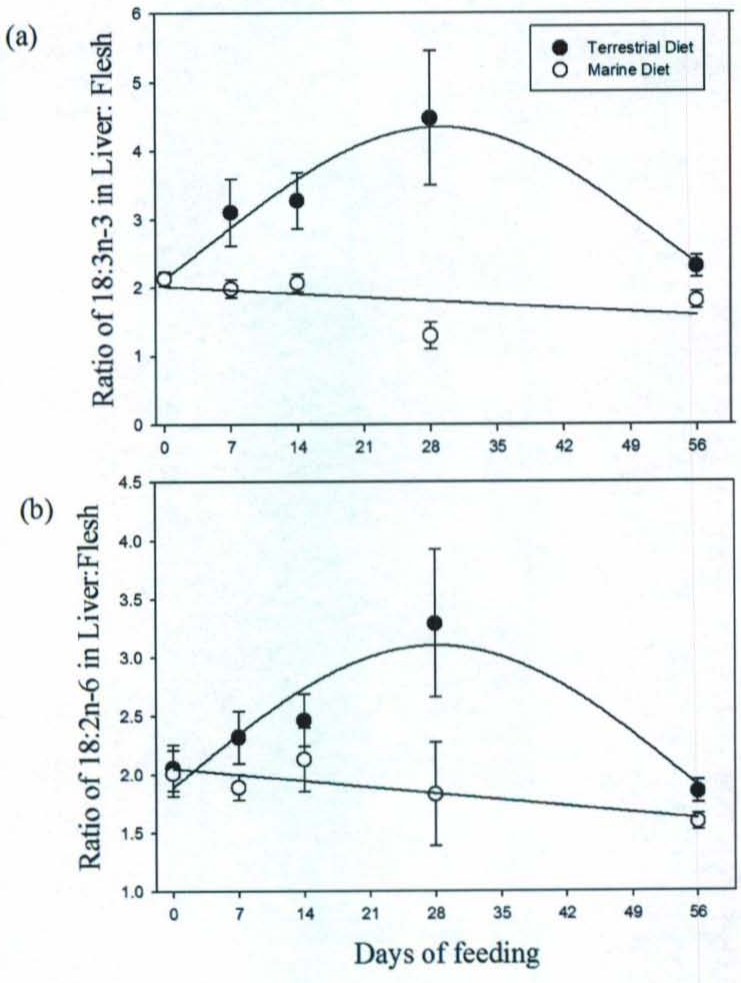
(a)
Ratio of 18:3n-3 in Liver: Flesh
Terrestrial Diet
Marine Diet
0
1
2
3
4
5
6
0
7
14
21
28
35
42
49
56
(b)
Ratio of 18:2n-6 in Liver:Flesh
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Days of feeding

Figure 5

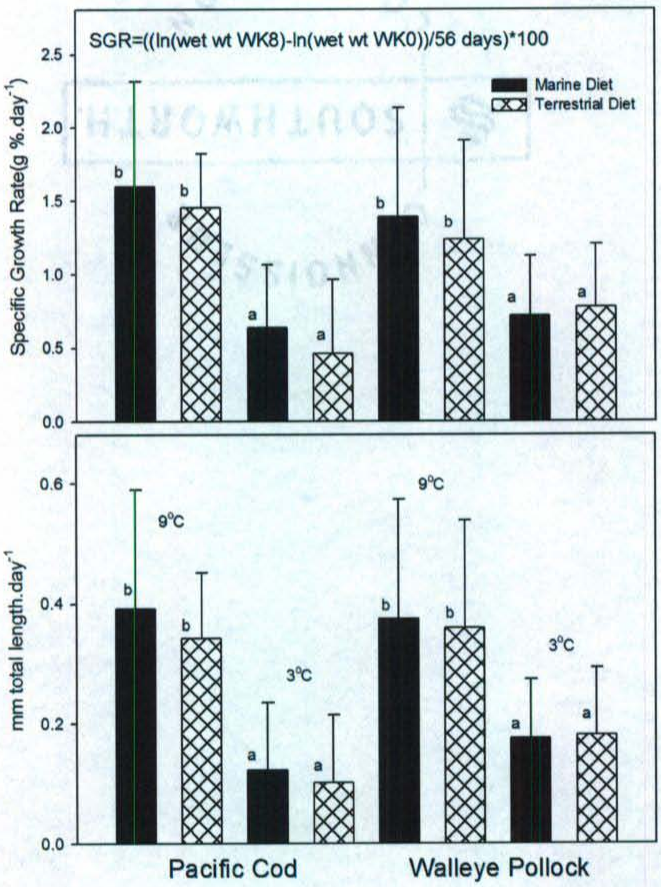
SGR=((ln(wet wt WK8)-ln(wet wt WK0))/56 days)*100
Marine Diet
Terrestrial Diet
Specific Growth Rate(g %.day-1)
2.5
2.0
1.5
1.0
0.5
0.0
b
b
a
a
b
b
a
a
mm total length.day-1
0.6
0.4
0.2
0.0
9°C
3°C
9°C
3°C
Pacific Cod
Walleye Pollock

Figure 6

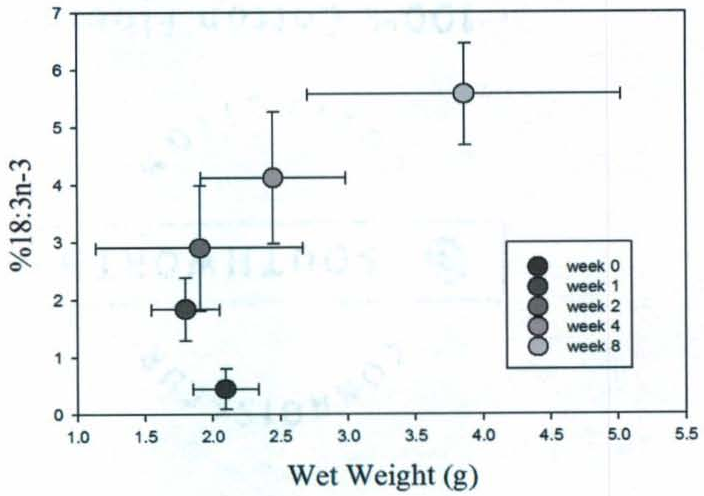
%18:3n-3
Wet Weight (g)
week 0
week 1
week 2
week 4
week 8
0
1
2
3
4
5
6
7
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5

Figure 7

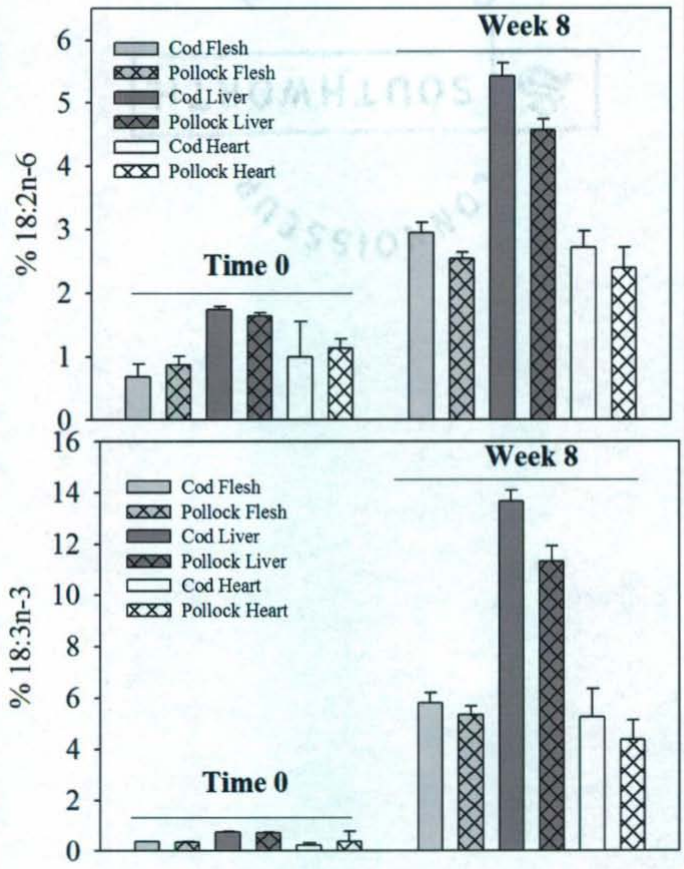
Week 8
Cod Flesh
Pollock Flesh
Cod Liver
Pollock Liver
Cod Heart
Pollock Heart
Time 0
% 18:2n-6
0
1
2
3
4
5
6
Week 8
Cod Flesh
Pollock Flesh
Cod Liver
Pollock Liver
Cod Heart
Pollock Heart
Time 0
% 18:3n-3
0
2
4
6
8
10
12
14
16

Figure 8

CHAPTER 6: Thesis Conclusions

Examination of the lipid classes in age-0 juvenile Atlantic cod at settlement showed a decrease in energy storage that was not evident from traditional condition factors that depend on wet weight (e.g. Fulton's K). The storage of lipids was variable and dependent on fish size; however, juvenile cod did not follow the estuarine model of increased neutral lipid storage prior to over-wintering. I found decreased lipids in the flesh of cod at settlement compared to pre-settlement, which indicated increased muscle water content. Reduction in the amount (lipid per wet wt) of all lipid classes in juvenile cod after settlement, coupled with decreased proportions of EFAs in the flesh of juvenile fish, indicated that the nutritional quality of zooplankton in eelgrass was not optimal. However, further studies will multiple years of dietary sampling are required to confidently confirm this statement. The decrease in lipid storage observed in juvenile cod during settlement in eelgrass habitat suggests an emphasis on growth during this critical period in development.

Lipid biomarkers, compound-specific isotopes, and principal components analysis demonstrated increased terrestrial carbon input into the diet of juvenile cod during fall settlement into eelgrass habitat. I used compound-specific isotopes and lipid classes to clarify the utility of Σ18:3n-3 & 18:2n-6 as an indicator of terrestrial carbon input and to distinguish terrestrial from eelgrass carbon sources. I found no evidence that eelgrass entered the diet of juvenile cod and therefore, I concluded that the functional significance of eelgrass to Atlantic cod juveniles is for refuge rather than increased nutrition. Given the indicated input of terrestrial carbon into the diet of juvenile cod at settlement, I

concluded that terrestrial buffer zones surrounding eelgrass habitat should also be considered in the evaluation of essential fish habitat for this species.

I examined the importance of EFAs during the critical first-feeding stage of Pacific cod larvae. Specifically, I showed that Pacific cod larvae are sensitive to changes in the ratios of essential PUFA in their prey and that optimum dietary levels are comparable to those in wild zooplankton. Further, like Atlantic cod, Pacific cod require high levels of dietary n-3 PUFA. I concluded that FA-limited growth should be considered as a contributory factor to variability in year-class strength in Pacific cod throughout the North Pacific.

I examined the abiotic and biotic factors that affected the rate of uptake of two C_{18} PUFAs in the tissues of two species of juvenile gadids, Pacific cod and walleye pollock. I found no effect of temperature or species on the rate of uptake of 18:3n-3 and/or 18:2n-6 in gadid flesh, suggesting that the rates I report can potentially be applied 1) to juveniles of other low-fat marine species and 2) throughout the range of temperatures experienced during gadid settlement in temperate, nearshore habitats. Tissue specificity led to temporal variation in the proportion of C_{18} PUFAs in liver relative to flesh tissue. This temporal variation shows promise for future studies that aim to determine timing of nearshore residency. I found maximal differences in liver:flesh ratios after 1-month of feeding with equilibration after 2 months of feeding. Laboratory rates that I report in this thesis, matched those previously found using $\delta^{13}C$ labeled FAs (Dalsgaard et al. 2004) as well as previous field reports for Atlantic juvenile cod (e.g., Chapter 3).

Within this thesis, I examined trophic ecology of Atlantic and Pacific gadids, during two critical periods in the first year of life. I combined both qualitative and quantitative lipid class and FA applications to study fish condition, trophic relationships, changes in sources of organic carbon, and the rate of uptake of FABM in relation to both biotic and abiotic factors. I have provided the first investigations of Pacific gadid early lipid nutrition as well as the first use of FABM in determining trophic relationships during settlement in the nearshore.

The use of FABM as a quantitative tool for determining diet composition in higher-level, endothermic consumers is now well established (Budge et al. 2006), however as Iverson et al. (2009) indicated, it is not known how these quantitative FA analyses can be applied to ectothermic fish, and even less is known about its potential application to primary consumers such as zooplankton. Given the requirements for quantitative FA dietary analysis (knowledge of the variability in the FAs of all prey items and conversion factors in the consumer); it may be impractical to take this same approach to studies of juvenile fish feeding ecology. The large number of zooplankton prey items, their small size, the difficulty in collecting and sorting individual species, as well as the time scale on which they are likely to vary their lipid composition makes this approach seem rather unrealistic.

Future applications of the FABM approach to juvenile fish feeding ecology should focus on utilizing laboratory-determined rates of uptake of FAs that are indicative of feeding habitats or oceanographic regimes, in order to provide information on connectivity in the wild. Laboratory rates that I determined in Chapter 5, will be applied

to an upcoming study of wild juvenile Pacific cod, in order to determine offshore-inshore nursery migrations. Based on the uptake rate of C_{18} PUFAs in the liver relative to the flesh it should be possible to determine the time scale over which juveniles reside within the nearshore.

Neither species nor temperature affected the rate of FABM uptake in the flesh of juvenile gadids, indicating that it may be able to generalize rates between similar types of fish (low-fat, high-fat marine juveniles). Attempts should be made to determine rates of FABM uptake through the use of existing literature. A large body of aquaculture nutrition data exists which represents over 30 years of research on EFA fish nutrition. Currently, knowledge about fish EFA metabolism is not efficiently communicated from aquaculture to fisheries ecology, likely because these sub disciplines frame their research questions differently. However, this extensive data set should be analyzed, synthesized, and published in an ecological journal in order to prevent redundant research projects.

Future work using FABMs should utilize complimentary indicators of diet such as: gut content analysis, bulk stable isotopes, selected samples for δ13C FA, and the use of lipid class analysis (i.e. sterol biomarkers). The utility of this approach is shown in Chapter 3, where I was only able to distinguish terrestrial from eelgrass carbon by using complimentary approaches. Future work on nearshore foodwebs should continue to take complementary approaches in order to tease apart the diverse sources of organic carbon present in these complex biogenic habitats.

REFERENCES

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22: 759-801

Dalsgaard J, St. John M (2004) Fatty acid biomarkers: validation of food web and trophic markers using 13C-labeled fatty acids in juvenile sand eel (*Ammodytes tobianus*). Canadian Journal of Fisheries and Aquatic Sciences 61: 1671-1680.

Iverson, S.J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. pp. 281-307 in *Lipids in Aquatic Ecosystems*. Arts, M.T., Brett, M.T., Kainz, M.J. (eds.). Springer, New York

APPENDICES

*All appendices relate to fish flesh and liver tissues discussed in Chapter 5.

Appendix 1: Total lipids and lipid classes for flesh of juvenile (a) Pacific cod and (b) walleye pollock fed either a marine or terrestrial diet for 8-wks (temperatures are pooled). Data are mean ± SEM, n=3 fish at time-0, n=6 fish at wk 8.

(a)	Cod Time 0	Cod Marine Wk 8	Cod Terrestrial Wk 8
Total Lipids per wet wt $mg.g^{-1}$	8.4 ± 0.7	25.4±16.5	10.4±1.2
% total lipids			
HC	0.8 ± 0.4	0.3 ± 0.3	0.7 ± 0.6
SE + EE + ME	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.3
TAG	1.4 ± 0.3	1.1 ± 0.4	1.6 ± 0.9
FFA	4.5 ± 2.0	0.7 ± 0.3	0.7 ± 0.1
ST	13.3 ± 1.8	10.8 ± 1.8	9.4 ± 0.5
DG	0.4 ±0.7	0.1 ± 0.1	0.1 ± 0.1
AMPL	5.1 ± 6.2	1.0 ± 0.1	0.9 ± 0.0
PL	73.9 ± 6.7	85.3 ± 2.0	85.6±4.3

(b)	Pollock Time 0	Pollock Marine Wk 8	Pollock Terrestrial Wk 8
Total Lipids per wet wt $\mu g.g^{-1}$	11.3 ± 2.4	9.6 ± 1.7	8.7 ± 1.6
% total lipids			
HC	6.7 ±0.9	0.0 ± 0.1	0.1 ± 0.3
SE + EE + ME	1.2 ± 0.3	1.0 ± 0.9	0.2 ± 0.5
TAG	2.2 ± 2.1	1.2 ± 0.8	1.2 ± 1.2
FFA	0.3 ± 0.3	1.8 ± 1.1	0.8 ± 0.4
ST	11.8 ± 1.2	10.1 ± 3.0	11.1 ± 3.6
DG		0.4 ± 1.1	0.2 ± 0.4
AMPL	2.0 ± 0.4	0.9 ± 0.3	1.1 ± 0.6
PL	75.9 ± 3.3	83.7 ± 4.6	84.8 ± 6.9

HC=hydrocarbons, SE = Steryl esters, EE= ethyl esters, ME= methyl esters, TAG= triacyclglycerols, FFA= free fatty acids, DG= diacylglycerols, AMPL = acetone mobile polar lipids, PL= phospholipids.

Appendix 2: Total lipids and lipid classes in the liver of juvenile (a) Pacific cod and (b) walleye pollock fed either a marine or terrestrial diet for 8-wks (temperatures are pooled). Data are mean ± SEM, n=3 fish at time-0, n=6 fish at wk 8.

(a)	Cod Time 0	Cod Marine Wk 8	Cod Terrestrial Wk 8
Total Lipids per wet wt $mg.g^{-1}$	115.2 ± 28.7	292.0 ± 57.7	364.6 ± 78.8
% total lipids			
HC		0.2 ± 0.1	0.7 ± 0.3
SE + EE + ME		0.1 ± 0.1	
TAG	49.9 ± 18.9	69.6 ± 4.9	68.9 ± 6.1
FFA	2.6 ± 1.7	10.6 ± 1.0	9.6± 0.9
ST	2.6± 1.4	2.2 ± 0.6	0.9 ± 0.1
DG		0.3 ± 0.3	
AMPL	9.4 ± 3.5	3.2 ± 0.8	2.2 ± 0.5
PL	21.4 ± 12.2	13.2 ± 3.5	17.7 ± 4.5

(b)	Pollock Time 0	Pollock Marine Wk 8	Pollock Terrestrial Wk 8
Total Lipids per wet wt $\mu g.g^{-1}$	386.2 ± 188.2	364.2 ± 46.1	542.2 ± 60.2
% total lipids			
HC	2.3 ±1.0	0.1 ± 0.1	0.1 ± 0.1
SE + EE + ME	0.1 ± 0.1		
TAG	82.9 ± 2.6	77.1 ± 0.6	80.3 ± 1.6
FFA	0.5 ± 0.5	12.4 ± 1.1	8.4 ± 0.6
ST	2.4 ± 1.2	0.6 ± 0.1	1.5 ± 0.5
DG			0.5 ± 0.5
AMPL	6.1 ± 1.4	1.5 ± 0.3	1.2 ± 0.3
PL	5.7 ± 1.8	7.9 ± 1.3	8.0 ± 0.9

Appendix 3: Fatty acid proportions in Pacific cod flesh after feeding on either a marine or terrestrial diet for 8-wks (temperatures pooled). Data are the mean ± SD, n=3 fish at time-0, n=6 fish at wk-8. All routinely identified FAs shown below in elution order.

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
	Avg	SD	Avg	SD	Avg	SD
14:0	1.8	1.3	1.4	0.4	1.2	0.4
14:1	0.0	0.0	0.0	0.0	0.0	0.0
i-15:0	0.1	0.0	0.1	0.0	0.1	0.0
ai-15:0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	0.4	0.3	0.2	0.0	0.2	0.0
15:1	0.0	0.0	0.0	0.0	0.0	0.0
i-16:0	0.1	0.0	0.0	0.0	0.0	0.0
16:0	26.7	19.8	14.3	0.5	14.0	0.4
16:1n-11?	0.0	0.0	0.1	0.0	0.1	0.0
16:1n-9?	0.7	0.5	0.3	0.0	0.3	0.0
16:1n-7	1.2	0.8	2.4	0.4	1.9	0.3
16:1n-5	0.1	0.1	0.2	0.0	0.2	0.0
i17:0	0.3	0.1	0.3	0.0	0.2	0.0
ai17:0	0.1	0.0	0.1	0.0	0.1	0.0
16:2n-4	0.4	0.3	0.6	0.1	0.5	0.1
17:0	0.4	0.3	0.2	0.0	0.2	0.0
16:3n-4?	0.0	0.0	0.1	0.0	0.0	0.0
17:1	0.5	0.2	0.5	0.1	0.4	0.1
16:4n-3?	0.0	0.0	0.2	0.2	0.3	0.2
16:4n-1	0.0	0.0	0.1	0.0	0.1	0.1
18:0	9.4	7.7	4.7	0.6	4.6	0.9
18:1n-11?	0.0	0.0	0.1	0.1	0.0	0.0
18:1n-9	11.9	0.5	13.0	0.4	12.8	1.3
18:1n-7	5.3	1.5	4.1	0.3	3.8	0.2
18:1n-6?	0.0	0.0	0.0	0.0	0.0	0.0
18:1n-5?	0.3	0.3	0.2	0.0	0.2	0.0
18:2n-6	0.7	0.5	1.4	0.3	2.9	0.6
18:2n-4	0.1	0.1	0.2	0.1	0.1	0.0
18:3n-6	0.1	0.0	0.1	0.0	0.1	0.0
18:3n-4	0.2	0.2	0.1	0.1	0.1	0.1
18:3n-3	0.4	0.0	0.5	0.1	5.6	1.4

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
18:4n-3	0.3	0.3	0.5	0.1	0.4	0.1
18:4n-1?	0.2	0.4	0.1	0.1	0.1	0.1
20:0	0.0	0.0	0.1	0.0	0.1	0.0
18:5n-3	0.0	0.0	0.0	0.0	0.0	0.0
20:1n-11?	0.5	0.4	0.8	0.1	0.8	0.2
20:1n-9	0.9	0.3	1.6	0.1	1.4	0.0
20:1n-7?	0.1	0.1	0.1	0.0	0.1	0.0
20:2a?	0.0	0.0	0.0	0.0	0.0	0.0
20:2b?	0.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	0.2	0.2	0.3	0.0	0.4	0.1
20:3n-6	0.0	0.0	0.1	0.0	0.1	0.0
20:4n-6	1.2	1.0	1.9	0.3	1.6	0.1
20:3n-3	0.1	0.1	0.2	0.0	0.4	0.2
20:4n-3	0.3	0.3	0.7	0.2	0.6	0.1
20:5n-3	10.6	9.1	16.4	1.0	14.8	0.9
22:0	0.0	0.0	0.1	0.1	0.0	0.0
22:1n-11(13)	0.3	0.1	0.6	0.2	0.5	0.2
22:1n-9	0.1	0.1	0.3	0.0	0.2	0.1
22:1n-7	0.0	0.0	0.1	0.0	0.0	0.0
21:5n-3?	0.2	0.2	0.4	0.1	0.3	0.1
23:0	0.0	0.0	0.0	0.0	0.0	0.0
22:4n-6?	0.0	0.0	0.1	0.0	0.1	0.0
22:5n-6	0.2	0.2	0.5	0.1	0.4	0.1
22:4n-3?	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-3	1.2	1.1	2.4	0.2	2.0	0.0
24:0	0.2	0.2	0.3	0.0	0.2	0.1
22:6n-3	20.5	16.5	24.9	1.8	23.9	2.8
24:1	1.3	1.1	2.0	0.5	1.4	0.5
Σ Bacterial	1.9	0.5	1.5	0.2	1.3	0.1
Σ SFA	39.2	29.6	21.4	0.4	20.5	0.4
Σ MUFA	23.3	0.8	26.3	0.5	24.3	1.3
Σ PUFA	37.0	29.3	51.8	0.5	54.7	1.7
Σ Terrestrial (18:3n-3+18:2n-6)	1.1	0.6	1.9	0.4	8.5	2.0

Appendix 4: Fatty acid proportions in Pacific cod liver after feeding on either a marine or terrestrial diet for 8-wks (temperatures pooled). Data are the mean ± SD, n=3 fish at time-0, n=6 fish at wk 8.

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
	Avg	SD	Avg	SD	Avg	SD
14:0	2.8	0.5	3.3	0.4	2.5	0.3
14:1	0.0	0.0	0.1	0.0	0.1	0.0
i-15:0	0.2	0.0	0.1	0.0	0.1	0.1
ai-15:0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	0.4	0.1	0.3	0.0	0.3	0.0
15:1	0.0	0.0	0.0	0.0	0.0	0.0
i-16:0	0.1	0.0	0.1	0.0	0.1	0.0
ai-16:0?	0.2	0.0	0.0	0.0	0.0	0.0
16:0	14.5	0.8	11.9	1.8	10.7	1.1
16:1n-11?	0.1	0.0	0.1	0.1	0.1	0.0
16:1n-9?	0.4	0.1	0.4	0.3	0.2	0.1
16:1n-7	4.8	0.4	7.9	0.9	6.0	0.5
16:1n-5	0.2	0.0	0.2	0.0	0.2	0.0
i-17:0	0.4	0.0	0.3	0.0	0.2	0.1
ai-17:0	0.2	0.0	0.2	0.0	0.2	0.0
16:2n-4	1.2	0.1	0.8	0.3	0.7	0.2
17:0	0.3	0.0	0.2	0.1	0.2	0.0
16:3n-4?	0.2	0.0	0.5	0.3	0.3	0.2
17:1	0.4	0.0	0.5	0.2	0.5	0.2
16:4n-3?	0.0	0.0	0.1	0.0	0.0	0.0
16:4n-1	0.2	0.0	0.5	0.1	0.3	0.1
18:0	4.4	0.3	2.0	1.0	2.5	0.5
18:1n-11?	0.1	0.1	0.9	1.6	0.0	0.0
18:1n-9	17.1	1.5	20.1	2.5	21.8	2.0
18:1n-7	5.9	0.3	4.8	0.7	4.4	0.7
18:1n-6?	0.1	0.0	0.0	0.0	0.0	0.0
18:1n-5?	0.3	0.0	0.3	0.0	0.3	0.0
18:2n-6	1.7	0.1	2.5	0.2	5.4	0.5
18:2n-4	0.2	0.0	0.4	0.0	0.2	0.0
18:3n-6	0.1	0.0	0.2	0.0	0.1	0.0
19:0	0.0	0.0	0.0	0.0	0.0	0.0
18:3n-4	0.1	0.0	0.1	0.1	0.1	0.1
18:3n-3	0.7	0.1	1.0	0.1	13.7	1.1

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
18:4n-3	1.3	0.3	1.9	0.2	1.1	0.6
18:4n-1?	0.1	0.0	0.2	0.0	0.1	0.0
20:0	0.0	0.0	0.0	0.0	0.0	0.0
18:5n-3	0.0	0.0	0.0	0.0	0.0	0.0
20:1n-11?	2.3	0.3	2.4	0.5	2.4	0.3
20:1n-9	1.9	0.2	2.4	0.4	1.7	0.3
20:1n-7?	0.2	0.0	0.3	0.0	0.2	0.0
20:2a?	0.0	0.0	0.0	0.0	0.0	0.0
20:2b?	0.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	0.3	0.0	0.4	0.1	0.4	0.0
20:3n-6	0.1	0.0	0.1	0.0	0.1	0.0
21:0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n-6	1.3	0.3	0.8	0.2	0.6	0.1
20:3n-3	0.2	0.0	0.2	0.0	0.3	0.1
20:4n-3	0.6	0.0	0.8	0.4	0.6	0.1
20:5n-3	12.5	1.4	12.9	1.0	8.7	0.8
22:0	0.0	0.0	0.0	0.0	0.0	0.0
22:1n-11(13)	1.9	0.2	1.5	0.7	1.4	0.7
22:1n-9	0.3	0.1	0.5	0.6	0.5	0.6
22:1n-7	0.0	0.0	0.1	0.1	0.0	0.0
21:5n-3?	0.6	0.0	0.5	0.3	0.4	0.0
23:0	0.0	0.0	0.0	0.0	0.0	0.0
22:4n-6?	0.0	0.0	0.1	0.0	0.0	0.0
22:5n-6	0.2	0.0	0.2	0.0	0.3	0.4
22:4n-3?	0.0	0.0	0.0	0.0	0.1	0.1
22:5n-3	1.7	0.1	2.1	0.4	1.3	0.2
24:0	0.0	0.0	0.0	0.0	0.0	0.0
22:6n-3	16.6	1.2	12.2	2.4	8.1	0.9
24:1	0.6	0.2	0.2	0.1	0.2	0.0
Σ Bacterial	2.1	0.2	1.8	0.3	1.6	0.2
Σ SFA	22.5	1.0	18.0	3.0	16.3	1.7
Σ MUFA	36.5	2.2	42.8	3.8	40.1	2.5
Σ PUFA	40.0	2.8	38.4	4.6	43.0	3.2
Σ Terrestrial (18:3n-3+18:2n-6)	2.5	0.3	3.5	0.2	19.1	1.6

Appendix 5: Fatty acid proportions in walleye pollock flesh fed either a marine or terrestrial diet for 8-wks (temperatures pooled). Data are the mean ± SD, n=3 fish at time-0, n=6 fish at wk 8.

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
14:0	1.5	0.6	1.5	0.1	1.0	0.2
14:1	0.0	0.0	0.0	0.0	0.0	0.0
i-15:0	0.1	0.0	0.1	0.0	0.1	0.0
ai-15:0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	0.3	0.1	0.3	0.0	0.2	0.0
15:1	0.0	0.0	0.0	0.0	0.0	0.0
i-16:0	0.1	0.0	0.0	0.0	0.0	0.0
ai-16:0?	0.0	0.0	0.0	0.0	0.0	0.0
16:0	21.2	9.8	15.2	0.3	14.3	0.8
16:1n-11?	0.0	0.0	0.0	0.0	0.1	0.1
16:1n-9?	0.4	0.3	0.2	0.0	0.2	0.0
16:1n-7	1.8	0.6	2.7	0.5	1.9	0.4
16:1n-5	0.1	0.0	0.2	0.0	0.1	0.0
i-17:0	0.3	0.1	0.3	0.0	0.2	0.1
ai-17:0	0.1	0.0	0.1	0.0	0.1	0.1
16:2n-4	0.3	0.2	0.5	0.3	0.3	0.3
17:0	0.3	0.2	0.2	0.1	0.2	0.0
16:3n-4?	0.1	0.0	0.1	0.0	0.1	0.2
17:1	0.6	0.2	0.4	0.0	0.4	0.2
16:4n-3?	0.0	0.0	0.2	0.1	0.2	0.2
16:4n-1	0.1	0.0	0.1	0.0	0.0	0.0
18:0	7.1	3.6	4.2	0.2	4.5	0.3
18:1n-11?	0.0	0.0	0.0	0.0	0.0	0.1
18:1n-9	12.6	1.7	12.4	0.8	13.2	0.5
18:1n-7	4.2	0.2	4.4	0.3	4.0	0.3
18:1n-6?	0.0	0.0	0.0	0.0	0.0	0.0
18:1n-5?	1.4	2.1	0.2	0.0	0.2	0.0
18:2n-6	0.9	0.4	1.5	0.1	2.5	0.2
18:2n-4	0.1	0.1	0.2	0.0	0.1	0.0
18:3n-6	0.1	0.0	0.1	0.0	0.0	0.0
19:0	0.0	0.0	0.0	0.0	0.0	0.0
18:3n-4	0.1	0.1	0.1	0.1	0.1	0.0
18:3n-3	0.3	0.1	0.6	0.1	5.3	0.8

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
18:4n-3	0.3	0.3	0.6	0.1	0.4	0.1
18:4n-1?	0.6	1.0	0.1	0.0	0.1	0.0
20:0	0.1	0.0	0.1	0.0	0.0	0.0
18:5n-3	0.0	0.0	0.0	0.0	0.0	0.0
20:1n-11?	0.8	0.5	0.9	0.1	0.8	0.2
20:1n-9	1.3	0.2	1.5	0.1	1.3	0.2
20:1n-7?	0.1	0.1	0.1	0.1	0.1	0.1
20:2a?	0.0	0.0	0.0	0.0	0.0	0.0
20:2b?	0.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	0.1	0.1	0.3	0.1	0.4	0.1
20:3n-6	0.0	0.0	0.1	0.0	0.1	0.0
21:0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n-6	1.5	0.5	1.7	0.1	1.5	0.1
20:3n-3	0.0	0.0	0.1	0.0	0.4	0.1
20:4n-3	0.3	0.3	0.7	0.1	0.5	0.1
20:5n-3	12.5	4.9	16.1	0.7	14.3	0.8
22:0	0.0	0.0	0.0	0.0	0.0	0.0
22:1n-11(13)	0.8	0.5	0.5	0.1	0.3	0.2
22:1n-9	0.2	0.2	0.3	0.0	0.2	0.1
22:1n-7	0.0	0.0	0.0	0.0	0.0	0.0
21:5n-3?	0.2	0.2	0.5	0.1	0.3	0.1
23:0	0.0	0.0	0.0	0.0	0.0	0.0
22:4n-6?	0.0	0.0	0.1	0.0	0.0	0.0
22:5n-6	0.2	0.2	0.4	0.0	0.4	0.2
22:4n-3?	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-3	1.7	0.8	2.6	0.1	2.3	0.1
24:0	0.4	0.3	0.1	0.1	0.1	0.1
22:6n-3	22.6	8.0	25.9	1.4	25.1	2.8
24:1	1.6	0.3	1.4	0.1	1.6	0.3
Σ Bacterial	1.7	0.3	1.4	0.2	1.3	0.3
Σ SFA	31.3	15.0	21.7	0.4	20.6	1.0
Σ MUFA	26.0	2.4	25.3	1.3	24.5	1.9
Σ PUFA	42.2	15.0	52.4	1.4	54.5	2.3
Σ Terrestrial (18:3n-3+18:2n-6)	1.2	0.5	2.1	0.2	7.9	1.0

Appendix 6: Fatty acid proportions in walleye pollock livers after fed on either a marine or terrestrial diet for 8-wks (temperatures pooled). Data are the mean ± SD, n=3 fish at time-0, n=6 fish at wk 8.

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
14:0	3.7	0.5	3.0	0.5	2.5	0.3
14:1	0.1	0.0	0.1	0.0	0.1	0.0
i-15:0	0.2	0.0	0.1	0.0	0.1	0.0
ai-15:0	0.0	0.0	0.0	0.0	0.0	0.0
15:0	0.4	0.0	0.3	0.0	0.2	0.1
15:1	0.0	0.0	0.0	0.0	0.0	0.1
i-16:0	0.1	0.0	0.1	0.0	0.1	0.0
ai-16:0?	0.1	0.0	0.0	0.0	0.0	0.0
pristanic?	0.0	0.0	0.0	0.0	0.0	0.0
16:0	15.7	2.2	8.8	5.1	11.3	1.2
16:1n-11?	0.0	0.0	2.6	5.8	0.0	0.0
16:1n-9?	0.2	0.2	0.3	0.4	0.3	0.5
16:1n-7	7.2	0.5	7.9	0.7	7.0	1.1
16:1n-5	0.2	0.0	0.2	0.0	0.1	0.0
i-17:0	0.3	0.0	0.3	0.0	0.2	0.0
ai-17:0	0.2	0.0	0.3	0.0	0.1	0.1
16:2n-4	1.1	0.1	0.5	0.3	0.6	0.1
17:0	0.3	0.0	0.2	0.1	0.2	0.0
16:3n-4?	0.4	0.0	0.5	0.1	0.3	0.2
17:1	0.5	0.0	0.6	0.1	0.5	0.2
16:4n-3?	0.0	0.0	0.1	0.0	0.0	0.0
16:4n-1	0.4	0.0	0.6	0.1	0.4	0.1
18:0	3.4	0.3	2.1	1.1	1.9	0.9
18:1n-11?	0.0	0.0	0.4	0.9	0.8	1.3
18:1n-9	20.3	2.3	21.3	2.3	21.9	2.4
18:1n-7	5.0	0.2	5.0	0.8	5.1	0.5
18:1n-6?	0.6	1.0	0.1	0.2	0.0	0.0
18:1n-5?	0.3	0.0	0.3	0.0	0.3	0.0
18:2n-6	1.6	0.1	2.2	0.1	4.2	1.0
18:2n-4	0.2	0.1	0.4	0.1	0.3	0.1
18:3n-6	0.2	0.0	0.1	0.1	0.1	0.0
19:0	0.0	0.0	0.0	0.0	0.0	0.0
18:3n-4	0.1	0.0	0.2	0.1	0.1	0.0
18:3n-3	0.7	0.1	0.9	0.1	9.8	4.2

Fatty acids	Week 0		Week 8 Marine Diet		Week 8 Terrestrial Diet	
18:4n-3	1.6	0.1	2.0	0.1	1.6	0.2
18:4n-1?	0.1	0.1	0.2	0.0	0.2	0.0
20:0	0.1	0.0	0.1	0.1	0.1	0.0
18:5n-3	0.0	0.0	0.0	0.0	0.0	0.0
20:1n-11?	4.0	1.0	3.0	1.0	2.8	0.7
20:1n-9	2.2	0.2	2.0	0.2	1.7	0.4
20:1n-7?	0.2	0.2	0.5	0.6	0.2	0.0
20:2a?	0.0	0.0	0.0	0.0	0.0	0.0
20:2b?	0.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	0.2	0.1	0.3	0.1	0.3	0.0
20:3n-6	0.0	0.0	0.1	0.0	0.1	0.0
21:0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n-6	0.7	0.0	0.9	0.3	0.8	0.3
20:3n-3	0.1	0.0	0.1	0.0	0.3	0.1
20:4n-3	0.6	0.1	0.8	0.1	0.6	0.1
20:5n-3	10.2	0.7	12.8	0.6	9.5	1.3
22:0	0.0	0.0	0.0	0.0	0.0	0.0
22:1n-11(13)	3.3	0.4	2.0	0.4	2.0	0.6
22:1n-9	0.6	0.0	0.5	0.2	0.4	0.2
22:1n-7	0.4	0.5	0.1	0.1	0.1	0.0
21:5n-3?	0.5	0.0	0.6	0.1	0.4	0.1
23:0	0.0	0.0	0.0	0.0	0.0	0.0
22:4n-6?	0.0	0.0	0.1	0.0	0.0	0.0
22:5n-6	0.2	0.0	0.2	0.1	0.2	0.1
22:4n-3?	0.1	0.0	0.1	0.1	0.0	0.0
22:5n-3	1.6	0.2	1.8	0.1	1.2	0.1
24:0	0.0	0.0	0.0	0.0	0.0	0.0
22:6n-3	9.6	0.6	11.6	2.9	8.3	3.7
24:1	0.3	0.0	0.4	0.1	0.3	0.1
Σ Bacterial	2.0	0.1	1.9	0.2	1.5	0.3
Σ SFA	23.5	3.1	14.8	4.6	16.2	2.0
Σ MUFA	45.3	2.3	47.3	6.8	43.8	5.0
Σ PUFA	30.3	0.9	37.0	3.5	39.4	6.0
Σ Terrestrial (18:3n-3+18:2n-6)	2.4	0.2	3.2	0.2	14.0	5.2