Fatty Acids as Dietary Tracers at the Base of Benthic Food Webs

by

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DEDICATION

To my parents,
and to Mathew,
without whom this would
not have been possible.

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ABSTRACT

Fatty acid (FA) analysis is a powerful ecological tool for examining trophic relationships among marine organisms. Its application in benthic food webs may be limited because many benthic organisms consume a highly mixed diet, and FA metabolism of benthic invertebrates may obscure dietary markers. This thesis examines the use of FA as dietary tracers for studying the diets of benthic invertebrates and the fate of primary production in rocky subtidal food webs. In Chapter 2, I review the use of FA for studying benthic marine food webs, and suggest that field studies using FA analysis should also include data from controlled feeding experiments, gut contents, or stable isotope analysis to provide more reliable results. In Chapter 3, I compare FA composition among sea urchins fed four natural algal diets in a controlled feeding study. These sea urchins substantially modified their dietary FA but differed in their overall FA composition according to diet. In Chapter 4, I use FA to trace the invasive alga *Codium fragile* ssp. *fragile* and the native kelp *Saccharina longicruris* through two trophic transfers in an experimental food web. Substantial signal attenuation occurred with each trophic transfer, suggesting that FA analysis may be of limited use for tracing benthic primary producers in field studies. In Chapter 5, I use FA in conjunction with stable isotope analysis and gut contents analysis to investigate the contribution of detrital kelp to the diet of sea urchins in habitats adjacent to kelp beds. FA analysis was unable to distinguish among sea urchins at different distances from the kelp bed, but the results of all analyses indicated that the availability of kelp detritus declines with distance from the kelp bed, and that sea urchins in low-productivity habitats rely on both kelp detritus and benthic diatoms. In Chapter 6, I summarize the overall findings and suggest experimental and statistical methods to address some of the problems associated with using FA analysis to study trophic relationships in benthic food webs.

LIST OF ABBREVIATIONS USED

FA	Fatty acid(s)
SFA	Saturated FA
MUFA	Monounsaturated FA
PUFA	Polyunsaturated FA
HUFA	Highly unsaturated FA
LCFA	Long-chain FA
SI	Stable isotope(s)
GI	Gonad index

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CHAPTER 1: INTRODUCTION

Understanding consumer diets and the fate of primary production is critical for tracing energy flow through food webs, modeling community dynamics, and predicting the impacts of disturbance (Pimm et al. 1991, Pauly et al. 1998, Cebrian 1999). In the marine environment, direct observation of animals feeding or transfer of primary production through grazing and detrital food webs is often difficult or impossible, so indirect methods for dietary analysis are common (Peterson 1999, Iverson et al. 2004). Gut contents and fecal pellets are commonly analyzed to estimate diets of marine consumers (Foale & Day 1992, Meidel & Scheibling 1998, Graeve et al. 2001, Britton-Simmons et al. 2009). However, these physical methods provide only short-term dietary information (Graeve et al. 2001, Pinnegar & Polunin 2000) and may underestimate the contribution of items that lack hard parts (Foale & Day 1992, Latyshev et al. 2004). Digestion rates may also vary with food quality and bias gut contents data toward less digestible food items (Foster & Hodgson 1998). In contrast, tracer methods such as stable isotope and fatty acid (FA) analysis rely on chemical tracers that accumulate in consumer tissues over a longer period and hence provide less biased, time-integrated dietary information, and may allow food sources to be traced through multiple trophic transfers (Peterson 1999, Pinnegar & Polunin 2000, Graeve et al. 2001, Iverson et al. 2004).

Fatty acid analysis has been used to trace the consumption of primary producers in pelagic food webs: major classes of phytoplankton have characteristic marker FA, and these markers are transferred to the herbivorous copepods that consume them (Graeve et al. 1994, Dalsgaard et al. 2003). Controlled feeding studies have shown that marker FA levels in copepods shift with dietary changes (Graeve et al. 1994). Phytoplankton markers have been used to examine seasonal changes (Sargent & Falk-Petersen 1988) and regional patterns (Kattner & Hagen 1995) in the diets of herbivorous copepods. The marker approach has also been applied in secondary consumers, as characteristic FA of herbivorous copepods have been used to detect their presence in the diets of ctenophores (Falk-Petersen et al. 2002).

At higher trophic levels, this simple "marker" method is less effective because FA metabolism by each consumer can obscure dietary signals, and the majority of marine FA cannot be attributed to a single food source (Budge et al. 2006). These problems have been addressed with

a "signature" approach that uses multivariate statistical analyses to compare the overall FA composition of foods and consumers (Navarro et al. 1995, Iverson et al. 1997). In vertebrate predators, the signature approach has been extended through the development of a statistical model that accounts for the effects of biosynthesis and estimates the contribution of various food sources to mixed diets (quantitative FA signature analysis (QFASA), Iverson et al. 2004). Following calibration in controlled feeding studies, the model has been used to quantitatively estimate the contribution of various prey items to the diets of harbour seals (Iverson et al. 2004), polar bears (Iverson et al. 2006) and seabirds (Iverson et al. 2007).

Trophic relationships near the base of benthic food webs have also been examined using FA analysis, but relatively few field studies have tested the assumption that dietary FA are transferred directly to consumer tissues (Cook et al. 2000, Hall et al. 2006). This assumption may be problematic in invertebrates, which vary in their capacity to modify their dietary FA (D'Souza & Loneragan 1999, Castell et al. 2004, Delaporte et al. 2005), but generally are capable of more extensive FA modification than vertebrate predators (Budge et al. 2006, Iverson 2009). Benthic consumers have access to a wide array of potential food sources including macroalgae, bacteria, and phytoplankton, and are likely to consume mixed diets (Peterson 1999). The food sources available to benthic consumers generally lack unique markers, although some FA may be useful as indicators if they are present at much higher abundance in one food source than others (Dalsgaard et al. 2003). These issues suggest the need for (1) controlled feeding experiments to determine which dietary FA are transferred without modification, and might therefore be useful as markers, and (2) a multivariate approach for comparing overall FA signatures in benthic food webs.

The rocky subtidal ecosystem off the Atlantic coast of Nova Scotia is an ideal model system in which to study the transfer to FA between benthic primary producers and consumers because the food web is relatively simple and trophic linkages are well documented. The dominant grazer, the green sea urchin *Strongylocentrotus droebachiensis*, is a generalist consumer and its feeding ecology is well known (reviewed by Scheibling & Hatcher 2007). Population dynamics of *S. droebachiensis* drive shifts in the system between two alternative stable states: kelp beds with a low density of sea urchins, and barrens dominated by sea urchins and encrusting coralline algae

(Scheibling 1986, Lauzon-Guay & Scheibling 2007). Most of the previous work on FA of sea urchins has focused on the impact of artificial diets on sea urchin FA composition in aquaculture applications (Liyana-Pathirana et al. 2002, Castell et al. 2004). Sea urchins in the kelp and barrens ecosystem states provided an opportunity to compare FA composition between animals on different natural diets. Sea urchins also live in low-productivity habitats tens to hundreds of meters offshore from kelp beds, but the relative importance of local production and detritus from kelp beds in the diets of these sea urchins is unknown. These sea urchins provided an opportunity to apply multivariate analysis of FA signatures to consumers of mixed diets.

An additional ecosystem state was introduced to the Atlantic coast of Nova Scotia in the mid-1990s when an invasive bryozoan caused extensive defoliation of kelp beds and allowed the establishment of monospecific stands of the invasive green alga *Codium fragile* ssp. *fragile* (Scheibling & Gagnon 2006, 2009). Biomass of *C. fragile* can be substantial but the fate of its production is largely unknown, as it contains a chemical which may deter herbivory and is a low-preference food for sea urchins (Lyons et al. 2007, Lyons & Scheibling 2007*a*). The presence of *C. fragile* in the rocky subtidal ecosystem provided an opportunity to test FA as tracers of a primary producer at higher trophic levels, and to apply FA analysis to studying the effects of an invasive species.

This thesis examines the use of FA as dietary tracers for studying the diets of benthic invertebrates and the fate of primary production in rocky subtidal food webs. In Chapter 2, I review the use of FA as a tool for studying trophic relationships in benthic marine food webs, and highlight the importance of using controlled feeding studies and multivariate analyses of FA data. The remainder of my thesis examines the utility of FA in the rocky subtidal ecosystem in Nova Scotia by comparing sea urchins fed various natural diets, tracing an invasive alga through multiple trophic transfers, and examining the diets of sea urchins in low-productivity habitats. In Chapter 3, I compare FA composition among sea urchins fed four natural algal diets in a controlled feeding study. In Chapter 4, I use FA to trace the invasive alga *Codium fragile* ssp. *fragile* and the native kelp *Saccharina longicruris* through two trophic transfers in an experimental food web. In Chapter 5, I use FA in conjunction with stable isotope analysis and gut contents analysis to investigate the contribution of detrital kelp to the diet of sea urchins in

habitats adjacent to kelp beds. In Chapter 6, I provide an overview of the conclusions of the previous chapters and make recommendations for future research in this area.

AUTHORSHIP

Chapters 3, 4 and 5 are multi-authored papers. I was responsible for collecting and processing samples for fatty acid analysis, analyzing the data, and writing the manuscripts for each of these chapters.

CHAPTER 2: FATTY ACIDS AS DIETARY TRACERS IN BENTHIC FOOD WEBS

2.1 ABSTRACT

Fatty acid (FA) analysis is a well-established tool for studying trophic interactions in marine habitats. However, the study of benthic food webs using FA analysis poses two particular challenges. First, unlike pelagic zooplankton, benthic consumers have access to a wide range of primary producers and may consume a highly mixed diet. Classes of benthic primary producers are distinct in their overall FA composition, but most do not possess unique marker FA that can be used to identify their contribution to higher trophic levels. Second, unlike mammalian predators, benthic invertebrates have the capacity to significantly modify their dietary FA and thereby obscure markers for food sources. Controlled feeding studies have been used to distinguish useful dietary tracer FA from those that are modified by the consumer in several benthic invertebrates, but more such studies are needed. Despite these challenges, FA have been used to study trophic structure in a variety of benthic habitats including the deep sea, polar regions, estuaries and the rocky subtidal zone. However, the complexity of benthic food webs and lack of unique markers impose uncertainties in the interpretation of FA data from field studies. Multivariate analyses are necessary for analyzing FA datasets, although univariate tests can be useful for comparing levels of informative FA among food sources and consumers. Combining FA analysis with other lines of evidence, such as stable isotope analysis, offers a more reliable approach to examining trophic interactions in benthic systems.

2.2 INTRODUCTION

Identifying the diets of animals is important for understanding their basic ecology, characterizing trophic interactions, and predicting community-level consequences of biotic and abiotic changes. In the marine environment, direct observation of feeding is often impractical or impossible, so ecologists have developed indirect methods for examining diet. Physical methods such as analysing gut contents or fecal pellets have been used extensively, but these can underestimate the importance of soft and highly digestible food items and overestimate that of recently consumed items (Graeve et al. 2001, Latyshev et al. 2004). These methods may also be inappropriate for invertebrates with unusual modes of feeding or digestion; for example, many sea stars feed extra-orally and are rarely observed with any gut contents (Howell et al. 2003).

Biochemical tracer methods such as stable isotope and fatty acid (FA) analysis have the advantage of potentially providing less biased, longer-term dietary information (Iverson et al. 2004).

Fatty acids have been used extensively in pelagic food webs, especially to identify phytoplankton and characterize the diets of zooplankton (reviewed by Dalsgaard et al. 2003). Diatoms and dinoflagellates are distinct in their FA composition, and zooplankton tend to retain dietary FA in their tissues. Calanoid copepods also synthesize a few relatively rare FA that can be used to identify their contribution to the diets of higher-order consumers (Sargent & Falk-Petersen 1988, Dalsgaard et al. 2003). Top predators have also been successfully studied using FA analyses; dietary FA are deposited in the blubber of marine mammals with relatively little modification, facilitating accurate characterization of their diets (Iverson et al. 2004, Budge et al. 2006).

Unlike pelagic systems, where phytoplankton is the major source of primary production, benthic food webs are supported by a diversity of primary producers that can include macroalgae, vascular plants, and bacteria in addition to phytoplankton (Dalsgaard et al. 2003, Kharlamenko et al. 2001). Although these primary producers have characteristic FA compositions, the larger number of potential food items can pose problems for distinguishing their contributions to diets, particularly when animals consume mixed diets (Kharlamenko et al. 2001, Alfaro et al. 2006). Some invertebrate taxa are capable of modifying dietary FA such that their FA composition may not directly reflect diet (Kelly et al. 2008, 2009). Most taxa lack a specific fat-storage organ, so other functions of lipid-rich tissues may influence their FA composition to a greater extent than does diet (Hughes et al. 2005). Despite these challenges, many studies have employed FA to better understand animal diets and trophic relationships in benthic habitats.

This review examines the utility of FA as bottom-up dietary tracers in benthic marine systems by comparing FA composition among benthic primary producers, summarizing laboratory-based controlled feeding studies, discussing field studies using FA in various benthic habitats, evaluating statistical analyses for FA data, and comparing stable isotope and FA analyses as techniques for studying benthic food webs. I do not cover extraction or chemical analysis of FA and refer readers to Budge et al. (2006) for advice on these processes.

2.2.1 Fatty Acid Nomenclature

Fatty acids consist of a terminal methyl group (CH_3), a carbon chain, and a terminal carboxyl group (COOH). Most naturally-occurring FA have an even number of carbons, usually between 14 and 24, although vascular plants commonly have long-chain FA of up to 28 carbons in their cuticular waxes (Gurr et al. 2002). Saturated FA (SFA) are those with no double bonds, and monounsaturated FA (MUFA) have one double bond. Polyunsaturated FA (PUFA) typically have 2-6 double bonds, and these are usually separated by a methyl group (CH_2) (Budge et al. 2006). Fatty acids are named using the notation A:Bn-x, where A is the number of carbons, B is the number of double bonds, and x is the position of the first double bond relative to the terminal methyl group.

2.3 Fatty Acids of Primary Producers

Phytoplankton are the base of pelagic food webs, and the main taxa of pelagic phytoplankton have distinct FA compositions (Dalsgaard et al. 2003). These microalgae also contribute to benthic food webs, and in some cases the presence of their characteristic FA in benthic organisms indicates a trophic connection between pelagic and benthic food webs. Benthic food webs also rely on macroalgae, vascular plants (such as mangroves and seagrass) and bacteria, and these sources of primary production also have characteristic FA compositions that can be used to identify their contributions to higher trophic levels (Dalsgaard et al. 2003).

I used multivariate techniques to analyse FA composition of primary producers from 27 published studies (Table 2.1). While the focus of this review is on benthic systems, I have included phytoplankton FA data in this analysis because they enter benthic food webs as phytodetritus or by being consumed by suspension feeders. There were significant differences in overall FA composition among vascular plants, diatoms, dinoflagellates, and green, brown and red macroalgae (analysis of similarity, ANOSIM $p < 0.001$; Fig 2.1). All pairwise comparisons were significant (ANOSIM $p < 0.001$). I used similarity percentage analysis (SIMPER) to identify characteristic FA of each group and found that several commonly-used indicators were identified as contributing significantly to within-group similarity for multiple groups: 20:5*n*-3 was identified for dinoflagellates, diatoms and brown and red macroalgae; 20:4*n*-6 for both

brown and red algae; 18:1*n*-9 for red algae, brown algae, dinoflagellates and vascular plants, and 18:3*n*-3 for green algae, vascular plants and brown algae (Table 2.2). This overlap indicates the need for caution when interpreting single FA as tracers for primary producers (Sargent et al. 1987, Dalsgaard et al. 2003).

2.3.1 Algae

De novo synthesis of algal FA occurs in the chloroplasts and results in the production of saturated FA (SFA) typically 16:0, from acetyl-CoA (Gurr et al. 2002). These SFA are modified through a series of elongation and desaturation steps to produce various unsaturated FA (Fig 2.2). Conversion from 18:1*n*-9 to 18:2*n*-6 and 18:3*n*-3 requires Δ12 and Δ15 desaturase enzymes found only in primary producers. These two polyunsaturated FA (PUFA) are therefore called "essential" FA because animals must obtain them from their diets rather than by modifying other FA (Dalsgaard et al. 2003). Further elongation and desaturation of 18:2*n*-6 and 18:3*n*-3 produce other common marine PUFA including 20:4*n*-6, 20:5*n*-3 and 22:6*n*-3 (Gurr et al. 2002; Fig 2.2). An alternative pathway, in which 16:0 is desaturated to 16:1*n*-7 and then further desaturated to 16:4*n*-1, is characteristic of diatoms, and both of these FA are commonly used as markers of diatoms (Dalsgaard et al. 2003).

The contribution of major microalgal taxa to benthic food webs can be estimated based on FA composition because diatoms and dinoflagellates have characteristic FA compositions that have been used as indicators in environmental samples (e.g. sediment or suspended matter) and in consumer tissues. Diatoms are frequently found in benthic environments as epiphytes (Nichols et al. 1986, Latyshev et al. 2004, Richoux & Froneman 2008), and both diatoms and dinoflagellates are captured from the water column by suspension feeders and from sediments by deposit feeders (Graeve et al. 2001, Howell et al. 2003, Guest et al. 2008). Diatoms are characterized by relatively high concentrations of 16:1*n*-7, 16:4*n*-1 and 20:5*n*-3, while dinoflagellates are typically rich in 22:6*n*-3 and 18:4*n*-3 (Budge & Parrish 1998, Dalsgaard et al. 2003). FA ratios are often used to distinguish between diatom and dinoflagellate dominance in phytoplankton samples: 16:1*n*-7/16:0 > 1 or 20:5*n*-3/22:6*n*-3 >1 indicate diatom dominance (Budge & Parrish 1998, Dalsgaard et al. 2003; Table 2.3). In my analysis of primary producer FA composition, diatoms and dinoflagellates are distinguished from each other by 16:1*n*-7 and

20:5*n*-3, which are more abundant in diatoms, and by 22:6*n*-3 and 18:1*n*-9, which are more abundant in dinoflagellates (Table 2.2).

Macroalgae are rich in PUFA, and contain especially high concentrations of *n*-6 PUFA as compared with microalgae (Dalsgaard et al. 2003). Red algae (Rhodophyta) typically contain a high concentration of 20:5*n*-3 (as high as 67% in Antarctic *Adouinella purpurea*, Graeve et al. 2002) and moderate levels of 20:4*n*-6, 18:1*n*-9 and 16:1*n*-7 (Graeve et al. 2002, Li et al. 2002, Vaskovsky et al. 2006). Rhodophyta is the oldest macroalgal lineage, and dominance of C_{20} PUFA is considered an ancestral characteristic (Graeve et al. 2002). Green algae (Chlorophyta) contain a higher concentration of C_{18} PUFA (18:3*n*-3, 18:2*n*-6, 18:4*n*-3) and lower levels of C_{20} PUFA than other macroalgae (Khotimchenko et al. 2002, Li et al. 2002, Vaskovsky et al. 2006). Certain green algae also contain high levels of 16:4*n*-3 (10 to 20% in Or. Ulvales; Fleurence et al. 1994, Vaskovsky et al. 1996, Khotimchenko et al. 2002). Chlorophyta is the most modern macroalgal lineage, and its FA composition is most similar to that of higher plants, which are generally dominated by C_{18} PUFA (Graeve et al. 2002). Green and red macroalgae were distinguished mainly by 20:5n-3, which was more abundant in red algae, and 18:3*n*-3, which was more abundant in green algae (Table 2.2). Brown algae (Phaeophyta) are characterized by relatively high concentrations of 20:5*n*-3, 20:4*n*-6, 18:1*n*-9 and 18:4*n*-3, with slightly lower concentrations of 18:2*n*-6 and 18:3*n*-3 (Vaskovsky et al. 1996, Graeve et al. 2002, Khotimchenko et al. 2002, Li et al. 2002). This mixed FA composition supports the intermediate phylogenetic position of the Phaeophyta between the older Rhodophyta and more modern Chlorophyta (Graeve et al. 2002). SIMPER analysis showed that the FA that contributed most to differences between brown and green macroalgae were 18:3*n*-3 (more abundant in green algae) and 18:1*n*-9 (more abundant in brown algae). Brown and red macroalgae were distinguished by 20:5*n*-3 and 16:0, which were more abundant in red algae, and by 18:4*n*-3, 20:4*n*-6 and 18:1*n*-9, which were more abundant in brown algae (Table 2.2).

2.3.2 Vascular Plants

Vascular plants (Tracheophyta) contribute to nearshore benthic food webs through direct consumption by grazers and via detrital pathways. As in green algae, PUFA in vascular plants consist mainly of 18:2*n*-6 and 18:3*n*-3 (Dalsgaard et al. 2003). Saturated, long-chain FA (LCFA)

with an even number of carbon atoms ($\geq C_{22}$) are found in the waxy leaf cuticle of higher plants; along with 18:2*n*-6 and 18:3*n*-3, these LCFA serve as indicators of vascular plant matter in sediments and suspended organic matter (Canuel et al. 1995, Budge et al. 2001). LCFA have also been used as trophic tracers for mangroves and halophytes in benthic invertebrates (Meziane et al. 1997, Meziane & Tsuchiya 2000, 2002), but they are not assimilated by all consumers and may therefore be of limited use as tracers in some food webs (Hall et al. 2006). SIMPER analysis showed that vascular plants were distinguished from green algae by 18:3*n*-3, 18:2*n*-6, and LCFA, all of which were more abundant in vascular plants (Table 2.2).

2.3.3 Marine Bacteria

Marine bacteria play an important role in many benthic food webs, as food sources for deposit and suspension feeders, and as endosymbionts of hydrothermal vent invertebrates (Kharlamenko et al. 1995, Pond et al. 1997, 2002, Meziane & Tsuchiya 2002, Howell et al. 2003). FA biosynthesis in bacteria produces odd-numbered and branched FA such as 15:0, 17:0, 15:1, 17:1 and *iso*- and *anteiso*-branched SFA. These FA, along with 16:1*n*-7 and 18:1*n*-7, are typically dominant in bacterial FA composition and are used as tracers for bacterial contribution to sediments, suspended organic material and animal diets (Kharlamenko et al. 1995, Meziane & Tsuchiya 2002, Dalsgaard et al. 2003). Bacteria generally do not produce PUFA, although PUFA synthesis has been shown in some deep-sea bacteria (Nichols et al. 1993, Russell & Nichols 1999). Because 16:1*n*-7 and 18:1*n*-7 are also produced by diatoms and macroalgae, these should only be considered bacterial indicators when odd-chain and branched FA are also present and PUFA are not abundant (Dalsgaard et al. 2003). Relative proportions of various bacterial FA are indicative of different taxonomic groups of bacteria (Sargent et al. 1987, Colaço et al. 2007; Table 2.3), and have been used to examine microbial community structure in marine sediments (Parkes & Taylor 1983, Gillan & Hogg 1984).

2.4 Controlled Feeding Studies

Controlled feeding studies in a laboratory setting can further our understanding of how FA are transferred from food to consumer, which FA are catabolised or excreted, and how specific FA affect consumer growth and physiology. Field studies in which diet is inferred from FA present in consumers generally assume that FA are transferred from food to consumer with minimal

modification. This is a reasonable assumption in some taxa – for example, mammalian predators have a very limited capacity to alter dietary FA (Budge et al. 2006). However, because benthic invertebrates have a greater ability to modify dietary FA, controlled feeding studies should be employed before using FA as dietary tracers in field studies.

2.4.1 Fatty Acid Transfer

Controlled feeding studies have identified FA that transfer reliably from food to consumer. These FA may be useful as indicators for identifying certain food sources in the diets of consumers if their concentrations are found to be (1) significantly higher in one food source than others and (2) significantly higher in consumers of the food source of interest as compared to consumers of other foods. Most studies that have demonstrated FA transfer in benthic invertebrates have used artificial diets enriched with high concentrations of certain FA. For example, sea urchin larvae (*Paracentrotus lividus*) fed dinoflagellates (*Dunaliella tertiolecta*) resembled their diet in having higher 18:3*n*-3, while larvae fed an artificial diet containing fish meal resembled their diet in having higher levels of 22:6*n*-3 (Liu et al. 2007). Larvae of mud crabs (*Scylla serrata*) fed rotifers enriched with 18:2*n*-6, 18:3*n*-3, 20:5*n*-3 or 22:6*n*-3 also contained higher concentrations of these FA corresponding to their diets (Suprayudi et al. 2004). FA 20:4*n*-6 and 18:4*n*-3 also were indicators of kelp *Saccharina longicruris* in *Strongylocentrotus droebachiensis* fed kelp compared to sea urchins fed artificial diets enriched with corn, linseed or menhaden oil, and which contained characteristic FA of these oils (Castell et al. 2004). The results of controlled feeding studies using artificial diets normally are not useful for determining the diets of animals in field studies, but Cook et al. (2000) found that 22:6*n*-3 in salmon-feed pellets transferred to sea urchins *Psammechinus miliaris* and was useful as an indicator for this salmon feed in diets of sea urchins in a field study conducted near salmon farm pens.

The results of comparisons among natural diets are likely more applicable to ecologists seeking tracers for use in field studies than are aquaculture-focused studies comparing artificial diets, but controlled feeding studies comparing natural foods are relatively few. Mussels (*Perna viridis*) fed rotifers (*Brachionus plicatilis*) contained indicator FA for rotifers (18:1*n*-7 and 20:4*n*-6), which were absent in mussels fed diatoms (Shin et al. 2008). Prawn larvae (*Penaeus* spp.) contained indicator FA for their microalgal diets: 16:1*n*-7 and 20:5*n*-3 for the diatom

Chaetoceros muelleri, and 18:3*n*-3 and for dinoflagellate *Dunaliella tertiolecta* (D'Souza & Loneragan 1999). When sea urchins *Strongylocentrotus droebachiensis* fed kelp were compared with those fed the green alga *Codium fragile*, 20:4*n*-6 and 18:4*n*-3 were tracers for kelp and 16:3*n*-3 and 18:3*n*-3 were tracers for *C. fragile* (Kelly et al. 2009). However, when *S. droebachiensis* were fed four different algal diets (including *Saccharina longicruris*) no tracers distinguished sea urchins among diet treatments (Kelly et al. 2008).

Tracking changes in FA composition over time during a controlled feeding experiment may also be useful for validating FA tracers for a diet of interest. When sea urchins (*Strongylocentrotus droebachiensis*) were switched from kelp to a grain-based diet, kelp indicators 18:4*n*-3, 20:4*n*-6 and 20:5*n*-3 decreased, and tracers for the artificial diet, 18:2*n*-6 and 18:1*n*-9, increased in sea urchin tissues (Liyana-Pathirana et al. 2002). Both mangrove leaves and the tissues of crabs *Parasesarma erythodactyla* fed mangrove leaves contained high levels of 18:2*n*-6 and 18:3*n*-3 (Hall et al. 2006). A feeding/starving regimen provided support for these FA as dietary tracers: levels of 18:2*n*-6 and 18:3*n*-3 increased when crabs were fed mangrove leaves, and decreased during starvation periods, showing that these FA were affected by diet. 18:3*n*-3 had similar tracer properties when tissues of *Parasesarma erythodactyla* were fed to another crab (*Portunus pelagicus*). However, both 18:2*n*-6 and 18:3*n*-3 are common in primary producers and may be of limited use in field studies (Hall et al. 2006).

2.4.2 Fatty Acid Biosynthesis and Selective Retention

Controlled feeding studies have revealed that many invertebrate taxa possess active elongase and desaturase enzymes that enable them to significantly modify dietary FA. Prawn larvae (*Penaeus* spp.) synthesized 20:5*n*-3 from 18:3*n*-3 when their diets were deficient in 20:5*n*-3 (D'Souza & Loneragan 1999). *Penaeus* spp. larvae contained *iso*18:0, 20:2*n*-6, 20:3*n*-3 and 22:6*n*-3 when these were absent from their diets, suggesting biosynthesis of these from dietary precursors (D'Souza & Loneragan 1999). The crabs *Parasesarma erythodactyla* and *Portunus pelagicus* appeared to synthesize 20:4*n*-6, 20:5*n*-3 and 22:6*n*-3 from dietary precursors 18:2*n*-6 and 18:3*n*-3 (Hall et al. 2006, Fig 2.2). Bell et al. (2001) used a radio-labelled diet to show that the sea urchin *Psammechinus miliaris* can convert 18:3*n*-3 to 20:5*n*-3, even when 20:5*n*-3 is present in the diet, but that the rate of conversion is very slow. Because marine fish and mammals cannot

convert 18:2*n*-6 and 18:3*n*-3 to highly unsaturated FA (HUFA) 20:4*n*-6, 20:5*n*-3 and 22:6*n*-3 and must obtain these FA from their diets, biosynthesis by invertebrates may represent an important link in marine food webs (Sargent et al. 1999, Hall et al. 2006).

Products of biosynthesis may be useful as dietary indicators if there is a clear relationship between levels of the precursor FA in the food and the product in the consumer. Sea urchins (*Strongylocentrotus droebachiensis*) fed artificial diets rich in 18:1*n*-9 had high concentrations of both 18:1*n*-9 and its elongation product 20:1*n*-9 (Castell et al. 2004). Levels of 20:4*n*-6 and 20:5*n*-3 in sea urchins were directly related to levels of precursor FA 18:2*n*-6 and 18:3*n*-3 in artificial diets that lacked 20:4*n*-6 and 20:5*n*-3 (Castell et al. 2004). Given the ability of invertebrates to alter their dietary FA, the use of products of FA biosynthesis as dietary indicators merits further research, as these may prove useful in field studies where few dietary tracer FA are transferred directly to consumer tissues.

Either biosynthesis or selective retention may be postulated when consumer tissues contain higher concentrations of certain FA than does the food (Castell et al. 2004). FA 20:4*n*-6 and 20:5*n*-3 each comprised 10 to 18% of total FA in sea urchin larvae (*Paracentrotus lividus*) cultured on foods containing 0 to 7% of these two FA (Liu et al. 2007). The presence of both FA in larvae fed algae that did not contain these FA suggests that *P. lividus* is capable of synthesizing them from precursors 18:2*n*-6 and 18:3*n*-3 (Liu et al. 2007). Sea urchin (*Strongylocentrotus droebachiensis*) tissues contained 5 to 10% 20:1*n*-15 when only trace amounts (< 0.1%) of this FA were present in its food (Castell et al. 2004, Kelly et al. 2008). *S. droebachiensis* also may either synthesize or retain 20:1*n*-9, 20:1*n*-11, and 22:1*n*-9, all of which were present at lower levels in their diet (<1%) than in their tissues (1 to 7%) (Castell et al. 2004). Sea urchins (*Psammechinus miliaris*) fed salmon-feed pellets contained 10-12 times more 20:4*n*-6 and 20:5*n*-3 than did their diet (Cook et al. 2000). *P. miliaris* fed a natural kelp diet contained higher levels of these HUFA than did urchins fed salmon feed, but lower levels than were present in kelp, indicating that they did not selectively retain HUFA when fed their natural diet (Cook et al. 2000). Juvenile shrimp (*Lysmata seticaudata*) had higher levels of HUFA (15%) than did their mixed-bivalve feed (3%), and the feed contained < 1% precursors for HUFA synthesis (18:2*n*-6 and 18:3*n*-3), suggesting selective retention of HUFA rather than biosynthesis

(Calado et al. 2005). Prawn larvae (*Penaeus* spp.) contained higher levels of 18:2*n*-6 than did their microalgal diets (e.g. 5% in *Dunaliella tertiolecta* vs. 10% in prawns fed *D. tertiolecta*) (D'Souza & Loneragan 1999). Synthesis of 18:2*n*-6 occurs only in primary producers, suggesting that prawns must selectively retain this FA.

2.4.3 Egested Fatty Acids

Consumers may egest certain FA rather than assimilating them directly or after modification. Mangrove leaves contained 17 to 24% long-chain FA (LCFA; 24:0, 26:0 and 28:0), but tissues of crabs (*Parasesarma erythodactyla*) fed mangrove leaves did not contain these FA (Hall et al. 2006). However, feces of *P. erythodactyla* contained over 15% LCFA, suggesting that the crabs may be unable to metabolise these FA (Hall et al. 2006). These results highlight the need for controlled feeding studies before FA are used as tracers in field studies: the lack of LCFA in *P. erythodactlya* tissues could otherwise appear to indicate that these crabs do not feed on mangrove leaves. Fecal pellets of deposit-feeding fiddler crabs *Uca vocans* and *Scopimera globosa* also contained LCFA that were absent from their tissues (Meziane et al. 2002). These feces also contained lower levels of diatom indicator 20:5*n*-3 and bacterial FA than did tissues and sediments, indicating that the crabs effectively assimilated diatoms and bacteria but not mangrove leaves (Meziane et al. 2002). Comparisons of FA profiles of consumer feces to those of food and consumer tissues may provide valuable information in other benthic systems and should be included in controlled feeding studies.

2.4.4 Effects of Fatty Acids on Physiology

Many studies that identify FA of benthic organisms fed a known diet have been undertaken for aquaculture-related purposes, such as maximizing growth (Merican & Shim 1996, Lim et al. 1997, Glencross & Smith 1999, 2001, Gonzalez-Felix et al. 2002, Castell et al. 2004, Calado et al. 2005), reproduction (Wen et al. 2002, Alava et al. 2007), or larval survival (D'Souza & Loneragan 1999, Alava et al. 2007). Diets in such studies often include feeds with high concentrations of specific FA, making it possible to isolate the effects of FA of interest (Castell et al. 2004, Calado et al. 2005). Sea urchin larvae (*Paracentrotus lividus*) provided with artificial feeds rich in HUFA did not grow faster than those fed with microalgae, possibly because those on the microalgal diet were able to meet their HUFA needs through elongation and desaturation

of dietary 18:2*n*-6 and 18:3*n*-3 (Liu et al. 2007). Juvenile sea urchins (*Strongylocentrotus droebachiensis*) fed oil-enhanced artificial diets grew faster than those fed kelp for the first 4 mo of a controlled feeding study, but the kelp-fed sea urchins grew faster after 4 mo (Castell et al. 2004). Crabs (*Eriocheir sinensis*) fed artificial diets with high 20:5*n*-3 content showed improved fecundity, and eggs of crabs supplemented with 22:6*n*-3 had a higher hatching rate as compared to diets with lower HUFA levels (Wen et al. 2002). The ratio of 18:2*n*-6 to 18:3*n*-3 was important for growth of juvenile prawns (*Penaeus mondon*), and imbalance between the two FA caused poor growth, possibly because excess 18:2*n*-6 or 18:3*n*-3 can suppress HUFA synthesis (Glencross & Smith 1999).

2.5 Fatty Acid Analysis of Benthic Food Webs

Fatty acid analysis has been used in field studies to address a variety of questions related to trophic structure in benthic habitats. One relatively common use is to infer diet from consumer FA. However, predicting consumer diets based on a few FA can be problematic, since most marine FA are ubiquitous and therefore rarely indicate the presence of a specific food item. Use of a single or few FA can also be misleading in that marker FA could be obtained by consuming a primary producer, or by preying on a consumer of that primary producer (Budge et al. 2006). FA are frequently used as markers in field studies without considering the role of consumer metabolism, even though many benthic invertebrates are capable of biosynthesis and selective retention of various FA. Field studies that use tracers validated in controlled feeding studies are rare (Cook et al. 2000). Such studies are important because they allow comparison between consumers fed a single diet and wild-caught animals that likely consumed a mixed diet, and provide some indication of which FA are affected by biosynthesis or selective retention. Ideally, field studies should employ multivariate analyses to examine overall differences among groups in addition to univariate comparisons of specific indicator FA.

Fatty acids are useful in habitats where direct observation would pose significant challenges, such as the deep sea (Howell et al. 2003, Drazen et al. 2009). FA tracers are also used to determine the fate of primary production, especially in habitats where the dominant primary producer is not directly consumed by dominant herbivores (Meziane & Tsuchiya 2000, Kharlamenko et al. 2001). Other field studies have employed FA analysis to address ecological

questions about how animal diets vary with habitat, season and anthropogenic effects (Takagi et al. 1980, Hughes et al. 2005, Fernandez-Jover et al. 2007).

2.5.1 Deep-sea and Hydrothermal Vent Food Webs

Deep-sea food webs are especially well-suited to study using FA analysis because direct observation is difficult at depth, and food webs are relatively simple because of the limited number of primary producers. Studying the diet of deep-sea species using more direct methods is problematic because the pressure changes experienced as animals are brought to the surface frequently causes regurgitation or stomach eversion, precluding analysis of stomach contents (sea stars, Howell et al. 2003; macrourid fishes, Drazen et al. 2009). Relative abundances of several diet-derived FA varied among 7 species of deep-sea sea cucumbers from the north-east Atlantic, suggesting a diversity of feeding modes or niches (Ginger et al. 2000). PCA separated deep-sea sea cucumbers and brittle stars from the north-east Pacific based on the higher proportions of algal-derived HUFA (20:5*n*-3 and 22:6*n*-3) in the deposit-feeding sea cucumbers, and copepod-derived MUFA (20:1 and 22:1) in the omnivorous brittle stars (Drazen et al. 2008*a*). One sea cucumber species (*Oneirophanta mutabilis*) contained higher levels of bacterial FA (18:1*n*-7 and odd-number and branched-chain FA) suggesting selective feeding on bacteria-rich detrital particles rather than algal detritus (Drazen et al. 2008*a*). When 8 species of deep-sea sea stars from the north-east Atlantic were classified using hierarchical cluster analysis and MDS, their categories corresponded with their known feeding types: suspension feeders contained higher proportions of tracer FA of diatoms (20:5*n*-3), dinoflagellates (22:6*n*-3) and copepods (20:1 monounsaturated FA, MUFA); mud-ingesting sea stars contained higher proportions of bacterial FA (Howell et al. 2003). Each of the 3 predator-scavenger species had a distinct FA composition, indicating specialization on (1) benthic invertebrates and foraminifera, (2) micro-infauna, and (3) echinoderms (Howell et al. 2003). The ratio of 18:1*n*-9 to 18:1*n*-7 is used as an indicator of carnivory because 18:1*n*-7 comes from bacteria and carnivores typically contain high levels of 18:1*n*-9 (Graeve et al. 1997, Drazen et al. 2008*b*). The presence of 18:1*n*-9 alone does not indicate carnivory, since animals are capable of synthesizing it *de novo* (Gurr et al. 2002). Drazen et al. (2008*b*) characterized the FA composition of deep-sea invertebrates in the north-east Pacific and found the highest 18:1*n*-9/18:1*n*-7 ratio in amphipods, suggesting that carrion is dominant in their diet. A caridean shrimp had an intermediate value, supporting a

carnivorous diet, while 3 polychaete taxa had lower ratios that indicated that both phytodetritus and animal material were important in their diets (Drazen et al. 2008*b*). Unusual FA present in echinoderms (23:1, 24:1, and C_{24} and C_{26} PUFA) were absent from FA of deep-sea macrourid fishes from the north-east Pacific, suggesting that despite their abundance, echinoderms are not an important food source for these fishes (Drazen et al. 2009). Comparison of macrourid fish FA profiles to those of their potential prey suggested that sunken carrion of epipelagic fishes and squid are a more important source of nutrition than is the deep-sea benthic food web (Drazen et al. 2009).

Chemoautotrophic bacteria provide the major food source for invertebrate communities at deep-sea hydrothermal vents, and their characteristic FA profile allows these bacteria to be traced through the food web. Deep-sea mussels *Bathymodiolus thermophilus* from both the East Pacific Rise and Galapagos hydrothermal vents contained high concentrations of 16:1*n*-7 and 18:1*n*-7, which in deep-sea habitats indicate bacterial food sources, especially when combined with presence of 20:1*n*-7 and 22:1*n*-7 and low concentrations of 20:5*n*-3 and 22:6*n*-3 (Ben-Mlih et al. 1992). Hydrothermal vent shrimp *Rimicaris exoculata* contained high proportions of bacterial FA 16:1*n*-7 and 18:1*n*-7, and also 16:2*n*-4 and 18:2*n*-4, which are desaturation products of 16:1*n*-7 (Pond et al. 1997). Colaço et al. (2007) classified invertebrates from deep hydrothermal vents at the Mid-Atlantic Ridge into two groups using PCA on their overall FA composition: (1) mussels, their commensal polychaetes, and whelks, which all contained a higher proportion of branched *n*-7 FA, indicating a reliance on sulphide-reducing bacteria; and (2) shrimp and crabs, which contained a higher proportion of *n*-7 MUFA and non-methylene-interrupted dienes (NMIDs), indicating a reliance on sulphate-oxidizing bacteria. The deposit-feeding polychaete *Amathys lutzi* contained indicator FA of both types of bacteria, indicating that it feeds non-selectively (Colaço et al. 2007). The hydrothermal vent clam *Calyptogena pacifica* from the Juan de Fuca Ridge obtains most of its nutrition from chemoautotrophic bacterial symbionts and accordingly was rich in 16:1*n*-7; specimens also contained NMIDs and 20:3 PUFA that were likely derived from conversion of 16:1*n*-7 (Allen et al. 2001). In addition to bacterial FA, shrimp *Alvinocaris markensis* from the same site contained high proportions of 20:5*n*-3 and 22:6*n*-3. These PUFA are often considered markers of phytodetritus (i.e. diatoms and dinoflagellates), but 22:6*n*-3 in particular may also indicate a scavenging diet, and appendages of *A. markensis* are

adapted for scavenging (Pond et al. 1997). Hydrothermal vent worms *Ridgeia piscesae* and *Protis hydrothermica* were rich in 16:1*n*-7 and 18:1*n*-7, but also in PUFA 20:5*n*-3 and 20:4*n*-6 (Pond et al. 2002). At 2200 m depth it is unlikely that hydrothermal vent worms – especially *R. piscesae*, which relies entirely on bacterial endosymbionts for nutrition – acquire PUFA from phytodetritus, but they possess the enzymes necessary to convert bacterially-derived 18:2*n*-6 and 18:3*n*-3 into 20:4*n*-6 and 20:5*n*-3 (Pond et al. 2002). However, Allen et al. (2001) concluded that the small proportions of 20:5*n*-3 and 22:6*n*-3 in clams (*C. pacifica*) from the same site were likely photosynthetically-derived and acquired by filter-feeding. Ben-Mlih et al. (1992) also attributed the low concentrations of 20:5*n*-3 and 22:6*n*-3 in the hydrothermal vent mussel *B. thermophilus* to filter-feeding.

Like their deep-water counterparts, shallow hydrothermal vent systems are host to chemoautotrophic bacteria (Kharlamenko et al. 1995). However, animals at shallow vent systems have access to a wider variety of potential food sources, including planktonic diatoms, particulate organic matter, microbial mats, sediments and macrophytes, and may therefore have more complex trophic pathways. Based on their overall FA profiles, 16 consumer taxa at a shallow hydrothermal vent off the Kurile Islands in the northwest Pacific were classified into 7 clusters that corresponded generally with feeding modes. Suspension feeders had high levels of diatom indicator 20:5*n*-3, while carnivorous polychaetes and corals clustered together and had high levels of 22:6*n*-3. Sea cucumbers *Eupentacta pseudoquinquesemita* and *Psolus* spp. had high bacterial FA concentrations, which point to deposit feeding, and also relatively high concentrations of 20:5*n*-3, suggesting diatoms as a likely food source. These species are suspension feeders, so the high concentration 20:5*n*-3 was expected, and dissection of gill tissues revealed symbiotic bacteria (rather than the microbial mat) as the source of bacterial FA (Kharlamenko et al. 1995).

2.5.2 Polar Food Webs

Fatty acid analysis of benthic fauna in polar habitats has improved understanding of their trophic interactions. In the Arctic, suspension-feeding brittle stars were rich in 18:1*n*-7 and 20:5*n*-3. Found together, these FA indicate that diatoms constitute an important food source (Graeve et al. 1997). Benthic decapod crustaceans in the Arctic contained high concentrations of diatom

indicators 16:1*n*-7 and 20:5*n*-3, and of carnivory indicators 18:1*n*-9 and 22:6*n*-3, suggesting that their prey consumed diatoms (Graeve et al. 1997). The FA 22:6*n*-3 also was elevated in a predatory brittle star *Ophiura sarsi* (Graeve et al. 1997). FA profiles of 6 species of Antarctic amphipods corresponded with their feeding modes (Graeve et al. 2001). The grazer *Oradarea edentata* was rich in macroalgal tracer 20:4*n*-6, and suspension feeder *Ampelisca richardsoni* was rich in phytoplankton tracer 18:4*n*-3. Predatory amphipod *Eusirus perdentatus*, deposit feeder *Epimeria georgiana*, sponge-predator *Echiniphimedia hodgsoni*, and scavenger *Waldeckia obesa* all contained relatively high levels of carnivory indicator 18:1*n*-9. *E. hodgsoni* was rich in 16:1*n*-7, 18:1*n*-7 and 20:5*n*-3, indicating that diatoms were an important food source for the sponges it consumes. In the Antarctic, FA compositions of benthic icefishes *Dolloidraco longedorsalis* and *Bathydraco marri* were similar to that of their benthic invertebrate prey in that all were rich in carnivory indicators 18:1*n*-9 and 22:6*n*-3 and diatom tracers 16:1*n*-7, 18:1*n*-7 and 20:5*n*-3 (Hagen et al. 2000). Copepod tracers (20:1 and 22:1 MUFA) comprised <5% of total FA in benthic icefishes, as compared with 10 to 15% in pelagic species (Hagen et al. 2000).

2.5.3 Estuarine Food Webs

Estuarine habitats support relatively diverse faunal assemblages, but few of the resident species directly consume the dominant primary producers (Meziane & Tsuchiya 2000, Kharlamenko et al. 2001). FA analysis has been used to trace the fate of these primary producers and to compare the relative contribution of mangrove and seagrass material to consumer diets with that of other food sources such as macroalgae, diatoms, dinoflagellates, zooplankton and bacteria. The diversity of potential food sources in these habitats requires that their FA be characterized before attempting to infer consumer diets, and the overlap in tracer FA among common food sources points to the need for multivariate analysis rather than interpretation of single FA. Indicators for food sources used in seagrass- and mangrove-dominated habitats include long-chain FA (LCFA) for mangroves; 16:1*n*-7, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3 for mangrove detritus; 18:2*n*-6, 18:3*n*-3, 18:4*n*-3 and 20:4*n*-3 for seagrass; 18:2*n*-6, 18:3*n*-3 and 18:3*n*-6 for green macroalgae; 18:3*n*-3 and LCFA for salt-marsh plants, *iso*- and *anteiso* 17:0 and 18:1*n*-7 for bacteria; 16:1*n*-7 and 20:5*n*-3 for diatoms; 22:6*n*-3 for zooplankton; and 18:1*n*-9 for brown macroalgae, cordgrass and salt marsh plants (Nichols et al. 1986, Meziane et al. 1997, Meziane & Tsuchiya 2000,

Kharlamenko et al. 2001, Bachok et al. 2003, Alfaro et al. 2006, Meziane et al. 2006, Richoux & Froneman 2008; Table 2.3).

Based on indicator FA present in consumer tissues, Kharlamenko et al. (2001) concluded that most animals in a seagrass bed in the Sea of Japan consumed seagrass as detritus or suspended particulate matter, and that diet of deposit feeders was more varied than that of grazers. Several molluscs in the study contained relatively high levels of 20:4*n*-6, which was attributed to consumption of fungi, even though fungi were not analyzed and 20:4*n*-6 was found only in very low levels in sediments and suspended particulate matter. Biosynthesis of 20:4*n*-6 from its precursor 18:2*n*-6 may have been a more likely source of this FA, and has been demonstrated in controlled feeding studies of the scallop *Pecten maximus* (Soudant et al. 1996).

Most consumers in a tropical mangrove- and seagrass-dominated estuary in northern New Zealand were found to utilize a variety of food sources including mangrove leaves, seagrass, microalgae, a brown macroalga, zooplankton and bacteria (Alfaro et al. 2006). The authors interpreted the presence of 18:3*n*-3 conservatively, since it was present in both seagrass and mangrove leaves. This relatively cautious approach to interpreting FA tracer data is particularly appropriate when controlled feeding data are not available for consumers, and therefore their capacity for FA modification is unknown. Data from Alfaro et al. (2006) show evidence of biosynthesis or selective retention of PUFA by some consumers. For example, the shrimp *Palaemon affinis* contained 17.7% 20:5*n*-3, while the highest concentration of this FA in any potential food source was 5.4% in sediment and 7.3% in the gastropod grazer *Turbo smaragdus*. Many invertebrates are capable of synthesizing 20:5*n*-3 using 18:3*n*-3 as a precursor (e.g. crab *Parasesarma erythodactyla*, Hall et al. 2006; sea urchin *Strongylocentrotus droebachiensis*, Castell et al. 2004). Relative abundance of 18:3*n*-3 in primary producers ranged from 3.0% in the brown macroalga *Hormosira banksii* to 27.4% in seagrass, but in consumers its maximum abundance was 2.1%. This suggests that 18:3*n*-3 was usually catabolised by consumers and was probably not useful as a marker; however, the authors interpreted all FA marker data without considering the possible role of consumer metabolism. This highlights the need for controlled feeding studies to distinguish FA that are reliable indicators from those that are affected by consumer metabolism.

Animals in an intertidal flat adjacent to a mangrove forest near Okinawa, Japan contained tracers for bacteria, diatoms, macroalgae and mangroves, while animals from a reference site without mangroves lacked the long-chain FA (LCFA) characteristic of mangroves (Meziane & Tsuchiya 2000). Based on the relative abundance of indicator FA for each food source, the authors concluded that bacteria and macroalgae comprise most of the diet of intertidal grazers, with a relatively minor contribution from mangrove detritus (Meziane & Tsuchiya 2000). The abundance of LCFA in sediments decreased with distance from mangroves, indicating that detrital export is limited to < 1 km (Meziane & Tsuchiya 2000, Meziane et al. 2006).

Food webs of temperate estuaries are similar to those in the tropics in that most consumers are trophic generalists, and most of the biomass of dominant primary producers enters the detrital food web rather than being directly grazed (Meziane et al. 1997, Richoux & Froneman 2008). Although 10 food sources in a temperate South African estuary had distinct FA compositions, relatively few tracers for these food sources were useful in distinguishing among 14 consumer species (Richoux & Froneman 2008). Multivariate analyses of FA composition separated consumers into two general groups based on dominance of either dinoflagellates or benthic diatoms in their diets (Richoux & Froneman 2008). Benthic diatoms appear to play an important role in temperate estuarine food webs: diatom indicators 16:1*n*-7 and 20:5*n*-3 comprise significant proportions of total FA in sediments and animals, but not in suspended material (Meziane et al. 1997, Richoux & Froneman 2008). Bacterial FA were also present in all consumers in these studies.

2.5.4 Rocky Subtidal Food Webs

Relatively few studies have used FA to analyze rocky subtidal food webs, and a majority of these studies have focused on identifying diets of sea urchins. FA composition of sea urchins *Strongylocentrotus droebachiensis* from a kelp bed off the Atlantic coast of Nova Scotia, Canada differed from that of sea urchins from nearby coralline algae-dominated barrens (Takagi et al. 1980). Most notably, both kelp and urchins from the kelp bed were rich in 20:4*n*-6, while sea urchins from barrens contained a higher proportion of *n*-3 PUFA. Similarly, Kelly et al. (2008) found that overall FA composition differed between *S. droebachiensis* from kelp beds and

barrens at another site along this coast. The two groups were distinguished mainly by 20:5*n*-3, which was elevated in coralline algae and sea urchins from barrens. NMID 20:2Δ15,13 was elevated in sea urchins from kelp beds and is synthesized from 18:2*n*-6 (Castell et al. 2004), which was abundant in brown macroalgae including kelps (Kelly et al. 2008). Intertidal and subtidal populations of the sea urchin *Psammechinus miliaris* off the west coast of Scotland differed in their FA composition, but these differences were not consistent between sites, suggesting that sea urchin diets are variable (Hughes et al. 2005). FA composition was not reported for the dominant macroalgae, but dominant taxa in the intertidal (fucoid algae) and subtidal (laminarian kelp) zones may not have been sufficiently distinct in their FA composition to allow differentiation at the next trophic level. Intertidal urchins at one site contained a higher concentration of carnivory indicator 22:6*n*-3, suggesting that invertebrate prey contributed to their diet (Hughes et al. 2005).

2.5.5 Aquaculture Effects on Food Webs

The effects of fish farms on animal diets and trophic interactions are well-suited to FA analyses because fish feed tends to contain tracer FA that distinguish them from other benthic food sources. Sea urchins (*Psammechinus miliaris*) living under a salmon pen off the west coast of Scotland contained high concentrations of 22:6*n*-3, which is a major constituent FA of the salmon feed (Cook et al. 2000). Sea urchins fed salmon feed pellets in the lab also contained high levels of 22:6*n*-3, confirming that 22:6*n*-3 is a valid tracer for this salmon feed in sea urchins. FA 18:4*n*-3 and 20:4*n*-6 were similarly validated as tracers for kelp and were elevated in sea urchins from kelp-dominated intertidal sites (Cook et al. 2000). Wild Mediterranean horse mackerel *Trachurus mediterraneus* that aggregated near fish farm pens off the south-east coast of Spain had similar FA composition to feed pellets, with elevated levels of 18:2*n*-6 and 18:1*n*-9 and lower levels of 22:6*n*-3 as compared to control populations distant from fish farms (Fernandez-Jover et al. 2007). Stomach contents analysis confirmed that while natural populations feed on cephalopods and juvenile fishes, *T. mediterraneus* associated with fish farms feed mainly on feed pellets (Fernandez-Jover et al. 2007).

2.6 Statistical Analysis of Fatty Acid Data

The large size of most FA datasets requires that overall patterns among samples be examined using a multivariate statistical approach. Similarity-based techniques may be most appropriate for FA data because they have fewer assumptions regarding data structure and can be used with small sample sizes. Univariate statistical methods can be used in conjunction with multivariate analyses to compare levels of individual tracer FA among consumers and/or food sources, but care should be taken to minimize Type I error associated with multiple comparisons. While a wide variety of statistical techniques could potentially be applied to FA data, I briefly review those that are most commonly used.

2.6.1 Multivariate Statistics

Multivariate analysis of variance (MANOVA) and discriminant function analysis (DFA) are used to examine differences in overall FA composition among *a priori* groups of samples – for example, different species (Budge et al. 2002) or functional groups (Piche et al. 2010) or consumers on different diets in a controlled feeding study (Navarro et al. 1995). MANOVA is used to test for significant differences in overall FA composition among groups of samples by examining whether mean differences among groups could have occurred by chance. DFA calculates the combination of FA that produces maximum multivariate distance among groups by creating uncorrelated linear equations of the original FA. Scores of samples on the resulting discriminant functions can be plotted to reveal relationships among samples. Interpretation of DFA results is relatively simple, and FA that are most important in separating groups along each function can usually be identified (Navarro et al. 1995, Budge et al. 2006). DFA can also be used to classify new samples into trophic groups based on FA composition (Hair et al. 2006).

Principal component analysis (PCA) is used as an exploratory technique to examine relationships among samples, and to simplify large FA datasets. PCA reduces large numbers of variables to a few components that represent most of the variance in the data by combining correlated FA into new components. This allows identification of FA that contribute most to separation among observed groups and those FA that are highly correlated (Best et al. 2003, Budge et al. 2006). The scores of each sample on the first 2 to 3 principal components can be plotted to visualize relationships among samples. PCA has been used in benthic habitats to compare FA signatures

of consumers to their potential food items (Guest et al. 2008, Drazen et al. 2009) and to classify consumers by feeding mode (Colaço et al. 2007, Drazen et al. 2008*a*, Richoux & Froneman 2008).

MANOVA and DFA assume that the data are multivariate normal and the covariance matrices are homogeneous (Hair et al. 2006). The assumptions of PCA are somewhat less stringent, although a multivariate normality is preferred and more samples than variables are required for reliable results (Hair et al. 2006). To meet the assumption of multivariate normality, proportional FA data require transformation (Budge et al. 2006). The transformation $x_{trans} = ln\ (x_i/c_r)$, where x_i is a given FA expressed as percent of total FA and c_r is the percentage of a reference FA, has been recommended for FA data (Budge et al. 2006). The reference FA for this transformation should be one that is present regardless of diet, and is reliably quantified (Budge et al. 2006). To avoid heterogeneity of covariance, the number of variables in FA datasets usually must be reduced to $n-1$ where n is the smallest number of samples in any group, and each group should contain at least 20 samples (Budge et al. 2006, Hair et al. 2006). The subset of FA retained for analysis should include those that are generally obtained from diet rather than from biosynthesis by the consumer, and those that are indicators of potential food items (Iverson et al. 2004, Budge et al. 2006). However, the selection of FA that are of dietary rather than biosynthetic origin is often difficult for benthic invertebrates because many species are capable of substantial FA biosynthesis (D'Souza & Loneragan 1999, Cook et al. 2000, Castell et al. 2004, Calado et al. 2005, Hall et al. 2006) and FA metabolism for most species is not well-studied. The cost of obtaining an adequate number of samples for these techniques may be prohibitive, especially in studies that aim to describe a food web rather than the diet of one or a few consumers of interest. Statistical techniques with fewer assumptions regarding data structure may therefore be more practical for most studies of trophic relationships in benthic habitats.

Cluster-forming methods such as classification and regression trees (CART) and hierarchical clustering do not assume homogeneity of covariances or multivariate normal data, and more importantly the number of independent variables (i.e. FA) is not limited by sample size (Smith et al. 1997, Budge et al. 2006). CART uses FA with the greatest variance to divide the samples into dichotomous groups. CART may be useful as an exploratory method because it does not require selection of a smaller subset of FA; however, the algorithm may use FA that are not biologically

meaningful to create tree nodes (Budge et al. 2006). For example, 16:0 is ubiquitous because most organisms are capable of synthesizing it *de novo*, but its relative abundance varies among taxa. CART also does not provide any indication of overall relationships among samples (Budge et al. 2006). Hierarchical clustering uses the overall similarity between samples to either link them into increasingly large groups or divide them into dichotomous groups (Hair et al. 2006). It is similar to CART in that it is an exploratory method with few assumptions regarding data structure, but in hierarchical clustering the distances between groups in the resulting dendrogram represent their dissimilarity (Budge et al. 2006). Hierarchical clustering does not test whether groups are significantly different from each other; however, the similarity profile (SIMPROF) analysis in the PRIMER software package (Clarke & Gorley 2006) can be applied to test whether each node of the dendrogram represents a statistically significant division (Clarke et al. 2008).

Similarity-based statistical techniques are especially useful for FA data because they do not require homogeneity of covariances or multivariate normality, can be used with a large number of variables, and include a variety of both exploratory and hypothesis-testing methods (Clarke 1993). FA data are often used in similarity-based analyses without transformation to avoid giving artificial weight to FA that are present in small quantities (Howell et al. 2003, Hall et al. 2006, Kelly et al. 2008, 2009), but it should be noted that in most cases, analyses on untransformed data effectively only use abundant FA. The data are used to calculate a triangular matrix of similarities (such as Bray-Curtis similarity) between each pair of samples. Multidimensional scaling (MDS) then uses the ranks of similarities in an iterative process to find the spatial arrangement of samples that best represents the relationships among them (Clarke & Green 1988, Hair et al. 2006). MDS is an exploratory analysis that does not take into account any *a priori* groups and so can be used to classify consumers with unknown diets (Howell et al. 2003, Guest et al. 2008) as well as to look for spatial (Hughes et al. 2005, Fernandez-Jover et al. 2007) and temporal (Wai et al. 2008) differences in overall consumer FA composition. It has also been used to analyze the results of controlled feeding studies, both for comparing overall FA composition of animals on different diets (Kelly et al. 2008, 2009) and for tracking changes to FA composition over time (Hall et al. 2006). While MDS provides a valuable visual representation of relationships among samples, an additional technique is necessary for hypothesis testing. Analysis of similarity (ANOSIM) is a non-parametric, similarity-based analogue to multivariate

analysis of variance (MANOVA) that tests for differences in overall FA composition through permutations of the rank similarity matrix (Clarke 1993). ANOSIM is frequently used in conjunction with MDS, both for comparing *a priori* groups and for testing whether clusters observed in the MDS plot differ significantly from each other, and for performing pairwise comparisons among groups (Hughes et al. 2005, Hall et al. 2006, Kelly et al. 2008, 2009, Wai et al. 2008). ANOSIM and MDS are both available in the PRIMER software package (Clarke & Gorley 2006).

While it is important to understand relationships among the overall FA compositions of samples in any study, the individual FA driving overall patterns may also be of interest. In studies of benthic food webs, both the characteristic FA of potential food sources and the FA contributing most to differences among consumer species or feeding groups may be informative. Similarity percentage analysis (SIMPER) measures similarity between each pair of samples and calculates average similarity within groups and dissimilarity between groups, and examines the contribution of each FA to average similarity (Clarke 1993, Clarke & Gorley 2006). SIMPER results can be used to identify potential indicator FA. For example, 22:6*n*-3 had the highest contribution to dissimilarity between horse mackerel *Trachurus mediterraneus* collected from reference areas and near fish farms: reference fish were rich in 22:6*n*-3 as compared with fish associated with fish farms (Fernandez-Jover et al. 2007). Where FA identified by SIMPER are known indicators of specific food sources, the results can indicate diet of consumers. In the previous example, 22:6*n*-3 was an indicator for juvenile fish and cephalopods, which were the likely prey of reference fish (Fernandez-Jover et al. 2007). In deep-sea sea stars, FA composition of suspension feeders was characterized by copepod indicator FA 20:1*n*-9 as well as phytoplankton indicators 20:5*n*-3 and 22:6*n*-3, which Howell et al. (2003) also attributed to feeding on copepods.

2.6.2 Univariate Statistics

Although multivariate statistical methods should be used to analyze most FA datasets, the additional use of univariate tests on indicator FA of interest is appropriate following a finding of significant differences among groups using multivariate procedures. Univariate procedures such as analysis of variance (ANOVA) are commonly used for testing whether the concentration of a

single FA differs among groups of samples, and several such comparisons may be performed on a single dataset if α is adjusted (e.g. using Bonferroni correction). ANOVA is commonly used in field studies to compare levels of specific FA in organic material or animals among sampling locations (Cook et al. 2000, Alfaro et al. 2006, Meziane et al. 2006), or seasons (Bachok et al. 2003, Meziane et al. 2006), and among consumer species (Kharlamenko et al. 2001, Alfaro et al. 2006, Meziane et al. 2006). However, there are often several possible sources for a given indicator FA found in a consumer, and ecological knowledge and common sense should be applied in their interpretation. If available, information from a controlled feeding study using the consumer of interest provides valuable insight as to which of the potential indicator FA in the food items are actually conserved in consumer tissues (Cook et al. 2000). ANOVA is also appropriate for evaluating levels of indicator FA in food and consumers in controlled feeding studies (Hall et al. 2006, Kelly et al. 2008, 2009). As with any statistical test, care should be taken to distinguish statistical significance from biological significance, as small within-group variance (especially common in controlled feeding studies) can produce a statistically significant result even when levels of the indicator are similar among groups (Budge et al. 2006).

2.7 Stable Isotope Analysis

Stable isotope (SI) analysis has been used extensively in ecological applications, including studies of benthic marine food webs. Briefly, the carbon, nitrogen, sulphur, hydrogen and oxygen present in any biological sample consists of more than one isotope of these elements, and the relative abundance of two isotopes can be measured using a mass spectrometer (Peterson & Fry 1987). Carbon, nitrogen and sulphur are most commonly used in ecological studies. Sulphur isotope ratios remain relatively stable with trophic transfers, and carbon isotopic ratios are enriched by 0-1‰ with each trophic transfer, so both can be used to identify sources of primary production at higher trophic levels (Peterson & Fry 1987). Nitrogen isotope ratios are enriched by 3‰ with each trophic transfer, making them useful for identifying the trophic level of a consumer (Peterson & Fry 1987). Sulphur isotopes are especially useful in salt marshes and seagrass communities because vascular plants assimilate isotopically light sulphides from the anoxic sediments such that the contributions of vascular plants to food webs can be clearly distinguished from other inputs (Peterson 1999). Problems with identifying food sources of consumers can arise when the food sources of interest are not well-separated in their isotopic

signatures, or when several potential food sources are present (Peterson 1999). Using multiple isotopic tracers can help to distinguish food sources, but in complex systems and in consumers with mixed diets, the ability of stable isotope analysis to identify trophic relationships can be limited (Peterson 1999).

2.7.1 Stable Isotopes vs. Fatty Acids

Stable isotope and FA analysis have similar advantages over estimating diet based on gut contents and direct observation, but the methods differ in their spatial variability and their ability to distinguish certain primary producers (Richoux & Froneman 2008, Guest et al. 2010). SI signatures of primary producers are most strongly influenced by environmental factors such as light, temperature and CO_2 availability, while FA signatures are most strongly influenced by physiological processes (Guest et al. 2010). As environmental and physiological factors vary at different scales, so too do stable isotope and FA signatures of primary producers (Guest et al. 2010). SI are well-suited to distinguishing among macroalgae and various classes of vascular plants (Peterson 1999), but they cannot be used to estimate bacterial contribution to a benthic food web because isotopic signatures of sediments tend to overlap with those of primary producers (Richoux & Froneman 2007, 2008). The carbon isotopic values of benthic diatoms also overlap with those of major macroalgal taxa, making their contribution to consumer diets difficult to estimate using SI alone (Newell et al. 1995). FA analysis can easily distinguish bacteria and diatoms from other primary producers, and so is well-suited for characterizing benthic food webs which may have significant bacterial and microalgal components (Sargent et al. 1987, Kharlamenko et al. 2001, Richoux & Froneman 2008). These complementary characteristics of SI and FA analysis have led to an increasing number of studies using both tracers (Guest et al. 2010).

2.7.2 Bulk Stable Isotope Analysis with Fatty Acid Analysis

Bulk SI analysis has been used in conjunction with FA analysis as a means of cross-validating results as well as to provide additional information on trophic level of consumer species (Guest et al. 2010). Carbon isotope ratios for abalone (*Haliotis rubra*) were closer to those of brown algae than red algae, and abalone tissues contained a high proportion of a FA tracer for brown algae (20:4*n*-6) (Guest et al. 2008). Carbon isotope ratios also have been used in neashore

benthic food webs to estimate the contribution of seagrass-derived carbon because seagrass tends to differ from other primary producers in its carbon isotopic composition (Kharlamenko et al. 2001, Alfaro et al. 2006). Levels of seagrass FA indicators 18:2*n*-6 and 18:3*n*-3 were relatively low in all consumers in a seagrass bed (Kharlamenko et al. 2001), but these FA are also precursors for PUFA synthesis. Carbon isotope ratios of consumers indicated that the majority of consumers derived at least half their carbon from seagrass, and that grazers consumed a combination of seagrass and epiphytes (Kharlamenko et al. 2001). A combination of FA and SI data indicated that deposit feeders have a more varied diet than do grazers and filter feeders (Kharlamenko et al. 2001). In an estuary in northern New Zealand, mangroves and seagrass were well separated using carbon isotope ratios, but the $\delta^{13}C$ values of most consumers fell between those of seagrass and brown algae, which were not well separated (Alfaro et al. 2006). Seagrass and brown algae were distinct in their tracer FA, however, and other food sources including diatoms and bacteria were also traceable using FA (Alfaro et al. 2006). In a temperate estuary in South Africa, a crab *Cleistostoma edwardsii* and gastropod *Haminoea alfredensis* both had highly enriched $\delta^{13}C$ values, suggesting seagrass, epibionts or surface sediments as a major carbon source (Richoux & Froneman 2007). FA analyses in a complementary study identified high levels of diatom indicator 20:5*n*-3 in these animals, indicating that epibionts were the most likely food source (Richoux & Froneman 2008). Because FA tracers can be ambiguous with regard to a consumer's trophic level, nitrogen isotopes are a particularly valuable source of additional information in FA studies of food webs. The FA signature of the polychaete *Perenereis nuntia vallata* contained high levels of PUFA, essential FA and diatom indicators, as well as elevated bacterial FA, which taken alone would likely indicate surface deposit feeding (Richoux & Froneman 2008). However, the $\delta^{15}N$ signature was also enriched, suggesting that *P. nuntia vallata* also consumes animal material and may obtain some of its tracer FA by consuming other deposit feeders (Richoux & Fronema 2007). Carbon and nitrogen isotope ratios of food sources and consumers in nearshore shallow habitats in Hong Kong revealed that whelks *Thais clavigera* prey primarily on suspension feeding bivalves *Saccostrea cucullata* and *Septifer virgatus* (Wai et al. 2008). FA data showed only that all 3 species had similar levels of indicators for dinoflagellates and zooplankton.

2.7.3 Stable Isotopes of Indicator Fatty Acids

Stable isotope analysis of individual FA can help to discern trophic pathways with greater resolution than is possible with bulk SI analysis or FA analysis alone. Pond et al. (2002) used $\delta^{13}C$ ratios of indicator FA to investigate the source of PUFA in deep-sea hydrothermal vent worms. In the tubeworm *Ridgea piscesae*, $\delta^{13}C$ values of 16:1*n*-7 and 18:1*n*-7 indicate a chemosynthetic origin for these FA, which is consistent with the reliance of *R. piscesae* on endosymbiotic bacteria (Pond et al. 2002). However, $\delta^{13}C$ values for 20:4*n*-6 and 20:5*n*-3 were closer to expected values for photosynthetic microplankton. Similarly, in a mixotrophic tubeworm *Protis hydrothermica,* $\delta^{13}C$ values for 20:5*n*-3 were consistent with a chemosynthetic origin, but values for 22:6*n*-3 suggested a photosynthetic origin. Although other studies have found PUFA synthesis by bacteria (Jøstensen & Landfald 1997), these results indicate that chemosynthetic bacteria are not the only PUFA source for *R. piscesae* and *P. hydrothermica* and that other sources, including synthesis by the tubeworms, should be investigated (Pond et al. 2002). Jack et al. (2009) compared diets of rock lobster *Jasus edwardsii* in 3 marine reserves in New Zealand using bulk SI analysis and $\delta^{13}C$ values of selected indicator FA. Bulk SI analysis revealed that rock lobsters in 2 of the 3 reserves use suspended particulate matter and macroalgae as their basal carbon sources, suggesting that they prey on the abundant filter-feeding mussel *Mytilus edulis galloprovincialis*. Carbon and nitrogen isotope signatures of rock lobsters from the third reserve suggested that carbon from terrestrial detritus or chemoautotrophic bacteria contributed significantly to their diets, and elevated 16:1*n*-7 indicated the presence of either diatoms or bacteria. The $\delta^{13}C$ value for 16:1*n*-7 from these lobsters was within the expected range for sulphur-oxidizing bacteria, and the authors concluded that the symbiont-bearing clam *Solemya parkinsonii* is the most likely food source for the rock lobsters in the third reserve (Jack et al. 2009). Ice algal diatoms in the Arctic were distinct from pelagic diatoms in their FA composition, but $\delta^{13}C$ values of diatom tracers 16:4*n*-1 and 20:5*n*-3 enabled the use of mixing models to quantitatively estimate the contribution of each diatom source to higher trophic levels (Budge et al. 2008).

2.8 CONCLUSIONS

Both the complexity of benthic food webs and the ability of consumers to modify dietary FA introduce sources of error to studies using FA in benthic habitats, but the reliability of results can be improved by considering these factors when designing a study. An effort should be made to characterize the FA composition of as many likely food sources as possible so that indicator FA in the consumer can be attributed to the correct source. Whenever possible, potential food sources should be collected from the same habitat as the consumers, rather than using literature values from another habitat, because indicator levels are variable even among closely-related species, and a single FA may have several sources in some habitats. Ideally, the transfer of indicator FA from food to consumer should be verified in controlled feeding experiments before indicators are used in field studies. This is especially important in invertebrates because several taxa have been shown to be capable of substantial modification of their dietary FA, and this modification can obscure dietary indicators. The lack of unique FA markers for any species means that interpretation of FA data is always somewhat uncertain, and that results are always stronger when corroborated with data from direct observation, gut contents, or SI analysis.

As with most ecological methods, appropriate allocation of sampling effort and use of statistical techniques are important in studies using FA analysis. FA of consumers may be highly variable among replicates, so relatively large sample sizes may be required to detect differences among groups. Similarity-based measures have relatively few constraints and are most appropriate for most FA datasets. Univariate statistics can be used to test for differences in FA levels among groups, but these should be corrected for multiple comparisons. Because of the limited resolution of FA data and complex FA metabolism of consumers, the results of FA studies in benthic systems thus far have been qualitative. Quantitative estimates of contents of mixed diets using FA analysis are possible in seabirds and marine mammals, but these techniques require careful calibration even for consumers with limited ability to modify dietary FA (Iverson et al. 2004) and may therefore be impractical for invertebrates.

The application of FA analysis to benthic food webs has revealed trophic relationships in habitats that are otherwise difficult or impossible to study, traced the fate of primary production through

detrital and other pathways, and confirmed linkages that were previously detected using other methods. Benthic communities pose certain challenges for FA analysis in that they often contain many potential food sources, and consumers may be capable of modifying their dietary FA. A balanced approach that includes field sampling and controlled feeding experiments, multivariate and univariate analyses, and cross-validation with other methods will generally yield the best results.

2.9 TABLES AND FIGURES

Table 2.1. Studies used for meta-analysis of primary producer fatty acid composition data.

Taxon	No. of species	Location	Reference
Bacillariophyta	14	Cultured	Dunstan et al. (1994)
(diatoms)	6	Darwin Harbour, Australia	Renaud et al. (1994)
	2	Gulf of Carpentaria, Australia	Renaud et al. (1999)
	2	Queensland, Aus	Renaud et al. (1999)
	1	SE South Africa	Richoux & Froneman (2008)
	6	Cultured	Viso & Marty (1993)
	4	Cultured	Volkman et al. (1989)
Dinoflagellata	5	Cultured	Mansour et al. (1999)
(dinoflagellates)	1	Penang, Straits of Malacca	Usup et al. (2008)
	3	Sebatu, Straits of Malacca	Usup et al. (2008)
	6	South China Sea	Usup et al. (2008)
	3	Cultured	Viso & Marty (1993)
Chlorophyta	2	Brittany Coast, France	Fleurence et al. (1994)
(green algae)	1	Antarctic	Graeve et al. (2002)
	1	Spitsbergen, Arctic	Graeve et al. (2002)
	1	Nova Scotia, Canada	Kelly et al. (2009)
	2	Indian Ocean	Khotimchenko (2003)
	1	Sea of Japan	Khotimchenko (2003)
	1	South China Sea	Khotimchenko (2003)
	1	Yellow Sea	Khotimchenko (2003)
	3	Northern California, USA	Khotimchenko et al. (2002)
	3	Bohai Sea	Li et al. (2002)
	1	Okinawa, Japan	Meziane & Tsuchiya (2002)
	1	Southern California, USA	Nelson et al. (2002)
	1	Northern Chile	Ortiz et al. (2009)
	1	SE South Africa	Richoux & Froneman (2008)
	1	Sea of Japan	Sanina et al. (2004)
	8	Yellow Sea	Vaskovsky et al. (1996)
Phaeophyta	1	Northern New Zealand	Alfaro et al. (2006)
(brown algae)	5	Brittany Coast, France	Fleurence et al. (1994)
	3	Antarctica	Graeve et al. (2002)
	3	Spitsbergen, Arctic	Graeve et al. (2002)
	1	Cultured	Honya et al. (1994)
	3	Nova Scotia, Canada	Kelly et al. (2008)
	4	Kurile Islands	Khotimchenko (1998)
	15	Sea of Japan	Khotimchenko (1998)
	2	Sea of Japan	Khotimchenko & Kulikova (1999)
	9	Northern California, USA	Khotimchenko et al. (2002)
	10	Bohai Sea	Li et al. (2002)
	1	Southern California, USA	Nelson et al. (2002)
	1	Cultured	Ortiz et al. (2009)
	2	Sea of Japan	Sanina et al. (2004)

Taxon	No. of species	Location	Reference
Phaeophyta (brown algae)	6	Yellow Sea	Vaskovsky et al. (1996)
Rhodophyta (red algae)	4	Brittany Coast, France	Fleurence et al. (1994)
	11	Antarctica	Graeve et al. (2002)
	4	Spitsbergen, Arctic	Graeve et al. (2002)
	1	Nova Scotia, Canada	Kelly et al. (2008)
	1	Sea of Japan	Khotimchenko (2005)
	7	Northern California, USA	Khotimchenko et al. (2002)
	9	Bohai Sea	Li et al. (2002)
	1	Southern California, USA	Nelson et al. (2002)
	1	Cultured	Ortiz et al. (2009)
	12	Yellow Sea	Vaskovsky et al. (1996)
Tracheophyta (vascular plants)	2	Northern New Zealand	Alfaro et al. (2006)
	1	NE Australia	Hall et al. (2006)
	1	Sea of Japan	Kharlamenko et al. (2001)
	1	Okinawa, Japan	Meziane & Tsuchiya (2002)
	4	SE South Africa	Richoux & Froneman (2008)
	2	Yellow Sea	Vaskovsky et al. (1996)

Table 2.2. Results of similarity percentage analysis (SIMPER) of primary producer data from 27 published studies listed in Table 2.1. All fatty acids with average contribution ≥0.1% were included, and data were not transformed prior to analysis.

Group	*FA*	*Average abundance*	*Contribution(%)*
Chlorophyta (62% average similarity)	16:0	29.96	36
	18:3*n*-3	20.42	26
	18:2*n*-6	7.24	7
	18:1*n*-7	7.15	6
	16:4*n*-3	6.44	4
Phaeophyta (70% average similarity)	16:0	21.85	25
	18:1*n*-9	14.15	14
	20:4*n*-6	10.53	11
	20:5*n*-3	11.73	11
	18:4*n*-3	10.89	10
Rhodophyta (66% average similarity)	16:0	34.66	44
	20:5*n*-3	30.34	31
	18:1*n*-9	7.37	7
	20:4*n*-6	8.40	5
	14:0	4.45	5
Dinoflagellata (48% average similarity)	16:0	26.92	42
	18:1*n*-9	13.70	15
	22:6*n*-3	13.14	12
	20:5*n*-3	10.39	10
	18:0	7.49	7
Bacillariophyta (65% average similarity)	16:1*n*-7	25.97	33
	16:0	19.42	21
	20:5*n*-3	15.19	15
	14:0	13.51	14
	16:3*n*-4	6.14	4
Tracheophyta (56% average similarity)	16:0	26.08	38
	18:3*n*-3	30.32	34
	18:2*n*-6	13.88	12
	18:1*n*-9	4.50	4
	18:0	3.48	4

Table 2.3. Summary of fatty acids used as dietary tracers in selected published studies of benthic food webs. NMID (non-methylene-interrupted dienes) are FA with two double bonds not separated by a methyl group (CH_2); MUFA are FA with one double bond; prefixes *i* and *ai* indicate a methyl branch at the second or third carbon from the terminal methyl group, respectively (i.e. branched fatty acids.

Habitat	Source	Dietary tracer FA	Location	References
Deep sea	Algal material	20:5*n*-3 + 22:6*n*-3	NE Pacific	Drazen et al. (2008a)
	Animal material	18:1*n*-9	(4100 m depth)	
	Animal material	20:1 MUFA		
	Copepods	22:1 MUFA		
	Carnivory	18:1*n*-9/18:1*n*-7 ratio > 1	NE Pacific	Drazen et al. (2008b)
	Copepods	20:1 and 22:1 MUFA	(4100 m depth)	
	Bacteria	18:1*n*-7	NE Atlantic	Howell et al. (2003)
	Bacteria	Odd-chain and branched FA	(1053-4840 m	
	Bacteria	NMID	depth)	
	Copepods	22:1*n*-11 + 20:1*n*-9		
	Deep-sea fishes and crustaceans	18:1*n*-9		
	Dinoflagellates	22:6*n*-3		
	Photosynthetic microplankton	PUFA		
	Protozoa and microeukaryotes	20:4*n*-6		
	Sediment	Saturated FA		
Deep hydrothermal vents	Chemoautotrophic bacteria	16:1*n*-7, 18:1*n*-7	Juan de Fuca Ridge	Allen et al. (2001)
	Methanotrophic bacteria	*n*-8 MUFA	(2200 m depth)	
	Bacteria	*n*-7 MUFA with low 20:5*n*-3 and 22:6*n*-3	East Pacific Rise and	Ben-Mlih et al. (1992)
	Phytoplankton	16:4*n*-3 + 18:4*n*-3 + 18:3*n*-3	Galapagos Rise	
	Zooplankton	20:1*n*-9 + 22:1*n*-9	(2600 m depth)	

Habitat	Source	Dietary tracer FA	Location	References
Deep hydrothermal vents	Methanotrophic bacteria	16:1*n*-5, 16:1*n*-6, 16:1*n*-8, 18:1*n*-6, 18:1*n*-8	Mid-Atlantic Ridge (840-3600 m depth)	Colaco et al. (2007)
	Phytoplankton	20:5*n*-3 + 20:6*n*-3		
	Sulphate reducing bacteria (*Desulfobacter*)	10Me16, cyc18:0		
	Sulphate reducing bacteria (*Desulfovibrio)*	i17:1*n*-7, i15:1*n*-7, i19:1*n*-7		
	Sulphur oxidizing bacteria	16:1*n*-7, 18:1*n*-7		
	Sulphur oxidizing bacteria	i17:1*n*-5, 18:1*n*-6		
	Bacteria	16:2*n*-4, 18:2*n*-4	Mid-Atlantic Ridge	Pond et al. (1997)
	Scavenging/necrophagy or phytodetritus	20:5*n*-3 + 22:6*n*-3		
	Sulphur oxidizing bacteria	16:1*n*-7, 18:1*n*-7	Juan de Fuca Ridge (2200 m depth) and East Pacific Rise (2600 m depth)	Pond et al. (2002)
Shallow hydrothermal vents	Bacteria	Branched FA	Kurile Islands	Kharlamenko et al. (1995)
	Bacteria	18:1*n*-7		
	Brown macroalgae	C_{18} + C_{20} PUFA		
	Diatoms and bacteria	16:0/16:1*n*-7 < 1		
	Diatoms and brown and red macroalgae	20:5*n*-3		
Arctic	Carnivory	18:1*n*-9/18:1*n*-7 ratio > 1	Spitsbergen, NE Greenland, and Barents Sea (50-570 m depth)	Graeve et al. (1997)
	Copepods	20:1*n*-9 + 22:1*n*-11		
	Diatoms	16:1n-7, 20:5n-3		
Antarctic	Cryptophytes or haptophytes (microalgae)	18:4*n*-3	Weddell Sea and Bransfield Strait	Graeve et al. (2001)
	Diatoms	16:1*n*-7, 20:5*n*-3		
	Necrophagy	18:1*n*-9		

Habitat	Source	Dietary tracer FA	Location	References
	Copepods	20:1 and 22:1 MUFA	Weddell and Lazarev Seas (200-1400 m depth)	Hagen et al. (2000)
Tropical estuaries	Bacteria	$\Sigma C_{15} + \Sigma C_{17}$	Northern New Zealand	Alfaro et al. (2006)
	Bacteria	18:1*n*-7		
	Brown macroalgae	18:1*n*-9		
	Diatoms	20:5*n*-3		
	Diatoms	16:1/16:0 > 1.6		
Tropical estuaries	Diatoms	$\Sigma C_{16}/\Sigma C_{18} > 2$		Alfaro et al. (2006)
	Diatoms	20:5*n*-3/22:6*n*-3 > 1		
	Dinoflagellates	22:6*n*-3		
	Dinoflagellates	20:5*n*-3/22:6*n*-3 < 1		
	Mangroves	LCFA (>24:0)		
	Red macroalgae	20:5*n*-3/20:4*n*-6 > 10		
	Seagrass	18:2*n*-6 + 18:3*n*-3		
	Zooplankton	20:1 + 22:1		
	Bacteria	Branched FA + 18:1*n*-7	Okinawa, Japan	Bachok et al. (2003)
	Copepods	20:1*n*-9, 20:1*n*-11		
	Diatoms	20:5*n*-3		
	Dinoflagellates	22:6n-3, 18:4n-3		
	Mangrove detritus	16:1*n*-7, 18:1*n*-9, 18:2*n*-6, 18:3*n*-3		
	Bacteria	i17:0, ai17:0	Sea of Japan	Kharlamenko et al. (2001)
	Bacteria	18:1*n*-7		
	Diatoms	16:1*n*-7		
	Diatoms	20:5*n*-3		
	Dinoflagellates, cryptomonads	18:4*n*-3		
	Fungi, protozoa, algae	20:4*n*-3		
	Seagrass	18:2*n*-6, 18:3*n*-3		
	Zooplankton	22:6*n*-3		

Habitat	Source	Dietary tracer FA	Location	References
	Bacteria	i15:0, ai15:0, i17:0, ai17:0, 18:1*n*-7	Okinawa, Japan	Meziane & Tsuchiya (2000)
	Diatoms	20:5*n*-3		
	Green macroalgae	18:2*n*-6, 18:3*n*-6		
	Vascular plants	26:0, 28:0, 30:0		
	Bacteria	i15:0, ai15:0, i17:0, ai17:0, 18:1*n*-7	Queensland, Australia	Meziane et al. (2006)
	Diatoms	20:5*n*-3		
	Mangroves	LCFA (>24:0)		
	Mangroves	18:2*n*-6, 18:3*n*-3		
	Microalgae	16:1*n*-7/16:0		
Temperate estuaries	Bacteria	Branched FA + 18:1*n*-7	NW France	Meziane et al. (1997)
	Diatoms	20:5*n*-3		
	Salt marsh plants	LCFA (>24:0), 18:3*n*-3		
	Bacteria	i15:0, ai15:0, i17:0, ai17:0	SE South Africa	Richoux & Froneman (2008)
	Diatoms	20:5*n*-3, 16:1*n*-7		
	Diatoms	16:1/16:0		
	Dinoflagellates	22:6*n*-3		
	Dinoflagllates	22:6*n*-3/20:5*n*-3		
	Salt marsh plants and green algae	18:1*n*-9		
	Vascular plants	18:2*n*-6, 18:3*n*-3		
Rocky subtidal	Kelp	20:4*n*-6	Nova Scotia, Canada	Takagi et al. (1980)
	Kelp	20:4*n*-6, 18:4*n*-3	Scotland	Cook et al. (2000)

Figure 2.1. Non-metric multidimensional scaling (MDS) plot of Bray-Curtis similarities of primary producer data from 27 published studies listed in Table 2.1 All fatty acids with average contribution ≥0.1% were included, and data were standardized to equal 100% prior to analysis.

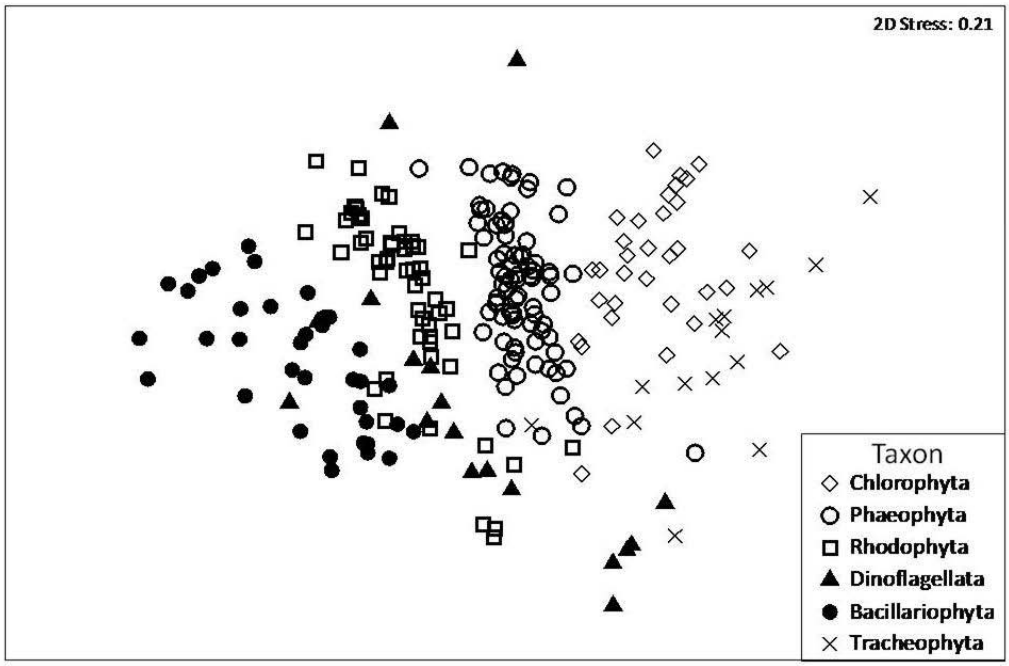
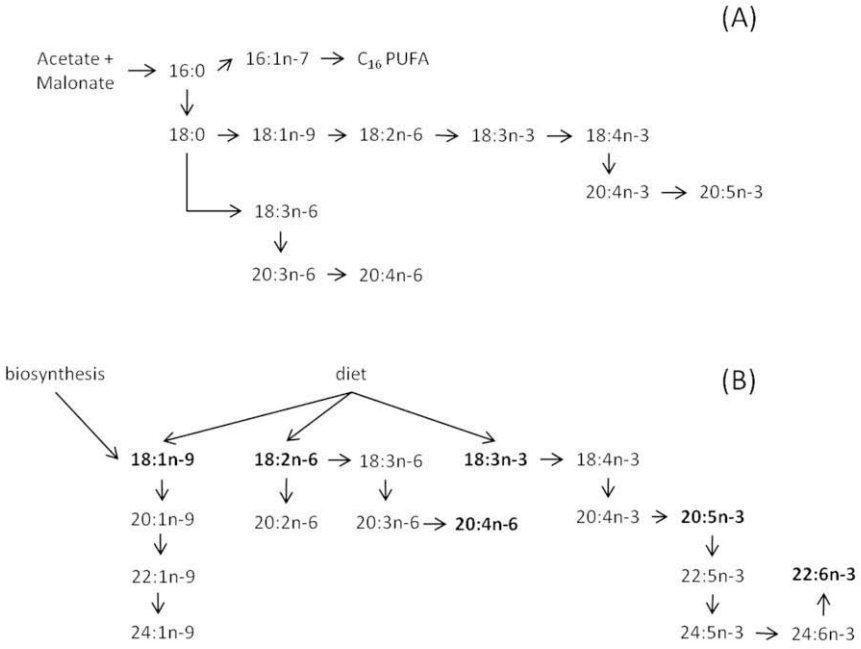


Figure 2.2. Major fatty acid biosynthetic pathways in plants and algae (A) and animals (B). Fatty acids shown in bold typeface in (B) tend to accumulate at relatively high concentrations in animal tissues. Horizontal arrows represent desaturation, downward vertical arrows represent 2-carbon chain elongation, and the upward vertical arrow represents β-oxidation. Re-drawn from Gurr et al. (2002).



CHAPTER 3: FATTY ACID PROFILES IN THE GONADS OF THE SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS* ON NATURAL ALGAL DIETS

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3.1 ABSTRACT

We examined fatty acid (FA) compositions of gonads of the green sea urchin (*Strongylocentrotus droebachiensis*) collected from a grazing aggregation (front) at the edge of a kelp bed and from coralline algae-dominated barrens, and those of urchins fed single algal diets in the laboratory. We compared these gonad FAs to those of the algal diets, which represented known urchin food sources in rocky subtidal habitats. Gonads of wild-collected urchins from both habitats and urchins fed kelp (*Saccharina longicruris*) in the laboratory contained more lipid than did urchins fed single diets of barrens macroalgae (*Agarum clathratum*, coralline red algae or *Desmarestia viridis*). Substantial biosynthesis of non-methylene-interrupted dienes and other FAs by urchins markedly affected their overall FA signatures. Although the FA compositions of gonads of laboratory-fed urchins did not clearly correspond with those of their diets, three clusters of urchins could be distinguished in multivariate space using MDS: (1) Urchins fed single diets of barrens macroalgae in the laboratory; (2) urchins fed *S. longicruris* in the laboratory or collected from the grazing front; and (3) urchins from the barrens. Characteristics of FA signatures found in urchins from the barrens suggested the occurrence of benthic diatoms in the diet of these urchins. Our results indicate that, while the FA signatures of urchin gonads are affected by diet and can be used to differentiate feeding groups of urchins in the laboratory and field, significant *de novo* biosynthesis and/or modification of FAs precludes simple correspondence of urchin FAs to that of their algal diets.

3.2 INTRODUCTION

Fatty acid (FA) analysis is a powerful ecological tool for discerning trophic relationships in terrestrial and aquatic ecosystems (Dalsgaard et al. 2003, Budge et al. 2006, Iverson 2009). Unlike traditional methods of studying diet, such as gut content analysis, FA profiles of consumers can provide information that is neither biased towards food items with hard parts, nor representative of only the last meal (e.g. Iverson et al. 1997, 2004, Budge et al. 2002, 2006). FAs were first used as dietary tracers in bottom-up studies of marine zooplankton (reviewed in Dalsgaard et al. 2003). These studies, and subsequent bottom-up studies of marine food webs, were facilitated by the disparate FA profiles of various classes of primary producers. Diatoms, dinoflagellates, bacteria, land plants and macroalgae can be identified by either unique classes of FAs or characteristic ratios of selected FAs (Sargent et al. 1988, Parrish et al. 2000). Generally, these FAs are passed up the food chain in a predictable manner such that a consumer's FA composition will be strongly influenced by that of its diet (e.g. Graeve et al. 1994, 2002, Rooker et al. 2006) and this potentially allows estimation of dietary composition, at least in higher order consumers (Iverson et al. 2004; Iverson 2009).

In marine systems, certain FAs can be used as indicators for the general type of primary producer in which they originated (Sargent et al. 1988). These marker FAs, when found in consumer tissues, can be used to characterize some food webs. For example, mangrove and seagrass ecosystems are well-suited to this type of marker analysis, as these inputs are distinguishable by the presence of long-chain FAs unique to angiosperms (Nichols et al. 1986, Kharlamenko et al. 2001, Alfaro et al. 2006, Hall et al. 2006, Meziane et al. 2006). In systems with several taxonomically similar primary producers, a higher level of resolution may be necessary to understand trophic relationships. In general, marine macroalgae can be identified to species level using FA analysis (Khotimchenko et al. 2002, Graeve et al. 2002, Nelson et al. 2002, Khotimchenko 2003), and further distinction among macroalgal sources of primary production may be possible at higher trophic levels, such as in fish consumers (Rooker et al. 2006).

In vertebrate predators, the array of FAs present (i.e., the "fatty acid signature", Iverson 1993) in fat stores can be used to indicate specific prey consumption (Kirsch et al. 1998, Iverson et al. 2004, 2007; Nordstrom et al. 2008). However, the same resolution may not be attainable with certain invertebrate herbivores, which are capable of greatly modifying or biosynthesizing FAs (Dalsgaard et al. 2003, Iverson 2009). Numerous studies have used field measurements of marker FAs in invertebrate herbivores to evaluate diet (Bottino 1974, Hayashi & Takagi 1977, Takagi et al. 1980) or to trace sources of primary production (Nichols et al. 1986, Pond et al. 1995, Kharlamenko et al. 2001, Reuss & Poulsen 2002, Alfaro et al. 2006, Meziane et al. 2006). Others have studied the effects of known diets on consumer FA composition (Graeve et al. 1994, Pantazis et al. 2000, Castell et al. 2004, Hall et al. 2006). Few studies have compared the FA composition of such organisms fed known diets in the laboratory to those collected from the wild in order to infer diet of wild-collected animals (Cook et al. 2000). The use of FAs as dietary tracers in invertebrate grazers requires controlled feeding experiments, employing naturally occurring diets, before the technique can be applied in the field.

The green sea urchin, *Strongylocentrotus droebachiensis*, is an ideal model organism to study the effects of dietary FAs on an invertebrate herbivore because it is a generalist grazer whose feeding ecology is well known from field studies (reviewed by Scheibling & Hatcher 2007). In the rocky subtidal ecosystem of Nova Scotia, the urchin plays a key role in driving phase shifts between two states: kelp beds (mainly *Saccharina longicruris* and *Laminaria digitata*) with a low urchin density, and barrens, dominated by urchins and encrusting coralline algae (*Phymatolithon* spp*., Lithothamnion glaciale, Clathromorphum circumscriptum*) (Scheibling 1986, Lauzon-Guay & Scheibling 2007). Dense feeding aggregations of urchins (fronts) destructively graze kelp beds, while more scattered populations of urchins consume coralline algae and microalgal films in barrens (Johnson & Mann 1982, Scheibling 1986, Scheibling et al. 1999, Lauzon-Guay & Scheibling 2007). Urchins in kelp beds and feeding fronts have a higher nutritional condition than those in barrens, and develop a larger gonadal mass (Meidel & Scheibling 1998).

Most lipid storage in sea urchins occurs in the gonads, and several studies have compared FA signatures of urchin gonads on kelp diets to those of urchins on a variety of artificial diets in fisheries and aquaculture applications (Cook et al. 2000, Liyana-Pathirana et al. 2002, Castell et al. 2004). While these studies have shown that FA compositions of urchins resemble those of their diets to some extent, the experimental diets were markedly different from each other and bore little resemblance to food normally encountered by urchins in the wild (i.e., experimental diets were composed of different nutrients and/or had higher dry matter and fat contents). Our study evaluates the use of FAs as dietary tracers at the base of naturally occurring rocky-subtidal food webs in Nova Scotia: from macroalgae to sea urchins. To determine the degree to which dietary FAs are deposited directly into urchin tissues, we compare FA compositions of four natural algal diets to those of gonadal tissue produced by urchins fed each diet. We also examine the FA compositions of urchins collected from two subtidal areas, a grazing front along the edge of a kelp bed and an adjacent urchin barrens, to compare these to the FA compositions of urchins fed the four algal diets in the laboratory.

3.3 Materials and Methods

3.3.1 Feeding Experiment

Our experiment was part of a broader study to examine growth, reproduction, and physical performance of *Strongylocentrotus droebachiensis* fed dominant species of macroalgae from urchin barrens, using kelp (*Saccharina longicruris*, a species absent in barrens but resulting in high rates of somatic and gonadic growth; Scheibling & Hatcher 2007) as a control. Urchins were collected by divers in February 2006 from a grazing front at the lower margin of a kelp bed (*S. longicruris* and *Laminaria digitata*) at Splitnose Point (44º28.609' N, 63º32.741' W), near Halifax, Nova Scotia. Urchins were maintained in 75-L glass aquaria supplied with flowing seawater and fed 50 g of kelp (*S. longicruris*) once or twice weekly (*ad libitum*) for 12 wk, and then starved for 2 wk until the start of the experiment. On 9 June, 20 urchins were placed in each of 5 replicate aquaria for each of 4 algal diets: 1) a kelp of low preference rank (*Agarum clathratum*), 2) crustose coralline algae (*Phymatolithon* spp*., Lithothamnion glaciale, Clathromorphum circumscriptum*), 3) a chemically-defended brown alga (*Desmarestia*

viridis), and 4) a highly preferred kelp (*S. longicruris*). Algae were collected by divers from Splitnose Point as needed throughout the experiment; *A. clathratum, D. viridis* and *S. longicruris* were cut off above the holdfast, and corallines were chipped off the seabed using hammers and chisels. Algae were maintained in aquaria supplied with flowing seawater. Urchins were fed each diet *ad libitum* (once or twice weekly) for 14 wk. Algal rations were blotted with paper towel for 40-60s and weighed before being added to urchin aquaria, with the exception of *D. viridis* which was blotted more rapidly (10-15s) to avoid release of sulfuric acid caused by contact with air. Uneaten pieces of algae were blotted, weighed and discarded before fresh rations were added so that consumption could be estimated for each feeding interval. At the end of the experiment, five urchins per tank were randomly selected for FA analysis.

In November 2007, 20 urchins were collected from the grazing front at Splitnose Point and another 20 from the coralline algae-dominated barrens about 10 m offshore of the front. Kelp biomass at the grazing front consisted of approximately equal proportions of *Saccharina longicruris* and *Laminaria digitata* (Lauzon-Guay & Scheibling 2007). Urchins were held in flowing seawater tanks for 24 h before being sacrificed for FA analysis.

3.3.2 Lipid Extraction

Algae were blotted to remove excess water and visually examined for epibionts, which were removed prior to processing. Whole thalli of *Desmarestia viridis* were homogenized using a hand-held immersion blender; kelps (*Saccharina longicruris*, *Agarum clathratum*) were coarsely chopped and homogenized into a paste using a mortar and pestle; fragments of coralline algae (chipped or pried off the bottom with a diver's knife) were crushed with mortar and pestle. 9.0 g samples of each alga (n = 2 for coralline algae and *D. viridis*; n = 3 for *A. clathratum* and *D. viridis*), each from a different thallus where applicable, were weighed before adding 20 ml chloroform and 10 ml methanol containing 0.01% butylated hydroxytoluene (BHT) as antioxidant.

Gonads were excised from 10 randomly-selected urchins per diet treatment and stored at -20°C for 30-90 d prior to lipid extraction. For lipid extraction, 1.5 g of gonad from each urchin was manually homogenized with 20 ml chloroform and 10 ml methanol containing 0.01% BHT.

Lipid extraction protocols were modified from Folch et al. (1957). The samples in solvent (urchin gonad or algae) were filtered (Fisher P8 filter paper) to remove any tissue before adding 7 ml 5% NaCl. Samples were shaken, then centrifuged for 20 min. The lipid-containing lower phase was filtered through $NaSO_4$ and evaporated to dryness under nitrogen at 25-30°C in a pre-weighed boiling tube. The boiling tube was re-weighed to calculate the amount of crude lipid for each sample.

3.3.3 Fatty Acid Methyl Ester (FAME) Preparation and Analysis

To prepare lipids for FA analysis, crude lipid was dissolved in 1.5 ml methylene chloride with 0.01% BHT and subjected to acidic transesterification (H_2SO_4 in methanol) to produce FA methyl esters (FAME), as described in Budge et al. (2006). FAME were purified to remove proteins and pigments using thin-layer chromatography (TLC). FAME were dissolved in hexane and spotted onto silica TLC plates (Sigma) and developed in a TLC chamber with 90:10:1 petroleum ether: ethyl ether: acetic acid for approximately 45 min. Plates were sprayed with 2,4 dichlorofluorescein and viewed under UV light to mark the FAME-containing band. The FAME-containing band was then scraped from the silica plate and transferred to a funnel packed with silane-treated glass wool. Chloroform containing 0.01% BHT was used to dissolve FAME into a pre-weighed tube. The sample was evaporated to dryness under nitrogen at 25-30°C and hexane was added to a final concentration of 50 mg ml^{-1}. Samples were stored at -20°C prior to analysis. Duplicate samples of FAME were analyzed and FAs identified using temperature-programmed gas chromatography (Iverson et al. 1997, 2004, Budge et al. 2006).

3.3.4 Statistical Methods

Lipid content of urchin gonads (% wet wt) was compared across the six treatments using one-way ANOVA. Lipid content data met ANOVA assumptions of normality and

homoscedasticity, and were not transformed prior to analysis. Post-hoc pairwise comparisons were made using Bonferroni adjustments for multiple comparisons.

Samples with a FAME weight ≥0.005 g (64 out of 80 samples; four samples from each diet group of laboratory-fed urchins were eliminated due to low FAME weight) were used in multivariate analyses. Only FAs with an overall mean contribution ≥0.1% were used and FA composition was normalized to 100% for all samples.

Levels of selected FAs were compared among algal species using one-way ANOVA following tests to confirm that data met ANOVA assumptions. Post-hoc pairwise comparisons were made with Bonferroni adjustments. FA compositions were compared among algae, and between urchin gonads and their respective diets using analysis of similarity (ANOSIM) on Bray-Curtis distances (Clarke & Warwick 1994) of untransformed data. FA composition of urchin gonads was compared across the six diet and habitat groups using multidimensional scaling (MDS) and ANOSIM on Bray-Curtis distances (Clarke & Warwick 1994). Homogenous clusters of diet and habitat groups were determined using pairwise comparisons in ANOSIM (Bonferroni-adjusted α=0.05). Urchin gonad data were then pooled according to these homogenous clusters, and SIMPER analysis was conducted on the clusters to determine the contribution of each FA to the average Bray-Curtis similarity within each cluster, and to the average Bray-Curtis dissimilarity among groups (Clarke & Warwick 1994). All multivariate analyses were conducted using PRIMER 5 software (Clarke & Warwick 1994).

3.4 RESULTS

Over the course of the 14-week feeding experiment, average consumption rates (calculated on a per-aquarium basis, n = 5 aquaria per algal diet) on the four algal diets were: 0.43 (±0.010 SE) g^{-1} urchin d^{-1} on *Agarum clathratum*, 0.013 (±0.004) g^{-1} urchin d^{-1} of coralline algae, 0.47 (±0.008) g^{-1} urchin d^{-1} on *Desmarestia viridis*, and 1.56 (±0.054) g^{-1} urchin d^{-1}on *Saccharina longicruris*.

The four algal diets (*Agarum clathratum*, coralline algae, *Desmarestia viridis,* and *Saccharina longicruris*) did not differ in lipid content ($F_{[3,6]}$ = 0.902, p = 0.493; Table

3.1), but differed in FA composition (ANOSIM, $p < 0.001$; Table 3.1). The saturated FA 16:0 was a major component of all algal diets, constituting between 13.4 and 25.0% of total FAs. Polyunsaturates 20:4*n*-6 and 20:5*n*-3 also were major components of all algal diets, making up 3.9 to 15.1% and 8.8 to 27.9% of total FAs, respectively. *A. clathratum* differed from other algal diets in having relatively high levels of 16:4*n*-1, and relatively low levels of 20:4*n*-6 and 20:5*n*-3. Coralline algae and *A. clathratum* both contained higher levels of 16:0 and 22:5*n*-3 than did *D. viridis* or *S. longicruris*. *S. longicruris* and *D. viridis* contained higher levels of 18:4*n*-3 than did coralline algae or *A. clathratum.* Relative to all other algal diets, corallines were rich in 16:0 and 20:5*n*-3 (ANOVA $p < 0.01$ for all comparisons among algae).

Lipid content of urchin gonads ranged from 0.7% (fed *Desmarestia viridis*) to 9.2% (fed *Saccharina longicruris*). Gonad lipid content differed significantly among groups of laboratory-fed and wild-collected urchins ($F_{[5,78]} = 9.791$, $p < 0.001$; Fig 3.1). Gonads of urchins fed coralline algae and *D. viridis* in the laboratory had significantly lower lipid content than those fed *S. longicruris* or collected from kelp bed or barrens habitats. Gonad lipid content of urchins fed *Agarum clathratum* did not differ significantly from that of other groups.

Urchin gonads contained all FAs in the respective algal diet, as well as a number of others that were absent from or present only in trace amounts in the diet, but contributed markedly to the overall FA signature of the gonads (Table 3.1). The FAs found only in urchins were primarily 20:1*n*-9, 20:1*n*-7, 22:1*n*-9, and several non-methylene-interrupted dienes (20:2Δ5,11, 20:2 Δ5,13, 20:2Δ5,11 and 20:2 Δ5,13). Several other FAs were found in urchin gonads at higher levels than in their diet (e.g. 14:0, 16:1*n*-7). Overall, the FA signatures of urchin gonads did not resemble those of their respective diets (ANOSIM, $p < 0.04$ for all diets; Table 3.1). However, urchins were separated into three homogenous clusters using MDS and ANOSIM: (1) urchins fed *Agarum clathratum*, coralline algae or *Desmarestia viridis* in the laboratory; (2) wild-collected urchins from the kelp bed and urchins fed *Saccharina longicruris* in the laboratory; and (3) wild-collected urchins from

the barrens (Fig 3.2). Within-group similarity for these clusters was 91%, 89% and 90%, respectively.

Average dissimilarity was 13.5% between Clusters 1 and 2, 15.7% between Clusters 1 and 3, and 15.8% between Clusters 2 and 3. The FAs that contributed most to dissimilarity among clusters included saturated FAs (14:0 and 16:0), monounsaturated FAs (16:1, 16:1*n*-7, 20:1*n*-15, 20:1*n*-9), polyunsaturated FAs (16:4*n*-1, 18:4*n*-3, 20:4n-6, 20:5*n*-3) and non-methylene-interrupted dienes (20:2Δ5,11 and 20:2 Δ5,13) (Table 3.2). Cluster 1 urchins were relatively rich in 20:1*n*-15, 20:2Δ5,11 and 20:2 Δ5,13; Cluster 2 urchins were relatively rich in 14:0 and 16:0, and Cluster 3 urchins were relatively rich in 16:4*n*-1 and 20:5*n*-3 (Table 3.2).

3.5 DISCUSSION

The FA compositions of the four algal taxa in this study were distinct (Table 3.1), and generally similar to those recorded in previous studies of the same or congeneric species: *Corallina pilulifera* (Kitamura et al. 1993), *Agarum cribosum* (= *clathrathum*) (Khotimchenko 1998), and *Saccharina longicruris* (Cook et al. 2000). The FA composition of *Desmarestia viridis* in our study differed from that reported by Li et al. (2002) in that it was lower in 16:0 and 18:1*n*-9. Likewise, urchins fed different algal diets had distinct gonadal FA compositions; however, these did not directly correspond to those of their diets. Substantial levels of non-methylene-interrupted dienes and other FAs present in urchin gonads but not in their diets, as well as higher levels of certain FAs in urchin gonads than in algae, indicate significant levels of FA biosynthesis in urchins. This level of biosynthesis clearly reduced the influence of dietary FAs, although the urchins were still separated by their dietary groups. Although the gonads are the key site of lipid storage in sea urchins, the gonads also have a dual function as a reproductive organ, which may thus experience greater biochemical and FA modification than other types of lipid storage areas (Hughes et al. 2006). Thus, although other invertebrate herbivores may also synthesize significant amounts of FAs, these effects may be particularly pronounced in urchins due to their specific site of lipid storage.

Although a correspondence between the FA compositions of urchin gonads and their diets has been documented in other studies (Cook et al. 2000, Liyana-Pathirana et al. 2002, Castell et al. 2004), these have compared a single algal diet to various synthetic diets of markedly different compositions. Urchins are known to convert some dietary FAs to new FA products through various biosynthetic pathways (Cook et al. 2000, Castell et al. 2004). While synthesized FAs cannot be used as direct dietary indicators, their levels may vary with diet. For example, Castell et al. (2004) found correlations between dietary levels of precursor FAs (18:2*n*-6 and 18:3*n*-3) and levels of synthesized FAs (20:4*n*-6, 20:5*n*-3, 22:6*n*-3 and 20:2Δ5,11) in urchin gonads. We did not find such correlations between dietary precursors and products of biosynthesis in urchins fed known algal diets.

We found that gonads of urchins fed *Agarum clathratum*, coralline algae and *Desmarestia viridis* in the laboratory contained relatively high amounts of the urchin-synthesized FAs 20:2Δ5,11 and 20:2 Δ5,13. The urchins fed these three non-kelp algal diets had low estimated intake rates, the lowest fat contents in their gonads (Fig. 3.1), and clustered together in MDS analysis (Fig. 3.2). The effect of a particularly low fat-intake diet should stimulate FA biosynthetic rates, possibly contributing further to the low apparent influence of dietary FAs. However, although the urchins fed kelp in the laboratory and those collected from on kelp grazing fronts formed a separate cluster in MDS analysis, there was no direct concordance between FAs in urchin gonads and their kelp diet (Table 3.1).

The gonad FAs of wild-collected urchins from barrens differed from those of all other groups, suggesting that the characteristic macroalgae of urchin barrens in eastern Canada – *Agarum clathratum*, coralline algae and *Desmarestia viridis*, which are all chemically or mechanically defended against urchin grazing (Himmelman & Nédelec 1990, Gagnon et al. 2003, Gagnon et al. 2005) – do not contribute significantly to the diet of urchins in this habitat. The polyunsaturates 16:4*n*-1 and 20:5*n*-3 were important in separating barrens urchins from all others, and accounted for an average of 1.8% and 9.0%, respectively, of total FAs in these urchins. The presence of these FAs suggests that urchins derive some FAs from microalgal films growing on coralline algae in barrens, as

16:4*n*-1 and 20:5*n*-3 are abundant in diatoms (Sargent et al. 1988). Diatoms and filamentous brown and red algae have been found in the gut of urchins from barrens in Nova Scotia (Chapman 1981).

Wild-collected urchins from a grazing front at the lower margin of a kelp bed and urchins fed *Saccharina longicruris* in the laboratory had similar gonad FA compositions, indicating that urchins in fronts derive the most of their nutrition from *S. longicruris* and similar kelp species. This is consistent with numerous field observations of dense urchin fronts that rapidly consume all kelp in their path (Chapman 1981, Scheibling et al. 1999, Gagnon et al. 2004, Lauzon-Guay & Scheibling 2007). Lauzon-Guay & Scheibling (2007) estimated that urchins at the grazing front at Splitnose Point consumed kelp at a rate of 0.027 g^{-1} urchin d^{-1}. Differences between the wild and laboratory urchins may be partly due to consumption of *Laminaria digitata*, which comprised approximately half of kelp biomass at this site (Lauzon-Guay & Scheibling 2007). The gonad FA compositions of both wild-collected urchins from a grazing front and urchins fed *S. longicruris* in the laboratory in this study were generally similar to FA signatures found by Liyana-Pathirana et al. (2002) and by Castell et al. (2004) using the same urchin and kelp species in controlled feeding experiments, and by Takagi et al. (1980) in wild-collected urchins from a Nova Scotian kelp bed.

As stated previously, gonads of urchins fed single diets of the characteristic macroalgae of barrens had lower lipid contents than gonads of urchins fed *Saccharina longicruris* and wild-collected urchins, suggesting that these barrens macroalgae, individually, are poor nutritional sources for urchins. Differences in sampling period between wild-collected (November) and laboratory-fed (September) urchins may account for some difference in lipid content between these groups, related to the gametogenic cycle and increasing gonad size in the fall (Meidel & Scheibling 1998). However, the similarity in gonad FA signatures between urchins collected from the kelp grazing front and those fed *S. longicruris* in the laboratory suggests that, although seasonal differences could have affected gonad size and lipid content, they did not affect the FA composition. Gender has

also been shown to affect gonad FA composition of urchins (Hughes et al 2006) and may account for some of the within-group variability in our study.

We have shown that the FA composition of urchin gonads is substantially influenced by *de novo* FA synthesis and does not completely resemble that of their algal diets. Therefore it is unlikely that algal FAs can be used as direct dietary tracers for sea urchins in the Nova Scotian system. However, our ability to distinguish urchins fed different algal diets by their gonad FA compositions indicates that FAs may serve as indirect dietary tracers in determining the source of primary production used by consumers at higher trophic levels. We also found evidence that urchins in barrens obtain much of their nutrition from non-macroalgal sources such as diatoms, and that these sources may be more nutritious than macroalgae typically found in barrens such as *Agarum clathratum,* corallines and *Desmarestia viridis*. Captive feeding experiments that include algae, herbivores (including those that can synthesize a significant portion of their FAs) and higher order consumers are required to determine the applicability of FA analysis to bottom-up tracking of energy flow in benthic marine food webs.

3.6 ACKNOWLEDGEMENTS

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3.7 TABLES AND FIGURES

Table 3.1. Relative fatty acid composition of algae used in feeding experiments, and sea urchin (*Strongylocentrotus droebachiensis*) gonads from laboratory-fed and wild-collected specimens. Lipid content values are mean % of total wet weight ± SE. Fatty acid values are mean % of total fatty acids ± SE of all fatty acids with overall mean concentration ≥ 0.1%.

	Algae				Laboratory-fed urchins				Wild-collected urchins	
	Agarum clathratum	Coralline algae	*Desmarestia viridis*	*Saccharina longicruris*	fed *A. clathratum*	fed coralline algae	fed *D. viridis*	fed *S. longicruris*	Barrens	Kelp front
n	3	2	2	3	5	6	9	11	12	21
% lipid	0.08 ± 0.03	0.04 ± 0.03	0.09 ± 0.01	0.02 ± 0.01	3.62 ± 0.62	2.95 ± 0.49	3.11 ± 0.31	5.16 ± 0.60	5.62 ± 032	5.69 ± 0.34
13:0	-	0.07 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	0.23 ± 0.02	0.28 ± 0.04	0.24 ± 0.01	0.16 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
iso14:0	0.11 ± 0.01	0.82 ± 0.29	0.53 ± 0.29	0.22 ± 0.15	0.03 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.11 ± 0.01	0.05 ± 0.00
14:0	2.88 ± 0.19	2.67 ± 0.41	5.53 ± 0.17	6.75 ± 0.49	10.39 ± 0.42	9.62 ± 0.52	8.55 ± 0.12	13.31 ± 0.74	9.66 ± 0.33	12.18 ± 0.38
14:1*n*-9	-	0.24 ± 0.23	-	0.01 ± 0.01	0.20 ± 0.01	0.16 ± 0.01	0.17 ± 0.00	0.17 ± 0.01	0.14 ± 0.00	0.18 ± 0.01
14:1*n*-5	0.06 ± 0.02	0.26 ± 0.06	0.11 ± 0.08	0.10 ± 0.01	1.58 ± 0.18	1.30 ± 0.17	0.96 ± 0.02	1.57 ± 0.17	0.74 ± 0.05	1.55 ± 0.13
iso15:0	0.08 ± 0.02	0.62 ± 0l.11	0.17 ± 0.04	0.13 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.18 ± 0.00	0.13 ± 0.02	0.45 ± 0.03	0.15 ± 0.01
anti15:0	0.01 ± 0.01	0.31 ± 0.00	-	-	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	0.06 ± 0.01	0.24 ± 0.02	0.07 ± 0.01
15:0	0.26 ± 0.05	0.39 ± 0.00	0.14 ± 0.01	0.25 ± 0.03	0.59 ± 0.05	0.63 ± 0.05	0.62 ± 0.01	0.53 ± 0.02	0.83 ± 0.04	0.43 ± 0.02
16:0	18.22 ± 0.31	24.95 ± 1.79	13.53 ± 0.05	13.40 ± 1.28	11.72 ± 0.32	11.87 ± 0.26	12.39 ± 10	14.17 ± 0.28	13.14 ± 0.30	14.31 ± 0.27
16:1*n*-11	1.84 ± 0.06	3.70 ± 0.27	1.37 ± 1.28	1.39 ± 0.02	0.28 ± 0.02	0.33 ± 0.02	0.36 ± 0.01	0.25 ± 0.02	0.42 ± 0.03	0.34 ± 0.02
16:1*n*-9	0.39 ± 0.13	0.23 ± 0.09	1.56 ± 1.35	0.13 ± 0.02	0.27 ± 0.01	0.26 ± 0.02	0.28 ± 0.00	0.21 ± 0.01	0.36 ± 0.01	0.24 ± 0.01
16:1*n*-7	5.66 ± 1.89	3.72 ± 0.47	2.61 ± 0.15	2.88 ± 0.35	5.96 ± 0.32	5.94 ± 0.32	5.83 ± 0.11	5.83 ± 0.19	5.94 ± 0.27	5.65 ± 0.39
16:1	0.76 ± 0.32	0.28 ± 0.01	4.53 ± 0.03	0.38 ± 0.03	3.71 ± 0.25	3.46 ± 0.30	2.62 ± 0.05	3.13 ± 0.18	2.18 ± 0.15	3.66 ± 0.18
16:2*n*-6	0.22 ± 0.04	0.02 ± 0.01	0.07 ± 0.00	0.34 ± 0.04	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.11 ± 0.01	0.14 ± 0.01	0.07 ± 0.00
iso17:0	0.03 ± 0.01	1.25 ± 0.90	-	-	0.07 ± 0.01	0.08 ± 0.02	0.10 ± 0.00	0.07 ± 0.01	0.41 ± 0.04	0.04 ± 0.01
16:2*n*-4	-	0.04 ± 0.01	-	0.01 ± 0.00	0.18 ± 0.03	0.25 ± 0.04	0.25 ± 0.01	0.14 ± 0.02	0.20 ± 0.01	0.12 ± 0.01
16:3*n*-6	1.99 ± 0.86	0.83 ± 0.12	0.21 ± 0.01	0.38 ± 0.02	0.11 ± 0.02	0.09 ± 0.01	0.11 ± 0.00	0.12 ± 0.01	0.54 ± 0.08	0.15 ± 0.02
17:0	0.09 ± 0.02	0.27 ± 0.02	0.05 ± 0.01	0.10 ± 0.03	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.00	0.09 ± 0.00	0.18 ± 0.01	0.09 ± 0.00
16:3*n*-4	1.83 ± 0.41	0.15 ± 0.05	0.07 ± 0.01	0.29 ± 0.02	0.09 ± 0.03	0.08 ± 0.01	0.09 ± 0.00	0.10 ± 0.01	0.81 ± 0.01	0.14 ± 0.02
17:1	0.75 ± 0.70	0.12 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.10 ± 0.08	0.10 ± 0.01	0.11 ± 0.00	0.09 ± 0.00	0.14 ± 0.29	0.09 ± 0.00
16:4*n*-3	-	-	-	-	0.12 ± 0.10	0.11 ± 0.01	0.16 ± 0.00	0.10 ± 0.01	0.16 ± 0.06	0.11 ± 0.01
16:4*n*-1	6.13 ± 1.45	0.08 ± 0.01	0.30 ± 0.01	0.56 ± 0.10	0.20 ± 0.05	0.12 ± 0.04	0.19 ± 0.01	0.18 ± 0.02	1.76 ± 0.02	0.30 ± 0.05
18:0	3.95 ± 1.04	5.87 ± 0.67	2.78 ± 1.18	6.40 ± 1.84	1.34 ± 0.10	1.50 ± 0.11	1.67 ± 0.04	1.28 ± 0.08	1.47 ± 0.13	1.25 ± 0.04
18:1*n*-13	-	0.17 ± 0.10	0.06 ± 0.03	0.05 ± 0.03	0.49 ± 0.05	0.47 ± 0.01	0.60 ± 0.01	0.42 ± 0.02	0.40 ± 0.11	0.44 ± 0.02
18:1*n*-9	11.32 ± 4.67	2.04 ± 0.85	5.25 ± 0.04	5.33 ± 0.29	2.96 ± 0.14	2.71 ± 0.15	2.90 ± 0.04	2.59 ± 0.09	2.10 ± 0.02	2.33 ± 0.11
18:1*n*-7	0.32 ± 0.02	1.70 ± 0.15	0.37 ± 0.05	0.34 ± 0.06	3.66 ± 0.11	3.94 ± 0.16	3.51 ± 0.04	3.30 ± 0.07	3.60 ± 0.08	3.88 ± 0.07

	Algae				Laboratory-fed urchins				Wild-collected urchins	
	Agarum clathratum	Coralline algae	*Desmarestia viridis*	*Saccharina longicruris*	fed *A. clathratum*	fed coralline algae	fed *D. viridis*	fed *S. longicruris*	Barrens	Kelp front
18:1*n*-5	0.10 ± 0.01	0.19 ± 0.05	0.32 ± 0.01	0.13 ± 0.03	0.31 ± 0.02	0.32 ± 0.02	0.26 ± 0.00	0.28 ± 0.01	0.21 ± 0.05	0.32 ± 0.01
18:2*n*-7	0.06 ± 0.01	0.02 ± 0.02	-	0.02 ± 0.01	1.08 ± 0.05	1.05 ± 0.18	1.09 ± 0.04	1.05 ± 0.09	0.69 ± 0.01	0.98 ± 0.07
18:2*n*-6	7.39 ± 3.05	2.82 ± 1.14	4.56 ± 0.04	6.27 ± 0.06	1.04 ± 0.08	0.97 ± 0.05	0.96 ± 0.02	1.19 ± 0.04	1.23 ± 0.05	1.10 ± 0.05
18:2*n*-4	0.64 ± 0.26	0.40 ± 0.02	0.01 ± 0.00	0.03 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.22 ± 0.01	0.08 ± 0.01
18:3*n*-6	0.42 ± 0.13	0.23 ± 0.05	0.74 ± 0.03	0.91 ± 0.06	0.20 ± 0.02	0.17 ± 0.01	0.20 ± 0.00	0.27 ± 0.01	0.44 ± 0.03	0.23 ± 0.01
18:3*n*-4	0.10 ± 0.03	0.02 ± 0.01	-	-	0.14 ± 0.02	0.15 ± 0.01	0.16 ± 0.00	0.12 ± 0.01	0.33 ± 0.03	0.12 ± 0.01
18:3*n*-3	5.44 ± 0.47	0.45 ± 0.07	11.33 ± 0.38	5.95 ± 1.07	1.11 ± 0.09	1.15 ± 0.07	1.02 ± 0.01	1.27 ± 0.04	0.88 ± 0.05	1.24 ± 0.07
18:3*n*-1	0.98 ± 0.37	0.13 ± 0.10	0.12 ± 0.03	0.33 ± 0.03	0.11± 0.01	0.12 ± 0.01	0.13 ± 0.00	0.11 ± 0.01	0.10 ± 0.02	0.10 ± 0.00
18:4*n*-3	2.53 ± 0.25	0.39 ± 0.00	15.64 ± 0.57	14.79 ± 2.69	2.66 ± 0.23	2.81 ± 0.29	2.25 ± 0.03	3.70 ± 0.16	2.28 ± 0.14	3.32 ± 0.18
18:4*n*-1	0.36 ± 0.11	-	0.27 ± 0.02	0.10 ± 0.03	0.12 ± 0.04	0.08 ± 0.01	0.12 ± 0.00	0.11 ± 0.01	0.49 ± 0.10	0.10 ± 0.02
20:0	0.18 ± 0.17	0.03 ± 0.01	-	0.01 ± 0.00	0.24 ± 0.04	0.29 ± 0.04	0.25 ± 0.01	0.36 ± 0.03	0.55 ± 0.04	0.43 ± 0.03
20:1*n*-15	0.01 ± 0.01	0.03 ± 0.02	-	0.03 ± 0.03	5.59 ± 0.19	5.89 ± 0.27	6.54 ± 0.07	4.53 ± 0.19	4.59 ± 0.13	4.18 ± 0.14
20:1*n*-11	0.01 ± 0.01	0.50 ± 0.04	0.05 ± 0.01	0.03 ± 0.00	1.41 ± 0.76	0.71 ± 0.03	0.80 ± 0.01	0.53 ± 0.03	0.69 ± 0.05	0.64 ± 0.03
20:1*n*-9	0.01 ± 0.00	0.04 ± 0.04	0.01 ± 0.00	0.02 ± 0.02	3.91 ± 1.00	5.25 ± 0.32	4.49 ± 0.06	4.35 ± 0.15	3.98 ± 0.14	5.27 ± 0.20
20:1*n*-7	-	0.14 ± 0.14	-	-	4.55 ± 0.13	4.20 ± 0.17	4.64 ± 0.13	3.33 ± 0.17	3.92 ± 0.13	4.06 ± 0.15
20:2Δ5,11	-	-	-	-	5.58 ± 0.23	5.37 ± 0.32	5.56 ± 0.04	4.59 ± 0.21	2.68 ± 0.15	4.54 ± 0.13
20:2Δ5,13	-	-	-	-	3.65 ± 0.25	3.63 ± 0.19	3.93 ± 0.10	2.62 ± 0.13	2.59 ± 0.15	2.90 ± 0.11
20:2*n*-9	-	-	-	-	0.25 ± 0.03	0.31 ± 0.01	0.23 ± 0.01	0.22 ± 0.02	0.18 ± 0.01	0.24 ± 0.01
20:1[a]	-	-	-	-	0.47 ± 0.06	0.47 ± 0.04	0.43 ± 0.01	0.52 ± 0.04	0.30 ± 0.03	0.53 ± 0.04
20:2*n*-6	0.04 ± 0.01	0.48 ± 0.14	0.03 ± 0.01	0.14 ± 0.04	1.03 ± 0.21	0.92 ± 0.09	1.03 ± 0.02	0.90 ± 0.07	0.88 ± 0.04	0.82 ± 0.04
20:3*n*-6	0.41 ± 0.19	1.47 ± 0.03	0.29 ± 0.01	0.35 ± 0.05	0.46 ± 0.03	0.43 ± 0.02	0.41 ± 0.00	0.64 ± 0.02	0.61 ± 0.03	0.64 ± 0.02
20:4*n*-6	3.90 ± 0.48	10.86 ± 0.29	8.09 ± 0.29	15.11 ± 0.88	5.01 ± 0.13	4.63 ± 0.16	5.39 ± 0.09	5.50 ± 0.30	4.63 ± 0.30	3.68 ± 0.15
20:0cyc[b]	-	-	-	-	2.27 ± 0.09	2.50 ± 0.07	2.45 ± 0.03	1.51 ± 0.10	1.87 ± 0.06	1.45 ± 0.05
20:3*n*-3	0.06 ± 0.01	0.11 ± 0.00	0.09 ± 0.01	0.09 ± 0.05	0.81 ± 0.06	0.92 ± 0.06	0.83 ± 0.01	0.85 ± 0.06	0.76 ± 0.03	0.96 ± 0.06
20:4*n*-3	0.17 ± 0.03	0.31 ± 0.01	0.95 ± 0.04	0.53 ± 0.04	0.65 ± 0.06	0.71 ± 0.07	0.51 ± 0.01	1.02 ± 0.06	0.65 ± 0.04	0.95 ± 0.05
20:5*n*-3	8.80 ± 0.13	27.93 ± 3.19	17.35 ± 0.70	14.38 ± 0.35	5.35 ± 0.24	5.10 ± 0.17	5.65 ± 0.10	6.01 ± 0.19	9.00 ± 0.46	6.04 ± 0.28
22:1*n*-11	-	-	-	-	0.09 ± 0.01	0.11 ± 0.02	0.11 ± 0.00	0.10 ± 0.02	0.20 ± 0.02	0.10 ± 0.01
22:1*n*-9	-	-	-	-	2.95 ± 0.16	3.11 ± 0.28	3.22 ± 0.04	2.27 ± 0.19	2.90 ± 0.16	2.35 ± 0.12
22:1*n*-7	-	-	-	-	0.18 ± 0.02	0.18 ± 0.02	0.20 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
22:2Δ7,13	-	-	-	-	0.61 ± 0.03	0.55 ± 0.03	0.67 ± 0.01	0.49 ± 0.02	0.39 ± 0.02	0.47 ± 0.02
22:2Δ7,15	-	-	-	-	1.67 ± 0.04	1.88 ± 0.11	1.86 ± 0.02	1.33 ± 0.08	1.69 ± 0.09	1.62 ± 0.14
22:4*n*-6	0.10 ± 0.01	0.39 ± 0.15	0.01 ± 0.01	0.13 ± 0.07	0.23 ± 0.10	0.25 ± 0.02	0.27 ± 0.01	0.30 ± 0.01	0.50 ± 0.03	0.36 ± 0.02
22:5*n*-6	0.01 ± 0.01	0.21 ± 0.15	0.34 ± 0.31	0.18 ± 0.08	0.14 ± 0.04	0.18 ± 0.03	0.17 ± 0.01	0.14 ± 0.02	0.27 ± 0.03	0.17 ± 0.01
22:5*n*-3	11.21 ± 2.99	1.38 ± 0.27	0.02 ± 0.01	0.08 ± 0.06	0.26 ± 0.10	0.27 ± 0.03	0.30 ± 0.01	0.32 ± 0.03	0.46 ± 0.03	0.32 ± 0.02
22:6*n*-3	0.04 ± 0.01	0.28 ± 0.03	0.27 ± 0.05	0.01 ± 0.00	0.73 ± 0.13	1.07 ± 0.12	1.08 ± 0.03	0.63 ± 0.09	1.78 ± 0.13	1.22 ± 0.10

[a]double bond position unknown; [b]tentatively identified as 20:0 with a cyclopropane ring

Table 3.2.*Strongylocentrotus droebachiensis*. SIMPER analysis for relative abundance of fatty acids in three clusters defined by ANOSIM pairwise comparisons. Cluster 1 contains urchins fed *Agarum clathratum*, *Desmarestia viridis* or coralline algae in the laboratory; Cluster 2 contains wild-collected urchins from the kelp bed and urchins fed *Saccharina longicruris* in the laboratory; and Cluster 3 contains wild-collected urchins from the barrens. Av. abun.: average relative abundance ± SE; av. dissim.: average Bray-Curtis dissimilarity; cum. dissim. (%): cumulative percentage of dissimilarity.

Fatty acid	Av. abun. Cluster 1	Av. abun. Cluster 2	Av. abun. Cluster 3	Av. dissim.	Cum. dissim. (%)
Clusters 1 and 2					
14:0	9.33 ± 0.29	12.57 ± 0.36		1.7	12.5
16:0	12.07 ± 0.18	14.26 ± 0.20		1.1	20.7
20:1*n*-15	6.11 ± 0.15	4.30 ± 0.12		0.9	27.4
20:4*n*-6	5.07 ± 0.21	4.30 ± 0.21		0.6	32.2
16:1*n*-7	5.90 ± 0.18	5.71 ± 0.26		0.6	36.6
20:2Δ5,11	5.51 ± 0.18	4.56 ± 0.11		0.6	40.9
20:5*n*-3	5.41 ± 0.16	3.45 ± 0.19		0.6	45.1
20:1*n*-9	4.57 ± 0.28	2.80 ± 0.16		0.6	49.1
Clusters 1 and 3					
20:5*n*-3	5.41 ± 0.16		9.00 ± 0.46	1.8	11.5
20:2Δ5,11	5.51 ± 0.18		2.68 ± 0.15	1.4	20.6
16:4*n*-1	0.17 ± 0.03		1.76 ± 0.29	0.8	25.7
20:1*n*-15	6.11 ± 0.15		4.59 ± 0.13	0.8	30.6
14:0	9.33 ± 0.29		9.66 ± 0.33	0.7	25.1
16:0	12.07 ± 0.18		13.14 ± 0.30	0.7	39.5
20:2Δ5,13	3.77 ± 0.15		2.59 ± 0.15	0.6	43.5
20:1*n*-9	4.57 ± 0.28		3.98 ± 0.14	0.6	47.0
16:1	3.14 ± 0.16		2.18 ± 0.15	0.5	50.5
Clusters 2 and 3					
14:0		12.57 ± 0.36	9.66 ± 0.33	1.5	9.8
20:5*n*-3		6.03 ± 0.19	9.00 ± 0.46	1.5	19.4
20:2Δ5,11		4.56 ± 0.11	2.68 ± 0.15	0.9	25.3
16:4*n*-1		0.26 ± 0.03	1.76 ± 0.29	0.8	30.2
16:0		14.26 ± 0.20	13.14 ± 0.30	0.8	35.0
16:1		3.48 ± 0.14	2.18 ± 0.15	0.7	39.3
16:1*n*-7		5.71 ± 0.26	5.94 ± 0.27	0.6	43.2
20:4*n*-6		4.30 ± 0.21	4.63 ± 0.30	0.6	47.2
18:4*n*-3		3.45 ± 0.13	2.28 ± 0.14	0.6	51.1

Figure 3.1. *Strongylocentrotus droebachiensis*. Lipid content of gonads from laboratory-fed (shaded bars) and wild-collected (unshaded bars) sea urchins. Data are mean lipid weight expressed as % of gonad wet weight + SE. Letters above bars indicate lipid contents that are not significantly different ($p < 0.05$).

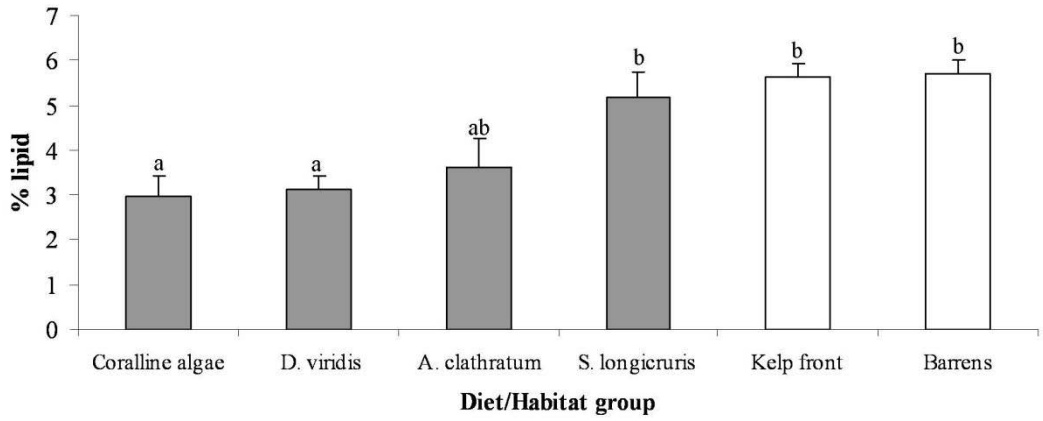
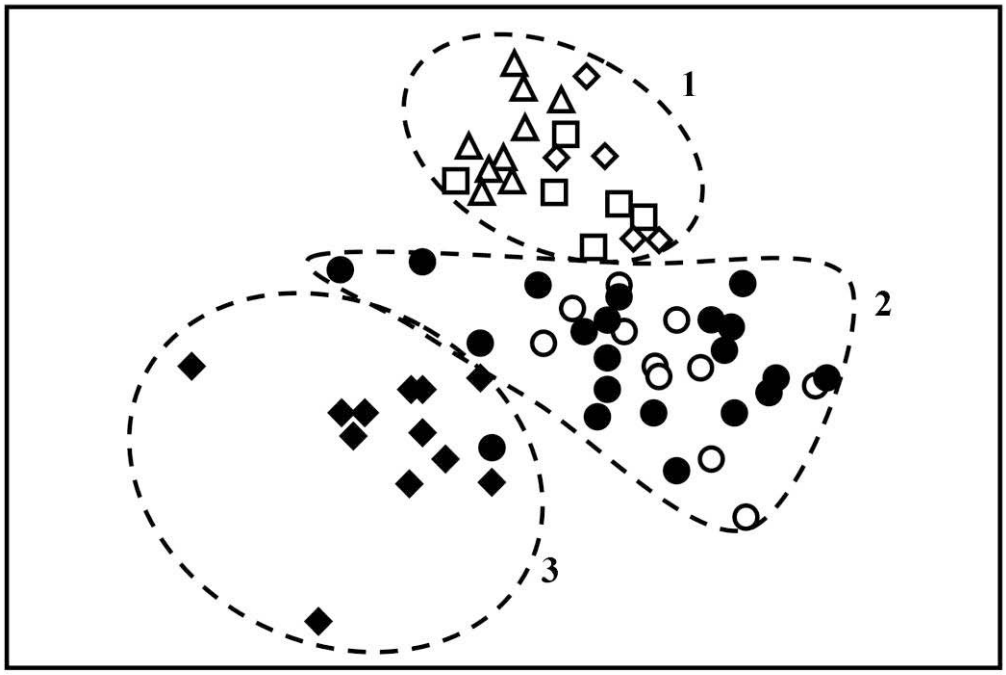


Figure 3.2. *Strongylocentrotus droebachiensis*. Multidimensional scaling (MDS) plot of Bray-Curtis similarities of fatty acid (FA) compositions of sea urchins in six diet/habitat groups, including all FAs with overall mean concentration ≥0.1%. Symbols indicate diet/habitat groups: urchins fed *Agarum clathratum* (◇), coralline algae (□), *Desmarestia viridis* (△), and *Saccharina longicruris* (○) in the laboratory, and wild-collected urchins from barrens (◆) and a kelp feeding front (●). Broken lines (--) indicate homogenous clusters 1-3 as determined by ANOSIM pairwise comparisons (Bonferroni-adjusted α=0.05)



CHAPTER 4: FATTY ACID TRACERS FOR NATIVE AND INVASIVE MACROALGAE IN AN EXPERIMENTAL FOOD WEB

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4.1 ABSTRACT

We assessed the potential of fatty acid (FA) markers for tracing primary production from an invasive green alga (*Codium fragile* ssp. *fragile*) and a native kelp (*Saccharina longicruris*) through two trophic levels in an experimental food web: a primary consumer, the green sea urchin (*Strongylocentrotus droebachiensis*), and two secondary consumers, the native rock crab (*Cancer irroratus*) and invasive green crab (*Carcinus maenas*). Urchins fed the two algal species had distinct gonadal FA compositions, and contained markers of each alga. Crabs of each species were then fed the gonads of urchins fed either *S. longicruris* or *C. fragile*, and their hepatopancreases analyzed. We were able to distinguish crabs of both species with *C. fragile* at the base of the food chain using the marker FAs 16:3*n*-3 and 18:3*n*-3. The overall FA composition of the hepatopancreas differed with diet in the green crab but not the rock crab. Our results suggest that 16:3*n*-3 may be a useful marker for tracing *C. fragile* production in grazers and some secondary consumers in rocky subtidal habitats in the Northwest Atlantic, but that signal attenuation with each trophic transfer will limit the utility of this approach in higher consumers.

4.2 INTRODUCTION

The development of dietary tracer techniques such as stable isotope (SI) and fatty acid (FA) analysis has enabled increasingly comprehensive studies of trophic relationships among marine organisms (Dalsgaard et al. 2003, Iverson et al. 2004, Budge et al. 2006, Rooker et al. 2006, Wai et al. 2008). Unlike analysis of gut contents or fecal pellets, these tracers can provide relatively long-term dietary data that are not biased toward food items with indigestible hard parts. Tracers may provide an additional advantage for studying food webs in that their signals may persist through multiple trophic transfers

(Hall et al. 2006). Although both SI and FA analysis have been used effectively in studies of marine food webs, FAs may be preferable for delineating trophic relationships in some systems because they can provide finer resolution and more discriminatory power than SIs (Kharlamenko et al. 2001, Iverson et al. 2004, Rooker et al. 2006).

Marine primary producers, including bacteria, diatoms, dinoflagellates, angiosperms and macroalgae, can be distinguished by characteristic "marker FAs", as well as combinations of FAs (Sargent et al. 1988, Graeve et al. 2002). These FAs are often conserved through trophic levels such that the FA patterns of consumers may be traced to their food sources (Graeve et al. 1994, Iverson et al. 1997). While quantitative transfer of whole combinations of FAs (the "FA signature"; Iverson et al. 1993) has been clearly demonstrated near the top of food webs – i.e. from prey to vertebrate predators (Iverson et al. 2004, 2007), this may be less likely at the base of food webs because of extensive FA biosynthesis and modification in invertebrate primary consumers. Nevertheless, FAs have been used to distinguish the sources of primary production consumed by grazers in a number of marine systems (Kharlamenko et al. 2001, Meziane et al. 2006, Rooker et al. 2006). In cases where a food source of interest is readily distinguishable based on the presence of a unique marker FA or an unusually large quantity of one or more FAs, it may be possible to trace primary producers through multiple trophic levels to higher-order consumers (Hall et al. 2006). Thus, FA analysis may be particularly useful for elucidating the role of non-native algal species in the food webs of invaded ecosystems if the non-native species differ from native species in their overall FA composition, or in their relative abundance of particular marker FAs.

The rocky subtidal ecosystem in Atlantic Canada presents an ideal model system for testing the transfer of FA markers across multiple trophic levels, and for tracking a non-native primary producer through a food web using FA analysis. This ecosystem historically has alternated between two states: kelp beds (mostly *Saccharina longicruris* and *Laminaria digitata*) with a low density of sea urchins (*Strongylocentrotus droebachiensis*), and barren grounds dominated by urchins and encrusting coralline algae (Scheibling 1986). Facilitative interactions between recently introduced non-native

species have brought about a third state of this ecosystem, in which an invasive green alga, *Codium fragile* ssp. *fragile* (previously *Codium fragile* ssp. *tomentosoides*, Provan et al. 2008; hereafter *C. fragile*) , has replaced kelp as the dominant canopy-forming macroalga off Nova Scotia and in the Gulf of Maine (Harris & Tyrell 2001, Chapman et al. 2002, Levin et al. 2002). The fate of *C. fragile* production is largely unknown, as it contains a chemical that may deter herbivores in the wild (Lyons et al. 2007, Lyons & Scheibling 2008). Although urchins consume *C. fragile* in a single-diet situation, it is a low-preference food that supports a low level of gonad production compared to kelp (Prince & LeBlanc 1992, Scheibling & Anthony 2001, Sumi & Scheibling 2005, Lyons & Scheibling 2007*a*, *b*). FA analysis may be useful for revealing the role of *C. fragile* in nearshore rocky subtidal food webs by determining whether *C. fragile* is consumed by the dominant grazers, and whether higher-order consumers prey on species that consume *C. fragile*.

Using FA signatures as dietary tracers in any system requires identification of FA markers in potential food species, and controlled feeding experiments to determine whether these markers are conserved in the consumer (Budge et al. 2006, Hall et al. 2006). A few studies have used FAs to trace the transfer of organic matter from primary producers to invertebrate consumers in nearshore benthic ecosystems (Nichols et al. 1986, Kharlamenko et al. 2001, Alfaro et al. 2006, Meziane et al. 2006), and Hall et al. (2006) identified marker FAs that could be traced through two trophic transfers, from primary producer to secondary consumer, in a controlled feeding experiment. Sea urchins (*Strongylocentrotus droebachiensis*) fed different algal diets in the laboratory can be distinguished based on their gonadal FA signatures, but these do not resemble the FA signatures of their food because of their extensive FA modification (Cook et al. 2000, Castell et al. 2004, Kelly et al. 2008). It may be possible, however, to distinguish the basal source of primary production in a multi-level food web including urchins by identifying markers of specific primary producers, or of urchins that consumed these primary producers, and finding these marker FAs in higher-order consumers. If FA markers can be used to distinguish organisms that use native kelp as their basal food source from those that use the non-native *Codium fragile*, this technique could be applied

in field studies to compare the relative contribution of each species to higher trophic levels.

In this study, we use FA analysis to trace two primary producers, non-native *Codium fragile* and native kelp (*Saccharina longicruris*) through an experimental food web. We address the following specific questions: (1) Are there individual FA markers or characteristic FA ratios that can be used to track the fate of either algal species in grazers and higher-order consumers? (2) How does FA composition differ between sea urchins fed one or the other algal species? (3) How are the nutritional status and FA composition of the next trophic level (crabs fed urchins) affected by the source of primary production utilized by their prey?

4.3 MATERIALS AND METHODS

4.3.1 Experimental Design

We constructed an experimental food web consisting of three trophic levels: 1) the native kelp *Saccharina longicruris* and the invasive green alga *Codium fragile* as primary producers; 2) the green sea urchin *Strongylocentrotus droebachiensis* as a herbivore that consumes either *S. longicruris* or *C. fragile*; and 3) the native rock crab (*Cancer irroratus*) and invasive green crab (*Carcinus maenas*) as predators that consume urchins fed either *S. longicruris* or *C. fragile*. These species co-occur in shallow (< 25 m depth) rocky habitats along the Atlantic coast of Nova Scotia and the Gulf of Maine (Harris & Tyrell 2001, Chapman et al. 2002, Levin et al. 2002). *S. longicruris* is a preferred food of sea urchins and supports high rates of their growth and reproduction (Scheibling and Hatcher 2007). *C. fragile* is a low-preference food, although urchins consume it at high rates in the absence of kelps or other fleshy algae (Sumi & Scheibling 2005, Lyons & Scheibling 2007*a*, 2008). Both crab species are generalist predators and scavengers. The rock crab commonly preys on urchins in the wild (Himmelman & Steele 1971, Scheibling 1984), while the green crab typically does not (Scheibling & Hamm 1991).

Experimental organisms were collected using SCUBA at sites near Halifax, Nova Scotia. *Saccharina longicruris* was collected at Splitnose Point (44º28’ N, 63º32’ W) and

Codium fragile was collected at Cranberry Cove (44º28'N, 63º56' W) as needed throughout the experiment. Algae were maintained in aquaria supplied with flowing seawater. Sea urchins were collected from a grazing front at the margin of the kelp bed at Splitnose Point, in June and August 2007. Urchins were maintained in flowing seawater and fed *ad libitum* either *S. longicruris* or *C. fragile*. 240 urchins collected in June were held in eight 75-L glass aquaria (30 per aquarium) for 12 weeks. By August, these urchins were beginning to show signs of stress (drooping spines, decreased feeding) associated with increasing water temperature (17-18 ºC; Lyons & Scheibling 2007*b*). Therefore, we collected an additional 400 urchins and held them in two adjacent 1900-L (150 x 150 x 85 cm) fiberglass tanks with greater water flow and thermal buffering capacity. In October, remaining urchins from the 75-L aquaria were transferred to the 1900-L tanks. Uneaten algal rations in all tanks were removed and replaced with fresh algae twice per week. The change in wet weight of algae in each aquarium each week (11 June to 22 August 2007) was measured to calculate consumption rates.

Rock crabs and green crabs were collected at Duncan's Cove (44º30'N, 63º31' W) in August 2007. Crabs were held without food in four fiberglass tanks supplied with flowing seawater for one week after collection. Individuals of both species were then allocated randomly to the four tanks (20 crabs per tank), and each diet (gonads of urchins fed either *Saccharina longicruris* or *Codium fragile*) was assigned randomly to two replicate tanks. Individual crabs were placed in plastic containers (1 L volume), perforated (5-mm diameter holes) to allow water flow, within the tanks. Crabs were fed once per week for 14 weeks with 1 g of freshly dissected urchin gonad. Gonad tissue from ≥2 urchins was fed to each crab at each feeding event, and we observed crabs until all urchin gonad tissue was consumed. From 28 August to 9 October, urchins from the 75 L aquaria (which had been fed a single algal diet for a minimum of 12 weeks) were used to feed crabs; thereafter, urchins from the larger tanks (which had been fed a single algal diet for a minimum of 10 weeks) were used to feed crabs. Urchins were selected haphazardly for crab feeding and, because populations of *Strongylocentrotus droebachiensis* have a 1:1 sex ratio (Meidel & Scheibling 1998), crabs likely consumed similar proportions of male and female urchin gonads.

4.3.2 Lipid Extraction and FA Analysis

Gonadal tissue was excised from 3-6 urchins from each algal diet at on 28 August, 4 September, 17 October, and 1, 15 and 28 November 2007. Hepatopancreases were excised from all crabs (euthanized by freezing) on 5-6 December 2007. To extract lipids from urchin gonads and crab hepatopancreas, a 1.5 g tissue sample from each animal was immediately transferred to a tube containing 20 ml chloroform and 10 ml methanol containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. Tissue samples were manually homogenized, flushed with nitrogen and stored at 4°C for 24-48h prior to lipid extraction. Algae were blotted to remove excess water and cleaned of epibionts before being chopped coarsely and manually homogenized using mortar and pestle. A 9.0 g sample from each algal thallus (n = 5 for each species) was weighed before adding 20 ml chloroform and 10 ml methanol containing 0.01% BHT.

Lipid extraction protocols were modified from Folch et al. (1957) as described in Kelly et al. (2008). The samples in solvent (algae, urchin gonad or crab hepatopancreas) were filtered (Fisher P8 filter paper) to remove any tissue before adding 7 ml 5% NaCl. Samples were shaken, then centrifuged for 20 min. The lipid-containing lower phase was filtered through $NaSO_4$ and evaporated to dryness under nitrogen at 25-30°C in a pre-weighed boiling tube. The boiling tube was re-weighed to calculate the amount of crude lipid for each sample.

Crude lipid samples were purified to remove proteins, pigments and cholesterol using thin-layer chromatography (TLC). Lipids were dissolved in hexane and spotted onto silica TLC plates (Sigma) and developed in a TLC chamber with 90:10:1 petroleum ether: ethyl ether: acetic acid for approximately 45 min. Plates were sprayed with 2,4 dichlorofluorescein and viewed under UV light to mark the lipid-containing band. The lipid-containing band was then scraped from the silica plate and transferred to a funnel packed with silane-treated glass wool. Chloroform containing 0.01% BHT was used to dissolve purified lipid into a pre-weighed tube. The sample was evaporated to dryness under nitrogen at 25-30°C, and the purified lipid was dissolved in 1.5 ml methylene

chloride with 0.01% BHT and subjected to acidic transesterification (H_2SO_4 in methanol) to produce FAME, as described in Budge et al. (2006). Following transesterification, hexane was added to a final concentration of 50 mg ml^{-1}. Samples were stored at -20°C prior to analysis. Duplicate samples of FAME were analyzed and FAs identified and quantified using temperature-programmed gas chromatography according to Iverson et al. (1997, 2004) and Budge et al. (2006). FAs are expressed as weight percent of total FAs and named as carbon number:number of double bonds and location (*n*-x) of the double bond nearest the terminal methyl group, where all additional double bonds are separated by a -CH2- group (i.e., "methylene-interrupted"). In non-methylene interrupted (NMI) FA, double bonds are separated by more than one methylene group and therefore named as each position (Δ) from the carboxyl end.

4.3.3. Statistical methods

Algal consumption rates, measured as the change in wet weight of algae in each aquarium each week (11 June to 22 August 2007) divided by the number of urchins in the aquarium, were compared between algal diets using repeated-measures ANOVA. Lipid content (% wet wt) of the two algal species, and of gonads of urchins on the two algal diets, was compared using t-tests. Lipid content of algae and urchins, and algal consumption data were homoscedastic (F-ratio test $p > 0.05$). Lipid content of crab hepatopancreas tissue was compared between crab species and diet treatments using two-way ANOVA. Crab lipid content data were heteroscedastic, so these were arcsine-transformed ($x' = \sin^{-1}\sqrt{x}$) to produce equal variances (Cochran's Q, $p > 0.05$).

Only FAs with an overall mean contribution ≥0.1% were used and FA composition was standardized to 100% for all samples prior to analysis. Comparisons of overall FA composition were made using one-way analysis of similarity (ANOSIM) and multidimensional scaling (MDS). ANOSIM and MDS were performed on Bray-Curtis distances (Clarke & Warwick 1994) of untransformed data using PRIMER 6 software (Clarke and Gorley 2006).

Levels of selected FAs were compared between algal species, between gonads of urchins on the two algal diets, and between hepatopancreases of each crab species on the two urchin diets using t-tests with Bonferroni-adjusted p-values (experimentwise $\alpha = 0.05$). The selection of these FAs was based on markers identified for *Codium fragile* (16:3*n*-3 and 18:3*n*-3; Khotimchenko 2003) and *Saccharina longicruris* (18:4*n*-3 and 20:4*n*-6; Cook et al. 2000). All FAs selected for pairwise comparisons were tested for homoscedasticity prior to performing t-tests (F-ratio test; $\alpha = 0.05$). Welch's t-test was used for data that did not meet the assumption of equal variances.

Levels of other FAs were compared across trophic levels; these FAs were selected based on known precursors for polyunsaturated FA (PUFA) synthesis (16:0, 18:0, 16:1*n*-7, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3; Dalsgaard et al. 1993). Individual FAs were compared between trophic levels using 2-way ANOVA with trophic level (algae vs. urchins, urchins vs. green crabs or red crabs) and primary production source (*Saccharina longicruris* or *Codium fragile*) as fixed factors. The total abundance of FAs found in urchins and crabs, but absent from algae, was compared between urchins on the two algal diets using a t-test, and between crabs using a 2-way ANOVA. These data were tested for homoscedasticity (Cochran's Q; $\alpha = 0.05$); the arcsine transformation was used where it produced equal variances, but untransformed data were used where transformation did not improve homoscedasticity.

4.4 RESULTS

Lipid content of algae samples ranged from 0.23% to 0.93% of wet weight in *Saccharina longicruris* and from 0.56% to 1.23% of wet weight in *Codium fragile*, but the two species were not significantly different (Table 4.1; Fig 4.1). FA composition of *S. longicruris* differed significantly from that of *C. fragile* (ANOSIM $p = 0.002$; Fig 4.2a). Average Bray-Curtis dissimilarity between algal species was 42%. *C. fragile* contained a significantly higher proportion of marker FAs 16:3*n*-3 and 18:3*n*-3 than did *S. longicruris*; *S. longicruris* contained a significantly higher proportion of markers 18:4*n*-3 and 20:4*n*-6 (Table 4.1, Fig 4.3).

Gonad lipid content of urchins fed *Codium fragile* was significantly higher than that of urchins fed *Saccharina longicruris* (Table 4.1, Fig 4.1). Urchins consumed *C. fragile* at a significantly greater rate than *S. longicruris*: from 11 June to 22 Aug 2007, mean ± SE consumption rates per urchin (calculated on a per aquarium basis, n = 4 per algal diet) were 1.53 ± 0.07 g d^{-1} on *C. fragile* and 1.09 ± g d^{-1} on *S. longicruris*. Algal species and time both had significant effects on consumption rate ($p = 0.011$ and 0.004, respectively) but there was no significant interaction ($p = 0.568$).

Gonad FA composition differed significantly between urchins fed *Codium fragile* and urchins fed *Saccharina longicruris* in both the 75-L (ANOSIM $p < 0.001$) and 1900-L tanks (ANOSIM $p = 0.029$), and sampling date was non-significant for each diet and tank size ($p > 0.05$); therefore data were pooled for further analyses. Within-group Bray-Curtis similarity was 88% for both urchins fed *C. fragile* and those fed *S. longicruris*. Between-group dissimilarity was 14% for urchins on the two algal diets.

All FAs present in algae were also present in both groups of urchins. The marker FAs identified as more abundant in *Codium fragile*, 16:3*n*-3 and 18:3*n*-3, also were more abundant in gonads of urchins fed *C. fragile* than in gonads of urchins fed *Saccharina longicruris* (Table 4.1, Fig 4.3). Of the marker FAs identified as more abundant in *S. longicruris*, 20:4*n*-6 was more abundant in gonads of urchins fed *S. longicruris*, but there was no difference related to diet for 18:4*n*-3 (Table 4.1, Fig 4.3).

Of the precursor FAs (16:0, 18:0, 16:1*n*-7, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3), all were significantly more abundant in algae than in urchins, except for 16:1*n*-7, which was more abundant in urchins (Table 4.2, Fig 4.4). The source of primary production (i.e. *Codium fragile* or *Saccharina longicruris*) was a significant factor for 18:0 and 18:2*n*-6, which were more abundant in *S. longicruris*, and for 16:0 and 18:3*n*-3, which were more abundant in *C. fragile* (Table 4.2, Fig 4.4). The interactive effect of primary production source and trophic level was significant for all precursor FAs except 16:1*n*-7 (Table 4.2). These precursor FAs comprised a greater proportion of the total FAs in *C. fragile* (58.5 ±

0.8% SE) than in *S. longicruris* (52.2 ± 3.0%), although this difference was marginally non-significant (Table 4.1).

Twenty-five additional FAs were present in urchin gonads but either absent from algae or present only in trace amounts (average < 0.10%), including 18-carbon PUFAs, 20-, 22- and 24-carbon monounsaturates, 20:2 and 22:2 NMI dienes, 21:1 and certain omega-3 and omega-6 PUFAs (Table 4.3). These FAs comprised a greater proportion of total FAs in urchins fed *Codium fragile* (32.5 ± 0.5%) than in urchins fed *Saccharina longicruris* (28.3 ± 0.9%) (Table 4.1).

The hepatopancreas of rock crabs contained slightly more lipid than that of the green crabs ($p = 0.057$) but there was no effect of diet on hepatopancreas lipid content ($p = 0.915$) and no interaction between crab species and diet ($p = 0.333$; Fig 4.1). FA composition of green crabs differed significantly between diet treatments ($p = 0.031$; Fig 4.2c); average Bray-Curtis dissimilarity between individuals in the two diet treatments was 15%. There was no effect of diet on overall FA composition of rock crabs ($p = 0.7$; Fig 4.2d); average Bray-Curtis dissimilarity between individuals in the two diet treatments was 20.2%.

All FAs present in urchin gonads also were present in the hepatopancreas of both crab species, and no additional FAs were detected in crab hepatopancreas tissues. Of the marker FAs identified in algae and urchins, the *Codium fragile* marker 18:3*n*-3 was significantly more abundant in crabs of both species in *C. fragile* food chains, and 16:3*n*-3 was significantly more abundant in green crabs in the *C. fragile* food chain than those in the *Saccharina longicruris* food chain (Table 4.1, Fig 4.3). The relative abundance of markers identified for *Saccharina longicruris* (18:4*n*-3 and 20:4*n*-6) was not affected by diet for crabs of either species (Table 4.1, Fig 4.3). The precursor FAs 16:0, 18:0, 16:1*n*-7, 18:1*n*-9 and 18:3*n*-3 were significantly more abundant in urchins than in either crab species (Table 4.2, Fig 4.4).

4.5 DISCUSSION

We examined the ability of FA data to distinguish sources of primary production contributing to higher trophic levels in an experimental food web based on two algal species (native *Saccharina longicruris* and invasive *Codium fragile*), with one primary consumer (sea urchins) and two secondary consumers (native rock crabs and invasive green crabs). We found two potential markers of *C. fragile* that persisted through both trophic transfers; however, their relative abundance decreased with each transfer, indicating the limited utility of these markers for larger-scale food web studies.

The two algal species in this study, *Saccharina longicruris* and *Codium fragile*, were distinct in their FA compositions, and these were similar to the FA compositions reported by Cook et al. (2000) for *S. longicruris* and by Khotimchenko (2003) for *C. fragile*. We identified 20:4*n*-6 and 18:4*n*-3 as potential marker FAs for *S. longicruris*, and 16:3*n*-3 and 18:3*n*-3 as potential marker FAs for *C. fragile*. FAs 20:4*n*-6 and 18:4*n*-3 are dominant in brown macroalgae and are useful as general markers for the group (Graeve et al. 2002), but would not distinguish *S. longicruris* from other kelps present in the rocky subtidal ecosystem in Atlantic Canada, such as *Laminaria digitata.* FA 18:3*n*-3 is both a major constituent in green macroalgae (Graeve et al. 2002, Khotimchenko 2003) and a precursor for a major highly unsaturated FA (HUFA) synthesis pathway, so its utility in distinguishing *C. fragile* in field studies may be limited. However, significant quantities of 16:3*n*-3 have been detected only in *C. fragile* and two other genera of green algae (Khotimchenko 1995, 2003), making it a potentially useful marker for *C. fragile*.

The higher consumption rate of *Codium fragile* than *Saccharina longicruris* by urchins has been reported previously (Scheibling & Anthony 2001, Sumi & Scheibling 2005, Lyons & Scheibling 2007*a*, *b*) and attributed to compensatory feeding on *C. fragile* because of its lower nutritional quality compared to kelp (Lyons & Scheibling 2007*a*). Gonads of sea urchins fed the two algal diets differed significantly in their FA composition, although differences between the urchins were less pronounced than differences between their diets. Urchins modify dietary FAs substantially such that their overall FA composition does not closely resemble that of their diets (Castell et al. 2004,

Kelly et al. 2008). The presence of FAs in urchin gonads that were absent from their diets indicates that FA biosynthesis occurred in urchins on both algal diets (Castell et al. 2004). Many marine organisms have a limited capacity for HUFA biosynthesis, so synthesis of these FAs by urchins from precursors in macroalgae may represent an important link in rocky subtidal food webs (Dalsgaard et al. 2003, Hall et al. 2006).

Certain saturated and monounsaturated FAs and the PUFAs 18:2*n*-6 and 18:3*n*-3 decreased in relative abundance from algae to urchins, likely indicating that these were used as precursors for synthesis of other FAs (Castell et al. 2004). The higher proportion of urchin-synthesized FAs found in gonads of urchins in the *Codium fragile* food chain may be a result of a higher proportion of precursor FAs found in *C. fragile* than in *Saccharina longicruris*. Data from Castell et al. (2004) support this hypothesis: urchins fed artificial diets high in precursor FAs (94-96% of total FAs) had a higher proportion of urchin-synthesized FAs in their gonads than those fed a similar diet with fewer precursors (55%) or kelp, with the lowest proportion of precursors (34%).

The source of primary production at the base of the food chain significantly affected overall FA composition of the invasive green crab but not of the native rock crab. Crustaceans generally have a limited ability to synthesize HUFAs such as 20:4*n*-6 and 20:5*n*-3 from C_{18} precursors such as 18:2*n*-6 and 18:3*n*-3 (Gonzalez-Felix et al. 2002). However, our results show an increase in the relative abundance of these HUFAs between urchins and crabs regardless of urchin diet or crab species. This suggests that crabs must either synthesize or preferentially retain these HUFAs. The presence of 20:2*n*-6 in both crab species suggests that dietary 18:2*n*-6 is converted to 20:4*n*-6, as 20:2*n*-6 is an intermediate in this metabolic pathway (Merican & Shim 1996). Further evidence of FA modification by crabs was provided by the lower levels of potential precursor FAs in crabs than in urchins.

The persistence of markers for *Codium fragile* but not *Saccharina longicruris* in the food web, and the distinct FA compositions of green crabs but not rock crabs on different diets, may be related to the feeding ecology of both urchins and crabs. The green sea

urchin has evolved to feed on native seaweeds, such as *S. longicruris*, and is unaccustomed to consuming the invasive alga *C. fragile*. Desaturase enzymes in the urchin may be present in quantities appropriate for native food sources, but insufficient for certain novel food items. Thus, *C. fragile* markers in urchin tissues may represent the quantity of 16:3*n*-3 and 18:3*n*-3 in *C. fragile* that exceeds the processing capacity of Δ-6 desaturase in the urchin. Similarly, markers present in the invasive green crab may represent FAs in excess of its enzymatic processing capacity, as it does not normally consume urchins. Because the rock crab is a natural predator of sea urchins, it may have the enzymatic capacity to process FAs present in urchins, resulting in no marker FAs in the kelp food chain and no overall difference between rock crabs on the two diets. This pattern merits further research, as it may suggest the utility of FA analysis for detecting invasive primary producers at higher trophic levels, and for analyzing the diet of invasive consumers.

Other studies that have traced FA markers through multiple trophic transfers have focused on a food chain with a single source of primary production (Hall et al. 2006, Shin et al. 2008). Our study is one of the few that have compared levels of potential marker FAs between animals on different diets in order to assess their validity as tracers in a particular food web. Our results concur generally with those of Hall et al. (2006) in that we also were able to detect several markers in the first trophic transfer and fewer in the second transfer, and the relative abundance of marker FAs decreased with each trophic transfer. Both Hall et al. (2006) and Shin et al. (2008) found an increase in the relative abundance of 20:4*n*-6 at each trophic transfer. Our results were similar for the second trophic transfer, indicating either synthesis or selective retention of this FA by both crab species.

We were able to detect *Codium fragile* as the primary producer using the FA markers for the invasive alga (16:3*n*-3 and 18:3*n*-3). Of the two markers, 18:3*n*-3 is less likely to be useful in field studies because it is a general marker for green algae and ubiquitous in marine systems, as well as being a common precursor for FA synthesis (Hall et al. 2006). Signal attenuation is also likely to limit the usefulness of FA markers in this food web, as

the magnitude of difference between diets decreased with each trophic transfer. Differences between crab species in the overall effect of diet on their FA composition indicates that the effects of primary producers may vary across consumer taxa, and thus specific food chains of interest should be tested in the laboratory before attempting to use FA analysis in field studies.

4.6 ACKNOWLEDGEMENTS

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4.7 TABLES AND FIGURES

Table 4.1. T-tests on relative abundance of fatty acid markers in organisms which differed in the source of primary production at their food web base (either *Codium fragile* or *Saccharina longicruris*). Precursor fatty acids were 16:0, 16:1*n*-7, 18:0, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3. Urchin-synthesized fatty acids included 25 fatty acids present in urchin gonads but absent from their algal diets. Results that were significant following Bonferroni correction (experimentwise $\alpha = 0.05$) are indicated with *.

	Variable	df	t	p	
Algae	% lipid	8	1.59	0.150	
	16:3*n*-3	4	12.33	0.000	*
	18:3*n*-3	5	12.07	0.000	*
	18:4*n*-3	5	-6.58	0.001	*
	20:4*n*-6	4	2.13	0.001	*
	Σ precursor FAs	4	2.36	0.078	
Urchins	% lipid	36	2.63	0.012	*
	16:3*n*-3	17	4.60	0.000	*
	18:3*n*-3	20	4.26	0.000	*
	18:4*n*-3	31	-2.03	0.052	
	20:4*n*-6	36	-3.98	0.000	*
	Σ precursor FAs	26	-0.70	0.492	
	Σ urchin-synthesized FAs	36	5.57	0.000	*
Green crabs	% lipid	32	1.32	0.196	
	16:3*n*-3	22	3.25	0.004	*
	18:3*n*-3	32	6.87	0.000	*
	18:4*n*-3	32	1.09	0.285	
	20:4*n*-6	32	-1.37	0.179	
Rock crabs	% lipid	39	-0.60	0.549	
	16:3*n*-3	37	2.41	0.021	
	18:3*n*-3	27	3.46	0.002	*
	18:4*n*-3	37	0.46	0.647	
	20:4*n*-6	37	-0.82	0.415	

Table 4.2. Two-way ANOVAs on relative abundance of selected fatty acids in organisms in the experimental food web with trophic level (primary producer, primary consumer or secondary consumer) and source of primary production at food web base (*Codium fragile* or *Saccharina longicruris*) as fixed factors. Results that were significant following Bonferroni correction (experimentwise $\alpha = 0.05$) are indicated with *.

		Algae vs. urchins			Urchins vs. *Carcinus maenas*			Urchins vs. *Cancer irroratus*		
	Source of variation	$F_{1,45}$	p		$F_{1,68}$	p		$F_{1,73}$	p	
16:0	Trophic level	103.45	0.000	*	180.86	0.000	*	133.97	0.000	*
	Primary producer	12.98	0.001	*	2.15	0.147		0.91	0.343	
	Trophic level x 1° producer	22.27	0.000	*	0.72	0.398		0.89	0.350	
16:1*n*-7	Trophic level	27.59	0.000	*	60.75	0.000	*	14.50	0.000	*
	Primary producer	0.82	0.370		0.86	0.356		0.19	0.664	
	Trophic level x 1° producer	0.38	0.543		3.37	0.071		1.95	0.167	
18:0	Trophic level	112.77	0.000	*	111.40	0.000	*	235.32	0.000	*
	Primary producer	7.22	0.010		0.28	0.596		1.30	0.258	
	Trophic level x 1° producer	19.71	0.000	*	4.05	0.048		1.68	0.199	
18:1*n*-9	Trophic level	100.70	0.000	*	186.50	0.000	*	100.99	0.000	*
	Primary producer	25.38	0.000	*	5.16	0.026		0.58	0.450	
	Trophic level x 1° producer	21.15	0.000	*	0.82	0.369		0.06	0.810	
18:2*n*-6	Trophic level	1171.46	0.000	*	5.73	0.019		0.31	0.581	
	Primary producer	90.27	0.000	*	0.48	0.493		0.80	0.376	
	Trophic level x 1° producer	82.57	0.000	*	0.20	0.653		0.12	0.728	
18:3*n*-3	Trophic level	852.08	0.000	*	48.03	0.000	*	73.97	0.000	*

	Algae vs. urchins			Urchins vs. *Carcinus maenas*			Urchins vs. *Cancer irroratus*		
Source of variation	$F_{1,45}$	p		$F_{1,68}$	p		$F_{1,73}$	p	
Primary producer	455.67	0.000	*	25.64	0.000	*	26.09	0.000	*
	346.34	0.000		10.75	0.002		13.88	0.000	*
Trophic level x 1° producer			*			*			

Table 4.3 Relative fatty acid composition of organisms in experimental food web: macroalgae (*Codium fragile* and *Saccharina longicruris*), sea urchin (*Strongylocentrotus droebachiensis*) gonads, green crab (*Carcinus maenas*) and rock crab (*Cancer irroratus*) hepatopancreases. Fatty acid values are mean % of total fatty acids ± SE of all fatty acids with overall mean concentration ≥0.1%.

	Algae				Sea urchin *(Strongylocentrotus droebachiensis)*				Green crab (*Carcinus maenas)*				Rock crab (*Cancer irroratus)*			
	Codium fragile		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*	
N	5		5		18		20		16		18		20		19	
12:0	1.35	0.06	0.19	0.02	0.04	0.01	0.04	0.01	0.02	0.01	0.02	0.02	0.07	0.03	0.07	0.04
13:0	0.01	0.01	0.01	0.01	0.05	0.04	0.04	0.03	0.03	0.01	0.02	0.01	0.05	0.03	0.03	0.02
14:0	1.45	0.08	7.52	0.61	10.39	1.56	14.23	2.98	3.73	1.39	3.45	1.89	3.41	1.70	3.59	1.38
14:1 *n*-9	0.07	0.02	0.04	0.01	0.13	0.02	0.15	0.04	0.05	0.03	0.06	0.04	0.12	0.07	0.10	0.05
14:1 *n*-5	1.14	0.28	0.09	0.03	0.95	0.28	1.59	0.69	0.28	0.14	0.26	0.18	0.22	0.16	0.24	0.14
*iso*15:0	0.22	0.01	0.06	0.01	0.21	0.08	0.14	0.05	0.13	0.04	0.10	0.04	0.20	0.09	0.17	0.09
15:0	0.13	0.00	0.28	0.02	0.49	0.13	0.37	0.10	0.37	0.09	0.32	0.07	0.38	0.10	0.34	0.11
iso16:0	0.06	0.00	0.01	0.01	0.05	0.04	0.05	0.02	0.53	0.42	0.71	0.38	0.48	0.58	0.51	0.43
16:0	23.49	0.55	18.77	0.96	15.31	1.68	15.97	1.12	11.71	0.81	11.89	1.02	11.59	1.33	11.60	1.80
16:1 *n*-11	4.78	0.77	1.30	0.19	0.41	0.14	0.21	0.05	0.21	0.07	0.17	0.05	0.26	0.12	0.26	0.15
16:1 *n*-9	0.33	0.03	0.12	0.03	0.32	0.05	0.24	0.04	0.24	0.05	0.24	0.07	0.23	0.05	0.22	0.07
16:1 *n*-7	3.66	0.47	2.92	0.32	5.84	1.43	6.55	1.03	4.30	0.71	4.07	1.05	5.09	2.06	4.77	1.62
16:1	0.18	0.01	0.27	0.05	2.91	0.65	3.23	0.67	1.42	0.41	1.30	0.47	0.97	0.48	0.98	0.43
iso17:0	0.78	0.13	0.02	0.00	0.04	0.02	0.04	0.02	0.16	0.10	0.17	0.11	0.30	0.25	0.24	0.13
16.3 *n*-6	0.09	0.02	0.20	0.02	0.44	0.15	0.38	0.13	0.14	0.08	0.09	0.07	0.12	0.07	0.11	0.06
17:0	0.13	0.01	0.14	0.01	0.10	0.03	0.07	0.03	0.20	0.06	0.21	0.07	0.23	0.10	0.22	0.09
16:3 *n*-4	0.11	0.02	0.16	0.03	0.29	0.13	0.23	0.07	0.06	0.034	0.06	0.04	0.07	0.04	0.07	0.04
16:3 *n*-3	11.91	0.96	0.12	0.02	1.35	1.17	0.09	0.02	0.28	0.12	0.17	0.06	0.34	0.13	0.25	0.12
16:4 *n*-3	0.11	0.03	0.00	0.00	0.11	0.03	0.08	0.04	1.69	0.98	2.14	1.17	1.42	1.07	1.50	1.04
16:4 *n*-1	0.05	0.03	0.26	0.05	1.07	0.44	0.88	0.36	0.21	0.08	0.20	0.08	0.19	0.09	0.18	0.11
18:0	2.32	0.18	3.54	0.38	1.49	0.20	1.25	0.21	2.85	0.67	3.26	1.16	2.85	0.56	2.87	0.60
18:1 *n*-13	0.11	0.01	0.01	0.00	0.46	0.08	0.42	0.11	0.52	0.10	0.48	0.12	0.66	0.30	0.71	0.38
18:1 *n*-9	5.79	0.48	13.36	2.94	1.90	0.32	2.22	0.35	4.84	1.64	5.57	1.09	7.87	3.29	8.48	3.33
18:1 *n*-7	1.33	0.06	0.56	0.16	3.87	0.49	3.72	0.60	4.17	0.36	4.41	0.32	6.42	1.79	6.60	2.08

	Algae				Sea urchin *(Strongylocentrotus droebachiensis)*				Green crab (*Carcinus maenas)*				Rock crab (*Cancer irroratus)*			
	Codium fragile		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*	
18:1 *n*-5	0.18	± 0.02	0.24	± 0.07	0.25	± 0.04	0.26	± 0.07	0.39	± 0.05	0.42	± 0.05	0.51	± 0.12	0.55	± 0.10
18:2 *n*-7	0.06	± 0.01	0.09	± 0.01	1.05	± 0.51	0.99	± 0.35	0.72	± 0.14	0.69	± 0.15	0.48	± 0.20	0.52	± 0.20
18:2 *n*-6	4.37	± 0.30	6.82	± 0.37	1.06	± 0.20	1.11	± 0.22	0.97	± 0.14	0.98	± 0.23	1.05	± 0.12	1.07	± 0.18
18:2 *n*-4	0.01	± 0.01	0.06	± 0.01	0.23	± 0.07	0.19	± 0.06	0.20	± 0.03	0.18	± 0.02	0.21	± 0.07	0.21	± 0.07
18:3 *n*-6	1.48	± 0.11	1.01	± 0.11	0.44	± 0.08	0.38	± 0.06	0.19	± 0.06	0.14	± 0.07	0.15	± 0.09	0.15	± 0.06
18:3 *n*-4	0.02	± 0.00	0.06	± 0.01	0.20	± 0.05	0.16	± 0.04	0.23	± 0.05	0.23	± 0.05	0.19	± 0.06	0.20	± 0.04
18:3 *n*-3	18.89	± 1.14	4.72	± 0.30	1.94	± 0.93	0.97	± 0.29	0.75	± 0.09	0.54	± 0.09	0.58	± 0.19	0.43	± 0.08
18:4 *n*-3	1.88	± 0.31	8.93	± 1.03	2.28	± 0.59	2.83	± 1.05	0.73	± 0.26	0.61	± 0.35	0.62	± 0.33	0.57	± 0.28
18:4 *n*-1	0.01	± 0.00	0.07	± 0.01	0.27	± 0.12	0.26	± 0.11	0.13	± 0.07	0.14	± 0.05	0.10	± 0.05	0.12	± 0.05
20:0	1.02	± 0.15	0.63	± 0.09	0.64	± 0.20	0.49	± 0.17	0.45	± 0.06	0.40	± 0.04	0.47	± 0.16	0.42	± 0.12
20:1A	0.04	± 0.01	0.01	± 0.01	4.25	± 0.61	3.56	± 0.84	4.59	± 1.14	3.54	± 1.05	3.40	± 1.89	2.91	± 1.54
20:1 *n*-11	0.10	± 0.01	0.09	± 0.02	0.67	± 0.18	0.50	± 0.14	0.70	± 0.20	0.63	± 0.21	1.31	± 0.86	1.37	± 0.68
20:1 *n*-9	0.03	± 0.00	0.01	± 0.00	4.32	± 0.87	4.22	± 0.83	4.14	± 0.78	3.95	± 0.82	3.92	± 1.55	3.99	± 1.41
20:1 *n*-7	0.00	± 0.00	0.00	± 0.00	3.70	± 0.59	3.47	± 0.67	3.29	± 0.81	2.68	± 0.97	3.51	± 1.26	3.24	± 0.86
20:2 Δ 5,11	0.01	± 0.00	0.00	± 0.00	3.89	± 0.64	3.51	± 0.77	2.82	± 0.68	2.46	± 0.75	1.82	± 1.03	1.77	± 0.95
20:2 Δ 5,13	0.00	± 0.00	0.00	± 0.00	2.67	± 0.82	2.45	± 0.71	2.21	± 0.59	1.73	± 0.53	1.65	± 0.73	1.56	± 0.65
20:2 *n*-9	0.33	± 0.03	0.00	± 0.00	0.18	± 0.08	0.19	± 0.05	0.25	± 0.06	0.25	± 0.03	0.22	± 0.10	0.22	± 0.10
20:1B	0.00	± 0.00	0.00	± 0.00	0.39	± 0.15	0.50	± 0.23	0.27	± 0.08	0.32	± 0.07	0.19	± 0.10	0.25	± 0.14
20:2 *n*-6	0.05	± 0.00	0.09	± 0.01	0.91	± 0.23	0.86	± 0.23	1.89	± 0.28	1.94	± 0.45	2.34	± 0.65	2.43	± 0.86
20:3 *n*-6	0.26	± 0.02	0.70	± 0.14	0.65	± 0.08	0.70	± 0.14	0.61	± 0.06	0.63	± 0.08	0.60	± 0.21	0.65	± 0.20
20:4 *n*-6	4.45	± 0.23	13.83	± 1.01	2.97	± 0.58	3.81	± 0.71	10.12	± 3.31	11.82	± 3.84	7.32	± 2.92	8.30	± 4.30
21:1	0.00	± 0.00	0.03	± 0.01	1.48	± 0.18	1.30	± 0.35	1.31	± 0.39	0.95	± 0.35	1.09	± 0.60	0.91	± 0.53
20:3 *n*-3	0.10	± 0.01	0.02	± 0.01	1.42	± 0.68	0.69	± 0.33	1.02	± 0.16	0.75	± 0.12	0.95	± 0.24	0.77	± 0.16
20:4 *n*-3	0.24	± 0.04	0.73	± 0.08	0.89	± 0.24	0.88	± 0.30	0.54	± 0.17	0.56	± 0.08	0.51	± 0.12	0.54	± 0.26
20:5 *n*-3	5.35	± 0.68	11.81	± 0.74	7.87	± 2.09	7.86	± 1.51	15.01	± 3.27	16.12	± 3.98	12.33	± 2.74	12.41	± 2.92
22:1 *n*-11	0.12	± 0.05	0.01	± 0.00	0.14	± 0.04	0.10	± 0.05	0.30	± 0.42	0.22	± 0.21	0.56	± 0.48	0.67	± 0.71
22:1 *n*-9	0.07	± 0.04	0.00	± 0.00	2.79	± 0.65	2.21	± 0.59	2.06	± 0.60	1.59	± 0.58	2.07	± 1.09	1.75	± 0.90
22:1 *n*-7	0.06	± 0.01	0.00	± 0.00	0.16	± 0.04	0.12	± 0.04	0.26	± 0.04	0.27	± 0.09	0.59	± 0.30	0.55	± 0.21
22:2 Δ 7,13	0.11	± 0.03	0.00	± 0.00	0.39	± 0.07	0.32	± 0.09	0.39	± 0.11	0.33	± 0.18	0.64	± 0.30	0.62	± 0.24
22:2Δ7,15	0.01	± 0.01	0.00	± 0.00	1.28	± 0.38	1.11	± 0.46	1.46	± 0.39	1.06	± 0.41	1.52	± 0.66	1.26	± 0.55

	Algae				Sea urchin *(Strongylocentrotus droebachiensis)*				Green crab (*Carcinus maenas)*				Rock crab (*Cancer irroratus)*			
	Codium fragile		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*		*Codium fragile*		*Saccharina longicruris*	
21:5 *n*-3	0.05	± 0.01	0.00	± 0.00	0.16	± 0.06	0.12	± 0.04	0.12	± 0.02	0.11	± 0.02	0.14	± 0.08	0.13	± 0.07
22:4 *n*-6	0.04	± 0.00	0.00	± 0.00	0.26	± 0.07	0.27	± 0.05	0.38	± 0.03	0.41	± 0.07	0.99	± 0.91	0.98	± 0.64
22:5 *n*-6	0.14	± 0.03	0.00	± 0.00	0.27	± 0.09	0.17	± 0.04	0.23	± 0.02	0.23	± 0.03	0.28	± 0.18	0.25	± 0.04
22:5*n*-3	0.92	± 0.07	0.05	± 0.01	0.39	± 0.13	0.31	± 0.07	0.77	± 0.25	0.81	± 0.20	1.35	± 0.86	1.36	± 0.62
22:6*n*-3	0.02	± 0.01	0.00	± 0.00	1.25	± 0.48	0.84	± 0.25	2.31	± 0.86	2.85	± 0.86	2.73	± 1.08	2.70	± 1.21

[a] double bond position currently unknown; [b] tentatively identified as 20:0 with a cyclopropane ring

Figure 4.1. Lipid content (% wet weight + SE) of algae (*Codium fragile* and *Saccharina longicruris*), gonads of sea urchins (*Strongylocentrotus droebachiensis*), and hepatopancreases of green crabs (*Carcinus maenas*) and rock crabs (*Cancer irroratus*). Unshaded bars represent organisms whose source of primary production was *C. fragile* (i.e. *C. fragile*, urchins fed *C. fragile*, and crabs fed gonads of urchins fed *C. fragile*). Shaded bars represent organisms whose source of primary production was *S. longicruris*.

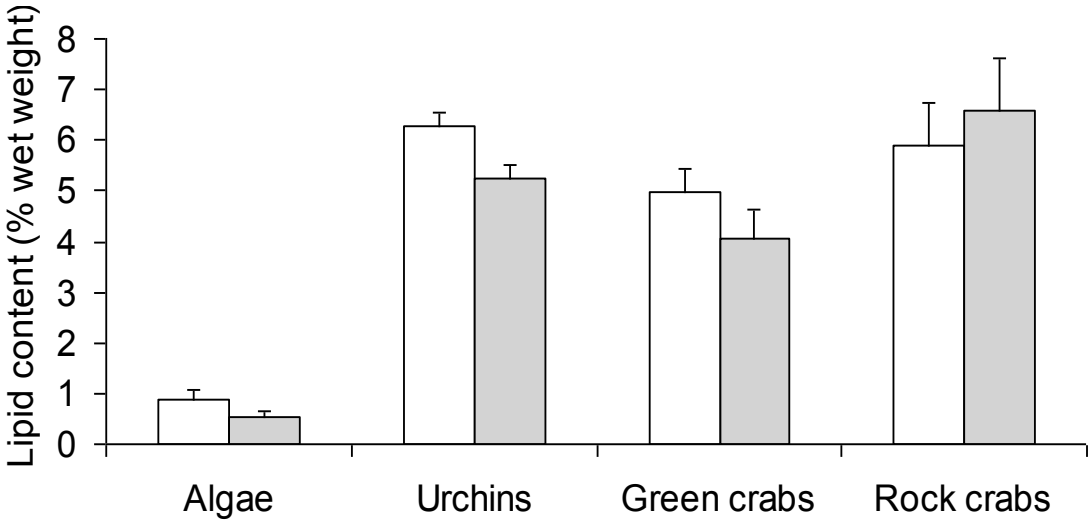


Figure 4.2. Three-dimensional multidimensional scaling (MDS) plot of Bray-Curtis similarities of fatty acid compositions of organisms with either *Codium fragile* (open circles) or *Saccharina longicruris* (filled circles) as the source of primary production. 3-D stress values are 0.01, 0.1, 0.03 and 0.05, respectively.

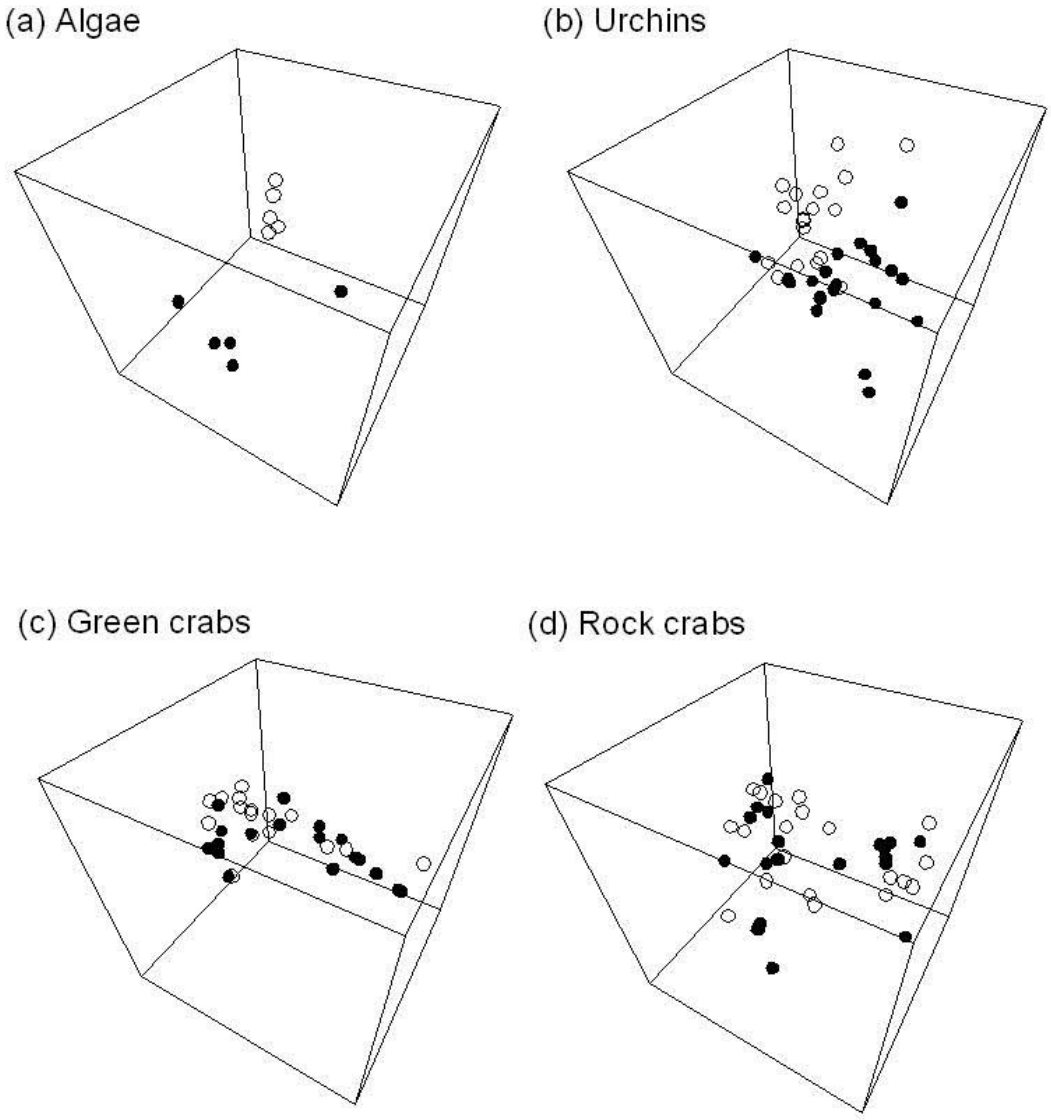


Figure 4.3. Abundance of selected marker fatty acids (as % of total FAs + SE) in algae (*Codium fragile* and *Saccharina longicruris*), gonads of sea urchins (*Strongylocentrotus droebachiensis*), and hepatopancreases of green crabs (*Carcinus maenas*) and rock crabs (*Cancer irroratus*). Unshaded bars represent organisms whose source of primary production was *C. fragile* (i.e. *C. fragile*, urchins fed *C. fragile*, and crabs fed gonads of urchins fed *C. fragile*). Shaded bars represent organisms whose source of primary production was *S. longicruris*.

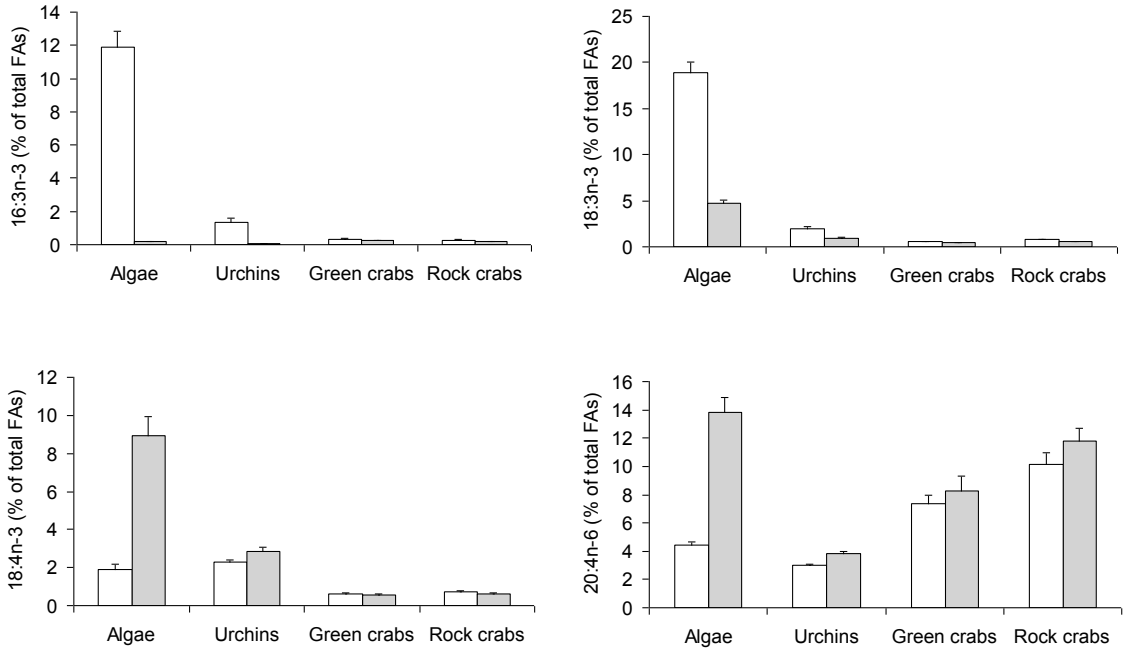
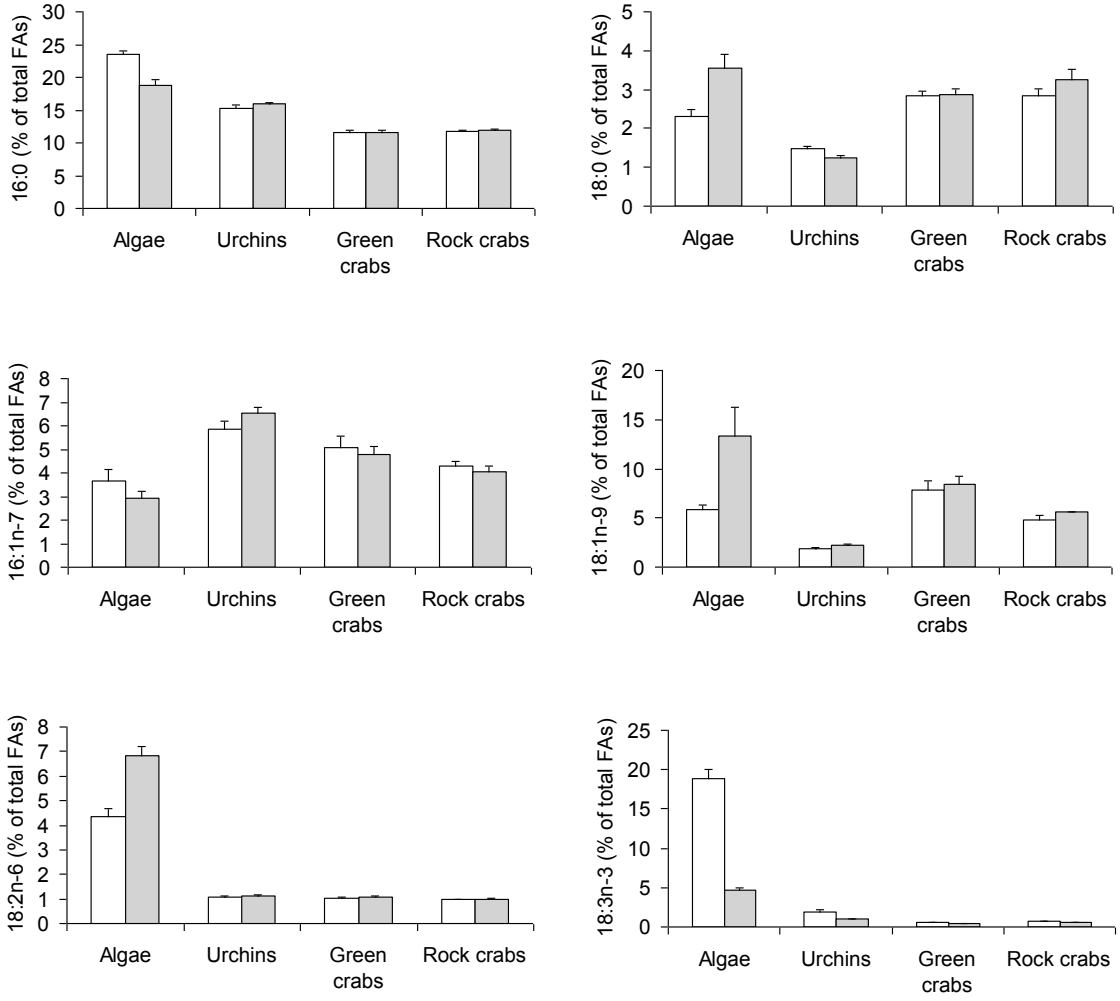


Figure 4.4. Abundance of selected precursor fatty acids (as % of total FAs + SE) in algae (*Codium fragile* and *Saccharina longicruris*), gonads of sea urchins (*Strongylocentrotus droebachiensis*), and hepatopancreases of green crabs (*Carcinus maenas*) and rock crabs (*Cancer irroratus*). Unshaded bars represent organisms whose source of primary production was *C. fragile* (i.e. *C. fragile*, urchins fed *C. fragile*, and crabs fed gonads of urchins fed *C. fragile*). Shaded bars represent organisms whose source of primary production was *S. longicruris*.



CHAPTER 5: DRIFT ALGAE SUBSIDIES TO SEA URCHINS IN LOW-PRODUCTIVITY HABITATS

This chapter has been submitted for publication to Marine Ecology Progress Series as:

Kelly JR, Krumhansl KA, Scheibling RE (2011) Drift algae subsides to sea urchins in low-productivity habitats.

5.1 ABSTRACT

Highly productive kelp beds off Nova Scotia, Canada export a large quantity of detrital material to adjacent low-productivity habitats. We used a combination of dietary tracers (fatty acids, stable isotopes, and gut contents) and gonad index to evaluate the importance and spatial extent of this energy subsidy to sea urchins (*Strongylocentrotus droebachiensis*) offshore from kelp beds along 240-m transects perpendicular to shore at 4 sites. Fatty acids, $\delta^{13}C$ values and gut contents indicated the presence of kelp in the diets of sea urchins collected up to 240 m offshore from kelp beds. We observed a corresponding decrease in gonad index with distance from the kelp at all sites but one, where patches of live kelp offshore from the main kelp bed provided an additional food source. Sea urchins feeding on a large pool of detrital kelp at another site had ~15% larger gonads than sea urchins at other locations. $\delta^{15}N$ values were more enriched for sea urchins at 160 and 240 m from the kelp bed, suggesting that these sea urchins consume more animal matter. Our findings suggest that drift kelp represents an important energy source for sea urchins in subtidal habitats on the scale of tens to hundreds of meters offshore from kelp beds, and that this resource is increasingly patchy in space and time with distance from the kelp bed.

5.2 INTRODUCTION

Trophic linkages between discrete habitats are a common feature of marine ecosystems (Kirkman & Kendrick 1997, Polis et al. 1997, Heck et al. 2008). Transfer of macrophyte detritus from a habitat with high productivity, such as kelp forests or seagrass beds, to one with lower productivity, such as mudflats, the rocky intertidal or the deep sea, can be an important determinant of community structure and secondary production in the subsidized habitat (Polis et al. 1997, Vetter 1998, Heck et al. 2008, Vanderklift &

Wernberg 2008). Detrital transport across the seafloor is wave- and current-driven and therefore affected by regional-scale oceanographic processes, seasonal storms, and bottom topography (Bustamante & Branch 1996, Vetter 1998, Vetter & Dayton 1999, Rodríguez 2003, Vanderklift & Wernberg 2008). Consequently, the availability of this resource to consumers in linked habitats can be patchy in space and time (Vetter 1998, Rodríguez 2003, Britton-Simmons et al. 2009).

Kelp forests are among the most productive habitats in the world, but the majority of kelp biomass is transported to other habitats as detritus rather than being directly grazed (Cebrian 1999, Krumhansl & Scheibling 2011). Drift from subtidal kelp beds is a major food source for consumers in intertidal and offshore habitats. In the Benguela Current system off South Africa, drift kelp increases secondary production in the intertidal zone by providing food for limpets (Bustamante et al. 1995, Bustamante & Branch 1996). On the Chilean coast, intertidal sea urchins feed preferentially on drift kelp where it is available, and sea urchins with access to this resource develop larger gonads than those feeding on intertidal algae (Rodríguez 2003). Kelps growing on offshore rocky reefs off the coast of western Australia provide a subsidy to adjacent seagrass beds (Wernberg et al. 2006) and other reefs kilometers away (Vanderklift & Wernberg 2008). On the coast of Washington, USA, sea urchins beyond the lower depth limit of kelp capture drift algae (Vetter & Dayton 1999) and develop gonads as large as those in shallow subtidal habitats (Britton-Simmons et al. 2009). However, the availability of drift algae and sea urchin gonad size both decrease with distance from shore in northern California (Rogers-Bennett et al. 1995).

Along the Atlantic coast of Nova Scotia, Canada, coralline algal-dominated barrens are found in the rocky subtidal below the depth limit of kelps. Although primary productivity is low in these barrens, they often support dense populations of the green sea urchin *Strongylocentrotus droebachiensis* (80-100 sea urchins $\cdot$ m^{-2} at 18-24 m depth; Himmelman 1986, Brady & Scheibling 2005). These sea urchins graze microalgal films and coralline algae and generally have smaller gonads and slower somatic growth than sea urchins feeding on kelp (Himmelman 1986, Brady & Scheibling 2006). Drift from

nearby kelp beds may provide a substantial energy subsidy to these sea urchin populations, as detrital kelp production can reach 1.7 kg dry weight · m^{-2} · $year^{-1}$ (Krumhansl & Scheibling 2011). Sea urchins in barrens opportunistically consume drift algae when it is available (Himmelman & Steele 1971, Johnson & Mann 1982, Himmelman 1986, Meidel & Scheibling 1998), but the abundance and spatial extent of this detrital material in offshore barrens, and its importance in sea urchin diets, are unknown.

Diets of marine invertebrates have been identified by analyzing gut contents and by using chemical tracers such as stable isotopes (SI) and fatty acids (FA). Gut contents provide information on an organism's diet in the relatively short term, but can overestimate the contribution of the most recently consumed food items and those that are digested more slowly (Foale & Day 1992). Chemical tracers may provide longer-term and less biased dietary information (Iverson et al. 2004) but their interpretation may be complicated if animals consume mixed diets or modify chemical signatures of their food (Peterson 1999, Kelly et al. 2008, 2009). Carbon isotopic ratios can be used to identify the sources of primary production contributing to higher trophic levels, while nitrogen isotopic ratios increase with each trophic transfer such that the relative trophic position of various consumers can be identified (Peterson & Fry 1987). Characteristic FA for various classes of primary producers are transferred to higher trophic levels conservatively, and can be used to identify food sources of consumers (Graeve et al. 1994, Dalsgaard et al. 2003). Some invertebrates are capable of substantial biosynthesis or selective retention of dietary FA, which dilute dietary signals, although consumers of different diets may still differ in their overall FA composition (Kelly et al. 2008, 2009). The results of chemical tracer analyses are most robust when corroborated by other lines of evidence (Peterson et al. 1999) and studies using a combination of analytical methods to study diet are common (Graeve et al. 2001, Kharlamenko et al. 2001, Rodríguez 2003, Crawley et al. 2009, Guest et al. 2010, Hanson et al. 2010).

We measured gonad production, gut contents, stable carbon and nitrogen isotopic signatures, and FA composition of sea urchins in rocky subtidal habitats offshore from

kelp beds to address the following questions: 1) What is the spatial extent of drift kelp subsidy to sea urchins in deeper-water habitats offshore from kelp beds? 2) What is the relative importance of drift kelp as compared to autochthonous production in the diets of sea urchins at increasing distance offshore from kelp beds? 3) Can gut contents, SI and FA be used to distinguish diets of sea urchins on the scale of tens to hundreds of meters?

5.3 Materials and Methods

5.3.1 Study Sites

Field collections took place in April 2009 at 4 sites on the Atlantic coast of Nova Scotia (Fig 5.1). Sampling was conducted in April to coincide with peak gonad development in sea urchins (*Strongylocentrotus droebachiensis*) in this region (Meidel & Scheibling 1998). Sites were chosen to include various depth profiles: Gill Cove (44°29.8'N, 63°31.7'W) and Duncan's Cove (44°29.2'N, 63°31.2'W) had a steep slope offshore from the edge of the kelp bed, while Black Rock (44°27.1'N, 63°31.8'W) and Splitnose Point (44°28.6'N, 63°32.7'W) had more gradual slopes (Fig 5.2). Depth was measured every 1.6 m along each transect using sonar (Humminbird 1198c Side Imaging Sonar).

Substratum type and macroalgae were recorded along each transect using a towed underwater video camera (Shark Marine SV-16HR Mini Colour Camera mounted on a JW Fisher DD1 deep dive wing) at a height of 1-2 m off the bottom and a speed of 0.25 to 0.5 $m \cdot s^{-1}$. At all sites, *Saccharina longicruris* and *Laminaria digitata* were the dominant canopy-forming kelps in the shallow subtidal zone. Other macroalgae, mainly *Desmarestia* spp., *Agarum cribosum*, *Alaria esculenta*, *Bonnemaisonia hamifera*, and *Palmaria palmata*, were present in low abundances at all sites. Below 8 m depth, attached fleshy macroalgae were rare and crustose coralline algae (mainly *Lithothamnion glaciale* and *Phymatolithon lenormandii*) were dominant on the rocky substratum until this graded to sand. The substratum at Black Rock consisted mainly of granite ledges and boulders along the length of the transect, except for a 25 m long patch of sand and boulders beginning 127 m from the kelp bed (Fig 5.2). Sparsely-distributed kelp was present to 40 m beyond the edge of the dense kelp bed, and a dense stand of kelp was present on a shoal (12-19 m depth) between 150 and 175 m from the main kelp bed.

Duncan's Cove was characterized by granite ledges and boulders that transitioned to mixed sand and cobbles around 47 m from the kelp bed, and then back to granite ledges and boulders at 142 m from the kelp bed. A large deposit of kelp detritus was present 40 m from the kelp bed. At Gill Cove, granite ledges and boulders were interspersed with small patches (3-14 m in length) of sand and boulders beginning around 70 m from the kelp bed. The substratum at Splitnose Point consisted of granite ledges and boulders along the entire transect. Duncan's Cove and Gill Cove both had relatively steep slopes from 0 to 40 m from the kelp bed, while Black Rock and Splitnose Point had more gradual, shallow slopes (Fig 5.2).

5.3.2 Sample Collection

At each site, sea urchins were collected using SCUBA along transects extending offshore from the edge of the kelp bed (perpendicular to shore). Sea urchins were collected at the edge of the kelp bed (0 m distance) and at 40, 80, 160 and 240 m along each transect ($n \geq 9$ sea urchins at each location). The two deepest locations (160 and 240 m from the kelp bed at Gill Cove) were beyond diving depth, so sea urchins were collected using lobster traps baited with fish. Bait was placed in mesh bags inside a perforated plastic container to prevent sea urchins from consuming it, and traps were retrieved after 24 h.

Dominant macroalgae were collected from the kelp bed and adjacent barrens for $\delta^{13}C$ and $\delta^{15}N$ SI characterization. Sea urchins and algae were transferred to the laboratory; algae were immediately prepared for SI analysis (see below) and sea urchins were held in aquaria with flowing seawater for up to 10 d before processing.

5.3.3 Sea Urchin Metrics

Test diameter of each sea urchin was measured using digital callipers. Wet weights of whole sea urchins (drained on paper towel for 30 s) and excised gonads were measured with a digital balance (0.01 g accuracy). Gonad index (GI) was calculated as (gonad wet weight/total wet weight) x 100. Sex was determined from a gonad smear using a compound microscope.

5.3.4 Gut Contents Analysis

Following excision of gonads, gut contents were removed from the digestive tract and stored in 70% ethanol for later analysis. Gut content particles were spread on a 5 x 5 cm grid with 121 intersection points and examined under a dissecting microscope. Items observed at intersection points were recorded. These included kelp, *Desmarestia* spp., other brown algae, green algae, red filamentous and fleshy algae, coralline algae, zoaria of the kelp-encrusting bryozoan *Membranipora membranacea*, parts of other invertebrates (mainly brittle stars, polychaete worms, and sea urchins), and unidentified material. The abundance of each item was calculated relative to the total number of intersection points where a food item was present. Because dissections were spread over 10 d following collection and sea urchins egested some of their gut contents during this interval, we measured the relative abundance of various food items but not the total volume of gut contents.

5.3.5 Stable Isotope Analysis

For SI analysis, algal material was cleaned of epiphytes, rinsed in distilled water, dried at 60°C for 48 hours to a constant weight, and ground to a fine, homogeneous powder using a mortar and pestle. Coralline algal samples were then acidified in 1N HCl to remove inorganic carbon (Boutton 1991). For sea urchins, the muscles of the Aristotle's lantern were extracted from 5 sea urchins for each distance at each site, rinsed in distilled water, acidified in 1N HCl, and ground (Rodríguez 2003). Algal and sea urchin samples were weighed into tin capsules and sent to the University of California Davis Stable Isotope Facility (USA) or Stable Isotopes in Nature Lab at University of New Brunswick (Canada) for analysis.

The isotopic value of each sample is reported in δ notation as: δX (‰) = $[(R_{sample} - R_{standard}/R_{standard})] \times 1000$, where $\delta X = \delta^{13}C$ or $\delta^{15}N$, and $R = {}^{13}C:{}^{12}C$ or ${}^{15}N:{}^{14}N$. Air and Vienna Pee Dee Belmenite were used as standards for nitrogen and carbon, respectively.

5.3.6 Lipid Extraction and Fatty Acid Analysis

For lipid extraction, 1.5 g of gonad from each sea urchin was manually homogenized with 20 ml chloroform and 10 ml methanol containing 0.01% BHT. The same solvent ratio was maintained for sea urchins with < 1.5 g total gonad. Lipid extraction protocols were modified from Folch et al. (1957), as described in Kelly et al. (2008). The samples in solvent (sea urchin gonad or algae) were filtered (Fisher P8 filter paper) to remove residual tissue before adding 7 ml of 5% NaCl. Samples were shaken and then centrifuged for 20 min. The lipid-containing lower phase was filtered through Na_2SO4 and evaporated to dryness under nitrogen at 25 to 30°C in a pre-weighed boiling tube. The boiling tube was re-weighed to calculate the amount of crude lipid for each sample.

To prepare lipids for FA analysis, crude lipid was dissolved in 1.5 ml methylene chloride with 0.01% BHT and subjected to acidic transesterification (H_2SO_4 in methanol) to produce FA methyl esters (FAME), as described in Budge et al. (2006). FAME were purified to remove proteins and pigments using thin layer chromatography (TLC). FAME were dissolved in hexane and spotted onto silica TLC plates (Sigma) and developed in a TLC chamber with 90:10:1 petroleum ether : ethyl ether : acetic acid for approximately 45 min. Plates were sprayed with 2,4 dichlorofluorescein and viewed under UV light to mark the FAME containing band. The FAME-containing band was then scraped from the silica plate and transferred to a funnel packed with silane treated glass wool. Chloroform containing 0.01% BHT was used to dissolve FAME into a pre-weighed tube. The sample was evaporated to dryness under nitrogen at 25 to 30°C and hexane was added to a final concentration of 50 mg ml^{-1}. Samples were stored at –20°C prior to analysis. Duplicate samples of FAME were analyzed and FA identified using temperature-programmed gas chromatography (Iverson et al. 1997, 2004, Budge et al. 2006).

5.3.7 Statistical Analysis

Sea urchin test diameter, GI, gut contents (% kelp and % coralline algae) and SI ($\delta^{13}C$ and $\delta^{15}N$) values were compared using two-way ANOVA with site and distance from the kelp bed as fixed factors. Significant two-way interactions were further examined using a one-way ANOVA for each site with distance from the kelp bed as a fixed factor. Gonad

lipid content (% wet weight) was also compared among distances at each site using one-way ANOVA because of missing values due to gonad samples that were too small for lipid analysis. Post-hoc comparisons were made following significant one-way results ($\alpha = 0.01$) using Tukey's HSD where Levene's test indicated equal variances, and Games-Howell tests where variances were unequal. Test diameter and SI data were untransformed; GI data were log-transformed; gonad lipid and gut contents data were arcsine-transformed. Transformations generally produced normal and homoscedastic data (Shapiro-Wilk and Levene's tests, $p > 0.05$); exceptions are noted in Table 5.2. ANOVA and pairwise comparisons were conducted using SYSTAT 12 software.

FA with an overall mean contribution >0.1% were included in statistical analyses, and FA composition was standardized to 100% for all samples prior to analysis. Overall FA composition was compared using permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) on Bray-Curtis distances of untransformed data with site and distance from the kelp bed as fixed factors. Homogeneity of variance of FA data was tested using permutational analysis of multivariate dispersions (PERMDISP, Anderson 2004). The significant interaction was further examined with one-way PERMANOVA for each site with distance as a fixed factor, and multidimensional scaling (MDS) plots for each site. Multivariate analyses were conducted using PRIMER 6 software with the PERMANOVA+ package (Clarke & Gorley 2006).

5.4 RESULTS

There was a general trend of decreasing test diameter and GI with distance from the kelp bed (Fig 5.3), although this effect varied somewhat across sites, as indicated by a significant interaction between site and distance for both variables (Table 5.1). Mean GI ranged from 22.4 at 40 m from the kelp bed at Duncan's Cove to 0.67 at 240 m from the kelp bed at Splitnose Point (Fig 5.3). GI decreased significantly with distance from the kelp bed at all sites except Black Rock (Table 5.2). At Black Rock, GI did not differ with distance from the kelp bed except 80 m, where it was significantly lower (Table 5.2).

There was a significant interactive effect of site and distance on both kelp and coralline algae in the gut contents (Table 5.1, Fig 5.3). The proportion of kelp in the gut contents generally decreased with distance from the kelp bed, except at Black Rock, where sea urchins 160 m from the kelp bed had significantly higher kelp content than sea urchins at other distances (Table 5.2, Fig 5.3). Sea urchins 40 m from the kelp bed at both Black Rock and Duncan's Cove also had high kelp content (Fig 5.3). The proportion of kelp in the gut contents did not differ significantly with distance from the kelp bed at Splitnose Point (Table 5.2). The proportion of coralline algae in the gut contents generally increased with distance from the kelp bed, except at Black Rock (Table 5.2, Fig 5.3). Gut contents of sea urchins from 240 m from the kelp bed were characterized by a high proportion of animal material (Table 5.3).

$\delta^{13}C$ of the two dominant kelp species ranged from -19.13 to -15.93‰ for *Laminaria digitata* and -20.40 to -15.38‰ for *Saccharina longicruris* (Table 5.4). Most other algal species were more depleted than kelps, with *Palmaria palmata* being the most depleted (-33.86‰). Coralline algae were more enriched than kelps (-8.21‰). $\delta^{15}N$ ranged from 3.80 to 6.80‰ for *L. digitata*, and 3.12 to 7.96‰ for *S. longicruris* (Table 5.4).

$\delta^{13}C$ for sea urchins generally was more depleted with distance from the kelp bed, except at Black Rock (Fig 5.4), and there was a significant interactive effect of site and distance (Table 5.1). $\delta^{13}C$ was most enriched in sea urchins 0 and 40 m from the kelp bed at Duncan's Cove, and 0 m from the kelp bed at Splitnose Point (Table 5.2). The greatest range in mean $\delta^{13}C$ was recorded at Splitnose Point, from -16.23‰ at the edge of the kelp bed to -18.4‰ at 240 m from the bed (Fig 5.4). Average $\delta^{13}C$ values for sea urchins fell within one standard error of kelp $\delta^{13}C$ values, except for sea urchins 240 m from the kelp bed at Duncan's Cove and Splitnose Point, which were more depleted, and sea urchins at the edge of the kelp bed at Splitnose Point, which were more enriched than kelp (Fig 5.4).

$\delta^{15}N$ for sea urchins generally was more enriched with distance from the kelp bed, except at Black Rock (Fig 5.4), and there was a significant interactive effect of site and distance (Table 5.1). At Duncan's Cove, Gill Cove and Splitnose Point, $\delta^{15}N$ was significantly

more enriched at sites distant from the kelp bed (Table 5.2). Mean $\delta^{15}N$ ranged from 6.69‰ at 40 m from the kelp bed at Black Rock to 9.98‰ at 240 m from the kelp bed at Gill Cove (Fig 5.4). Average $\delta^{15}N$ of sea urchins was 1.6 to 4.9‰ more enriched than that of kelp.

Lipid content of sea urchin gonads ranged from 1.5 to 9.7% by weight (pooled mean ± SD = 4.5 ± 1.5) and did not differ among sites or distances from the kelp bed (Table 5.2). There was a significant interactive effect of site and distance from the kelp bed on overall FA composition of sea urchin gonads (Table 5.1). FA composition differed among distances from the kelp bed at Black Rock, Duncan's Cove and Gill Cove but not at Splitnose Point (Table 5.2). Multivariate dispersion was homogeneous across sites and distances (PERMDISP $p > 0.5$). Samples did not cluster by distance from the kelp bed and no spatial pattern was evident in MDS of FA composition (Fig 5.5). FA identified as markers for kelp (18:1*n*-9, 18:4*n*-3, 20:4*n*-6) and diatoms (16:1*n*-7, 20:5*n*-3) in other studies (Dalsgaard et al. 2003, Kelly et al. 2008, 2009) were present in sea urchins at all sites and distances, but there was no detectable spatial pattern in their relative abundance (Table 5.5).

5.5 DISCUSSION

Stable isotope, FA, and gut content analyses indicate that drift algae comprises a substantial proportion of diets of sea urchins in deeper barrens offshore from kelp beds at our study sites, but the quantity of this trophic subsidy appears to decline with distance from the kelp bed. Carbon isotopic signatures of sea urchins up to 160 m offshore from the kelp bed at all sites and 240 m from the kelp bed at Gill Cove and Black Rock overlapped with those of kelps, indicating reliance of these sea urchins on drift algae. Sea urchins 240 m from the kelp bed at Duncan's Cove and Splitnose Point had slightly more depleted carbon isotopic signatures, suggesting the presence of an additional food source. Nitrogen isotopic signatures indicated increased trophic level with distance from kelp bed, suggesting that sea urchins distant from the kelp bed consume more animal material. Gut contents indicated a more mixed diet than did carbon isotopic signatures, although the higher contribution of animal material to the diets of sea urchins distant from the kelp

bed corresponded with the higher nitrogen isotopic signatures of these sea urchins. FA analysis did not show any spatial patterns in sea urchin diets, but the relative abundance of characteristic kelp FA in all sea urchins supports the conclusion that drift kelp is important in the diets of sea urchins at the scale of tens to hundreds of meters offshore from kelp beds.

Mean GI of sea urchins in this study mostly fell within the range (3 to 12) reported by Brady & Scheibling (2006) for sea urchins at 12 to 24 m depth and approximately 10 to 40 m from the adjacent kelp bed. Sea urchins 160 and 240 m from the kelp bed at Gill Cove and Duncan's Cove, and 240 m from the kelp bed at Splitnose Point, all had a mean GI < 1. Sea urchins raised on a diet of coralline algae in the laboratory generally do not develop gonads (Meidel & Scheibling 1999). Therefore, the development of gonads, although small, at 160 to 240 m from the kelp bed suggests these sea urchins had occasional access to drift algae. Gonad development and somatic growth are directly related to the quantity of kelp available (Meidel & Scheibling 1999). The pattern we observed in GI and test diameter is consistent with a reduction in drift kelp availability with distance from the kelp bed at all sites except Black Rock. The presence of an offshore stand of kelp 160 m from the kelp bed at Black Rock likely mitigated any effects of distance on drift kelp availability over the length of the transect, and explains the greater test diameter, GI, and kelp in the gut contents of sea urchins distant from the main kelp bed at this site. The highest mean GI value, 22.4 at 40 m from the kelp bed at Duncan's Cove, was comparable to the maximum GI recorded for sea urchins in kelp beds (Meidel & Scheibling 1998). The large accumulation of drift kelp present 40 m from the kelp bed at Duncan's Cove provided sea urchins with an abundant food source, which likely enabled these sea urchins to develop larger gonads. Degrading kelp has a lower C:N ratio than fresh kelp (Norderhaug et al. 2003), so the higher nutritional quality of this food source may also have contributed to the high GI of sea urchins at this location. Given that the patterns we observed with distance from sources of kelp occurred at sites with different depth profiles, depth was likely less important than distance from kelp in determining drift kelp availability to sea urchins in the barrens.

Kelp was present in the gut contents of 89% of sea urchins in our study. Similarly, Britton-Simmons et al. (2009) found kelp material in 97% of red sea urchins (*Strongylocentrotus franciscanus*) in deep barrens (23 m depth) adjacent to kelp beds in Washington, USA. Most specimens of *S. franciscanus* had only algal material in the gut contents (Britton-Simmons et al. 2009), as compared with the mixed gut contents of all sea urchins in this study. Sea urchins in our study also ingested variable amounts of coralline algae, sediment, and bryozoan. Their gut contents were comparable to those reported by Meidel & Scheibling (1998) for urchins in shallow barrens. This suggests that *S. franciscanus* in Washington may receive sufficient drift algae to rely exclusively on this trophic subsidy, while urchins at our study sites receive less drift algae and therefore consume other foods.

A wide range of carbon and nitrogen isotopic values have been reported for laminarian kelps (Simenstad et al. 1993, Fredriksen 2003) and the values for *Saccharina longicruris* and *Laminaria digitata* in our study fall within that range. $\delta^{13}C$ values of sea urchins that likely rely on a drift kelp subsidy tend to fall within the range of values for local kelp (Rodríguez 2003, Wing et al. 2008, Vanderklift & Wernberg 2010), as did $\delta^{13}C$ values of most urchins in our study. The lowest values for $\delta^{15}N$ enrichment between urchins and kelp in our study were similar to the expected value of 1.6‰ (± 0.4 SE) for herbivorous sea urchins in a meta-analysis of 27 published studies (Vanderklift et al. 2006). This indicates a mainly herbivorous diet of sea urchins near the kelp bed. Omnivorous sea urchins *Centrostephanus tenuispinus* and *Phyllacanthus irregularis* had greater $\delta^{15}N$ enrichment relative to kelp (5.5 and 5.7‰ respectively) than did the most $\delta^{15}N$-enriched urchins in our study (4.9‰ in urchins 240 m from the kelp bed at Gill Cove). Animal material made up approximately 10% of gut contents of *C. tenuispinus* and *P. irregularis* (Vanderklift et al. 2006), as compared to nearly half of gut contents of urchins 240 m from the kelp bed at Gill Cove in our study. If the gut contents we observed were representative of the longer-term diet of these urchins, we would expect greater $\delta^{15}N$ enrichment relative to kelp. This discrepancy suggests that diets of sea urchins in barrens are variable in time and that animal material contributes less to the overall diet than was reflected in the gut contents that we sampled. Differences between the results of gut

contents and SI analyses may be relatively common in grazers that utilize a patchy food source like drift kelp (Bustamante et al. 1995, Bustamante & Branch 1996, Vanderklift et al. 2006). The contribution of the preferred food (i.e. kelp) to the diet may also be underestimated using gut contents because lower-preference foods have a longer gut residence time (Boolootian & Lasker 1964, Foale & Day 1992, Sauchyn & Scheibling 2009). The lag time between collection and dissection for gut contents in our study may also have contributed to an overall underestimation of kelp contribution, although the presence of kelp in the gut contents of 89% of urchins in our study suggests that this was not an important factor.

A shift in the SI signature of decaying drift kelp may provide an alternative explanation for the spatial patterns of δ^{15}N-enrichment and δ^{13}C-depletion we observed with increasing distance from the kelp bed. Isotopic signatures of *Saccharina longicruris* shifted by an average of +1.1‰ for δ^{15}N and -3.0‰ for δ^{13}C over a 16-wk degradation period (Krumhansl & Scheibling, unpublished data). δ^{13}C values of urchins 240 m from the kelp bed at Duncan's Cove and Splitnose were < 1‰ more depleted than those of *S. longicruris*, on average. A shift in δ^{13}C due to kelp degradation could account in part for the more depleted values of urchins distant from the kelp bed. However, the magnitude of δ^{15}N enrichment due to degradation is insufficient to explain the more enriched δ^{15}N values of these urchins, indicating that animal material likely contributed to their diet.

Marker FA for kelp identified in urchins fed single diets (Kelly et al. 2009) showed no spatial pattern with distance from the kelp bed in our study. The presence of marker FA for kelp and diatoms in sea urchins from all sites and distances from the kelp bed suggests that urchins feed on drift kelp when it is available, and microalgal films at other times. The carbon isotopic values for benthic diatoms typically overlap with those of kelp and thus are difficult to distinguish using SI analysis (Newell et al. 1995), but their characteristic FA make diatoms relatively easy to identify using FA analysis (Dalsgaard et al. 2003, Alfaro et al. 2006). The lack of spatial pattern may be attributed to modification of dietary FA by sea urchins, which may obscure dietary information (Kelly et al. 2008). Selective retention of highly unsaturated FA 20:4*n*-6 and 20:5*n*-3 could also

have masked spatial patterns in the relative abundance of kelp and microalgae in urchin diets. FA markers in sea urchins may be more useful for identifying the presence of a unique food item (Cook et al. 2000, Castell et al. 2004, Kelly et al. 2009) than in distinguishing among mixed diets with varying proportions of the same food types.

Overall FA composition may be useful in identifying dietary differences among sea urchins (Kelly et al. 2008, 2009), but the lack of spatial pattern in overall FA composition of sea urchins in our study suggests that there were no dietary differences associated with distance from the kelp bed. Reduced lipid deposition during times of very low food availability could also have biased the results of FA analysis. If urchins distant from the kelp bed only deposited lipid when food was abundant (i.e. drift kelp was available), their FA composition would be similar to that of urchins nearer the kelp bed, which would deposit lipid at a higher rate, but with similar composition. The lower GI of urchins distant from the kelp bed is consistent with lower lipid deposition rates in these urchins. FA signatures can be highly variable among individuals because they reflect metabolic processes that vary with age, food availability or reproductive status, while SI signatures tend to be less variable among individuals because they reflect environmental factors (Guest et al. 2010).

The results of this study indicate that influence of drift kelp extends at least 240 m from the kelp bed at our study sites. Recent video surveys at nearby sites have documented patches of drift kelp, often with clusters of sea urchins, up to 500 m offshore at depths of 30 to 85 m (K. Filbee-Dexter and R.E. Scheibling, unpublished data), and individual kelp fronds have been observed, also with sea urchins, at depths of up to 3000 m on the continental slope off Nova Scotia (A. Metaxas, personal communication). Other studies have found that drift kelp provides a substantial trophic subsidy to habitats kilometers away from kelp beds (Kirkman & Kendrick 1997, Vanderklift & Kendrick 2005, Vanderklift & Wernberg 2008). Given the decrease in drift kelp availability within the spatial scale of our study, it is unlikely that drift kelp provides a consistent trophic subsidy to more distant habitats. However, large quantities of detrital material probably are transported greater distances in winter storms. The spatial extent of this subsidy may

depend on bottom topography, as negatively-buoyant drift kelp is transported along the sea floor by wave-driven currents and deposited in depressions (Vetter & Dayton 1999). Our results suggest that a relationship between substratum type and drift kelp accumulation may exist, with higher kelp detritus availability in depositional areas with a relatively flat, sandy bottom. Outside of such depressions, it is likely that drift kelp becomes an increasingly patchy resource with distance from the kelp bed.

If sea urchin diets were uniform over time, all methods used in this study should have produced similar spatial patterns with respect to the contribution of drift kelp. The patchiness of drift kelp subsidy to the barrens may have contributed to discrepancies among the results of gut contents, SI and FA analyses, since these methods differ in the temporal scale over which dietary information is integrated. It is likely that, if examined at the scale of years, there would be a strong spatial pattern of decreasing drift kelp availability with distance from the kelp bed. In this study, only the SI signatures of muscle tissue could have reflected a longer-term pattern, and this method showed a strong spatial pattern. Sea urchin gonad FA should reflect diet since the last spawning (~ 1 yr, Meidel & Scheibling 1998), and gut contents should represent diet on the scale of days (Sauchyn & Scheibling 2009). Given the potential for SI analysis to capture longer-term patterns, a combination of approaches that includes SI and one or more other methods that captures shorter-term variability is likely to provide the most robust results in trophic studies of sea urchins in rocky subtidal ecosystems.

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5.7 TABLES AND FIGURES

Table 5.1. Results of two-way tests with site and distance from the kelp bed as fixed factors. Test diameter, gonad index, % kelp and coralline algae in gut contents, and stable isotope values were compared using two-way ANOVA. Fatty acid composition was compared using two-way permutational multivariate analysis of variance (PERMANOVA).

	Source	df	MS	*F*	p
Test diameter	Site	3	1113.823	26.627	< 0.001
	Distance	4	2093.277	50.042	< 0.001
	Site x distance	12	194.985	4.661	< 0.001
	Error	221	41.831		
Gonad index	Site	3	13.73	26.933	< 0.001
	Distance	4	29.779	58.411	< 0.001
	Site x distance	12	7.908	15.511	< 0.001
	Error	218	0.51		
% kelp in gut contents	Site	3	0.218	4.972	0.002
	Distance	4	0.735	16.732	< 0.001
	Site x distance	12	0.254	5.78	< 0.001
	Error	199	0.044		
% coralline algae in gut contents	Site	3	0.218	4.972	0.002
	Distance	4	0.735	16.732	< 0.001
	Site x distance	12	0.254	5.78	< 0.001
	Error	199	0.044		
Muscle $\delta^{13}C$	Site	3	1.07	2.387	0.074
	Distance	4	4.136	9.225	< 0.001
	Site x distance	12	1.608	3.587	< 0.001
	Error	93	0.448		
Muscle $\delta^{15}N$	Site	3	13.034	51.242	< 0.001
	Distance	4	9.943	39.09	< 0.001
	Site x distance	12	1.141	4.486	< 0.001
	Error	93	0.254		
FA composition	Site	3	149.74	1.402	0.15

Source	df	MS	*F*	p
Distance	4	454.57	4.256	0.001
Site x distance	8	200.7	2	0.002
Error	150	106.82		

Table 5.2. Results of one-way tests for each site with distance from the kelp bed as a fixed factor. Test diameter, gonad lipid content, gonad index, % kelp and coralline algae in gut contents, and stable isotope values were compared using one-way ANOVA. Post-hoc comparisons are presented with levels in ascending order of magnitude; locations with common underlining are not significantly different from each other ($\alpha = 0.01$). Tukey's HSD was used where Levene's test indicated equal variances, and Games-Howell tests were used in cases of unequal variance (indicated by *). All data were normal except gonad index at Splitnose Point (Shapiro-Wilk p = 0.045). Fatty acid composition was tested using one-way permutational multivariate analysis of variance (PERMANOVA) with pairwise comparisons. Locations with common underlining are not significantly different from each other ($\alpha = 0.01$).

	Black Rock	Duncan's Cove	Gill Cove	Splitnose Point
Test diameter				
F-ratio	$F_{4,48} = 12.07$	$F_{4,67} = 37.54$	$F_{4,45} = 9.19$	$F_{4,61} = 16.89$
P	< 0.001	< 0.001	< 0.001	< 0.001
pairwise	0 40 160 80 240	0 40 80 160 240	0 40 80 160 240	0 40 80 160 240*
Gonad lipid content				
F-ratio	$F_{2,29} = 2.69$	$F_{4,49} = 0.47$	$F_{3,30} = 0.19$	$F_{3,42} = 0.56$
P	0.091	0.757	0.900	0.642
Gonad index				
F-ratio	$F_{4,48} = 10.83$	$F_{4,64} = 51.37$	$F_{4,45} = 15.11$	$F_{4,61} = 22.98$
P	< 0.001	< 0.001	< 0.001	< 0.001
pairwise	0 40 160 240 80*	40 0 80 160 240	0 40 80 160 240	0 40 80 160 240
% kelp in gut contents				
F-ratio	$F_{4,39} = 10.67$	$F_{4,61} = 27.69$	$F_{4,43} = 9.08$	$F_{4,56} = 0.86$
P	< 0.001	< 0.001	< 0.001	0.492
pairwise	160 40 0 80 240	0 40 80 160 240*	0 40 160 80 240	n/a
% coralline algae in gut contents				
F-ratio	$F_{4,39} = 13.08$	$F_{4,61} = 9.33$	$F_{4,43} = 2.37$	$F_{4,56} = 13.64$
P	< 0.001	< 0.001	0.068	< 0.001
pairwise	80 0 240 40 160*	160 240 80 0 40	n/a	240 160 80 40 0

	Black Rock	Duncan's Cove	Gill Cove	Splitnose Point
Muscle $\delta^{13}C$				
F-ratio	$F_{4,22} = 1.24$	$F_{4,26} = 8.13$	$F_{4,18} = 2.51$	$F_{4,27} = 9.98$
P	0.325	< 0.001	0.078	< 0.001
pairwise	n/a	40 0 80 160 240	n/a	0 40 80 160 240
Muscle $\delta^{15}N$				
F-ratio	$F_{4,22} = 5.63$	$F_{4,26} = 22.79$	$F_{4,18} = 9.35$	$F_{4,27} = 14.31$
P	0.003	< 0.001	< 0.001	< 0.001
pairwise	80 240 160 0 40	240 160 80 40 0	240 160 80 40 0	240 160 80 40 0
Fatty acid composition				
pseudo-*F*	$F_{2,29} = 5.87$	$F_{4,49} = 2.29$	$F_{3,30} = 2.60$	$F_{3,42} = 1.76$
P	0.001	0.011	0.003	0.039
pairwise	0 40 160	n/a	0 160 40 80	n/a

Table 5.3 Average contribution (%) of food items to gut contents at varying distances from the kelp bed at each site. Standard error is given below each mean in italics.

	Black Rock					Duncan's Cove					Gill Cove					Splitnose Point				
Distance from kelp (m)	0	40	80	160	240	0	40	80	160	240	0	40	80	160	240	0	40	80	160	240
N	7	8	11	10	8	10	4	10	14	28	9	11	10	9	9	6	9	26	10	9
Kelp	31.8	51.5	16.5	64.4	30.0	56.1	60.3	42.9	9.6	11.8	56.2	44.5	21.1	38.7	5.0	31.1	15.1	19.3	17.9	10.1
	7.4	*3.9*	*4.5*	*5.4*	*7.9*	*7.8*	*4.4*	*5.2*	*3.3*	*1.8*	*8.8*	*7.6*	*5.4*	*9.7*	*3.3*	*11.0*	*5.2*	*3.7*	*6.3*	*3.0*
Coralline algae	20.6	9.0	36.5	2.0	25.1	7.6	0.4	10.0	34.0	26.5	16.5	13.8	21.1	24.3	37.8	5.4	12.0	18.1	37.1	46.4
	5.0	*5.5*	*4.8*	*0.6*	*6.0*	*4.0*	*0.4*	*3.9*	*6.6*	*3.0*	*8.8*	*4.8*	*3.5*	*6.0*	*7.6*	*2.1*	*2.9*	*1.9*	*7.4*	*6.2*
Desmarestia spp.	5.3	0.0	2.0	0.4	0.0	8.1	0.0	7.6	12.3	8.2	6.2	2.6	2.4	3.5	2.7	2.8	25.7	20.8	8.7	11.4
	4.7	*0.5*	*2.3*	*2.9*	*3.8*	*1.0*	*6.8*	*1.1*	*5.1*	*2.3*	*0.9*	*2.7*	*3.4*	*4.3*	*1.4*	*2.1*	*1.9*	*1.0*	*2.5*	*2.6*
Other brown algae	0.4	0.0	1.5	0.0	0.0	0.3	0.0	0.4	0.4	1.2	0.5	0.1	1.7	0.1	0.2	0.0	0.1	0.5	1.2	0.0
	4.3	*4.0*	*3.5*	*1.4*	*4.2*	*1.6*	*3.1*	*3.2*	*5.2*	*3.9*	*1.7*	*1.8*	*3.8*	*7.0*	*9.9*	*4.3*	*2.3*	*2.2*	*2.9*	*2.0*
Green algae	4.5	5.5	0.6	1.2	2.3	8.6	0.4	0.4	0.3	2.2	5.2	2.7	1.4	1.6	0.0	23.7	3.0	1.8	0.7	0.9
	1.6	*2.5*	*0.4*	*0.5*	*1.4*	*3.6*	*0.4*	*0.4*	*0.2*	*0.9*	*2.9*	*0.9*	*0.5*	*0.6*	*0.0*	*15.7*	*1.2*	*0.7*	*0.5*	*0.7*
Red algae	12.2	20.1	21.0	18.9	23.6	12.0	5.6	24.5	16.3	13.2	7.6	24.5	29.4	12.3	2.4	27.5	28.1	26.3	13.6	16.5
	1.6	6.7	5.3	3.9	4.1	2.7	3.9	6.5	3.3	2.2	2.4	4.5	8.7	6.2	0.9	10.2	6.5	3.3	4.6	4.8
Membranipora	0.6	4.5	0.3	2.7	0.6	0.1	0.0	0.3	0.0	0.0	2.4	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0
	0.2	*0.0*	*1.1*	*0.0*	*0.0*	*0.3*	*0.0*	*0.3*	*0.3*	*0.4*	*0.3*	*0.1*	*0.7*	*0.1*	*0.2*	*0.0*	*0.1*	*0.3*	*1.0*	*0.0*
Other invertebrates	12.5	8.4	14.9	5.8	10.6	5.3	22.0	10.4	13.4	22.6	3.3	6.4	12.8	11.2	49.4	7.4	9.0	10.2	14.4	9.7
	1.8	*0.0*	*1.0*	*0.4*	*0.0*	*2.7*	*0.0*	*2.3*	*3.2*	*1.3*	*3.6*	*1.6*	*1.5*	*2.0*	*1.3*	*2.8*	*4.0*	*2.8*	*1.7*	*3.4*
Other/ unidentified	12.1	1.0	6.7	4.6	7.7	1.9	11.4	3.4	13.7	14.3	2.1	5.4	10.1	8.2	2.4	2.1	6.9	2.9	6.4	4.9
	0.6	*3.5*	*0.3*	*1.7*	*0.4*	*0.1*	*0.0*	*0.2*	*0.0*	*0.0*	*2.4*	*0.0*	*0.0*	*0.1*	*0.0*	*0.0*	*0.1*	*0.1*	*0.0*	*0.0*

Table 5.4. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures (mean ± SE, ‰) of macroalgae.

		n	$\delta^{13}C$	$\delta^{15}N$
Phaeophyta				
Saccharina longicruris	Black Rock	3	-18.66 ± 0.89	4.03 ± 1.00
	Duncan's Cove	2	-17.36 ± 0.18	7.29 ± 0.35
	Gill Cove	3	-17.67 ± 1.26	6.14 ± 1.52
	Splitnose Point	3	-17.11 ± 0.88	6.71 ± 0.42
Laminaria digitata	Black Rock	2	-17.71 ± 1.21	4.59 ± 0.79
	Duncan's Cove	2	-14.96 ± 0.97	6.68 ± 0.13
	Gill Cove	1	-17.01	4.92
	Splitnose Point	2	-17.62 ± 1.51	5.02 ± 1.81
Agarum cribrosum	Duncan's Cove	3	-23.57 ± 0.68	6.84 ± 0.45
	Gill Cove	3	-22.63 ± 0.90	4.88 ± 0.30
Alaria esculenta	Duncan's Cove	3	-20.30 ± 0.66	5.88 ± 0.57
Desmarestia aculeata	Splitnose Point	3	-32.99 ± 0.12	6.47 ± 0.10
Desmarestia viridis	Splitnose Point	3	-33.01 ± 0.04	6.10 ± 0.04
Rhodophyta				
Bonnemaisonia hamifera	Splitnose Point	3	-32.69 ± 0.28	5.50 ± 0.22
Crustose coralline algae	Splitnose Point	4	-8.21 ± 0.49	6.58 ± 0.18
Palmaria palmata	Gill Cove	3	-33.86 ± 0.39	5.79 ± 0.09

Table 5.5 Relative fatty acid composition of sea urchin gonads from transects offshore from kelp beds at 4 sites. Fatty acid values are mean percent of total fatty acids ± SE of all fatty acids with overall mean concentration ≥0.1%.

	Black Rock									Duncan's Cove										
Distance from kelp bed (m)	0			40			160			0			40			80			160			240		
n	12			10			10			10			10			2			14			18		
14:0	12.25	±	0.54	11.66	±	0.68	12.51	±	0.87	11.07	±	0.66	10.58	±	0.41	14.11	±	0.38	11.18	±	0.66	12.66	±	0.55
14:1*n*-9	0.22	±	0.01	0.16	±	0.01	0.27	±	0.03	0.21	±	0.02	0.17	±	0.02	0.28	±	0.08	0.17	±	0.02	0.19	±	0.01
14:1*n*-5	1.89	±	0.11	1.38	±	0.14	2.27	±	0.33	1.65	±	0.22	1.26	±	0.13	2.18	±	0.63	1.36	±	0.18	1.73	±	0.12
iso15:0	0.12	±	0.01	0.28	±	0.04	0.21	±	0.07	0.16	±	0.02	0.22	±	0.03	0.14	±	0.04	0.25	±	0.04	0.20	±	0.02
15:0	0.49	±	0.04	0.62	±	0.06	0.55	±	0.09	0.52	±	0.04	0.62	±	0.03	0.43	±	0.11	0.57	±	0.07	0.53	±	0.04
16:0	12.53	±	0.45	12.70	±	0.50	11.93	±	0.31	12.17	±	0.64	12.01	±	0.44	14.15	±	1.01	12.33	±	0.34	13.08	±	0.30
16:1*n*-11	0.32	±	0.02	0.31	±	0.05	0.34	±	0.04	0.38	±	0.04	0.24	±	0.02	0.36	±	0.02	0.36	±	0.06	0.33	±	0.03
16:1*n*-9	0.24	±	0.01	0.26	±	0.02	0.24	±	0.01	0.25	±	0.01	0.25	±	0.01	0.22	±	0.01	0.25	±	0.01	0.24	±	0.01
16:1*n*-7	6.55	±	0.36	4.72	±	0.27	5.86	±	0.48	5.91	±	0.48	4.48	±	0.32	6.53	±	0.06	5.21	±	0.36	5.93	±	0.27
16:1	4.48	±	0.16	3.22	±	0.20	4.71	±	0.31	3.72	±	0.41	3.25	±	0.21	4.63	±	1.04	3.57	±	0.30	3.88	±	0.17
16:2*n*-4	0.17	±	0.02	0.13	±	0.03	0.12	±	0.03	0.19	±	0.03	0.09	±	0.02	0.16	±	0.01	0.13	±	0.03	0.13	±	0.02
16:3*n*-6	0.09	±	0.02	0.14	±	0.03	0.09	±	0.03	0.12	±	0.03	0.13	±	0.03	0.09	±	0.00	0.15	±	0.03	0.20	±	0.04
16:3*n*-4	0.08	±	0.03	0.11	±	0.03	0.10	±	0.05	0.11	±	0.02	0.10	±	0.02	0.06	±	0.01	0.16	±	0.03	0.18	±	0.05
17:1	0.09	±	0.01	0.15	±	0.02	0.10	±	0.01	0.10	±	0.01	0.13	±	0.01	0.10	±	0.01	0.15	±	0.05	0.13	±	0.02
16:4*n*-1	0.15	±	0.07	0.28	±	0.07	0.22	±	0.09	0.25	±	0.08	0.26	±	0.07	0.12	±	0.03	0.29	±	0.06	0.40	±	0.12
18:0	1.04	±	0.10	1.40	±	0.08	1.09	±	0.14	1.07	±	0.09	1.38	±	0.08	1.12	±	0.11	1.65	±	0.26	1.19	±	0.07
18:1*n*-13	0.44	±	0.02	0.49	±	0.04	0.43	±	0.02	0.48	±	0.04	0.46	±	0.02	0.63	±	0.02	0.46	±	0.03	0.45	±	0.03
18:1*n*-9	2.77	±	0.13	2.41	±	0.21	2.21	±	0.15	2.45	±	0.12	2.27	±	0.12	2.88	±	0.43	2.37	±	0.21	2.68	±	0.17
18:1*n*-7	3.94	±	0.15	3.00	±	0.15	3.93	±	0.14	3.87	±	0.27	3.14	±	0.15	3.60	±	0.24	3.55	±	0.17	3.43	±	0.12
18:1*n*-5	0.32	±	0.02	0.28	±	0.02	0.38	±	0.02	0.28	±	0.02	0.28	±	0.02	0.41	±	0.14	0.32	±	0.03	0.28	±	0.02
18:2*n*-7	1.18	±	0.10	0.74	±	0.10	0.89	±	0.11	0.90	±	0.11	0.58	±	0.06	0.97	±	0.22	0.67	±	0.10	0.99	±	0.10
18:2*n*-6	1.03	±	0.04	1.14	±	0.12	0.92	±	0.04	1.00	±	0.07	1.10	±	0.08	1.08	±	0.10	1.10	±	0.08	1.20	±	0.09
18:3*n*-6	0.20	±	0.01	0.16	±	0.02	0.18	±	0.02	0.19	±	0.01	0.16	±	0.01	0.16	±	0.00	0.26	±	0.08	0.24	±	0.02
18:3*n*-4	0.10	±	0.01	0.12	±	0.02	0.11	±	0.02	0.12	±	0.01	0.13	±	0.02	0.08	±	0.00	0.15	±	0.04	0.11	±	0.01
18:3*n*-3	1.29	±	0.05	1.66	±	0.22	1.14	±	0.08	1.38	±	0.10	1.65	±	0.17	1.50	±	0.16	1.50	±	0.16	1.61	±	0.10

Distance from kelp bed (m)	Black Rock									Duncan's Cove										
	0			40			160			0			40			80			160			240		
18:4*n*-3	3.56	±	0.23	4.27	±	0.69	3.32	±	0.34	3.79	±	0.27	4.18	±	0.39	3.86	±	0.49	3.84	±	0.43	3.90	±	0.21
20:0	0.30	±	0.03	0.39	±	0.05	0.25	±	0.03	0.35	±	0.05	0.40	±	0.07	0.33	±	0.15	0.44	±	0.05	0.37	±	0.02
20:1A	4.26	±	0.15	4.01	±	0.16	3.98	±	0.27	4.45	±	0.29	4.90	±	0.22	3.77	±	0.01	4.03	±	0.17	3.78	±	0.16
20:1*n*-11	0.68	±	0.02	0.55	±	0.07	0.60	±	0.05	0.76	±	0.05	0.62	±	0.05	0.44	±	0.00	0.57	±	0.06	0.42	±	0.04
20:1*n*-9	4.93	±	0.23	4.02	±	0.15	4.44	±	0.27	5.04	±		4.87	±	0.25	4.14	±	0.25	4.25	±	0.22	4.41	±	0.19
20:1*n*-7	4.24	±	0.23	4.58	±	0.28	4.56	±	0.27	4.67	±		4.90	±	0.16	5.00	±	0.03	4.52	±	0.33	4.57	±	0.22
20:2Δ5,11	5.23	±	0.22	2.86	±	0.23	4.26	±	0.28	4.85	±	0.33	3.36	±	0.28	3.88	±	0.06	3.40	±	0.29	3.05	±	0.17
20:2Δ5,13	3.74	±	0.26	2.60	±	0.27	2.78	±	0.13	3.88	±	0.26	2.69	±	0.15	3.05	±	0.14	2.74	±	0.21	2.42	±	0.08
20:2 *n*-9	0.30	±	0.03	0.23	±	0.02	0.28	±	0.02	0.26	±	0.02	0.27	±	0.03	0.28	±	0.04	0.22	±	0.02	0.24	±	0.01
20:1B	0.59	±	0.04	0.45	±	0.06	0.49	±	0.04	0.49	±	0.05	0.48	±	0.03	0.59	±	0.05	0.35	±	0.05	0.54	±	0.06
20:2*n*-6	1.56	±	0.05	1.17	±	0.19	1.24	±	0.18	1.62	±	0.12	1.63	±	0.12	0.94	±	0.05	1.51	±	0.10	1.20	±	0.10
20:3*n*-6	0.51	±	0.02	0.49	±	0.04	0.53	±	0.03	0.50	±	0.04	0.54	±	0.03	0.52	±	0.00	0.48	±	0.03	0.55	±	0.03
20:4*n*-6	3.03	±	0.19	4.67	±	0.40	4.52	±	0.40	3.05	±	0.27	4.81	±	0.54	3.11	±	0.26	4.70	±	0.36	4.25	±	0.39
21:1	1.66	±	0.14	1.41	±	0.10	1.25	±	0.11	1.58	±	0.10	1.49	±	0.10	0.97	±	0.05	1.31	±	0.07	1.28	±	0.05
20:3*n*-3	0.87	±	0.04	1.05	±	0.10	0.88	±	0.07	1.16	±	0.09	1.27	±	0.06	0.82	±	0.10	1.15	±	0.09	1.02	±	0.09
20:4*n*-3	0.78	±	0.05	1.18	±	0.18	0.77	±	0.07	0.96	±	0.12	1.25	±	0.13	1.02	±	0.23	1.03	±	0.10	1.04	±	0.09
20:5*n*-3	4.49	±	0.32	10.94	±	0.99	7.09	±	0.76	5.56	±	0.72	9.77	±	0.95	5.19	±	0.53	8.81	±	0.77	7.59	±	0.66
22:1*n*-11	0.11	±	0.01	0.11	±	0.02	0.13	±	0.03	0.13	±	0.01	0.13	±	0.02	0.08	±	0.01	0.11	±	0.03	0.10	±	0.02
22:1*n*-9	2.31	±	0.12	2.01	±	0.26	1.66	±	0.13	2.32	±	0.21	2.08	±	0.24	1.86	±	0.35	2.10	±	0.10	2.08	±	0.11
22:1*n*-7	0.13	±	0.01	0.12	±	0.01	0.11	±	0.01	0.13	±	0.01	0.12	±	0.02	0.11	±	0.01	0.11	±	0.01	0.12	±	0.01
22:2Δ7,13	0.56	±	0.02	0.40	±	0.05	0.48	±	0.04	0.64	±	0.05	0.51	±	0.04	0.40	±	0.03	0.39	±	0.04	0.39	±	0.03
22:2Δ7,15	1.49	±	0.12	1.22	±	0.15	1.88	±	0.22	1.66	±	0.26	1.61	±	0.11	1.01	±	0.25	1.36	±	0.12	1.25	±	0.08
22:4 *n*-6	0.24	±	0.01	0.31	±	0.05	0.37	±	0.03	0.28	±	0.04	0.28	±	0.03	0.21	±	0.01	0.34	±	0.04	0.29	±	0.04
22:5*n*-6	0.13	±	0.02	0.12	±	0.03	0.18	±	0.05	0.19	±	0.02	0.12	±	0.01	0.13	±	0.02	0.20	±	0.06	0.14	±	0.02
22:5*n*-3	0.22	±	0.02	0.49	±	0.10	0.40	±	0.04	0.32	±	0.05	0.43	±	0.08	0.20	±	0.01	0.47	±	0.09	0.35	±	0.05
22:6*n*-3	1.00	±	0.18	1.44	±	0.16	1.52	±	0.24	1.48	±	0.23	1.10	±	0.12	1.22	±	0.06	1.64	±	0.47	1.26	±	0.15

Distance from kelp bed (m)	Gill Cove				Splitnose Point			
	0	40	80	160	0	40	80	160
n	10	10	4	10	10	10	16	10
14:0	10.19 ± 0.44	12.57 ± 0.79	14.12 ± 0.30	11.93 ± 0.45	11.58 ± 1.05	12.00 ± 0.50	11.44 ± 0.56	11.73 ± 0.67
14:1*n*-9	0.23 ± 0.03	0.24 ± 0.03	0.20 ± 0.01	0.24 ± 0.01	0.24 ± 0.02	0.20 ± 0.01	0.17 ± 0.02	0.19 ± 0.02
14:1*n*-5	1.94 ± 0.26	2.03 ± 0.26	1.59 ± 0.18	1.90 ± 0.10	2.13 ± 0.30	1.61 ± 0.17	1.45 ± 0.17	1.68 ± 0.17
iso15:0	0.16 ± 0.01	0.18 ± 0.04	0.18 ± 0.03	0.11 ± 0.01	0.15 ± 0.02	0.17 ± 0.03	0.24 ± 0.03	0.14 ± 0.03
15:0	0.63 ± 0.04	0.58 ± 0.07	0.45 ± 0.04	0.46 ± 0.04	0.51 ± 0.04	0.49 ± 0.03	0.58 ± 0.04	0.46 ± 0.04
16:0	10.49 ± 0.51	11.12 ± 0.49	14.10 ± 0.42	10.66 ± 0.36	11.55 ± 0.42	12.12 ± 0.77	12.54 ± 0.21	11.03 ± 0.37
16:1*n*-11	0.32 ± 0.02	0.30 ± 0.03	0.36 ± 0.06	0.28 ± 0.02	0.34 ± 0.05	0.28 ± 0.02	0.34 ± 0.02	0.25 ± 0.01
16:1*n*-9	0.25 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.23 ± 0.01
16:1*n*-7	5.24 ± 0.42	5.11 ± 0.37	5.73 ± 0.59	5.31 ± 0.31	5.47 ± 0.30	5.73 ± 0.51	6.38 ± 0.68	5.20 ± 0.34
16:1	3.34 ± 0.20	4.29 ± 0.36	3.88 ± 0.27	4.00 ± 0.21	4.34 ± 0.48	3.98 ± 0.29	3.56 ± 0.26	3.87 ± 0.31
16:2*n*-4	0.15 ± 0.02	0.12 ± 0.02	0.16 ± 0.03	0.08 ± 0.02	0.14 ± 0.05	0.09 ± 0.02	0.17 ± 0.02	0.09 ± 0.02
16:3*n*-6	0.14 ± 0.04	0.12 ± 0.03	0.16 ± 0.04	0.07 ± 0.00	0.10 ± 0.02	0.09 ± 0.01	0.27 ± 0.06	0.08 ± 0.01
16:3*n*-4	0.14 ± 0.04	0.14 ± 0.05	0.15 ± 0.05	0.04 ± 0.00	0.12 ± 0.03	0.07 ± 0.01	0.27 ± 0.07	0.06 ± 0.02
17:1	0.13 ± 0.01	0.11 ± 0.00	0.12 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.15 ± 0.01	0.10 ± 0.01
16:4*n*-1	0.38 ± 0.17	0.34 ± 0.15	0.27 ± 0.10	0.07 ± 0.01	0.26 ± 0.08	0.17 ± 0.04	0.47 ± 0.11	0.12 ± 0.04
18:0	1.13 ± 0.08	1.24 ± 0.22	1.42 ± 0.08	0.96 ± 0.03	1.01 ± 0.14	1.17 ± 0.18	1.21 ± 0.07	1.17 ± 0.13
18:1*n*-13	0.41 ± 0.02	0.46 ± 0.05	0.53 ± 0.09	0.44 ± 0.03	0.45 ± 0.04	0.51 ± 0.04	0.46 ± 0.02	0.42 ± 0.02
18:1*n*-9	2.46 ± 0.13	2.25 ± 0.15	3.03 ± 0.24	2.98 ± 0.23	2.19 ± 0.21	2.50 ± 0.18	2.14 ± 0.17	2.73 ± 0.10
18:1*n*-7	3.43 ± 0.23	3.37 ± 0.11	3.28 ± 0.20	3.42 ± 0.18	3.71 ± 0.11	3.58 ± 0.23	3.64 ± 0.18	3.44 ± 0.18
18:1*n*-5	0.25 ± 0.01	0.32 ± 0.01	0.31 ± 0.03	0.31 ± 0.02	0.34 ± 0.04	0.32 ± 0.02	0.32 ± 0.02	0.33 ± 0.02
18:2*n*-7	0.63 ± 0.10	0.69 ± 0.12	0.96 ± 0.12	0.87 ± 0.14	0.97 ± 0.14	0.99 ± 0.16	0.73 ± 0.06	0.70 ± 0.12
18:2*n*-6	1.11 ± 0.09	1.02 ± 0.07	1.10 ± 0.11	1.37 ± 0.13	0.97 ± 0.09	1.05 ± 0.06	1.00 ± 0.07	1.20 ± 0.07
18:3*n*-6	0.22 ± 0.02	0.21 ± 0.02	0.17 ± 0.04	0.23 ± 0.02	0.20 ± 0.03	0.16 ± 0.02	0.19 ± 0.02	0.18 ± 0.02
18:3*n*-4	0.12 ± 0.01	0.11 ± 0.02	0.12 ± 0.02	0.08 ± 0.00	0.11 ± 0.02	0.09 ± 0.01	0.15 ± 0.02	0.09 ± 0.01
18:3*n*-3	1.46 ± 0.09	1.41 ± 0.09	1.46 ± 0.16	1.72 ± 0.11	1.30 ± 0.13	1.35 ± 0.12	1.42 ± 0.08	1.55 ± 0.12
18:4*n*-3	4.07 ± 0.23	3.95 ± 0.30	3.79 ± 0.66	4.72 ± 0.27	4.02 ± 0.48	3.50 ± 0.37	3.66 ± 0.20	3.90 ± 0.26
20:0	0.34 ± 0.05	0.30 ± 0.06	0.48 ± 0.06	0.26 ± 0.02	0.23 ± 0.04	0.27 ± 0.05	0.37 ± 0.03	0.34 ± 0.05
20:1A	5.00 ± 0.15	4.28 ± 0.29	3.75 ± 0.27	4.09 ± 0.15	3.89 ± 0.22	4.24 ± 0.39	3.95 ± 0.19	4.36 ± 0.29

Distance from kelp bed (m)	Gill Cove				Splitnose Point			
	0	40	80	160	0	40	80	160
20:1*n*-11	0.64 ± 0.04	0.64 ± 0.13	0.52 ± 0.10	0.52 ± 0.03	0.57 ± 0.03	0.46 ± 0.03	0.51 ± 0.04	0.50 ± 0.05
20:1 *n*-9	5.05 ± 0.17	4.62 ± 0.25	4.34 ± 0.51	4.61 ± 0.14	5.00 ± 0.41	4.26 ± 0.17	4.07 ± 0.31	4.86 ± 0.34
20:1*n*-7	4.42 ± 0.30	5.08 ± 0.26	4.91 ± 0.34	4.72 ± 0.25	5.00 ± 0.27	5.68 ± 0.23	4.82 ± 0.27	4.81 ± 0.24
20:2Δ5,11	4.70 ± 0.35	3.68 ± 0.24	2.99 ± 0.44	4.55 ± 0.26	4.37 ± 0.28	3.83 ± 0.32	3.06 ± 0.20	4.16 ± 0.32
20:2Δ5,13	2.80 ± 0.29	2.65 ± 0.21	2.70 ± 0.47	2.67 ± 0.29	2.81 ± 0.28	3.05 ± 0.21	2.68 ± 0.16	2.53 ± 0.17
20:2*n*-9	0.30 ± 0.04	0.28 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.28 ± 0.03	0.28 ± 0.03	0.25 ± 0.02	0.31 ± 0.04
20:1B	0.35 ± 0.04	0.45 ± 0.06	0.58 ± 0.05	0.44 ± 0.06	0.52 ± 0.07	0.55 ± 0.06	0.43 ± 0.05	0.51 ± 0.05
20:2*n*-6	1.84 ± 0.10	1.48 ± 0.14	0.97 ± 0.13	1.76 ± 0.12	1.76 ± 0.22	1.18 ± 0.08	1.41 ± 0.11	1.59 ± 0.14
20:3*n*-6	0.50 ± 0.05	0.49 ± 0.04	0.55 ± 0.10	0.56 ± 0.02	0.53 ± 0.04	0.51 ± 0.04	0.50 ± 0.03	0.49 ± 0.04
20:4*n*-6	4.80 ± 0.54	4.18 ± 0.36	3.27 ± 0.55	5.09 ± 0.30	4.07 ± 0.42	4.40 ± 0.45	3.79 ± 0.17	5.65 ± 0.38
21:1	1.76 ± 0.10	1.29 ± 0.10	1.10 ± 0.06	1.38 ± 0.10	1.28 ± 0.14	1.51 ± 0.12	1.33 ± 0.05	1.53 ± 0.13
20:3*n*-3	1.32 ± 0.08	1.10 ± 0.11	0.86 ± 0.12	1.20 ± 0.11	1.19 ± 0.17	0.98 ± 0.09	1.05 ± 0.07	0.96 ± 0.08
20:4*n*-3	0.88 ± 0.08	0.85 ± 0.09	1.20 ± 0.24	1.02 ± 0.05	0.93 ± 0.10	0.91 ± 0.09	1.03 ± 0.07	0.89 ± 0.08
20:5*n*-3	7.15 ± 0.82	8.17 ± 0.85	6.54 ± 0.62	7.38 ± 0.52	7.09 ± 0.75	8.44 ± 1.27	9.67 ± 1.06	8.89 ± 1.12
22:1*n*-11	0.15 ± 0.02	0.14 ± 0.03	0.11 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.01	0.14 ± 0.03	0.08 ± 0.02
22:1*n*-9	2.31 ± 0.19	1.83 ± 0.19	2.46 ± 0.23	1.81 ± 0.09	2.02 ± 0.28	2.23 ± 0.17	2.07 ± 0.11	1.97 ± 0.14
22:1*n*-7	0.15 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.00	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.10 ± 0.01
22:2Δ7,13	0.66 ± 0.05	0.55 ± 0.05	0.37 ± 0.02	0.51 ± 0.04	0.59 ± 0.05	0.45 ± 0.03	0.41 ± 0.04	0.40 ± 0.05
22:2Δ7,15	2.00 ± 0.18	1.78 ± 0.22	0.91 ± 0.04	1.51 ± 0.18	1.69 ± 0.17	1.27 ± 0.08	1.28 ± 0.12	1.51 ± 0.14
22:4*n*-6	0.36 ± 0.04	0.36 ± 0.04	0.28 ± 0.03	0.36 ± 0.04	0.35 ± 0.05	0.28 ± 0.02	0.32 ± 0.03	0.28 ± 0.02
22:5*n*-6	0.20 ± 0.02	0.14 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.15 ± 0.02	0.11 ± 0.01	0.15 ± 0.01	0.11 ± 0.01
22:5*n*-3	0.39 ± 0.06	0.43 ± 0.06	0.27 ± 0.02	0.29 ± 0.03	0.37 ± 0.05	0.31 ± 0.03	0.48 ± 0.08	0.30 ± 0.06
22:6*n*-3	1.33 ± 0.20	1.36 ± 0.15	1.43 ± 0.37	1.08 ± 0.18	1.37 ± 0.29	0.95 ± 0.14	1.47 ± 0.20	0.99 ± 0.09

Figure 5.1. Study site locations off the Atlantic coast of Nova Scotia, Canada. Inset shows starting point (●) and direction (→) of 240-m transect at each study site. BR: Black Rock; DC: Duncan's Cove; GC: Gill Cove; SP: Splitnose Point. Arrow size not to scale.

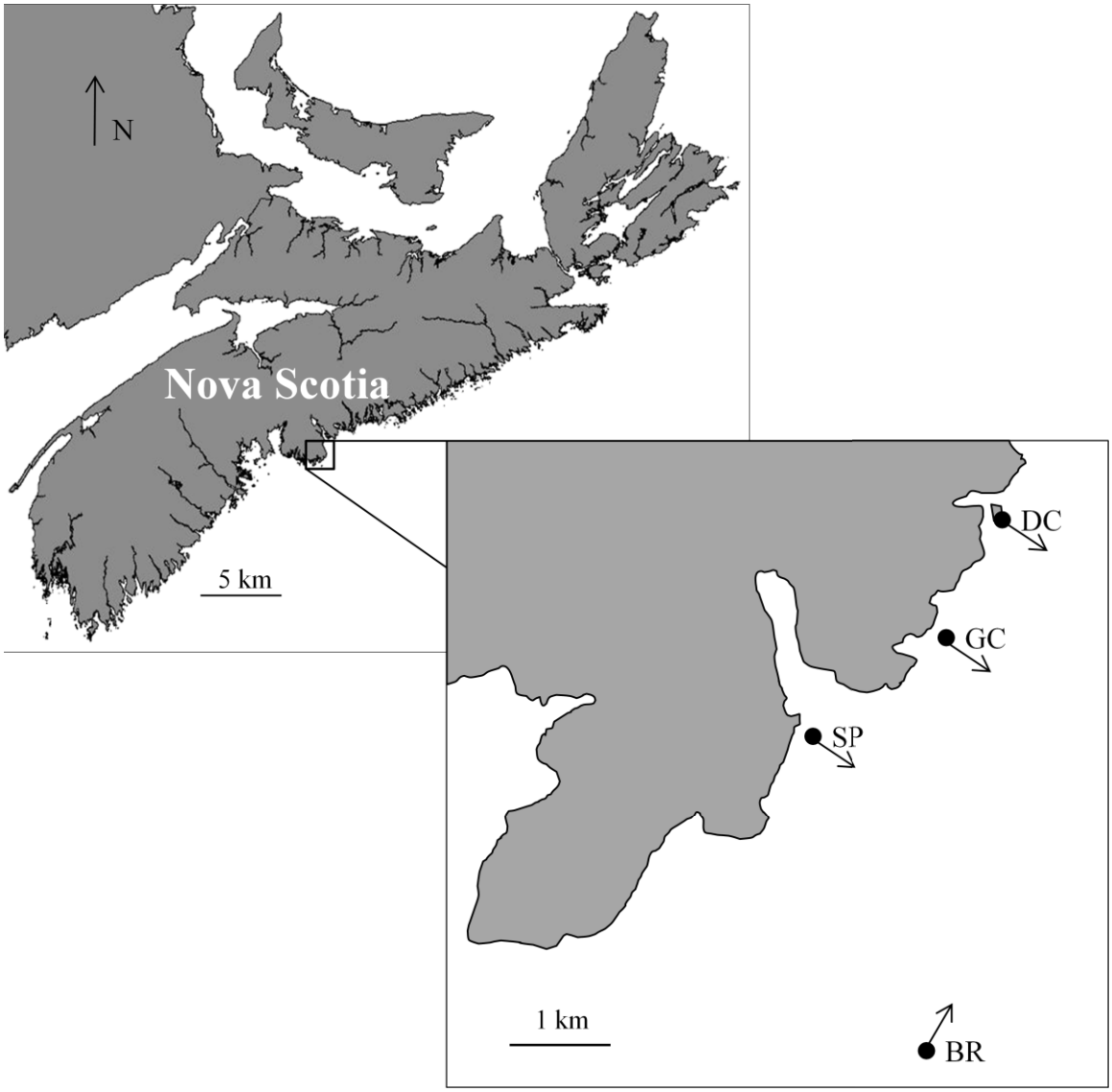


Figure 5.2. Transects along which sea urchins (*Strongylocentrotus droebachiensis*) were collected at 4 study sites. Sea urchins were collected at the edge of the kelp bed (0 m), and 40, 80, 160 and 240 m along the transect. (a) Depth profiles and (b) substratum type are shown for each transect.

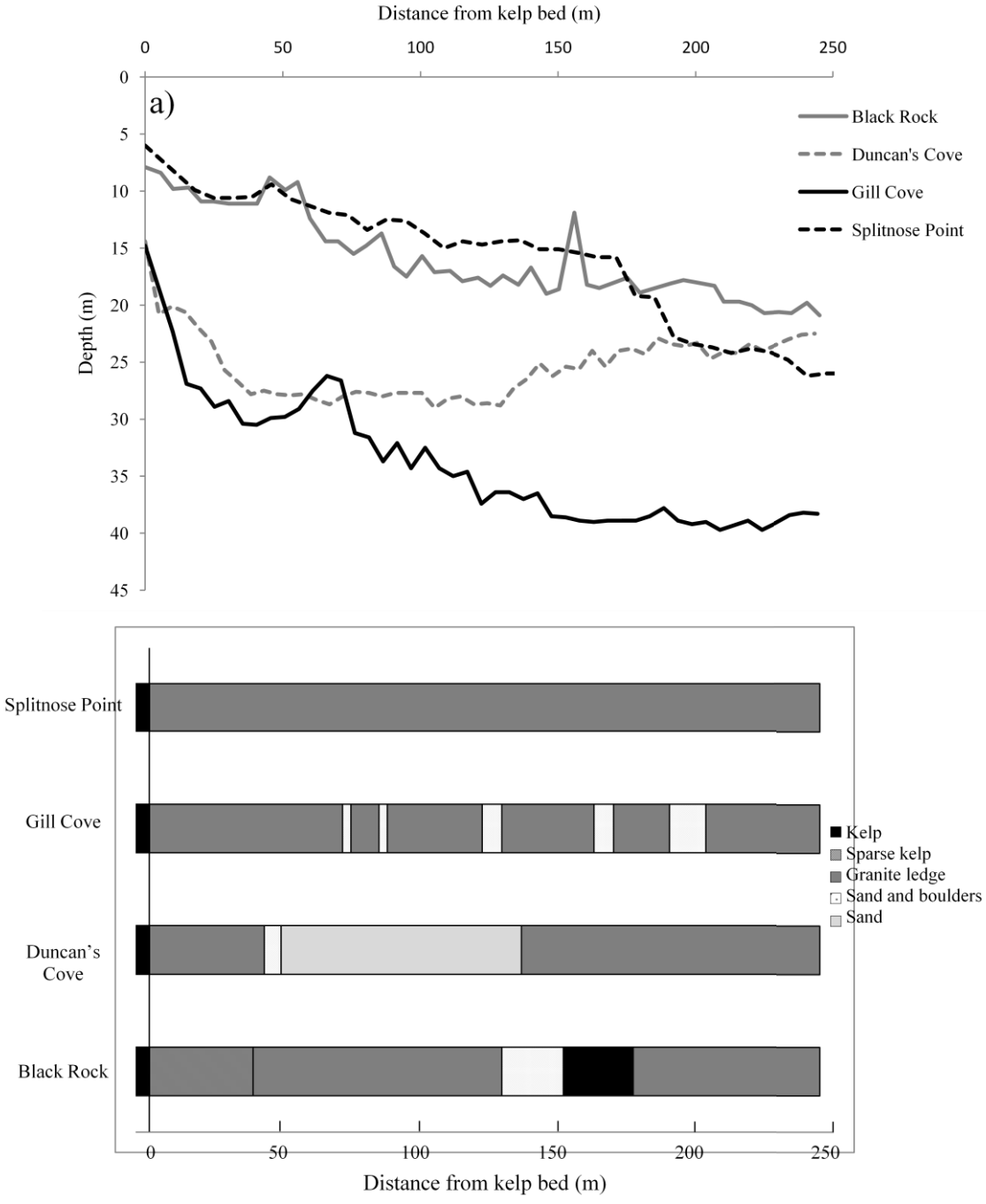


Figure 5.3. *Strongylocentrotus droebachiensis*. (a) Test diameter, (b) gonad index, (c) percent kelp in gut contents, and (d) percent coralline algae in gut contents of sea urchins along transects offshore from kelp beds. Data shown are mean + SE; n ≥ 9 sea urchins at each location.

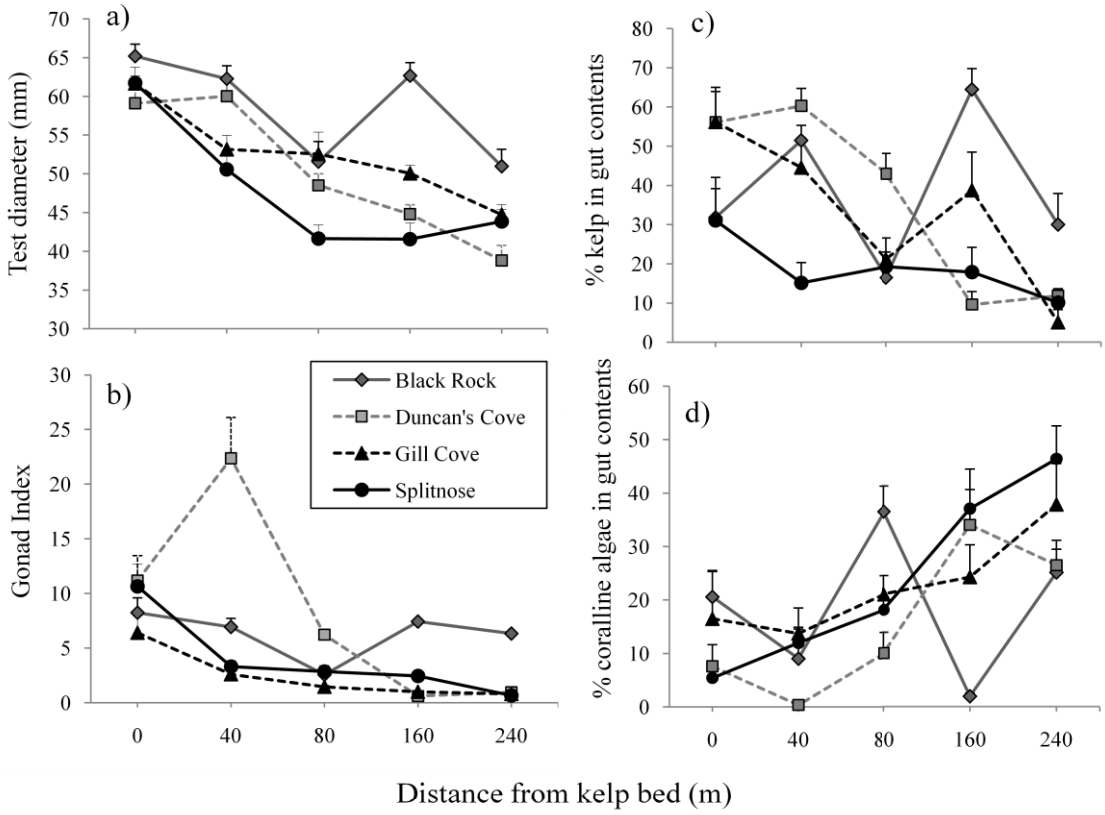


Figure 5.4. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values for sea urchins (*Strongylocentrotus droebachiensis*) along transects offshore from kelp beds, and for the two dominant kelp species (*Saccharina longicruris* and *Laminaria digitata*). Numbers next to symbols indicate distance from kelp bed (m). Data shown are mean + SE; $n \geq 5$ sea urchins at each location.

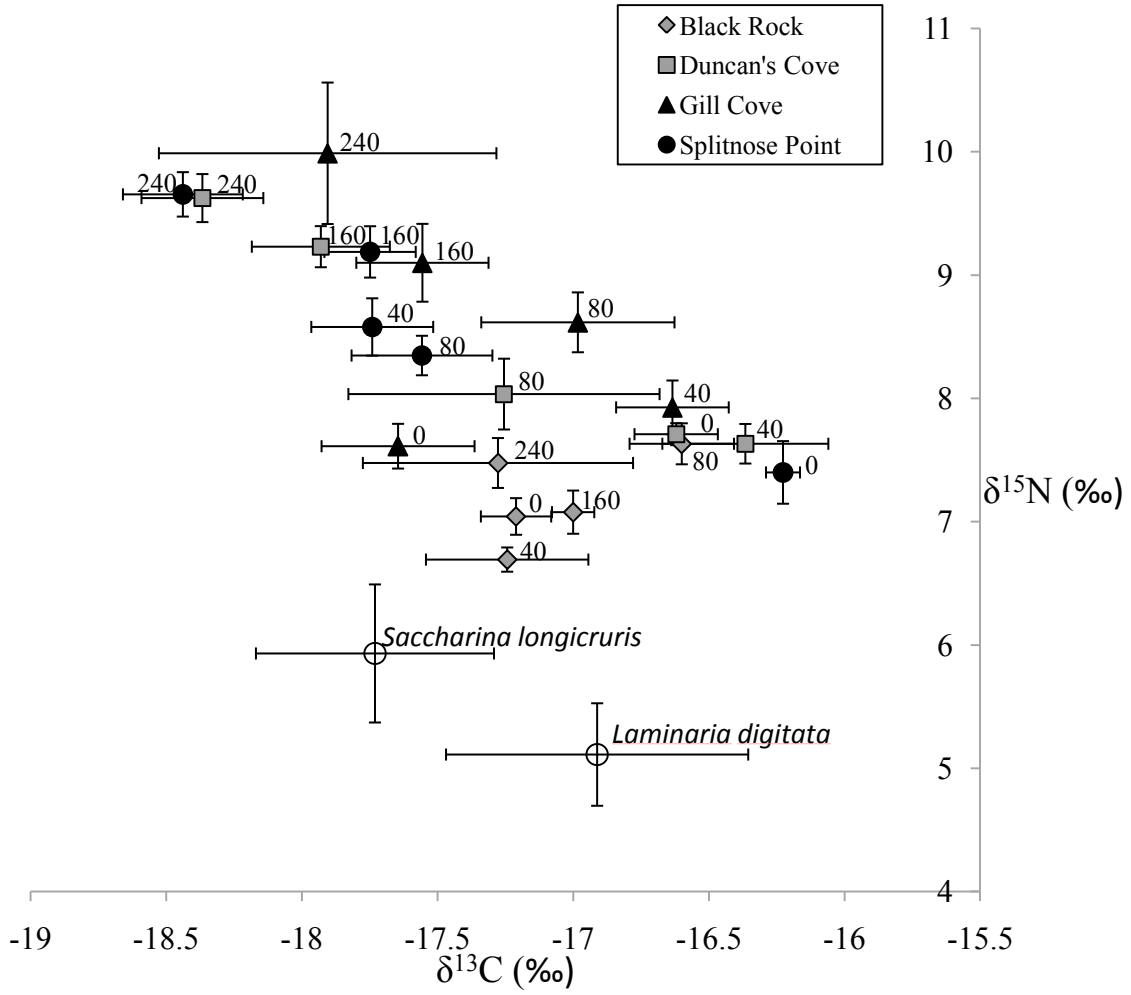
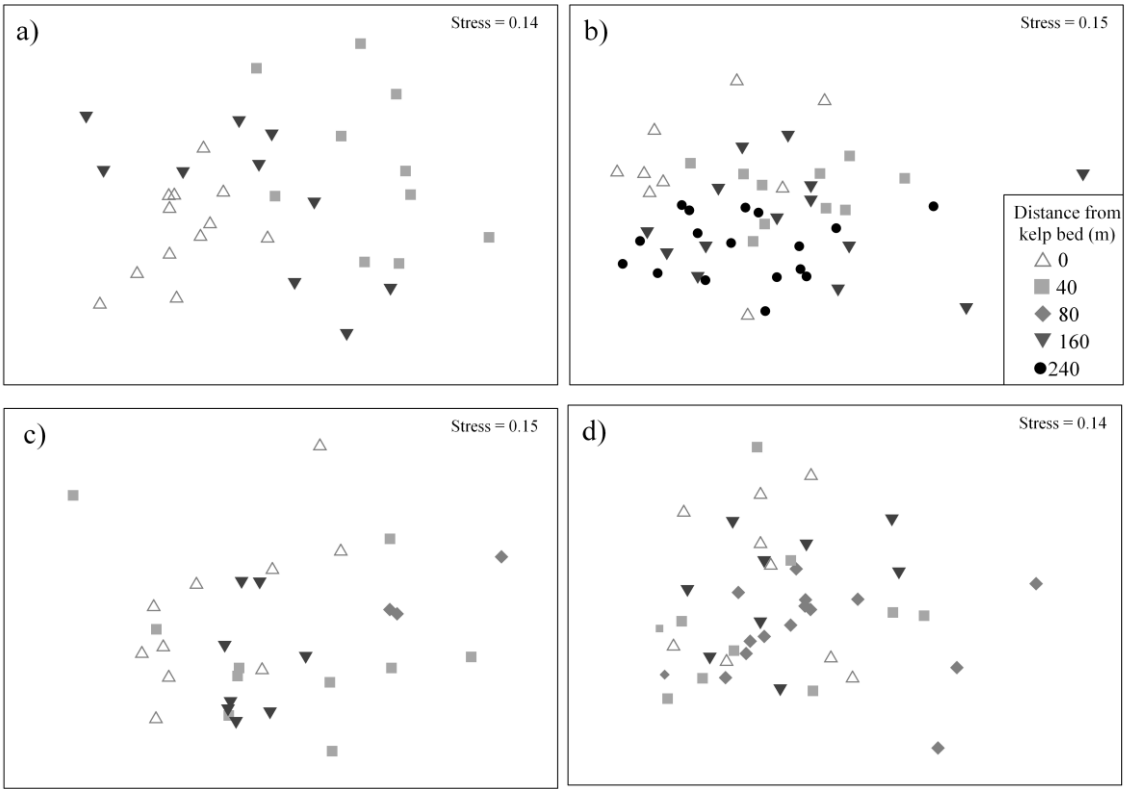


Figure 5.5. *Strongylocentrotus droebachiensis*. Two-dimensional non-metric multidimensional scaling (nMDS) plots of Bray-Curtis similarities of fatty acid compositions of sea urchins along transects offshore from kelp beds at 4 sites: (a) Black Rock, (b) Duncan's Cove, (c) Gill Cove, and (d) Splitnose Point.



CHAPTER 6: DISCUSSION

Fatty acid analysis may be a useful tool for studying certain trophic interactions in benthic food webs, but its application is more limited than in pelagic food webs or in vertebrate predators. FA present in benthic consumers can rarely be traced unambiguously to a single food source, both because most sources of primary production contributing to benthic food webs lack unique markers, and because many benthic consumers are capable of substantial FA biosynthesis.

My review of the literature (Chapter 2) indicates that FA metabolism of benthic consumers varies among taxa and may depend on diet. Controlled feeding studies have demonstrated direct transfer, modification, selective retention, and egestion of dietary FA in benthic consumers. Because consumer metabolism can change the relative abundance of individual FA obtained from the diet, I recommend multivariate analysis of overall FA composition. Comparisons of individual FA that drive overall differences can also be informative, but these should be interpreted with caution because there is considerable overlap among FA used as tracers for different primary producers. This overlap and the tendency of benthic consumers to modify dietary FA can be problematic for interpreting the results of field studies using FA data, but examples from various benthic habitats demonstrate that FA can provide qualitative dietary information in many cases. I recommend that field studies of benthic food webs using FA analysis include at least one other line of evidence, either from controlled feeding studies or another method of dietary analysis such as stable isotopes or gut contents.

The ability of benthic consumers to modify dietary FA composition through metabolic processes reinforces the need for controlled feeding studies using the foods and consumers of interest before FA analysis is used to estimate diet in field studies. Chapter 3 presents an example of this type of study, using sea urchins and four algal diets that are normally available to urchins in the rocky subtidal habitat. These sea urchins did not have abundant "marker" FA corresponding to their diets, suggesting that FA metabolism of sea urchins results in significant modification of dietary FA. However, sea urchins differed

in their overall FA composition depending on their algal diets, suggesting that a multivariate signature approach might be more appropriate than the marker approach for sea urchin FA analysis. The overall FA composition of sea urchins that were actively grazing on kelp in the wild was similar to that of sea urchins fed kelp in the laboratory, but the FA composition of sea urchins in the barrens did not resemble that of sea urchins fed barrens macroalgae. The abundance of indicator FA for diatoms in sea urchins from the barrens suggests that grazing on microalgal films could have driven the difference in FA composition between the laboratory-fed and wild-collected sea urchins.

The successful classification of sea urchins on different diets based on their overall FA signatures in Chapter 3 suggested that, despite the lack of unique markers in benthic primary producers, FA analysis could be used to distinguish not only sea urchins fed different diets, but also predators of sea urchins fed different diets. In this way, FA analysis could be used to trace the contribution of a primary producer of interest – in this case, an invasive alga – to higher trophic levels. In Chapter 4, I compared the FA signatures native kelp and the invasive *Codium fragile*, sea urchins fed each alga, and crabs (*Carcinus maenas* and *Cancer irroratus*) that consumed sea urchins fed each alga. Multivariate analysis showed differences between consumers with kelp and *C. fragile* as the basal food source. Biosynthesis and selective retention of certain FA occurred in sea urchins and crabs, such that FA composition of consumers did not match that of their food, but indicator FA for the invasive alga were detectable through two trophic transfers. Signal attenuation with each trophic transfer suggests limited applicability of the marker approach for detecting contribution of primary producers to higher trophic levels in field studies. However, the transfer of indicator FA for *C. fragile* suggests that indicator FA of unfamiliar foods may be detected in consumers more easily than FA of familiar foods. Results of other controlled feeding studies using artificial foods (Cook et al. 2000, Castell et al. 2004) also support this hypothesis. The use of FA analysis for detecting the contribution of artificial foods (such as excess feed from aquaculture operations, Cook et al. 2000) or invasive species to higher trophic levels should be further investigated using controlled feeding studies and field experiments.

The “baseline” FA signature of sea urchins feeding on kelp and its clear separation from that of sea urchins collected in the barrens (Chapter 3) suggested that the FA signatures of sea urchins along a gradient of decreasing kelp availability might reflect a gradual dietary shift from kelp to microalgae. In Chapter 5, I analyzed the diets of urchins collected at varying distances offshore from kelp beds using gut contents, stable isotopes, and fatty acid analysis. Stable isotope analysis and gut contents suggest that kelp detritus is an important resource for these sea urchins, but overall FA composition of sea urchins did not differ among urchins at increasing distance from the kelp bed. These results suggest that FA analysis may therefore not be useful for distinguishing among sea urchins consuming mixed diets. The effects of mixed diets on consumer FA composition should be investigated using controlled feeding studies to determine whether it is possible to distinguish among mixed diets consisting of different proportions of the same foods. The effects of food quality and quantity on FA composition of consumers fed a mixed diet should also be investigated, since it is likely that lipid deposition only occurs when food quality or quantity is high, and therefore FA composition only reflects the higher-quality food or food consumed in quantities that exceed basic metabolic requirements. Compound-specific stable isotope analysis of indicator FA (Pond et al. 2002, Budge et al. 2008) for kelp and diatoms could also be used to identify the sources of primary production that contribute to the diet of sea urchins at different distances from the kelp bed.

My thesis provides a detailed investigation of the use of FA analysis at the base of benthic food webs by testing underlying methodological assumptions and identifying limitations imposed by the primary producers and consumers in benthic habitats. In particular, I have underscored the lack of unique markers among benthic primary producers and the considerable effects of consumer metabolism on dietary FA. I have suggested experimental and statistical methods to address some of these problems, but I recommend that FA analysis be used in conjunction with other methods of studying trophic relationships in benthic food webs.

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