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Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*

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

KEY WORDS: Juvenile Atlantic cod · Eelgrass · Fatty acids · Lipid classes · Stable isotopes · Terrestrial input

INTRODUCTION

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

Stocks of Atlantic cod collapsed in the early 1990s, and despite a commercial fishing moratorium (Myers et al. 1996), there has been no population recovery (Lilly et al. 2003). Little is known about the relative input of different primary producers to the diet of juvenile Atlantic cod, or if these contributions change fol-

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lowing cod settlement. Examination of dietary carbon sources could result in expansion of critical habitat for juvenile Atlantic cod to include terrestrial buffer zones draining into eelgrass beds.

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

Lipids play a fundamental role in fish as a source of energy, as important structural components for cell membranes, and as precursors for biologically active compounds (Sargent et al. 1989, Arts et al. 2001, Copeman et al. 2002). Fatty acids (FAs) provide information on dietary intake and food constituents leading to sequestration of lipid reserves over a long period of time (St John & Lund 1996, Auel et al. 2002). C_{20} and C_{22} polyunsaturated FAs (PUFAs) are particularly important in coldwater marine systems, allowing animals to maintain cell membrane fluidity (Cossins et al. 1977, Cossins & Lee 1985). Marine fish generally cannot synthesise adequate quantities of long-chain PUFAs from shorter chain precursors to satisfy their metabolic requirements and therefore rely on dietary input for normal physiological function. PUFAs are formed in primary production and are transferred and concentrated in consumers throughout the food web (Sargent 1995, Arts et al. 2001, Budge et al. 2001, Copeman & Parrish 2003). Other sources of PUFAs in the nearshore marine environment include shorter chain C_{18} PUFAs from terrestrial sources; however, these are of lower nutritional value than longer chain PUFAs (Sargent et al. 1989).

Lipid biomarkers have been defined as compounds that can be used as signatures of a species, groups of organisms, or environmental processes (Parrish et al. 2000). FA biomarkers are normally synthesized at low trophic levels and ideally remain unchanged when transferred through food webs (Reuss & Poulsen 2002, Dalsgaard et al. 2003). They have been used for determining sources of terrestrial and anthropogenic carbon as well as to assess health of ecosystems (Colombo et al. 1997, Budge & Parrish 1998). Specific FAs have been correlated with various sources of primary production such as diatoms, bacteria, dinoflagellates, and terrestrial run-off both in plankton and sediments (Fraser & Sargent 1989, Mayzaud et al. 1989, Canuel et al. 1995, Budge & Parrish 1999, Meziane & Tsuchiya 2000, Parrish et al. 2000).

Bulk stable carbon isotopes have previously been widely used in food web studies to determine sources of dietary primary production at different trophic levels (Fry & Sherr 1984). Here we used compound-specific isotopes of FAs in select primary producers, secondary consumers, and juvenile fish to elucidate food web linkages. Previous analyses of eelgrass for bulk carbon-isotope composition has shown that eelgrass has much higher values (–15 to –7‰) than other sources of primary production in the nearshore (Kharlamenko et al. 2001, Cloern et al. 2002, Jaschinski et al. 2008). Further, little change in the $^{13}C/^{12}C$ ratio occurs with each successive trophic level, making it possible to estimate the importance of various sources of organic carbon to secondary consumers (DeNiro & Epstein 1978). The use of either FA or bulk $^{13}C/^{12}C$ ratios alone can have limited power for deducing linkages, but the combination of multiple biomarkers with multivariate statistics is a powerful approach to clarifying trophic relationships (Grahl-Nielson & Mjaavatten 1991, Canuel et al. 1997, Parrish et al. 2000, Ramos et al. 2003). Using this approach, we investigated temporal changes in the dietary composition of juvenile Atlantic cod during settlement into nearshore eelgrass. These data will be important in expanding the definition of critical habitat for juvenile Atlantic cod.

MATERIALS AND METHODS

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

Horizontal surface net tows (n = 36) were taken over shallow sites (10 m) in Newman Sound (Table 1). Sampling was concentrated over 2 eelgrass sites (Dockside and Mistaken Cove), except in late November when additional eelgrass locations were added. Triplicate tows were taken at each station using a 10 µm mesh plankton net over a distance of 640 m. Plankton was collected in a cod end (meshed collection pouch) and samples were backwashed into clean plastic vials and kept on ice until filtration (within 7 h). Prior to filtration, 3 size fractions were separated (>220, 80–220, and 10–80 µm), and samples were collected for lipid and weight determinations. Sub-samples (100 ml) from each size class of plankton were preserved for identifi-

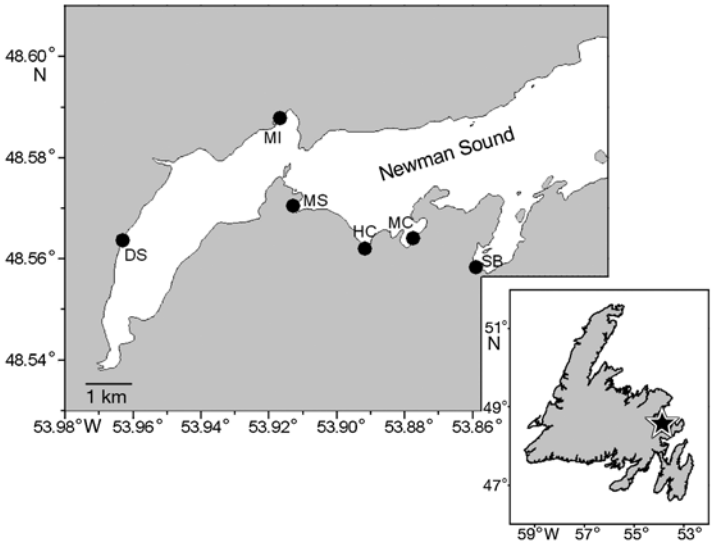

Fig. 1. Sample collection sites in Newman Sound, Bonivista Bay, Newfoundland (DS: Dockside; MI: Mistaken Cove; MS: Mt Stanford; HC: Heffern's Cove; MC: Minchin's Cove; SB: South Broad Cove)

cation by adding 1 ml of Lugol's iodine and 1 ml of 10% buffered formaldehyde. Plankton samples used for weight and lipid determination were collected on pre-combusted, pre-weighed glass fiber filters (Whatman GF/C). Filters were washed with 5 ml of 3% ammonium formate to remove salt, dried at 75°C for 24 h, weighed, and then ashed at 450°C for 24 h and re-weighed. Lipid samples were placed in clean glass vials, immersed in chloroform in the lab, and stored under nitrogen at –20°C until extraction.

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

Epibenthic invertebrates, amphipods *Gammarus* sp., and mysids *Mysis stenolepis*, were taken at beach seine sites during fish sampling (Table 1). Amphipods were collected using dip nets by dragging the nets along the bottom at a depth of <2 m while mysids were by-catch from seine hauls. Animals were placed in clean plastic bags filled with filtered seawater and stored at 4°C for 24 h to depurate. Animals were then washed in filtered seawater and placed in lipid clean tubes under chloroform and nitrogen at –20°C until extraction. The number of individuals per sample decreased as the season progressed: fewer individuals of a larger size were required for lipid analysis. These numbers ranged from a high of 25 amphipods per sample in August (0.5–1.5 mg wet wt ind.$^{-1}$) to a low of 12 in November (0.8–22.9 mg wet wt ind.$^{-1}$). Similarly, numbers of mysids per sample decreased from 20 in August (5.2–12.3 mg wet wt ind.$^{-1}$) to 6 in November (16.5–27.2 mg wet wt ind.$^{-1}$).

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

Lipids were extracted in chloroform/methanol according to Parrish (1988) using a modified Folch procedure (Folch et al. 1957). Lipid classes were determined using thin-layer chromatography with flame ionization detection (TLC-FID) with a Mark V Iatroscan (Iatron Laboratories) as described by Parrish (1987). Extracts were spotted on silica gel-coated Chromarods, and a

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

Location Date	Plankton tows (n)	Epibenthic prey samples (n)	Fish samples (n)
Dockside			
28–29 Aug	3	6	7
10–12 Sept	3	4	8
3–4 Oct	3	6	10
27 Oct – 8 Nov	3	8	2
Mistaken Cove			
28–29 Aug	3	2	5
10–12 Sept	3	0	6
3–4 Oct	3	6	10
27 Oct – 8 Nov	3	6	13
South Broad Cove			
27 Oct – 8 Nov		4	2
Minchin's Cove			
27 Oct – 8 Nov	3	4	4
Heffern's Cove			
27 Oct – 8 Nov	3	3	3
Mt Stanford			
27 Oct – 8 Nov	3	3	1

3-stage development system was used to separate lipid classes. After each separation, rods were scanned and the 3 chromatograms were combined using T-data scan software (RSS). The signal was quantified using lipid standards (Sigma).

FA methyl esters (FAMEs) were prepared from total lipid extracts by transesterification with 10% BF_3 in methanol at 85°C for 1 h (Morrison & Smith 1964, Budge 1999). FAMEs were injected in a Varian 3400 GC equipped with an 8100 autosampler (Varian) and an Omegawax 320 column (30 m, 0.32 mm i.d., 0.25 µm film thickness; Supelco). Hydrogen was the carrier gas, and the flow rate was 2 ml min^{-1}. The column temperature profile was: 65°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C min^{-1}, and hold at 220°C for 0.75 min after ramping at 2°C min^{-1}. The injector temperature increased from 150 to 250°C at 200°C min^{-1}. Peaks were detected by flame ionization with the detector held at 260°C. FA peaks were integrated using Varian Star Chromatography Software (version 4.02), and identification was made with reference to standards (PUFA 1 and 37 Component FAME Mix, Supelco).

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

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

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

Statistics. Select lipid classes and FAs in zooplankton, epibenthic prey, and fish were compared between months or (for fish) pelagic versus settled using a 1-way analysis of variance (ANOVA) with Tukey's pairwise comparisons. Residuals versus fitted values were examined to check for normality and heteroscedasticity, and certain percentage data were arcsine square-root transformed in order to meet these assumptions.

Principal component analysis (PCA) was used to simplify multivariate FA and lipid class data by transforming correlated variables into a set of uncorrelated principal components (Minitab, version 10.5; Meglen 1992). This technique was employed using 10 to 12 highly discriminatory lipid variables (based on previously run PCA analysis) from net tows, epibenthic prey, and fish samples. The first 2 principal components (PC1, PC2) accounted for 54 to 72% of the variance among samples, which allowed a display of the major trends within the data set without significant loss of the total original variation. PCA lipid loading coefficients are defined as the correlation coefficients between the original lipid variables and the PCA axis. PCA scores are defined as the position of the original variables along the new PCA axes (Meglen 1992). Lipid variables were chosen based on biological significance and the degree of variance explained by a given lipid class or FA. Addition of other lipid classes did not change the clustering of observations, indicating that the models were robust.

RESULTS AND DISCUSSION

Net tows

The net tow sampling locations and dates are shown in Fig. 1 and Table 1. At each sampling, 2 net tows from each size class were examined microscopically in order to determine zooplankton availability for settling juvenile cod. The smallest plankton (10–80 µm) had over ~80% of the field-of-view covered with an amorphous matrix and no recognizable phytoplankton cells. Fecal pellets and detritus were noted, and microscopically these samples resembled re-suspended sedimentary 'fluff.' Therefore, the smallest fraction is referred to as seston rather than plankton.

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

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

No *Calanus* spp. was identified in any of the samples taken from Newman Sound during 2002. Grant &

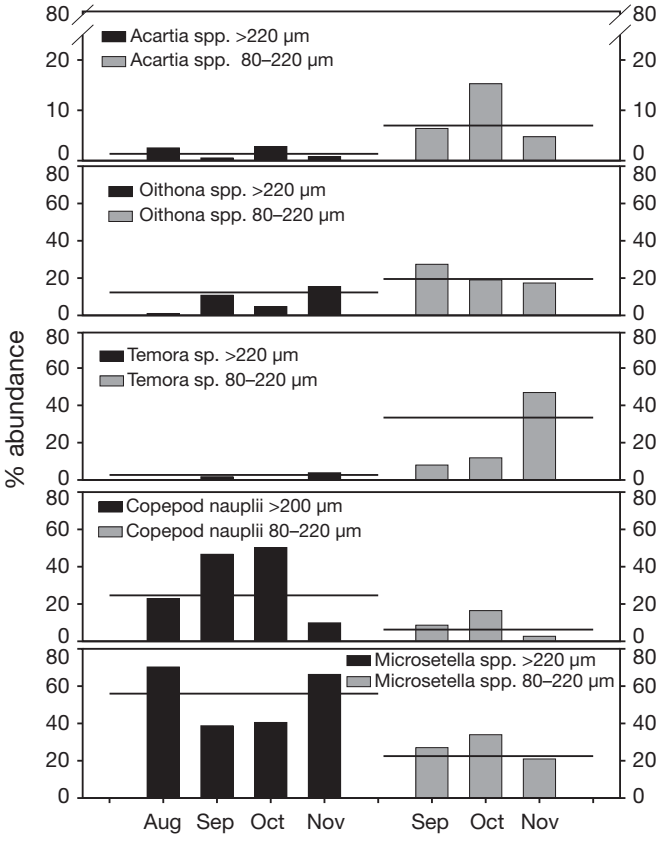

Fig. 2. Major zooplankton species abundance (>5%) in 2 size classes of plankton collected during the late summer and fall of 2002 (n = 2)

Brown (1998) previously found *Calanus* to be important in the diet of juvenile cod. This prey item was only present in their diet in October (21.5% relative importance) and November (5.8%) and was not found in either September or December. *Calanus* spp. form large globules of wax ester that are easily digested by juvenile fish and provide high amounts of energy (Sargent et. al 1999). A decrease in juvenile cod condition has been noted when larger *Calanus* spp. disappear from the diet (Grant & Brown 1999). The absence of *Calanus* spp. in our samples may indicate that food quality was not optimal during 2002.

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

Both sizes of zooplankton, discussed above, had significantly higher levels of lipid per dry weight than found in seston ($p < 0.001$, Table 2). The major lipid classes in both sizes of zooplankton were triacylglycerols (TAGs, 20–27%) and phospholipids (PLs, 34–42%), while seston had significantly higher levels ($p < 0.001$) of hydrocarbons (HCs) and acetone mobile polar lipids (AMPLs) than zooplankton (~2.5 and 8.5%, respectively).

Saturated FAs (SFAs), monounsaturated FAs (MUFAs), and PUFAs comprised 29–31, 19–25, and 44–52%, respectively, of total FAs in both sizes of zooplankton. Seston had lower levels of PUFAs (32%) and elevated levels of SFAs (39%) and MUFAs (29%). Terrestrial FAs (18:3n-3 and 18:2n-6) accounted for ~6% of total FAs in all size fractions. High levels of 18:4n-3 were found in net tows, with large zooplankton having the most elevated proportions (5.2 ± 1.4%). Levels of bacterial FAs in the 2 sizes of zooplankton were ~4.7%, with seston (10–80 µm) having the highest proportions (7.9 ± 2.6%, Table 2).

Our study was designed to look at the food web of eelgrass beds in relation to diet of settling juvenile Atlantic cod. Therefore, net tow data presented here, from late summer and fall, are probably not representative of plankton at other times of the year. The typical seasonal pattern of phytoplankton abundance in cold North Atlantic nearshore waters shows diatom lipid markers dominating in spring, followed by terrestrial plant material, small flagellates, zooplankton, and bacterial sources becoming more important in late summer and fall (Mayzaud et al. 1989, Parrish et al. 1995, 2000). Seasonal changes in lipid composition of particulate matter can be caused by changes in taxonomic source or physiological changes within a taxonomic group (Mayzaud et al. 1989). Algal lipid composition may be influenced by a number of physical factors, including irradiance, temperature, and nutrients (Thompson et al. 1990, 1992).

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

Table 2. Major lipid classes (>4% in at least one group) and fatty acids (>1.5%) in net tows and primary producers collected from August to November 2002 over *Zostera marina* beds in Newman Sound. n = 25 to 30 for net tows, n = 7 to 12 for primary producers, mean ± SD. Bacterial fatty acids were: Σ15:0, *ai*15:0, *i*15:0, *i*16:0, *ai*16:0, 15:1, 17:0, 17:1. SFA: saturated fatty acid (FA); PUFA: polyunsaturated FA; MUFA: monounsaturated FA; DHA: EPA: docosahexaenoic acid:eicosapentaenoic acid

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

nificant proportion of the late fall plankton community. However, they noted a distinct fall diatom bloom, which we did not observe in 2002.

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

Bacterial markers in Newman Sound seston were elevated, averaging 7.9% of identified FAs (Table 2). These levels were similar to those found in other shallow sheltered embayments, such as Gilbert Bay (~5%, Copeman & Parrish 2003) and Notre Dame Bay (~5.5%, Budge et al. 2001). Values of bacterial FA markers for net tows taken at the same latitudes but in deep fjord-like systems, such as Trinity Bay (2%, Budge & Parrish 1998) and Conception Bay (2–4%, Parrish et al. 2005), Newfoundland, were much lower. Elevated organic matter in eelgrass sediments provides a substrate for bacterial production. Likely sources of sedimentary carbon include seagrass detritus (Holmer & Nielsen 1997), root organic contributions (Moriarty et al. 1986), and benthic microalgae (Boschker et al. 2000). In Newman Sound, eelgrass sites were very shallow (<10 m) and

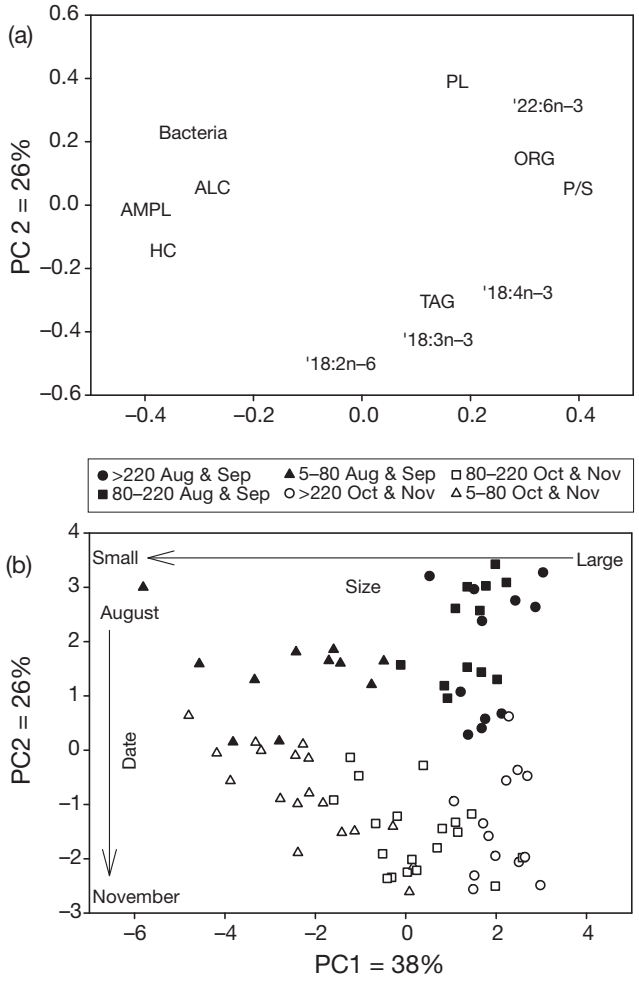

Fig. 3. Principal component (PC) analysis of lipid class and fatty acid proportions in plankton tows collected in Newman Sound during summer and fall 2002. (a) Lipid loading coefficients for PC1 and PC2. AMPL: acetone mobile polar lipids; ALC: alcohols; HC: hydrocarbons; PL: phospholipids; TAG: triacylglycerols; ORG: organic material per dry wt (mg g^{-1}); P/S: PUFA/SFA. (b) Different-sized net tows scores for PC1 and PC2

high levels of resuspension of degraded material from sediments likely contributed to elevated levels of bacteria observed in net tow samples (Table 2).

PCA allows the simplification of a large number of correlated FA variables into a few uncorrelated axes, which explain most of the original variance among the samples (Manly 1986). PCA of 11 FA and lipid class variables allowed us to view the lipid composition of net tows in terms of seasonal trends and size differences (Fig. 3). PC1 explained 38% of the variance, and examination of the lipid loading coefficients indicated that this axis represents a freshness axis with high levels of PUFAs, organic matter, 22:6n-3, and PLs positively loaded (Fig. 3a). Lipid classes typical of degradation (Parrish 1998) and bacterial FAs loaded negatively (Fig. 3a). Scores for net tows showed that larger zooplankton were associated with fresh material while seston was negatively loaded (Fig. 3b).

PC2 explained 26% of the variance and represented a seasonal axis showing that lipid parameters associated with marine lipids such as 22:6n-3 were positively loaded while those associated with terrestrial sources (18:2n-6 and 18:3n-3) were negatively loaded (Fig. 3a). PLs and TAGs are not diagnostic for marine or terrestrial sources; however, in this study it is clear that PLs are associated with marine derived lipids while TAGs are associated with terrestrially derived lipids. The distribution of the sample scores along PC2 shows a definite seasonal trend with samples taken in August and September positively located and those from the later in the fall negatively loaded (Fig. 3b).

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

Higher proportions of 18:4n-3 were found in large zooplankton (>200 µm) compared to seston (Table 2). The origin of this FA has previously been a source of some debate (Ramos et al. 2003); however, it has been linked to bacterial (Murphy & Abrajano 1994) and dinoflagellate sources (Dalsgaard et al. 2003). Further, this FA is likely an intermediate step in the chain elongation and desaturation pathway that copepods such as the harpacticoid *Tisbe holothuriae* use to form 22:6n-3 and 20:5n-3 from 18:3n-3 (Norsker & Stottrup 1994). The position of 18:4n-3 close to terrestrial markers in PCA analysis and away from other bacterial markers is evident in Fig. 3a. This indicates that elevated 18:4n-3 in copepods is associated with chain elongation of terrestrial material within zooplankton and not due to an increase in bacterial or dinoflagellate sources. Compound-specific isotope data, presented below, support the origin of significant amounts of 18:4n-3 as being from a terrestrial carbon source.

Primary producers

The lipid composition of 3 photoautotrophs (eelgrass blades, macroalgae, and eelgrass epiphytes) is shown in Table 2. The 2 major lipid classes in eelgrass blades were PLs (32%) and AMPLs (34%), while the major FAs were 16:0 (22%), 18:2n-6 (13%), and 18:3n-3

(27%). Levels of these FAs agree with previous studies on FA signatures of eelgrass blades and roots (Kharlamenko et al. 2001). Epiphytic algae had increased proportions of 16:1n-7 (diatom marker) and high levels of bacterial markers (8.3%), while macroalgae were characterized by elevated levels of 20:4n-6 relative to other sources of primary production (Table 2).

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

Epibenthos

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

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

Using 12 lipid class and FA variables, we were able to explain 54% of the variation in zooplankton and epibenthic prey collected from August to November in Newman Sound (Fig. 5) in the first 2 PCA axes. The lipid coefficient distribution showed that FAs associated with marine sources were loaded positively on PC1 (32% of the variance, Fig. 5a), while those associated with terrestrial material were negatively loaded. PC2 explained 22% of the variation, and FAs associated with the benthos, including benthic diatoms (16:1n-7) and macroalgae (20:4n-6), were negatively positioned. Conversely, FAs and lipid classes associated with water column terrestrial (18:2n-6 and 18:3n-3, HCs, and AMPLs) and marine sources (docosahexaenoic acids, DHAs and PLs) were positively loaded.

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

Along the pelagic to benthic axis (PC2, Fig. 5b) zooplankton were located positively, indicating they had

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

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

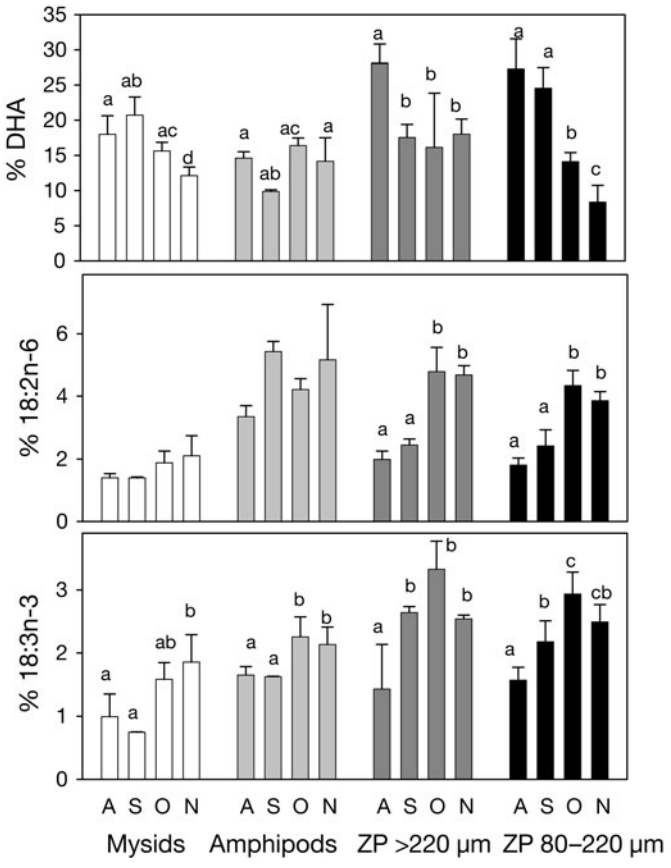

Fig. 4. Seasonal changes in polyunsaturated fatty acid (PUFA) levels in 2 types of epibenthic and 2 sizes of zooplankton during the late summer and fall of 2002. DHA: docosahexaenoic acid; ZP: zooplankton. A: August; S: September; O: October; N: November; 1-way ANOVA, p = 0.05

dietary input from pelagic terrestrial and marine sources and not from macroalgae and benthic diatoms. In contrast, mysids had a negative position along PC2, which indicates that benthic diatoms and macroalgae were major sources of dietary carbon. Amphipods showed an intermediate position along PC2, and this may demonstrate consumption of a mixture of organic material.

Juvenile cod

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

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

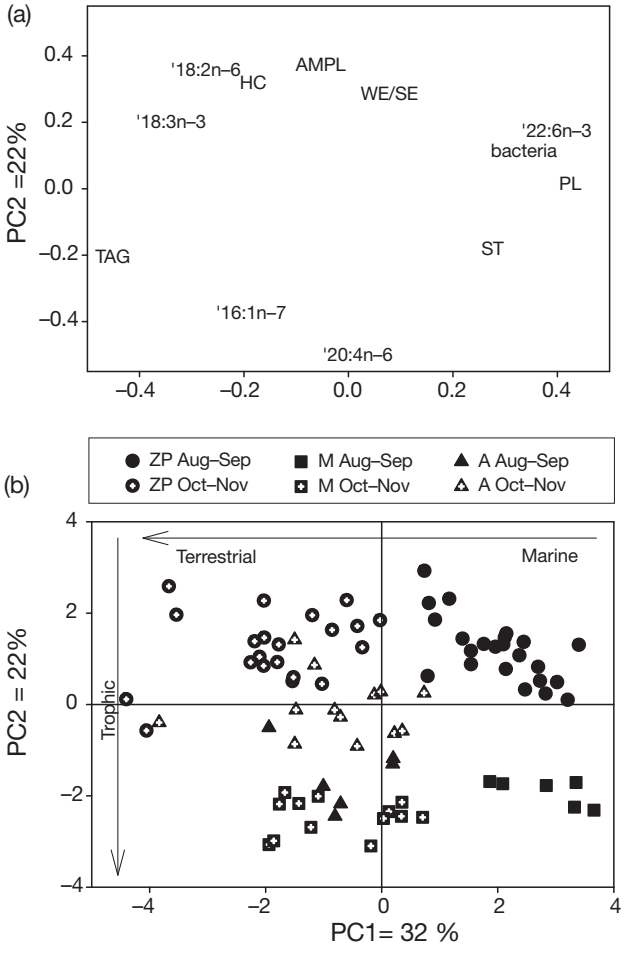

Fig. 5. Principal component (PC) analysis of epibenthic prey and zooplankton available from August to November 2002 in Newman Sound. (a) Lipid loading coefficients for PC1 and PC2. WE/SE: wax and steryl esters; ST: sterols. Other abbreviations as in Fig. 3. (b) Scores of summer and fall zooplankton (ZP), mysids (M), and amphipods (A) for PC1 and PC2

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

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

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

pelagic juveniles (Table 4). When pelagic juveniles were compared across 4 mo, fish collected in August showed significantly lower levels of terrestrial FAs than pelagic fish collected later in the season (Table 4).

PCA of specific FAs and lipid classes in both settled and pelagic juvenile cod from August until November is given in Fig. 6. PC1 explained 51% of the variance, and examination of the lipid loading coefficients indicates that the marine FAs found in cod phospholipids are positively loaded on PC1 while terrestrial FAs that are normally stored in neutral lipids are negatively loaded. When the scores for fish are examined, the August pelagic juveniles are located positively on PC1 with pelagic juveniles from later in the season moving to the negative side of the axis. Most negatively loaded onto this axis are the settled juveniles from November that have the highest relative amounts of terrestrial FAs and the lowest level of lipid as a proportion of wet weight. This confirms that fish mimic both pelagic and benthic prey items with increased terrestrial makers in November compared to August. Higher levels of C_{18} PUFA in both zooplankton and epibenthic prey likely also decreased food quality for fish in November.

Lipid classes and FAs from juvenile cod are shown as a function of month and settlement status (Table 4). Total lipids were 1.3 to 2.1% of wet weight, while previous reports on Newfoundland adult cod lipid composition showed that the flesh had 0.6% lipids while the liver contained 24% (Copeman & Parrish 2003). Settlement has previously been defined as 60 mm in standard length (SL) based on behavioral and pigmentation characteristics (Methven & Bajdik 1994, Laurel et al. 2003a) and more recently based on biochemical characteristics (Copeman et al. 2008). Table 4 shows that pelagic juvenile cod had low levels of total lipids and high proportions of PLs relative to TAGs, indicating few lipid reserves were present in the liver during settlement in the nearshore. Further, Copeman et al. (2008) found a significant break point in the regression of lipid class parameters with SL that occurred at the time of settlement. Cod also increased use of PLs and sterols and decreased relative amounts of lipid in the flesh at ~60 mm SL. Continued growth, with reduced energy storage, indicates that gape-limited predation pressure was a significant factor driving lipid utilization during settlement. This theory is supported by high predation

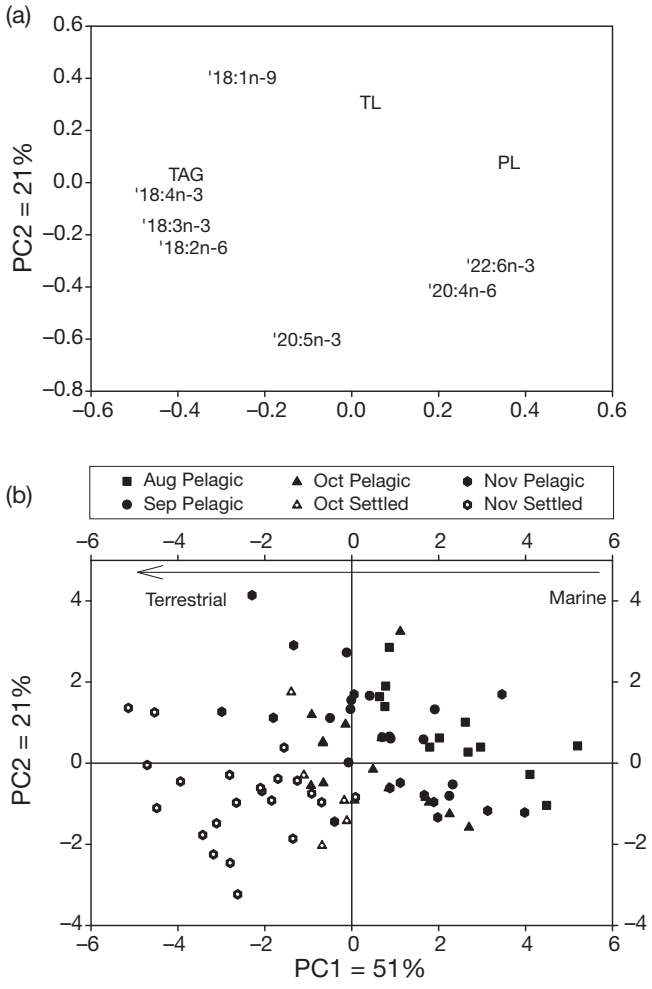

Fig. 6. *Gadus morhua.* Principal component (PC) analysis of lipid class and fatty acid proportions in juvenile cod collected in Newman Sound during summer and fall 2002. (a) Lipid loading coefficients for PC1 and PC2. TAG: triacylglycerols; PL: phospholipids; TL: total lipids per wet wt (mg g^{-1}). (b) Scores for pelagic and settled fish for PC1 and PC2

rates measured over eelgrass habitat in Newman Sound (Linehan et al. 2001, Laurel et al. 2003a).

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

The main SFAs, MUFAs, and PUFAs in the whole bodies of cod were 16:0, 18:1n-9, and 20:5n-3 + 22:6n-3, respectively (Table 4). Levels of these FAs are in agreement with amounts previously found in whole-animal analysis of cod (Kirsch et al. 1998). Like most organisms, fish can carry out de novo biosynthesis of SFAs and MUFAs with chain lengths up to C_{18}; however, synthesis of longer chain C_{20} + C_{22} PUFAs is inadequate to meet physiological demand (Henderson & Sargent 1985, Sargent et al. 1989). Therefore, high levels of preformed long-chain PUFAs are required in the diet.

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

Compound-specific isotope analysis

Fig. 7a shows the stable carbon isotope values for specific FAs found in various primary producers in eelgrass habitat. Unfortunately, samples of epibenthic algae from eelgrass were too weak to be analyzed using this method. For other primary producers, an early August sample and a November sample were analyzed. Early values were more enriched in ^{13}C compared to later values. When the $\delta^{13}C$ weighted mean was calculated for all identified FAs, the following ranges were found: eelgrass (early: –13.50‰, late: –14.6‰), macroalgae (early: –20.7‰, late: –22.9‰), and seston (early: –23.1‰, late: –24.7‰). Values for $\delta^{13}C$ in the smallest net tow fraction were in agreement with literature values for plankton sampled in late October in Notre Dame Bay, Newfoundland (Budge et al. 2001). 18:3n-3 and 18:2n-6 had lower $\delta^{13}C$ values in seston and zooplankton (Fig. 7).

The stable carbon isotope values for specific FAs found in fish (n = 6), zooplankton (n = 2), epibenthic prey (n = 4), and literature values for Newfoundland marine plankton and a terrestrial plant are given in

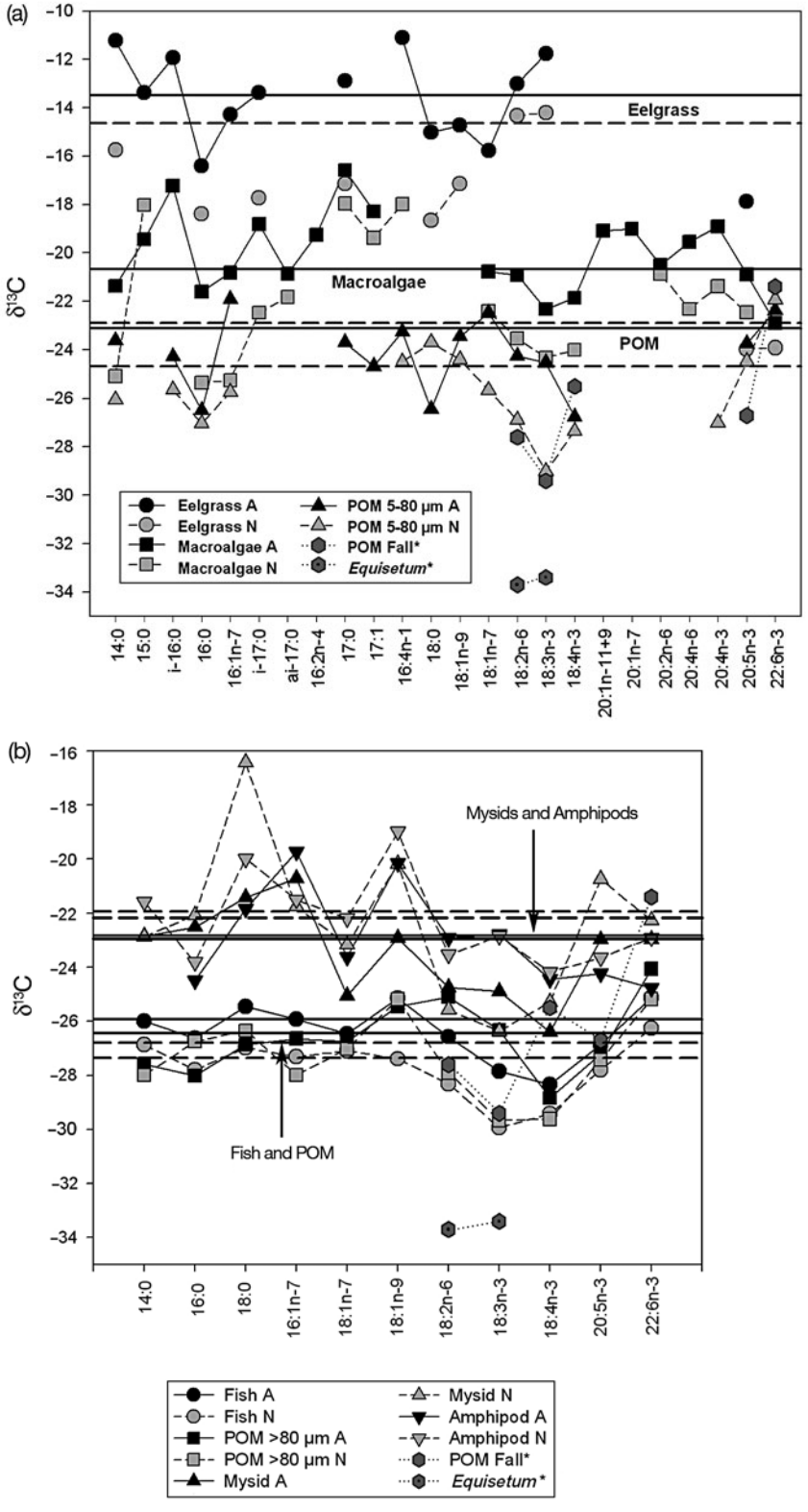

Fig. 7. Compound-specific fatty acid isotope data for (a) primary producers and (b) secondary consumers in August (A) and November (N) during 2002. POM: particulate organic matter. Lines represent the weighted averages for all fatty acids in a given sample. *Literature data for net tow and terrestrial plants (*Equisetum*) are from Budge et al. (2001)

Fig. 7b (Budge et al. 2001). Mysids and amphipods had more ^{13}C enriched values than zooplankton and fish, while *Equisetum*, a terrestrial plant, showed the most depleted values for the terrestrial markers (18:2n-2 and 18:3n-3). Similar values for $\delta^{13}C$ in 18:4n-3 and 18:3n-3 found in copepods (Fig. 7b) further support the assertion that they have similar origins.

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

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

Recently, Boschker et al. (2005) examined the phospholipid-derived FAs in the plankton community along a salinity gradient in a North Sea estuary. Their results varied with salinity (1 to 32), but at salinities similar to ours (31) they found levels of $\delta^{13}C$ in their green algae marker (18:3n-3) to be –26‰. We report values of 18:3n-3 in the fall as depleted as –29‰. Further, Boschker et al. (2005) reported very low concentrations of freshwater algae-derived FAs (18:3n-3, 18:2n-6, 18:4n-3) at salinities >5 compared to samples collected at <5. Also, our identification of net tow samples showed no fresh phytoplankton cells, with our smallest size fraction domi-

nated by fecal pellets and detritus. Therefore, freshwater algae are not a likely source of the depleted FAs (18:3n-3, 18:2n-6, 18:4n-3) that we observed in increasing proportions during fall.

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

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

CONCLUSIONS

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

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