Food web structure within an estuary of the southern Gulf of St. Lawrence undergoing eutrophication


Abstract: This study compared food web structure in eutrophied Ulva lactuca-dominated areas within an estuary in the Southern Gulf of St. Lawrence, with Zostera marina beds within the same estuary. The estuarine food web consisted only of primary producers, mesograzers, and secondary grazers, with the absence of piscivorous top predators. It was hypothesized that the altered plant habitat would lead to structural changes in the food web and the dominance of benthic carbon. Stomach contents from mummichog (Fundulus heteroclitus), fourspine stickleback (Pogonichthys quadracus), and American eel (Anguilla rostrata) showed that only mummichog had significant differences in prey items between the different habitats. Stable isotope showed that there were no significant differences in the food web structure and individual species’ δ13C values. A 13C spike in particular organic matter during the onset of anoxia in July, presumably due to bacterial blooms, indicated the complete dominance of benthic carbon the pelagic food web during this month. Thus, blooms of heterotrophs during anoxic events may have the greatest influence on nutrient cycling in estuaries undergoing eutrophication.

Résumé : L'étude compare la structure du réseau trophique dans des zones eutrophisées dominées par la laitue de mer (Ulva lactuca) d'un estuaire de la partie sud du golfe du Saint-Laurent et dans des lits de zostère marine (Zostera marina) dans ce même estuaire. Le réseau trophique estuarien ne comprend que des producteurs primaires, des mésoprédateurs et des brouteurs secondaires, les prédateurs piscivores de plus haut niveau trophique en étant absents. Il était postulé que l'habitat végétal modifié se traduirait par des changements structuraux au réseau trophique et une prédominance du carbone benthique. L'analyse des contenus stomacaux de choquemorts (Fundulus heteroclitus), d'épinettes à quatre épines (Pogonichthys quadracus) et d'anguilles américaines (Anguilla rostrata) a révélé que seuls les choquemorts présentaient des différences significatives selon l'habitat sur les éléments de proie. L'analyse des isotopes stables a, quant à elle, révélé que les valeurs de δ13C ne variaient pas de manière significative selon la structure du réseau trophique ou l'espèce. Un pic de δ13C dans la matière organique partielle au début de l'anoxie en juillet, vraisemblablement dû à des proliférations d'algues, indique une domination totale du carbone benthique dans le réseau trophique pêlagique durant ce mois. Ainsi, les proliférations d'hétérotrophes durant les épisodes anoxiques pourraient exercer la plus grande influence sur les cycles des nutriments dans les estuaires en voie d'eutrophisation. [Traduit par la Rédaction]

Introduction

Eutrophication, caused by excess nutrients entering an aquatic system, causes a shift in estuarine vegetation from seagrasses to macroalgae; this is a problem in coastal waters around the world (Valiela et al. 1997; Cloern 2001). With increased nutrient levels, macroalgae such as sea lettuce (Ulva lactuca) generally grow faster than seagrasses like eelgrass (Zostera marina) and, thus, out-compete (e.g., by shading) light-limited seagrasses (Valiela et al. 1990). Altered plant habitat has been found to significantly influence fish communities. Previous research has found higher fish abundance and species richness at sites with eelgrass than at sites with macroalgae (Hughes et al. 2002; Wyda et al. 2002).

As estuarine eutrophication progresses, the benthic environment degrades, and the filter feeders, largely mollusks, are the first to disappear in macrobenthic succession (Pearson and Rosenberg 1978). In areas where a large biomass of sea lettuce has accumulated and the benthic environment has become anoxic, the changes in the relative abundance of filter feeders, such as mussels and clams, as compared with grazers and detritivores, such as amphipods and snails, may be reflected in changes in the food web as a result of shifts in fish diet. Stable isotope values of carbon and nitrogen have commonly been used as a tool to delineate food webs by tracking trophic nutrient transfer. Since the proportion of 15N increases an average of 3.4% with each trophic level, nitrogen isotopes provide information about an organism's food chain position (Minagawa and Wada 1984; Post 2002). The carbon isotope value of an animal reflects the relative contribution of pelagic and benthic primary producers because pelagic algae typically have a smaller proportion of 13C than benthic plants or algae (DeNiro and Epstein 1978; Peterson and Fry 1987; Post 2002).

Prince Edward Island (PEI), Canada, has high (~50%) agricultural land use, and estuarine eutrophication is evidenced by dense sea lettuce blooms, the resultant loss of Zostera, and anoxic events since the late 1990s (Raymond et al. 2002; Bugden et al. 2013).
Anoxic events have been most severe on the north shore of PEI because of the low tidal amplitude and reduced flushing (Bugden et al. 2013). This eutrophication is a direct result of high nitrogen loads associated with row crop farming that uses primarily nitrate-based fertilizers (Finley et al. 2010, 2013). Schein et al. (2012) found significantly different fish communities in areas with eelgrass beds than in areas dominated by macroalgae within a single PEI estuary. In particular, there were shifts to higher abundance of resident estuarine fish that feed heavily on omnivores, with the mummichog (Fundulus heteroclitus) more abundant in Ulva habitat, and the threespine stickleback (Gasterosteus aculeatus) more abundant in Zostera habitat. In contrast, there were no changes in the relative abundance of the fourspine stickleback (Apeltes quadricaudus), one of the most abundant invertebrate grazers. The only resident predator, the American eel (Anguilla rostrata), also increased in relative abundance in Ulva beds.

The empirical purpose of this study was to document the spatial and temporal nature of the food web in a shallow, microtidal estuary on PEI using both stable isotope and stomach contents techniques. It was hypothesized that since plant habitat was previously observed to alter the fish community structure, the structure of the food web would also be altered. Specifically, the overall hypothesis was that at Ulva-dominated stations, there would be a reduction in filter-feeding invertebrates and an increase in consumers of Ulva. This in turn would lead to an overall change in the food web structure and would result in a greater proportion of benthic carbon in the grazer and predatory fishes. A single estuary undergoing a gradient of eutrophication was chosen for the study to minimize confounding factors between estuaries. A wide range of species was sampled in 2007 to get a preliminary look at the overall food web structure, while in 2008 the key species were chosen for a more in-depth examination of the temporal patterns of isotopic variability. To further evaluate changes in food web structure the stomach contents of the three fish species mummichog, fourspine stickleback, and American eel were examined.

Materials and methods

Study area

The Stanley River estuary complex empties into New London Bay on the north side of PEI (Fig. 1). Detailed land use and estuarine descriptors have been previously published (Schein et al. 2012). This estuary is in the process of undergoing eutrophication as measured by the upstream to downstream transition from eelgrass beds to sea lettuce mats as the primary plant habitat (Schein et al. 2012). This makes this estuary ideal for such a study, as both eutrophied and less eutrophied conditions exist within a single estuary, eliminating confounding factors of tidal and land use differences when comparing between estuaries.

Samples were collected for stable isotope analysis in five areas with differing vegetation: one in each of the three tributaries to the main estuary, Trout (TR), Granville (GR), and Founds Mills (FM), and two in the area of their confluence: Point (PT) and Stanley (ST) (Fig. 1). The three upstream stations (TR, GR, and FM) were chosen so as to be central within those sub-estuaries, and because they had large mats of Ulva and no Zostera beds (only isolated plants were present), whereas the two stations PT and ST had extensive eelgrass beds of greater than 100 m in length and limited amounts of sea lettuce. The maximum depth at the stations ranged from 4.5 to 6.5 m.

Sampling procedure

At each of the five stations, primary producers, invertebrates, and fishes were collected for stable isotope analysis by beach seining (30 m x 1.5 m seine, 3 mm mesh, and 1.2 m bags) or digging in the sediment (clams and polychaetes). In 2007 and 2008, enough individuals of each species or invertebrate taxon were collected for six replicates for each station. Fish were frozen in Whirl-Pak bags at -20 °C after the removal of the stomach and intestines. Invertebrates were kept in water for 24 h so they could clear their guts of food and were then frozen at -20 °C. Sea lettuce and eelgrass were frozen in Whirl-Pak bags at -20 °C.

The 2007 field season was used as a preliminary examination of the food web, to better design the methods for the following year of study. From 14–16 August 2007, sea lettuce, eelgrass (P. paludosum), sand shrimp (P. vulgata), grass shrimp (G. septemspinus), snails (N. obsoleta), clams (M. arenaria and M. balteata), isopods (I. balitkia), amphipods (G. sp.), mummichog, fourspine stickleback, threespine stickleback, nine-spined stickleback (L. mullus), Atlantic silverside (M. m. blanci), and northern pipefish (S. fuscus) were collected. On 18 September 2007, American eels were collected from fyke nets of a commercial fisherman (2 to 10 eels per station). Skinless dorsal muscle fillets were taken from the eels and frozen at -20 °C. Zooplankton were collected in August 2007 from just below the water surface with a 20 μm mesh, 0.5 m conical net towed from a boat. The collected material was filtered through a 250 μm sieve, and the material that did not go through was kept as zooplankton and frozen at -20 °C. On 18 September 2007, water was collected in plastic bottles from just below the surface at each station. This water was filtered through a 125 μm sieve, and then approximately 3 L of water was filtered onto three separate prewetted glass fibre (GF/C) filter papers per station, creating three replicates of particulate organic matter (POM) samples. POM was used as a
measure of phytoplankton for stable isotope analysis (Jeffrey et al. 1983).

In 2008, eelgrass, sea lettuce, Macoma balthica, polychaetes (Nereis diversicolor), mummichog, and fourspine stickleback were collected at each station monthly from April to August, because these were determined to be the numerically dominant resident estuarine species in the food web after the 2007 sampling. Between 23 April and 27 August 2008, zooplankton samples were collected nine times using a 153 μm mesh, 0.5 m conical net towed from a boat. In the laboratory, water from the plankton tow was filtered through 2 μm, 500 μm, and 125 μm sieves, and then filtered by the material filtered by the 125 μm sieve was kept as zooplankton and frozen at −20°C. On the same days, water was collected nine times in brown bottles from just below the surface for POM samples and chlorophyll analysis. In the laboratory, approximately 3 L of the surface water per station was filtered through a 75 μm sieve and then filtered onto three separate precut firm filters (GF/C filter paper, creating three replicates of POM samples. One litre of water per station was filtered onto a precut filter paper, which was then stored in 5 mL of acetone at −80°C until processing for chlorophyll analysis.

**Water chemistry and chlorophyll analysis**

Water chemistry and chlorophyll analysis was conducted over time in 2008 to relate these values to the temporal changes in food web isotopes. The primary hypothesis surrounding these measurements was that as the oxygen becomes depleted, isotopic changes would occur in the planktonic component of the food web. Furthermore, chlorophyll would indicate if the isotopic changes were associated with blooms of autotrophic organisms. Water temperature, salinity, dissolved oxygen, and pH were measured with a YSI MDS 650 multiparameter water quality meter equipped with a model 600QS sond (YSI Inc., Yellow Springs, Ohio, USA) on 20 August 2007 at 1 m below the surface of the water in the middle of the estuary and during each seining event in 2008 in the middle of the water column, approximately 5–10 m from shore (representing the middle of the seined area). In addition, water column profiles were measured at a water quality monitoring site parallel to each seine station in the middle of the estuary, nine times from 23 April to 27 August 2008, at 30 cm intervals from the bottom to the surface. Secchi depth was also measured at these locations.

Chlorophyll analysis was performed by high-performance liquid chromatography (HPLC) as previously described (Schein et al. 2012). Briefly, chlorophyll extracted with acetone was chromato- graphed with C18 reverse-phase HPLC. Chlorophyll was quantified against pure standards of chlorophyll a and b (Sigma) either at a fixed absorbance wavelength of 430 nm or using 430 and 650 nm fluorescence excitation and emission wavelengths, respectively.

**Stable isotope analysis**

Sea lettuce and eelgrass were washed with distilled water to remove any clinging debris, and epiphytes were scraped off. Shrimp were cut down the middle and the shell was removed and discarded. Clam shells were opened and discarded; the whole clam including the foot muscle was analysed. Snails were pulled out of their shells with forceps. Isopods, amphipods, zooplankton, and polychaetes were placed into vials whole. A dorsal muscle fillet (skin removed) was taken from the fish for analysis. In most cases there were six replicate individuals (except for small invertebrates, when two or three individuals were pooled) for each time and station. Exceptions were POM and zooplankton, where only one replicate per site and date was used in each case. For POM, three replicates were run on three occasions to test for repeatability; the mean coefficient of variation of these replicates was 0.0072 for δ13C and 0.0066 for δ15N. For zooplankton, six replicates from 10 June 2008 were run for each station; the mean coefficient of variation of these replicates was 0.0091 for δ13C and 0.018 for δ15N.

All samples were placed in 7 mL glass scintillation vials, dried in an oven at 60°C for 24–48 h, and ground into a fine powder using a mortar and pestle. Eelgrass was ground in a ball mill grinder because it was too tough to grind by hand. Ground plant (1.0 mg) and animal (0.2 mg) tissues were weighed into tin cups, while slices of filter paper with POM on them were put directly into the tin cups. These samples were combusted to gas using either a Carlo Erba NC2500 or a Costech ECS 4010 elemental analyser. A continuous flow of helium sent the resulting gases to a Finnigan Delta Plus or a Finnigan Delta Plus XP isotope-ratio mass spectrometer. Isotope ratios were expressed as a difference from an international standard (Vienna Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen) and calculated as parts per thousand (‰) with the following equation:

\[ \Delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \]

where \( X = {^{15}}\text{N} \) or δ13C and \( R = {^{15}}\text{N} / {^{12}}\text{N} \) or δ13C/δ12C. Working standards (bass muscle, bovine liver, niacinamide) that have been calibrated against the International Atomic Energy Agency standards CH6, CH7, N1, and N2 were used to correct the sample data. Over all runs, a commercially available standard (acetanilide, Elemental Microanalysis, Ltd.) had a coefficient of variation of 0.007 and 0.14 for δ13C and δ15N, respectively (n = 125).

**Stomach contents analysis**

Immediately after capture, the stomachs were removed from 20 mummichogs and 20 fourspine sticklebacks at each station every month in 2008 except April, when stomachs were collected from only 10 fish of each species. The stomachs were placed in vials with ethanol to preserve them until they were studied under a dissection microscope to determine the stomach contents of each fish. The number of each food item and the number of fish with empty stomachs, or with unidentified digested matter in their stomachs, was recorded. A bulk mass for each food item from all fish at a station was obtained for each species in each sampling month to calculate the relative contribution of each item to the total mass of stomach contents. Food items were identified to the most easily quantifiable level, ranging from class (Polychaeta) to species (Mya arenaria). Stomach contents of eels were obtained nonlethally by pumping water into their stomachs using a garden spray apparatus with a plastic pipette to flush out the contents. Stomach contents were only acquired from sea lettuce locations because eels were not frequently caught in eelgrass or they had empty stomachs.

**Data analyses**

Prior to parametric analysis, assumptions were assessed using probability plots for normality and Levene’s and Brown–Forsythe test for homogeneity of variance. Data were log transformed to meet the assumptions of parametric statistics when necessary. To test for differences in the overall pattern of carbon and nitrogen stable isotope values between sea lettuce and eelgrass stations, multivariate analysis of variance (MANOVA) was run for each time period, with vegetation type as the independent variable and δ13C and δ15N for every species evaluated as dependent variables. Wilks’ lambda was used as the multivariate test statistic. Alpha was set at \( p = 0.05 \), The maximum trophic position was calculated as \( 1 + (\delta^{15}N \text{organism} – \delta^{15}N \text{POM}) / 3.4 \) according to Post et al. (2000).

To determine whether the diet of mummichog and fourspine stickleback differed by station and across months, the multivariate statistics program PRIMER, version 6, with PERMANOVA was used in a two-way analysis with habitat type for each station nested within month (Clarke and Warwick 2001, PRIMER-E Ltd., Plymouth, UK). As this is a permunational ANOVA, it is distribution free, thus it does not have the same assumptions as parametric tests. Total cumulative biomass of each prey item for each
station was used as the dependent variable without transformation using a Bray–Curtis similarity matrix. To find out which food items were most responsible for causing significant differences between sea lettuce and eelgrass stations where they were observed, a SIMPER (similarity percentages) test was performed. To see whether there were differences in fish length among sea lettuce and eelgrass stations (a factor that may influence stomach contents), separate one-way nested ANOVAs were performed for each month, with vegetation and station nested in vegetation as factors.

Results

Water quality

Within a given month in 2008, stations varied little in water environmental parameters (Schein et al. 2012). The mean (±SD) difference in salinity between the furthest upstream and downstream stations during a given sampling period was 2.9 ± 1.8. In 2008, water quality variables were evaluated at stations at the deepest area immediately adjacent to the seine location and are summarized according to plant habitat (Figs. 2A–2C). Bottom dissolved oxygen decreased throughout summer, reaching anoxic levels by early August in the areas adjacent to sea lettuce-dominated habitat. Chlorophyll a showed peaks corresponding to a small phytoplankton bloom in the first half of June and a larger bloom in late August. Secchi depth indicated a decrease in water clarity from April till June in both sea lettuce and eelgrass sites, but a second decrease in water clarity only at the sea lettuce sites in late August, corresponding exactly with the observed increase in chlorophyll a.

Isotopic temporal variability

Short-lived organisms showed very significant temporal variation in isotope values (Figs. 2D and 2E). POM had a carbon value that ranged from −26.58% to −10.99% across all stations over the course of the summer. The 14 July 2008 samples were particularly heavy in δ¹³C, and POM δ¹³C was between −15.36% and −10.99%. To attempt to explain this, the POM samples from 14 July, along with controls from 10 June, were acid-treated with 1.0 mol/L HCl to see if carbonaceous algae were causing the less-negative δ¹³C value. The acid treatment had no effect on the δ³¹C of samples from either day (<1% difference). The zooplankton δ¹³C roughly mirrored that of POM and ranged from −27.27% to −16.78% over the sampling season, whereas on 14 July 2008 it was between −17.76% and −16.78%, indicating that the zooplankton were likely consuming the δ¹³C-heavy POM. The δ¹⁵N of POM and zooplankton did not fluctuate as much over time. It ranged from 3.07% to 6.71% for POM and from 6.05% to 9.45% for zooplankton. The enrichment of POM δ¹³C did not correspond with phytoplankton blooms (chlorophyll a on 10 June and 4 August) but fell in between those events. The peak enrichment of δ¹³C corresponded well with the beginning of declines in oxygen leading to an anoxic event at the sea lettuce sites.

Food web structure

A greater number of species were analysed from August 2007 than 2008 to get a preliminary assessment of the food web (Fig. 3). Sea lettuce and eelgrass had higher δ¹³C values than pelagic POM, though POM had higher δ¹³C values than expected, especially given the lower δ¹³C value of the zooplankton. Among the molluscs, the clam Mya arenaria received more nutrients from the pelagic food web (as suggested by low δ¹³C values) than the clam Macoma balthica, while the snail Ilyanassa obsoleta was the most benthic-feeding (highest δ¹³C values). The amphipods and isopods were closely linked to sea lettuce and eelgrass. The fish and shrimp species clumped together at the top of the food chain. Ninespine stickleback was at the top of the food web, followed by fourspine and threespine stickleback, though all three species were not a full trophic level higher than the other fishes. Based on
Fig. 3. Mean $\delta^{13}C$ and $\delta^{15}N$ of food web organisms collected at all sites in August 2007 in the Stanley River estuary, PEI. Diamonds, primary producers; circles, invertebrates; triangles, fish. POM, particulate organic matter; EG, eelgrass; SL, sea lettuce; ZP, zooplankton; MA, Mya arenaria (clam); MB, Macoma balthica (clam); GA, Gammarus spp. (amphipod); IB, Idotea balthica (isopod); IO, Nucula obsoleta (snail); GS, grass shrimp; SS, sand shrimp; PF, northern pipefish; AS, Atlantic silverside; MC, mummichog; EL, American eel; 3S, threespine stickleback; 4S, ninespine stickleback; 4F, fourspine stickleback. n ranged from 5 to 30 depending on how many individuals of each species could be caught at each station. Error bars are standard error.

$\delta^{15}N$ values for the ninespine stickleback in 2007, the maximum trophic position in this estuary was 3.15. The fourspine and threespine stickleback appear more linked than the ninespine stickleback to the benthic food web. Atlantic silverside and pipefish received more nutrients from the pelagic food web than other fish species.

Comparison of the overall food web structure between sea lettuce and eelgrass habitat conducted with MANOVA on a similar subset of organisms in August 2007, April 2008, and August 2008 showed no overall significant differences between the isotopic food web structure (Fig. 4). The only significant individual organism differences in $\delta^{13}C$ were in April and August 2008 (Table 1); with the exception of sea lettuce itself, eelgrass, zooplankton, and fourspine stickleback showed the opposite of what would be expected if benthic carbon were enriched in the sea lettuce habitat, with significantly higher $\delta^{13}C$ values found in the eelgrass habitat.

### Table 1. Statistically significant MANOVA univariate results comparing $\delta^{13}C$ and $\delta^{15}N$ values of different species between sea lettuce and eelgrass stations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isotope value and station type</th>
<th>$\delta^{13}C$</th>
<th>$\delta^{15}N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea lettuce</td>
<td></td>
<td>+2.1</td>
<td>+5.6</td>
</tr>
<tr>
<td>Eelgrass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zooplankton</td>
<td></td>
<td>+2.5</td>
<td>+0.7</td>
</tr>
<tr>
<td>Macoma balthica</td>
<td></td>
<td></td>
<td>+1.7</td>
</tr>
<tr>
<td>Gammarus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourspine</td>
<td></td>
<td>+2.1</td>
<td>+3.2</td>
</tr>
<tr>
<td>August 2008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eelgrass</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Note: In the table, numbers are the per mil (%) difference between the average value found at the sea lettuce stations and the average value at the eelgrass stations. The plus (+) denotes whether there was an increased value at sea lettuce or eelgrass stations.

Fish stomach contents

Approximately 23% of the 350 mummichog dissected had stomachs that were empty or only contained undigestible material. Mummichog from eelgrass habitat had a greater diversity of diet classes in their stomachs than mummichog from sea lettuce, with 11 and eight food categories represented, respectively (Table 2). The most abundant food item for mummichog from both habitats was gammarid amphipods. However, when examined by biomass, mussels comprised over half of the diet in mummichog from eelgrass habitat. Multivariate analysis (PERMANOVA) showed a significant difference overall in mummichog stomach contents between habitats nested within months and no significant effect of sampling month (Global $R = 0.542$). Of the four groups of organisms contributing most to the between-habitat variability (SIMPER), mussels comprised more of the diet at eelgrass stations, and amphipods, polychaetes, and snails contributed more at sea lettuce stations. Mummichog from sea lettuce stations were significantly longer than those from eelgrass stations in April (7.1 vs. 5.8 cm), but in all other months, the sizes did not differ significantly between vegetation types.

Fourspine stickleback was almost entirely reliant on gammarid amphipods that were found in 79% of the fish that had identifiable prey items. Amphipods also contributed the largest mass of any food item (Table 2). Unlike in mummichog, eggs were the second most prevalent food item (11%) in the stomachs of fourspine stick-
Fig. 4. Mean δ15N and δ18O of food web organisms collected in August 2007, April 2008, and August 2008 from the Stanley River estuary. POM, primary producers; circles, invertebrates; triangles, fish. POM, particulate organic matter; EG, eelgrass; SL, sea lettuce; PZ, zooplankton; MB, Macoma balthica; GA, Gammarus spp.; MC, mummichog; 4S, fourspine stickleback. n ranged from 3 to 18 at sea lettuce stations and 2 to 12 at eelgrass stations. Error bars are standard error; sometimes they are smaller than the symbol.

Fourspine stickleback showed no significant differences in diet biomass between plant habitat nested within month or between months (PERMANOVA). The mean length of f ourspine stickleback at sea lettuce stations was 4.2 cm, while the mean length at eelgrass stations was 4.4 cm.

A total of 28 eels (mean length ± SD = 42.7 ± 19.2 cm) caught at sea lettuce stations had identifiable stomach contents; no eels were caught at eelgrass stations. Forty-six percent of these eels had clams in their stomachs, while 32% had amphipods, and 4% had eggs. Stomach contents not as prevalent included mummichog (11%), unidentified fish pieces (11%), Uta (11%), shrimp (7%), and snails (4%). Biomass estimates showed that the stomach contents were 45.7% fish, 18.1% clams, 10% amphipods, 2.6% shrimp, 2.1% Uta, and 0.2% snails.

Discussion

The food web in the Stanley River estuary was short, having no more than three trophic levels: primary producers, mesograzers, and secondary grazers, with an absence of fish top predators. As indicated by analysis of the overall food web pattern and comparison of absolute isotopic values of individual organisms in the food web, the estuarine isotopic food web structure was not different between sea lettuce and eelgrass stations. Stomach content analysis did support the hypothesis that some mummichog in eelgrass habitat consumed a higher proportion of filter feeders (mussels) than grazers (amphipods). Unique findings of this study were the dramatic shift in the δ15N of POM and zooplankton across all sites in July, from a pelagic (autotrophic) to a benthic (presumed heterotrophic) carbon isotopic signature and the apparent absence of fish top predator species, as indicated by isotopic patterns.

The short food web in the estuary in question was likely a function of both the size of the system and the ability for piscivorous predators to survive and thrive in a relatively harsh environment. While no comprehensive review of the length of estuarine food webs has ever been conducted, a study of 25 northern temperate lakes showed that the value of the maximum trophic position metric ranged between 3.5 and 5.5 (Post et al. 2000). In those freshwater systems, maximum trophic position was associated with size (volume) and not with productivity. The comparatively low value of this metric in the current study (3.15) driven by the absence of a resident piscivorous fish species in the Stanley River estuary. Of the 15 species of fish captured in the estuary, only cunner (Tautogolabrus adspersus), brook trout (Salvelinus fontinalis), and American eel attain sufficient size to be dominant piscivorous predators (Schein et al. 2012). Of these, cunner and brook trout were very rare (<0.01% of total captures), and only eels are thought to be resilient enough to low dissolved oxygen (Lindman et al. 2005) to be resident in the estuary all year. Based on stomach contents data, even relatively large eels appear to be omnivorous rather than piscivorous, in agreement with their position in the food chain as determined isotopically.

Differences that existed in the trophic resolution between stomach contents and isotope data in mummichog were likely due to the differing temporal resolution of the two techniques. Tissue isotopes integrate the isotopic nature of the food consumed over a longer period, whereas stomach contents reflect diet only in the immediate period before sampling. Thus, differences in the mummichog isotope and stomach contents results can be reconciled when we consider that mussels were the only filter feeder observed to comprise a large component of the diet. Mussels of suitable size were likely not available for a sufficiently long period so as to cause changes in tissue isotope ratios. Thus, the strength of one technique over the other would likely be highly specific to the system studied and the sampling design. For example, in freshwater systems, isotope techniques have been demonstrated to show higher resolution of trophic level than stomach contents (Vinson and Budy 2011), whereas a study of
Table 2. The relative biomass and abundance (%) of each food item over all sampling months and stations within a vegetation type separated by sea lettuce and eelgrass stations.

| Diet item | Mummichog | | | | | Fourspine stickback | | |
|-----------|-----------|---|---|---|---|---|---|---|---|
|           | Relative biomass | Sea lettuce | Elgrass | Sea lettuce | Elgrass | Sea lettuce | Elgrass | Sea lettuce | Elgrass |
| (n = 129) | (n = 96) | (n = 129) | (n = 96) | (n = 93) | (n = 84) | (n = 93) | (n = 84) |
| Amphipods | 33.6 | 22.6 | 56.5 | 45.8 | 73.2 | 76.6 | 81.7 | 75.0 |
| Snails    | 29.1 | 0.8 | 39.5 | 4.0 | 8.7 | 7.4 | 3.2 | 6.0 |
| Polychaetes | 12.1 | 14.6 | 8.5 | 18.8 | 1.7 | 2.2 | 2.2 | 1.2 |
| Fishes    | 11.1 | 0.4 | 7.8 | 1.0 | 0.5 | 2.1 | 1.1 | 3.6 |
| Uvula     | 7.2 | 3.5 | 7.2 | 3.5 | 1.0 | 2.1 | 1.0 | 3.6 |
| Mussels   | 3.9 | 55.4 | 7.8 | 32.3 | 11.7 | 9.1 | 14.0 | 8.3 |
| Clams     | 4.3 | 3.1 | 3.1 | 3.1 | 4.1 | 1.1 | 3.1 | 4.1 |
| Eggs      | 0.5 | 0.5 | 3.1 | 1.0 | 1.0 | 1.1 | 1.0 | 1.1 |
| Beetles   | 0.1 | 0.1 | 1.0 | 1.0 | 0.1 | 0.1 | 0.1 | 0.1 |
| Diptera   | 0.1 | 0.1 | 1.0 | 1.0 | 0.1 | 0.1 | 0.1 | 0.1 |
| Eelgrass  | 1.3 | 2.1 | 1.3 | 2.1 | 3.1 | 3.1 | 3.1 | 3.1 |
| Shrimp    | 0.7 | 3.1 | 0.8 | 4.8 | 0.7 | 3.1 | 0.8 | 4.8 |
| Isopods   | 0.7 | 3.1 | 0.8 | 4.8 | 0.7 | 3.1 | 0.8 | 4.8 |
| Ants      | 0.7 | 3.1 | 0.8 | 4.8 | 0.7 | 3.1 | 0.8 | 4.8 |
| Mayflies  | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

Note: Only fish containing identifiable prey items (n) were included.

Estuarine food webs demonstrated that stomach contents gave more trophic resolution (Winemiller et al. 2007). Mummichog stomach contents and isolation values were consistent with their position in the food web as a generalist secondary benthic consumer. Many stomach contents studies have found that mummichog feed mainly on benthic organisms, including amphipods, polychaetes, snails, clams, and other small crustaceans, as well as plant and algal material (Kneib and Stiven 1978; Kelso 1979; Graham et al. 1998). All these items were found in mummichog guts in this study, although amphipods, mussels, and snails were most common. Despite the limited quantities of sea lettuce present in the eelgrass beds, the presence of Uvula in the stomachs of mummichog suggests that even in habitat dominated by eelgrass, mummichog may still prefer to forage for food items grazing on, or present upon, sea lettuce.

Fourspine stickback were found to be predominantly benthic feeders. Amphipods dominated the guts of the fourspine stickback in the present study. Similarly, Delbeek and Williams (1987) found gammarid amphipods and chironomid larvae to be the most important food items in fourspine stickback in a study that took place near St. Andrews, New Brunswick, Canada. Furthermore, fourspine stickback have been shown to be more efficient at eating gammarid amphipods than other stickleback species (Delbeek and Williams 1988). Eggs were more prevalent in the fourspine stickback than the mummichog, which could be why they were at a slightly higher trophic level than mummichog.

The temporal changes in isotopes of POM and zooplankton demonstrate a mid-summer period where the pelagic food web is dominated by benthic and not pelagic carbon in the entire upper estuary. This observation indicated a shift from autotrophs (phytoplankton) to heterotrophs (bacteria) during the period when Uvula is being decomposed. Live sea lettuce and eelgrass likely had comparable isolate values to their detritus, as shown in other studies (Fry 1984; Vizzini and Mazzolla 2004). The very 13C-enriched 14 July 2008 sample in which 13C was between -15% and -11% closely approximates the range of values of sea lettuce. Since the spike in heavier 13C did not correspond to an increase in chlorophyll a nor was it caused by inorganic carbon, it is most likely that this was caused by a bloom of bacteria. This was also supported by a decrease in Secchi depth just prior to this event and by the observation that zooplankton were consuming the material. It is not unusual for estuaries on PEI to turn completely white during an anoxic event, presumably due to blooms of bacteria utilizing the benthic carbon source from decomposing sea lettuce.

Subsequent work using flow cytometry and 16S rDNA sequencing in a number of estuaries undergoing anoxia has verified dominant communities of bacteria during anoxic or hypoxic events (James 2013).

Overall, eutrophication-induced plant habitat changes altered the fish community structure (Schein et al. 2012) but did not change the relative position of organisms within the food web. The fish community differs between the sea lettuce and eelgrass areas in this estuary, with mummichog and American eel being dominant in sea lettuce, while Atlantic silverside, threespine stickleback, and pipefish were associated with eelgrass (Schein et al. 2012). Fish abundance and species richness were significantly greater at eelgrass stations in August 2008 during an anoxic event that was more severe at sea lettuce stations (Schein et al. 2012). However, neither the absolute isotopic values of component species nor their relative position in the food web changed between the two plant habitats. In terms of the absolute isotopic values, this could be due in part to the strong influence that decomposing sea lettuce appears to exert on carbon flow in the entire estuary during mid-summer hypoxic or anoxic periods. However, this interconnectivity of nutrient flow would not influence the detection of substantial dietary shifts, as indicated by the relative position of organisms in the food web. Comparison of eutrophic estuaries with less impacted estuaries would be required to reveal the global importance of these observations with regards to nutrient cycling and eutrophication.

Acknowledgements

Field work would not have been possible without the help of Jennifer van der Lee, Christina Pater, and Joubin Safrany. Members of the Stable Isotopes in Nature Laboratory, University of New Brunswick, shared their knowledge about processing stable isotope samples and ran the samples. Funding was provided by a NSERC postgraduate scholarship to AS, an NSERC Undergraduate Student Research Award to KAC, and Canada Research Chair funding to MV.

References


PMID:10890443.


