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Sea Lamprey Carcasses Exert Local and Variable Food Web Effects in a Nutrient-limited Atlantic Coastal Stream.

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Abstract

Resource flows from adjacent ecosystems are critical in maintaining structure and function of freshwater food webs. Migrating sea lamprey *Petromyzon marinus* deliver a pulsed marine-derived nutrient subsidy to rivers in spring when the metabolic demand of producers and consumers are increasing. However, the spatial and temporal dynamics of these nutrient subsidies are not well characterized. We used sea lamprey carcass additions in a small stream to examine changes in nutrients, primary productivity, and nutrient assimilation among consumers. Algal biomass increased 57–71% immediately adjacent to carcasses, however broader spatial changes from multiple-site carcass addition may have been influenced by canopy cover. We detected assimilation of nutrients (via $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) among several macroinvertebrate families including Heptageniidae, Hydropsychidae, and Perlidae. Our research suggests that subsidies may evoke localized patch-scale effects on food webs, and the pathways of assimilation in streams are likely coupled to adjacent terrestrial systems. This research underscores the importance of connectivity in streams, which may influence sea lamprey spawning and elicit varying food web responses from carcass subsidies due to fine scale habitat variables.

Key words: *Petromyzon marinus*; sea lamprey; nutrient dynamics; marine-derived nutrient subsidies; streams; food webs

Introduction

Energy and nutrient flows across ecosystem boundaries can influence the structure and function of recipient ecosystems, alleviate nutrient limitation, and increase primary and secondary productivity (Vanni 2002; Polis et al. 2004; Lamberti et al. 2010). In aquatic systems, carbon, nitrogen, and phosphorus (an important subset of potential nutrient subsidies), may alleviate bottom-up constraints on productivity by facilitating in-stream production, and/or alter top-down effects if received directly by consumers (Rosemond et al. 1993; Lamberti 1996; Kiernan et al. 2010). Thus, stream production and food-web structure are determined largely by resource availability and assimilation through both autotrophic and heterotrophic pathways.

The effect of nutrient subsidies varies with the magnitude and duration of the resource, as well as the environmental and community-level processes of recipient systems (Marczak et al. 2007; Zhang and Richardson 2011). Pulsed nutrient subsidies may be sporadic or predictable, large or small in magnitude, but are often short lived and may alleviate nutrient limitations and stimulate productivity (Odum 1971; Yang et al. 2010; Weber and Brown 2013). Additionally, habitat variables (i.e., temperature, substrate, flow) may fluctuate across spatial and temporal scales influencing the effects of subsidies on food web structure (Roberts et al. 2007; Kohler et al. 2012). Thus, the pathways by which nutrient subsidies are utilized are specific to the context of the recipient ecosystem.

Migratory fish are vectors of nutrients and energy, and synchronous spawning events provide resource subsidies to ecosystems that support production of their offspring. Nutrients in the form of excretion, gametes, and carcasses may influence recipient stream food webs through various pathways (Gende et al. 2002; Tiegs et al. 2011; Childress and McIntyre 2015). Subsidies may be assimilated at the base of aquatic food webs in the form of inorganic nutrients, thereby

increasing algal biomass and primary productivity (Claeson et al. 2006; Kohler et al. 2008), or the production of heterotrophic microbes (Ruegg et al. 2011). Alternatively, subsidies may enter food webs through direct consumption by consumers (e.g., macroinvertebrates and fish; Lessard and Merritt 2006; Wipfli et al. 2003; Guyette et al. 2014); therefore nutrient response pathways vary and may be further modified by stream characteristics.

In Atlantic coastal waters, sea lamprey *Petromyzon marinus* spawning migrations deliver a pulse of marine-derived nutrient subsidies to freshwater streams and rivers in the spring. The decay rates of sea lamprey carcasses and subsequent water enriching effects of nitrogen and phosphorus occur over a relatively short period of several weeks (Weaver et al. 2015). At this time rising water temperatures and increased photoperiod stimulates primary productivity and increases the metabolic demand of consumers including young-of-the-year fish and macroinvertebrates (Hall 1972; Gustafson-Greenwood and Moring 1990; Nislow and Kynard 2009). During this period of nutrient and energy limitation, nutrient subsidies from sea lamprey received by Atlantic coastal waters are likely to be critical in maintaining structure and function of stream food webs.

We sought to quantify the spatial and temporal dynamics of sea lamprey nutrient subsidies on primary productivity and nutrient assimilation of stream organisms. We describe and present the results of two studies, the first was a carcass addition experiment in 2013 designed to determine temporal changes in primary productivity, which helped inform the design of a second experiment conducted in 2014 to investigate the spatial and temporal effects of nutrient subsidies in more detail. To address our objective, we sought to quantify changes in (1) stream nitrogen and phosphorus, (2) stream nutrient limitations, (3) spatial and temporal patterns

of primary productivity attributed to sea lamprey carcass nutrients, and (4) the assimilation of nutrients among select macroinvertebrates and juvenile sea lamprey (ammocoetes).

Materials and Methods

Study area

We conducted carcass addition experiments in 2013 and 2014 in Sedgeunkedunk Stream, a 3rd order tributary flowing into the Penobscot River at river kilometer (rkm) 36.5 (Figure 1; A and B). Two dams were removed on the stream, Mill Dam in 2008 and Meadow Dam in 2009, restoring 5.3 km and connectivity to the ocean. In subsequent years, spawning sea lamprey were regularly observed during spring in Sedgeunkedunk Stream (Gardner et al. 2012; Hogg et al. 2013). However, we selected study reaches where we observed no sea lamprey, evidence of nest building, or post-spawned carcasses during our experiments.

We collected pre-spawn sea lamprey for carcass addition experiments in 2013 from Veazie Dam (rkm 45.0) and in 2014 from Milford Dam (rkm 61.0) on the main-stem Penobscot River. Collection took place in May during migration, but before sea lamprey commenced nest building and spawning activities. All collected fish were measured for mass (± 0.1 g) and total length (± 1 mm), then stored frozen at -10°C until experimental addition.

Single Site Carcass Addition Experiment

In 2013, we selected a 20-m reach comprising of two riffle-run sequences that were similar in stream and riparian habitat (Figure 1; A). Fifty carcasses were placed in mesh bags, and randomly assigned to one of three 2.5-cm mesh metal cages (to discourage scavengers), and staked in the mid channel of the stream. The average individual carcass mass was 0.758 kg ($\pm$

0.023 SE); the total carcass mass added to the stream was 37.9 kg. Carcasses were deployed on 20 July, 2013.

Multiple Site Carcass Addition Experiment

Our results from 2013 directed our experimental design for 2014. In 2014 we chose a reach upstream from the previous year (Figure 1; B). We delineated 10 sites along an approximate 150 m reach (Figure 1; inset). Each site was comprised of a riffle and subsequent run that ended at the beginning of the next riffle; the average length for each site was 12 m. The two uppermost sites (1 and 2) were designated as reference sites. The following six downstream sites (3–8) were designated to receive 20 sea lamprey carcasses (120 carcasses total). Finally, the two lowermost sites (9 and 10) received no carcasses. Carcasses were caged similar to methods described above and were anchored at the upstream most end of each site. The average individual mass among all carcasses was 0.767 kg (± 0.02 SE); the mass added to each site averaged 15.3 kg (± 0.08 SE) and totaled 92.0 kg throughout the experimental reach. Carcasses were deployed on 25 June, 2014.

The numbers of carcasses we added to Sedgeunkedunk Stream during each experiment represent ecologically realistic densities that may be deposited after spring spawning. Mean estimates of sea lamprey spawning run densities after dam removal in Sedgeunkedunk Stream ranged 223–242 (47–51 fish/km; Hogg et al. 2013). Nislow and Kynard (2009) estimated 30–136 (100–453 fish/km) spawning sea lamprey in a 300-m reach in Fort River, a tributary to the Connecticut River, similar in width to Sedgeunkedunk Stream. Generally, however, population estimates of spawning sea lamprey throughout the Northeastern United States are not well characterized.

130 *Water chemistry*

131 During our multiple site carcass addition experiment (2014), we sampled stream water
132 for soluble nitrogen and phosphorus. Samples were taken 0.5 m from the right and left banks
133 and the mid channel at each of the 10 sites prior to the addition of carcasses, then after 12 h, days
134 1–4, then every other day until day 14. With the exception of our sample taken after 12 h, all
135 samples were collected during the same time of day. Approximately 60 mL of water was filtered
136 through 25-mm, 0.45- μm mixed cellulose ester membranes (Millipore Corp., Billerica,
137 Massachusetts, U.S.A.) with a syringe into an acid-washed bottle. Samples were stored frozen
138 until analysis. Samples were analyzed for dissolved inorganic nitrogen as ammonium (NH_4^+)
139 and nitrate (NO_3^-) by flow injection analysis (O.I. ALPKEM Flow Solution FS3000, College
140 Station, Texas, U.S.A.), and total soluble phosphorus by inductively coupled plasma optical
141 emission spectrometry (Thermo iCAP 6000, Thermo Fisher Scientific, Marietta, OH, U.S.A) by
142 the University of Maine Analytical Laboratory and Soil Testing Service. A filtered 60 mL
143 sample of deionized water, serving as a blank, was run periodically among sets of samples.
144 Detection limits for ammonium and nitrate were 0.002 and 0.0005 $\text{mg}\cdot\text{L}^{-1}$ respectively and 1.55
145 $\mu\text{g}\cdot\text{L}^{-1}$ for total soluble phosphorus.

146

147 *Primary productivity*

148 We used nutrient diffusing substrates to quantify changes in algal biomass and
149 subsequent nutrient limitation (Tank and Dodds 2003; Tank et al. 2006). Our nutrient solution
150 treatments consisted of 0.5M NH_4NO_3 , 0.25M KH_2PO_4 , 0.5M NH_4NO_3 + 0.25M KH_2PO_4 , and a
151 control (hereafter referred to as N, P, N + P, and C respectively). Solutions were amended with
152 2% agar and poured to the top of 60 mL polypropylene screw-cap bottles. The bottles were

153 topped with 2.5 cm diameter, 0.7 μ m glass microfiber filters (GE Healthcare Life Sciences,
154 Pittsburgh, Pennsylvania, U.S.A.). We bore holes through the caps, which were fastened over
155 the filters, securing them flush against the nutrient augmented agar solution.

156 We constructed arrays to house diffusers using 2.54-cm polyvinyl chloride (PVC) pipe to
157 serve as a rectangular base with 3.8-cm angled steel slats on top. Bottles were cable-tied to the
158 slats. In 2013 we constructed arrays that contained nine replicates of each nutrient treatment for
159 a total of 36 diffusers per array ($N = 72$ total diffusers). We deployed one array in the riffle
160 downstream of the carcasses and the other array in the riffle upstream of the carcasses. In 2014
161 we constructed arrays containing 3 replicates of each nutrient treatment for a total of 12 diffusers
162 per array. Three arrays were deployed at each site ($N = 360$ total diffusers). Arrays were
163 deployed downstream of carcasses at each site but required a minimum depth of 18 cm to
164 become fully submerged. All arrays remained submerged throughout both experiments. The
165 downstream distance between the added carcasses and the arrays varied, but were approximately
166 1–2 m. We did not exclude grazing invertebrates from our nutrient diffusing substrate arrays
167 during both carcass addition experiments; however, similar to Tank and Dodds (2003), we did
168 not observe invertebrate colonization among the arrays.

169 Replicates of each nutrient treatment within each array were sampled at one, two, and
170 three weeks after carcass addition as the majority of decomposition occurred during an initial
171 three week period (Weaver et al. 2015). Filters were lifted gently off the bottles with forceps,
172 placed into labeled 1.5 mL polyethylene tubes, and kept on ice in the dark. In the lab, filters
173 were stored at -10°C until extraction and analysis. Filters were homogenized using a 90%
174 acetone solution and a mortar and pestle. Extracted samples were analyzed for chlorophyll a ,
175 corrected for pheophytin using spectrophotometry (Strickland and Parsons 1972; APHA 1999)

with a Thermo Aquamate spectrophotometer (Thermo Fisher Scientific, Marietta, OH, U.S.A). During both years we lost a few diffusing substrata replicates, particularly in 2014 during high flows from a spate. We analyzed chlorophyll *a* in 57 samples in 2013 and 311 samples in 2014.

Stream habitat variables

During both experiments, temperature loggers (Onset, Hobo Pendant UA-001-08, Cape Cod, Massachusetts, U.S.A) were placed in the stream reach and retrieved at the conclusion of the experiment. Loggers continuously recorded temperature at one-hour intervals. During the multiple site carcass addition experiment (2014) we measured stream habitat characteristics at each of the ten sites. We measured total stream depth and mean column velocity at 0.5-m increments along one cross-sectional transect during base flow with a top-set wading rod and Swiffer model 2100 current velocity meter (Swiffer Instruments, Seattle, Washington, U.S.A). Percent overstory density was measured at each nutrient diffusing substrate array location with a spherical crown densiometer (model-A, Forestry Suppliers, Jackson, Mississippi, U.S.A), as described by Lemon (1956).

Fish and Invertebrate Collection

We caged individuals of a freshwater mussel, Eastern elliptio *Elliptio complanata* (Lightfoot 1786), and juvenile sea lamprey (ammocoetes) that we sampled before and after carcass addition for analysis of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopes. Ammocoetes and Eastern elliptio were collected approximately 2 km downstream of our experimental reach. Fifty ammocoetes were collected with backpack electrofishing and kept in aerated buckets prior to caging. Forty-two Eastern elliptio were collected by hand. We observed no mortality during

sampling and transport, and all individuals appeared to recover after capture. Ammocoetes were caged in a 0.25 x 0.25 m crate lined with fine screened mesh filled with fine sand obtained from adjacent areas in the stream. We observed ammocoetes bury immediately into the sediment upon addition to each cage. The tops of each cage were left open and were positioned in the stream so that the top was slightly above water. Eastern elliptio were placed into submerged 0.25 m diameter circular mesh pens. Half of the individuals of each species were placed at site 2, one of the upstream reference sites, and the other half at site 9, downstream of all 120 carcasses (Figure 1). We nonselectively sampled ammocoetes prior to carcass addition, then after three weeks. Eastern elliptio were nonselectively sampled prior to carcass addition, then after three and seven weeks. Macroinvertebrate samples representing several functional feeding groups were collected with a kicknet prior to carcass addition, and then after three and seven weeks at sites 1, 5, and 10 (Figure 1). Functional groups included scrapers (Ephemeroptera: Heptageniidae), predators (Megaloptera: Corydalidae; Plecoptera: Perlidae), and collector/gatherers (Trichoptera: Philopotamidae, Hydropsychidae).

Samples of adult sea lamprey tissue were taken prior to carcass addition. After euthanasia, a 1-cm² section of muscle tissue was extracted from the left dorsolateral side of six individuals. All macroinvertebrate and fish samples were stored at -80° C until sample preparation and stable isotope analysis.

Stable isotopes analysis

Stable isotope samples were prepared and analyzed at the University of New Brunswick Stable Isotopes in Nature Laboratory. Whole bodies of insects and ammocoetes, and the soft body component of Eastern elliptio extracted from the shell were used for analyses.

Macroinvertebrate gut contents were not removed, therefore stable isotope values reflect the whole body and food recently ingested. Samples were oven dried at 60° C for 24–48 h, and then ground into a fine powder with a mortar and pestle. Approximately 0.5 mg of each macroinvertebrate, mussel, and fish was weighed in tin capsules and combusted using a Costech 4010 Elemental Analyzer. Measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were performed using a Delta XP continuous flow isotope-ratio mass spectrometer (CF-IRMS; Thermo-Finnigan; Bremen, Germany). Stable isotope values were expressed in parts per thousand or permil (‰) and calculated as: $\delta X = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \cdot 1000$, where X is ^{13}C or ^{15}N , and R is the ratio of the heavy isotope to the light isotope ($R = ^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$ *sensu* Jardine et al. 2003). International standards were used to calculate R_{standard} values, which included Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen. Standard deviations of standard and sample repeats were approximately 0.1 ‰ or less for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Statistical analysis

We analyzed changes in chlorophyll *a* from the addition of sea lamprey nutrient subsidies from the single site carcass addition experiment using multi-factor analysis of variance (ANOVA). Residuals did not conform to a normal distribution (Shapiro-Wilk W test: $p < 0.05$; Zar 1999), therefore we applied a log transformation to chlorophyll *a* values, which satisfied normality assumptions. We modeled chlorophyll *a* as a function of week, nutrient treatment, and reach (treatment or reference) and all associated interactions. Furthermore, we determined whether carcass subsidies significantly increased chlorophyll *a* by modeling the chlorophyll *a* values of the control nutrient diffusers as a function of week and reach. Main effects and interactions were deemed significant at $p < 0.05$.

We analyzed changes in chlorophyll *a* and stream water nutrient concentrations from the multiple site carcass addition experiment using multi-factor ANOVA. For each time period (i.e. week for chlorophyll *a*; day for stream water nutrients), we averaged response values of the two reference sites (1, 2). We then subtracted mean reference values from each of the remaining downstream sites (3–10) for that particular time period. Residuals did not conform to a normal distribution (Shapiro-Wilk *W* test: $p < 0.05$; Zar 1999). We added a constant integer to each value so that negative numbers (i.e., treatments that were lower on average than the mean reference) were above zero. The value of the constant integer was chosen so that the lowest value was raised slightly above zero. Then, a log transformation was applied to the mean adjusted response (i.e., chlorophyll *a*, or stream nutrient concentration), which satisfied normality assumptions. We modeled chlorophyll *a* as a function of site, week, nutrient treatment, and the interaction of week and nutrient treatment. We modeled stream water nutrient concentrations as a function of site and day. Time was treated as a factor in both models. We conducted post-hoc pairwise tests for significant main effects.

We did not measure stream or terrestrial environmental variables continuously throughout the experiment, and could not include them in the multi-factor ANOVA models. We conducted a separate linear regression to analyze mean chlorophyll *a* values for each diffuser array (averaged across nutrient treatment and time) as a function of site-specific local overstory density.

Finally, we tested for spatial autocorrelation for chlorophyll *a* and stream nutrient concentrations among all treatment sites using a Mantel test. Two distance matrices were generated: one containing linear distances between each of the sites and another containing distances between our chlorophyll *a* values or stream nutrient concentrations. The Mantel test

computed the correlation of the two distance matrices, then calculated 1000 permutations to generate a p -value; p -values less than 0.05 allowed us to reject the null hypothesis that the spatial and response distances were unrelated (i.e., no autocorrelation).

We determined stream nutrient limitations during the single site and multiple site carcass addition experiments from samples collected at the reference sites using a multi-factor ANOVA. We modeled chlorophyll a as a function of week and the addition of nitrogen, phosphorus, nitrogen and phosphorus, or no nutrient addition (control). Significant main effects or interactions ($p < 0.05$) allowed us to infer nutrient limitation or colimitation (Tank and Dodds 2003).

We analyzed mean stable isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) with multivariate analysis of variance (MANOVA) with the Pillai's trace test to test for spatial and temporal differences in isotopic values among macroinvertebrate taxa, ammocoetes, and Eastern elliptio. We modeled the isotope values as a function of stream location and week. Data were tested for multivariate normality with Mardia's test (Mardia 1970), and for homogeneity of covariance matrices with a Box M test. Among taxa, data were multivariate normal, but did not meet the equal covariance assumption despite log transformation. The Pillai's trace test was selected because it is the most robust multivariate analysis when the assumption of equal covariance (i.e., heteroscedasticity) is not met (Johnson and Field 1993). Post-hoc multiple comparisons tests were conducted for those taxa with significant stream location main effects ($p < 0.05$) to determine differences in isotopic values between the reference site (Site 2) and two downstream treatment sites (Sites 5, and 9). All analyses were performed with the statistical package RStudio, (Version 0.99.491).

Results

Single Site Carcass Addition Experiment

Mean daily stream temperatures were 22.5 °C (± 0.25 SE) and ranged 20.0–26.6 °C. There were higher concentrations of chlorophyll *a* among all nutrient treatments downstream of the carcasses compared to upstream ($p < 0.001$; Figure 2). Our multi-factor ANOVA found differences among nutrient treatment by week, indicating that the nutrient treatments were responding differently among each sampling period from the addition of sea lamprey carcasses ($p = 0.038$). This may be due to the N and N + P treatments downstream of the carcasses, which exhibited a greater rate of change between the first and second weeks and second and third weeks compared to the rates of change upstream of the carcasses. Among control diffusers, our multi-factor ANOVA found chlorophyll *a* concentrations were 57–71% higher downstream of carcasses compared to the upstream reference over three weeks ($p < 0.05$; Figure 2).

Multiple Site Carcass Addition Experiment

The ten sites in our experimental reach were similar in physico-chemical stream habitat variables characteristic of a 3rd order stream (Table 1). However, forest overstory density varied among all ten sites. We estimated relatively higher overstory density among sites 3–6, and 9, and relatively lower overstory density among the other sites (Table 1). Our Mantel tests suggested spatial autocorrelation in that chlorophyll *a*, ammonium, and nitrate concentrations may have been influenced by adjacent sites ($p < 0.05$).

Daily stream temperature during the course of the experiment averaged 24.2 °C (± 0.42 SE), and ranged 17.6–29.0 °C. During the first week of our experiment, stream temperatures increased approximately 6 °C. During the second week of our experiment, a spate, from hurricane Arthur passed through the watershed and our experimental reach. This reduced stream

temperatures by an average of 8.0 °C, and increased mean stream velocities throughout the reach from 0.18 ms⁻¹ ($\pm$ 0.02 SE; baseflow) before the spate to 0.60 ms⁻¹ ($\pm$ 0.02 SE) after the spate. Temperatures were relatively constant during the third week; however the stream remained above base flow conditions for the remainder of the experiment.

We observed changes in stream nutrients during the course of our experiment. The multi-factor ANOVA identified differences in concentrations of all three nutrients across the sampling period ($p < 0.001$; Figure 3). We found differences in ammonium concentrations among sites ($p < 0.001$), but did not observe similar trends among nitrate or total soluble phosphorus. Stream ammonium concentrations at sites downstream of the carcasses increased from days 1–3 compared to the upstream reference sites (Figure 3; left panel). We selected days 2–4 to depict nutrient patterns along all ten sites as during this time we expected carcasses to liberate high concentrations of nutrients (Figure 3; right panel; Weaver et al. 2015). During days 2–4 the concentrations of ammonium in stream water appear to increase linearly going downstream, with the exception of site 8. This trend becomes absent by day 8 as concentrations appear similar among sites, coincidental with increased flows and runoff associated with the spate. For comparison, we observed no directional trends in nitrate or total soluble phosphorus concentrations during days 2–4 (Figure 3).

Chlorophyll *a* concentrations at sites 3–6 and 9 were lower than the average concentrations of the reference sites, while concentrations at sites 7–8 and 10 were higher than the average of the reference sites (Figure 4). Results from our multi-factor ANOVA show differences among all factors including site, week, nutrient treatment, and the interaction between week and nutrient ($p < 0.05$; Figure 4). The post-hoc test on the factor “site” revealed sites 3–6, and 9 were different than sites 7, 8, and 10 for all weeks and nutrient treatments. In

parallel with these trends, we found that sites 3–6, and 9 also had 20% higher overstory density than the other sites (Table 1). Chlorophyll *a* concentrations and percent overstory density among the nutrient diffusing substrate arrays were functionally related ($p < 0.05$). Percent overstory density explained 23% of the variation in chlorophyll *a* concentrations, and generally we observed lower concentrations at sites with higher overstory density (Table 1).

Our multi-factor ANOVA showed significant main effects of nitrogen and phosphorus on chlorophyll *a* in our reference sites for both carcass addition experiments ($p < 0.05$). These results suggest nitrogen and phosphorus colimitation during our experiments (Tank and Dodds 2003). Generally, samples from the N+P treatment had the highest chlorophyll *a* concentrations (Figure 2, 4).

Adult sea lamprey used in this experiment provided an enriched isotopic signal for ^{15}N and ^{13}C (mean $\pm$ SE; $\delta^{15}\text{N} = 12.16 \pm 0.22$; $\delta^{13}\text{C} = -17.96 \pm 0.19$) relative to the freshwater macroinvertebrates, ammocoetes, and Eastern elliptio sampled. We found that differences in stable isotope values varied among taxa attributed to subsidies delivered by carcasses as well as temporal changes in isotopic enrichment not related to the subsidies (Table 2; Figure 5). We observed significant isotope enrichment, primarily $\delta^{13}\text{C}$, among sampled individuals of all macroinvertebrate taxa over the three week period (Time main effect: $p < 0.05$; Table 2). Among Heptageniidae, Hydropsychidae, and Perlidae, we observed greater enrichment in stable isotope values in the treatment sites relative to the reference site (Site main effect: $p < 0.05$; Table 2; Figure 5). There was a significant time by site interaction among Heptageniidae, suggesting that the magnitude of the treatment effect changed over time. We observed enrichment in ^{13}C among ammocoetes, however we found no differences among reference or

treatment sites. Among Eastern elliptio, we observed depletion in both isotopes during the course of the experiment.

Discussion

We sought to quantify the spatiotemporal effects of sea lamprey carcass subsidies in an Atlantic coastal stream food web. We observed immediate downstream increases in primary productivity from carcass subsidies. Over the addition of carcass subsidies at multiple sites we observed varying responses of stream nutrients and reduced or increased algal biomass compared to reference values. Overstory canopy density partially contributed to the patterns we observed. The differences we found among sites may have reflected variability associated with other environmental variables that we did not measure. We observed stable isotope enrichment among a limited group of stream consumers but did not observe increased enrichment from multiple-site carcass addition. Thus, nutrient subsidies from sea lamprey carcasses evoke largely short-term localized effects limited to areas adjacent to the carcasses as demonstrated by Pacific salmon (Claeson et al. 2006). Furthermore, we suggest that the pathways by which nutrients are assimilated in food webs may be coupled to stream environmental variables, adjacent terrestrial systems, and flow disturbances that may alter subsidy delivery and community structure (Fisher et al. 1982; Power et al. 1988; Chaloner et al. 2004).

During our multiple-site carcass addition experiment we found spatial autocorrelation among chlorophyll *a*, ammonium, and nitrate concentrations. Sites that were closer to each other had more similar concentrations than those farther apart. The presence of spatial autocorrelation may violate the assumption of independently and identically distributed residuals, which may inflate the type I error rate, or the incorrect rejection of a true null hypothesis (Legendre 1993).

Therefore we must use some caution when interpreting our ANOVA results. Our results suggest that chlorophyll *a* concentrations were, in part, driven by canopy cover. Thus, riparian vegetation at one site may also have influenced an adjacent site. Among stream nutrient concentrations we might expect spatial autocorrelation as only a small portion of nutrients liberated from carcasses may be taken up and utilized by local stream organisms while the remainder flows downstream.

The quantitative input of nutrient subsidies to recipient systems may not correspond to concurrent responses in consumer biomass. The spatial pattern of ammonium concentrations increased from upstream to downstream (Figure 3; right panel). However, the spatial pattern of algal biomass is partly reflective of canopy cover and light availability (Table 1; Figure 4). A delivered pulse of nutrient subsidies may initially stimulate consumer biomass in recipient systems. A larger pulse of subsidies, however, may not elicit correspondingly larger effects. Consumer biomass may asymptote as organisms are constrained by assimilation efficiency, limited by another nutrient or resource (e.g., phosphorus), or, as our results suggest, influenced by environmental variables and habitat heterogeneity.

We found primary productivity was co-limited by nitrogen and phosphorus in Sedgeunkedunk Stream during both experiments. Other studies have generally concluded that temperate Eastern streams are phosphorus limited (Peterson et al. 1983; Newbold et al. 1983; Pringle and Bowers 1984), although see Norris (2012), while temperate Western streams are nitrogen limited (Grimm and Fisher 1986; Hill and Knight 1988; Tank and Dodds 2003). Productivity can vary across climatic and geologic regions (Minshall 1978), which may explain nutrient limitations and the role that nutrient subsidies play in alleviating those limitations. The stoichiometric ratios of nutrient subsidies (e.g., N:P) may elicit varying effects on the food webs

of nutrient limited systems (Elser et al. 1996). Sea lamprey carcasses have N:P ratios that range 20:1–22:1 (Weaver et al. 2015). Therefore, sea lamprey carcass subsidies may serve to alleviate nutrient limitations in Atlantic coastal streams during the spring. The pre-spawn carcasses we used in our experiments likely contain more energy and nutrients (e.g., gametes) than post-spawn carcasses as demonstrated with Pink salmon (*Oncorhynchus gorbuscha*; Gende et al. 2004). Thus the pre-spawn carcasses we used may have amplified concentrations of dissolved nutrients and corresponding effects on food webs.

Disturbance can influence food web structure (Sousa 1984; Ledger et al. 2008). High flows associated with a spate during 2014 may have influenced nutrient subsidy dynamics and food webs. In 2014 we observed flows three times greater than base flow conditions and high turbidity conditions associated with the spate. High flow disturbance events may have scoured periphyton from our nutrient diffusing substrates, and reduced light availability to primary producers through swifter turbid flowing waters, which likely suppressed algal biomass (Power et al. 1988; Grimm and Fisher 1989; Hall et al. 2015). Furthermore, elevated stream flows likely accelerated carcass breakdown and nutrient liberation to a time period shorter than a few weeks (Weaver et al. 2015). Our carcasses were caged to discourage scavengers and promote retention within the experimental reach rather than allow downstream displacement, which would likely happen in a natural environment (Gende et al. 2004; Williams et al. 2010). Thus, high flow disturbances may influence the balance between nutrient retention and transport; during high flows, transport is favored (Meyer and Likens 1979; Doyle 2005; Hall et al. 2009).

The patterns of algal biomass we observed may exemplify the coupled relationship of streams and adjacent terrestrial systems. Sea lamprey migrate in the spring when tree canopies have just begun to fill. These fish subsequently die in late spring and early summer when

canopies are completely full. Primary producers face seasonal shifts in light and nutrient limitations, while consumers face increased metabolic demands from rising water temperatures (Hall 1972; Hill et al. 2001). Stream organisms may depend upon the seasonal arrival of these nutrient subsidies. The temporal differences in executing our experiments (i.e., July 2013 versus June 2014) may have resulted in disparate food web responses, however environmental conditions such as temperature, canopy cover, and nutrient limitation were similar between experiments.

Similar to other studies, we observed reduced primary productivity among sites with relatively high percentages of overstory density (Lowe et al. 1986; Hill and Knight 1988; Table 1; Figure 4). The arrival of pulsed subsidies from sea lamprey may alleviate nutrient limitations among primary producers thereby strengthening bottom-up effects (Lamberti 1996). Conversely, areas imposed with light limitations may have lower primary production and consumers receive the subsidy directly (Kiernan et al. 2010; Rosemond et al. 1993). Thus, nutrient subsidies may influence stream food webs disparately and depend largely upon deterministic seasonal processes, and environmental characteristics of streams and adjacent riparian habitats (Chaloner et al. 2004).

We observed macroinvertebrates assimilate nutrients from sea lamprey carcasses, as demonstrated similarly with Pacific salmon carcasses (Claeson et al. 2006), and carcass analogs (Guyette et al. 2014). Nutrient subsidy assimilation among macroinvertebrates varied, likely due to differences among the functional feeding groups (Cummins 1974). Perlidae and Hydropsychidae, a predator and collector-gatherer respectively, may have fed directly on sea lamprey tissue, while Heptageniidae, a scraper, may have assimilated nutrients from biofilms enriched by nutrient subsidies. Sea lamprey nutrient subsidies may be important to heptageniid

mayflies during increased photoperiod and rising temperatures, which facilitate algal growth. Conversely, Lessard and Merritt (2006) found nutrient subsidies from fall spawning salmon did not benefit heptageniid mayflies during periods of increased flows, and declining photoperiod and temperature, which reduce algal growth. We observed no assimilation among Corydalidae and Philopotamidae, a predator and collector-gatherer, respectively. Therefore the assimilation of nutrient subsidies was not equivalent across the functional feeding groups. Furthermore, the response of stream organisms to nutrient subsidies may need to be placed in the context of subsidy arrival (i.e. fish phenology) and seasonally-variable environmental conditions.

Ammocoetes and Eastern elliptio are both filter feeders, and may assimilate nutrients from decomposing sea lamprey carcass tissue as detritus. However, we detected no enrichment in ammocoetes or Eastern elliptio attributed to carcass subsidies. Conversely Eastern elliptio demonstrated isotope depletion during the experiment. We did not conduct preliminary experiments to determine the effects of caging on these two species, therefore we cannot conclude whether the cage affected their behavior or if these species do not utilize carcass subsidies. Ammocoetes reside in silt beds and areas of slow-moving water within rivers and streams adjacent to suitable adult spawning habitat (Potter 1980) and thus it is plausible that they assimilate subsidies from adult carcasses or subsidy-enriched diatoms (Moore and Beamish 1973). In addition, ammocoetes reside in streams for up to eight years (Beamish 1980), and may have multiple opportunities to assimilate carcass subsidies delivered to recipient streams precluding assimilation by other freshwater consumers.

Migrating fish serve as vectors of energy and nutrients among ecosystems. Many populations have declined due to pervasive damming, habitat loss, and overfishing (Saunders et al. 2006; Limburg and Waldman 2009; Hall et al. 2011) which have reduced the delivery of

subsidies to resource limited ecosystems (Polis et al. 2004). Dam removal will facilitate anadromous fish passage and restore linkages between marine and freshwater ecosystems (Hall et al. 2011; Penobscot River Restoration Trust 2015). Our results suggest that nutrient subsidies demonstrate local and variable responses that may be influenced by finer scale habitat variables. The removal of barriers facilitates the movement of spawning adults into the upper reaches of streams and watersheds (Gardner et al. 2012; Hogg et al. 2013). Therefore, carcass subsidies may evoke varying effects on food webs influenced by local habitat and land-use characteristics.

Pulsed nutrient subsidies from anadromous sea lamprey may be important for Atlantic coastal waters. The pathways by which subsidies are utilized may depend on the environmental characteristics of the recipient system. We suggest that effects from sea lamprey nutrient subsidies are relatively localized to areas adjacent to carcasses and further influenced by multiple deterministic and stochastic mechanisms. Our research adds to a growing body of knowledge that characterizes the fate and efficacy of cross-ecosystem subsidies.

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669 62.
- 670

671 Table 1. Stream width and mean ($\pm$ SE) total depth and water velocity along cross-sectional
 672 transects at each site and mean ($\pm$ SE) percent overstory density among three nutrient diffusing
 673 substrate arrays placed at each site at base flow prior to the addition of sea lamprey carcasses.

674

Site	Stream width (m)	Average depth (m)	Average velocity (ms^{-1})	Overstory density (%)
1	7.5	0.20 (0.01)	0.35 (0.15)	77.8 (9.2)
2	8.0	0.20 (0.02)	0.27 (0.07)	67.1 (8.7)
3	7.2	0.23 (0.06)	0.30 (0.10)	83.7 (0.7)
4	5.3	0.23 (0.02)	0.46 (0.10)	87.5 (0.6)
5	4.8	0.23 (0.04)	0.34 (0.09)	94.1 (0.9)
6	6.6	0.27 (0.03)	0.23 (0.02)	92.7 (2.4)
7	7.9	0.22 (0.01)	0.38 (0.14)	75.0 (8.8)
8	6.8	0.24 (0.07)	0.33 (0.03)	66.7 (5.2)
9	7.8	0.24 (0.03)	0.21 (0.04)	91.0 (1.5)
10	5.4	0.21 (0.03)	0.35 (0.13)	72.6 (5.0)
Average	6.7	0.23	0.32	80.8

Table 2. Pillai’s trace, F , and P statistics from MANOVA results for macroinvertebrate taxa and *P. marinus* ammocoetes treating $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as dependent variables in the model. Bolded values indicate significant main effects or interactions at $p < 0.05$.

	Pillai’s trace	d.f.	F	P
Heptageniidae				
Time	1.256	4,60	25.3	<0.001
Site	0.551	4,60	5.7	<0.001
Interaction	0.730	8,60	4.3	<0.001
Hydropsychidae				
Time	0.766	4,60	9.3	<0.001
Site	0.407	4,60	3.8	0.007
Interaction	0.138	8,60	0.6	0.810
Philopotamidae				
Time	0.968	4,58	13.6	<0.001
Site	0.134	4,58	1.0	0.394
Interaction	0.289	8,58	1.2	0.301
Perlidae				
Time	0.716	4,58	8.1	<0.001
Site	0.410	4,58	3.7	0.009
Interaction	0.233	8,58	1.0	0.478
Corydalidae				
Time	0.172	4,50	1.2	0.332
Site	0.105	4,50	0.7	0.601
Interaction	0.108	8,50	0.4	0.939
<i>E. complanata</i>				
Time	0.847	4,64	11.8	<0.001
Site	0.087	2,31	1.5	0.244
Interaction	0.053	4,64	0.4	0.782
<i>P. marinus</i>				
Time	0.100	2,19	1.1	0.368
Site	0.198	2,19	2.4	0.122
Interaction	0.180	2,19	2.1	0.152

Figure captions

Figure 1. Location of study reaches for experimental sea lamprey carcass addition during 2013 (A), and 2014 (B) on Sedgeunkedunk Stream, Maine. Circles indicate former or current obstacles to fish passage. Inset depicts locations of ten sites within the experimental reach during 2014. Shaded boxes indicate sites that received additions of sea lamprey carcasses. Map source data were obtained from the United States Department of Agriculture Geospatial Data Gateway.

Figure 2. Mean ($\pm$ SE) chlorophyll *a* concentrations from nutrient diffusing substrates among four nutrient treatments over a three week period upstream and downstream of sea lamprey carcass addition in Sedgeunkedunk Stream, Maine, 2013. *F* and *P* statistics are presented for a model testing for the effects of reach, week, and nutrient treatment (see text for explanation).

Figure 3. Mean ($\pm$ SE) ammonium (a), nitrate (b), and total soluble phosphorus (c) stream water concentrations over a two week period among the average of the upstream reference sites (1,2) , two mid-reach sites (5,6), and the two lower most downstream sites (9,10; left panels), and during days 2–4 among all sites (right panels) following sea lamprey carcass addition in Sedgeunkedunk Stream, Maine, 2014. Time zero indicates samples taken before the addition of carcasses. The y-axis scales differ among nutrients. *F* and *P* statistics are presented for a multi-factor ANOVA model testing for the effects of site and day (see text and Figure 1 for site locations and descriptions).

702 Figure 4. Mean ($\pm$ SE) chlorophyll *a* concentrations following sea lamprey carcass addition
703 among sites downstream of carcasses adjusted for average chlorophyll *a* concentrations from
704 upstream reference sites from nutrient diffusing substrates among four nutrient treatments over
705 three weeks (a–c) in Sedgeunkedunk Stream, Maine, 2014. *F* and *P* statistics are presented for a
706 multi-factor ANOVA model testing for the effects of site, week, and nutrient treatment (see text
707 and Figure 1 for site locations and descriptions).

708

709 Figure 5. Mean ($\pm$ SE) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope values among six macroinvertebrate taxa and *P.*
710 *marinus* ammocoetes before carcass addition (white), and at weeks 3 (gray) and 7 (black) after
711 carcass addition on Sedgeunkedunk Stream, Maine, 2014. The triangle symbol corresponds to
712 site 2 (reference), the circle to site 5 (mid-reach), and the square to site 9 (downstream). The
713 hexagon in the upper right hand corner of each plot is the stable isotope signature of adult sea
714 lamprey carcasses used in this experiment (mean $\pm$ SE $\delta^{15}\text{N} = 12.16 \pm 0.22$; $\delta^{13}\text{C} = -17.96 \pm$
715 0.19). The x- and y-axis scales differ among taxa.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.