Received: 21 September 2017 | Revised: 26 February 2018 | Accepted: 1 May 2018

DOI: 10.1002/rra.3291

RESEARCH ARTICLE

WILEY

Subsidies from anadromous sea lamprey (*Petromyzon marinus*) carcasses function as a reciprocal nutrient exchange between marine and freshwaters

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Funding information
Maine Agriculture and Forest Experiment Station, Grant/Award Number: Publication Number 3589; U.S. Department of Agriculture National Institute of Food and Agriculture, Grant/Award Number: ME0-8367-0H

Abstract

Nutrient and energy flows across ecosystem boundaries subsidize recipient communities and influence bottom-up processes in food webs. Migratory fish such as anadromous sea lamprey provide a pulse of marine-derived nutrients and energy to Atlantic coastal streams in spring when organisms would otherwise be subject to limiting resources. We conducted sea lamprey carcass addition experiments to characterize the role of subsidies on producer and consumer trophic pathways by manipulating subsidy quantity and light exposure. We demonstrated that producer and decomposer productivity is constrained by nutrients during spring; however, these limitations were reduced in producers as light limitations intensified through riparian shading. We observed no significant effects of increasing carcass subsidies on producer and decomposer biomass. Our results suggest that high densities of carcass subsidies may stimulate primary productivity; however, these effects are mediated by the degree of riparian shading, which demonstrated a onefold to fourfold difference in biomass accrual. In addition, sea lamprey carcass nutrients were captured by larval conspecifics. Stable isotopes analysis demonstrated that adult sea lamprey carcass tissue was relatively enriched in ^{15}N and ^{13}C isotopes compared with larvae. We observed significant enrichment in the ^{13}C isotope among larvae sampled after 2 and 4 weeks of exposure to adult carcass nutrients. Our work suggests that a portion of sea lamprey subsidies serve as a reciprocal exchange between freshwaters and the ocean. We highlight that this cross-ecosystem linkage is likely influenced by subsidy quantity from donor systems and is mediated by environmental characteristics affecting the recipient system.

KEYWORDS

ammocoete, cross-ecosystem, nutrients, reciprocal exchange, sea lamprey, streams, subsidy

1 | INTRODUCTION

Resource flows from donor ecosystems subsidize recipient ecosystem productivity and therefore drive the structure and function of communities (Anderson, Alexander Wait, & Stapp, 2008; Bartels et al., 2012; Polis, Anderson, & Holt, 1997; Richardson & Sato, 2015). For example, islands and coastal lands are subsidized by nutrient exchanges from surrounding oceans via sea wrack, birds, and mammals (Anderson & Polis, 1999; Farina, Salazar, Wallem, Witman, & Ellis, 2003; Polis & Hurd, 1996), and reciprocal exchanges occur between temperate forests and headwater streams by leaf litter and riparian insects (Fisher & Likens, 1973; Richardson, Zhang, & Marczak, 2010). Migratory

animals, such as fish, also serve as an important link between ecosystems separated by great distances and exemplify cross-boundary exchanges of nutrients and material (Flecker et al., 2010; Harding & Reynolds, 2014; Lamberti, Chaloner, & Hershey, 2010).

Migratory fish serve as a predictable source of nutrients and materials, representing a functional link across marine, freshwater, and terrestrial ecosystem boundaries (Gende, Edwards, Willson, & Wipfli, 2002; Naiman, Bilby, Schindler, & Helfield, 2002). For example, Pacific salmon (*Oncorhynchus* spp.) influence upland-freshwater streams from the deposition of post-spawned carcasses by increasing dissolved nutrients, biofilm and macroinvertebrate production (Janetski, Chaloner, Tiegs, & Lamberti, 2009; Rex & Petticrew, 2008), and growth rates of juvenile conspecifics (Kiernan, Harvey, & Johnson, 2010; Lang, Reeves, Hall, & Wipfli, 2006; Rinella, Wipfli, Stricker, Heintz, & Rinella, 2012) and resident fish (Collins, Baxter, Marcarelli, & Wipfli, 2016). Pacific salmon also subsidize terrestrial ecosystems when moved by large terrestrial carnivores, thereby affecting terrestrial plants and invertebrates (Hocking & Reimchen, 2002; Hocking & Reynolds, 2011). Migratory fish, through the connection of marine, freshwater, and terrestrial ecosystems, create complex feedbacks and food-web interactions across large spatial scales (Helfield & Naiman, 2001; Wilzbach, Harvey, White, & Nakamoto, 2005).

Anadromous sea lamprey (*Petromyzon marinus*) provides a marine-derived nutrient resource that subsidize Atlantic coastal rivers and streams (Nislow & Kynard, 2009; Weaver, Coghlan, & Zydlewski, 2016; Weaver, Coghlan, Zydlewski, Hogg, & Canton, 2015). Sea lamprey spends 1–2 years in the ocean feeding as a top parasitic predator and migrates into freshwater streams during the spring to spawn and then die (Beamish, 1980; Figure 1). Larval sea lamprey may spend 2–12 years in freshwaters occupying a relatively low trophic position in stream food webs as deposit-feeding detritivores (Beamish, 1980; Manion & Smith, 1978; Morkert, Swink, & Seelye, 1998; Sutton & Bowen, 1994). Larvae may consume material from dead or decaying organisms such as lamprey carcasses. Thus, it is plausible that sea lamprey subsidies may function as a reciprocal resource flux with larval conspecifics between freshwater and marine ecosystems.

The spring arrival of spawning sea lamprey likely coincides with a period of limiting nutrients and resources for stream organisms. During spring, terrestrial organic matter remaining from the fall has declined in quality (nutrient concentration) and quantity in streams (Petersen & Cummins, 1974; Suberkropp, Godshalk, & Klug, 1976). Producer and consumer productivity is constrained now through nutrient (e.g., nitrogen [N], phosphorus [P], and carbon [C]) limitations (Elser et al., 2007; Tank & Webster, 1998) and is relieved as increasing light limitations intensify through shading as trees leaf-out (Hill, Ryon, & Schilling, 1995; Figure 1). Sea lamprey carcass nutrients subsidize the growth of producers (Weaver et al., 2016) and consumers (Guyette, Loftin, Zydlewski, & Cunjak, 2014). However, these effects on food-web dynamics are likely influenced by the quantity of the subsidy (number of adult sea lamprey spawners) and light limitation imposed by riparian shading from the adjacent terrestrial ecosystem.

We conducted field experiments to examine producer and consumer pathways in stream ecosystems by manipulating subsidies of sea lamprey carcasses. We predict that carcass subsidies influence reciprocal freshwater to marine exchanges through assimilation by larval conspecifics that migrate to the ocean. In addition, we hypothesize that effects of carcass subsidies in recipient freshwater streams are influenced by the quantity of the subsidy and shading from riparian growth. Specifically, our objectives were to characterize (1) changes in algal and fungal biomass driven by subsidies provided by experimental addition of sea lamprey carcasses; (2) the influence of light limitation from riparian shading on biofilm responses to subsidies; and (3) assimilation of nutrient subsidies from adult sea lamprey by larval conspecifics.

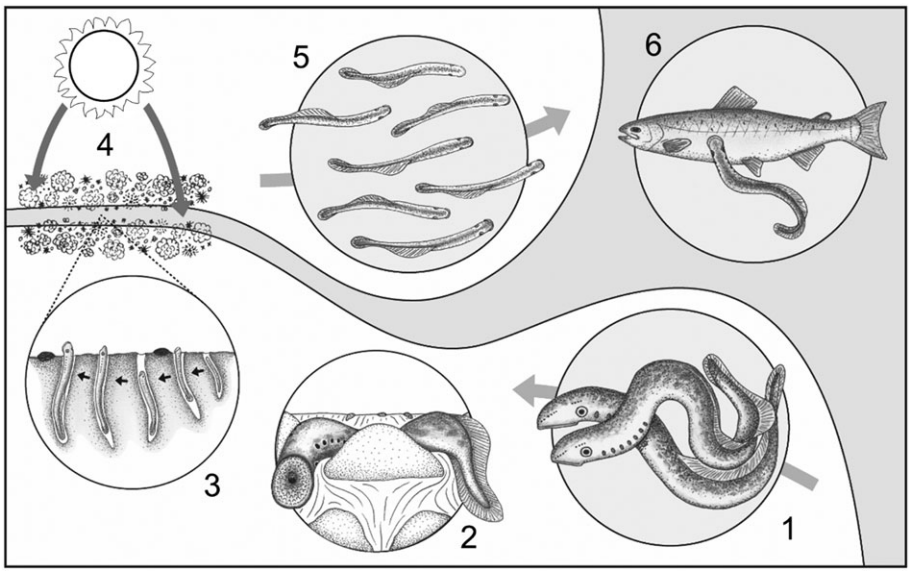

FIGURE 1 Diagram depicting sea lamprey life cycle and resource flux linking marine, freshwater, and terrestrial ecosystems: (1) migration during the spring into freshwaters, (2) post-spawning carcass nutrients in freshwaters, (3) larvae growth and assimilation of carcass nutrients in freshwaters, (4) the role of light and terrestrial ecosystems influencing food web processes, (5) juvenile migration to the ocean, and (6) juvenile parasitism of marine fish and other vertebrates

2 | MATERIALS AND METHODS

2.1 | Study area and fish collection

We conducted carcass addition experiments during the summers of 2015 and 2016 in Kenduskeag and Great Works Streams, tributaries to the Penobscot River, Maine, located at river kilometre (rkm) 40 and 59, respectively. Historically, Kenduskeag Stream has had uninterrupted connectivity to the Penobscot River and Atlantic Ocean, whereas Great Works Stream has recently re-established connectivity with the removal of two main-stem Penobscot River dams (Great Works Dam in 2012 and Veazie Dam in 2013; see Opperman, Royte, Banks, Day, & Apse, 2011). We collected pre-spawn sea lamprey from the Penobscot River (at Milford Dam, rkm 61) during spring migration. All collected fish were sacrificed according to Institutional Animal Care and Use Committee protocols, measured for mass (±0.1 g) and total length (±1 mm), then stored frozen at −10 °C until experimental addition.

2.2 | Experimental design

In 2015, we selected two 300-m study reaches in Great Works Stream to examine changes in stream biofilms and larval lamprey to an increasing gradient of lamprey carcasses. We observed no spawning sea lamprey or their carcasses at either reach. One reach contained a mix of mature deciduous and coniferous trees that contributed to stream shading (i.e., "closed canopy"), whereas the other reach was open and generally lacked large riparian trees (i.e., "open canopy"). The closed canopy reach was approximately 1 km upstream of the open canopy reach.

In-stream physical characteristics such as stream width, sediment size, and discharge were qualitatively similar. Within each reach, we delineated four cross-sectional transects located at a riffle-run habitat sequence to serve as replicates for carcass addition treatments. Along each of the four cross-sectional transect, we randomly assigned points at 2-m width intervals to receive one of the following carcass treatments: 0, 1, 3, or 6 carcasses. Transects were spaced approximately 25 m apart. Carcasses were placed in 2-cm polyethylene mesh bags and secured to the stream with steel wire mesh and rebar to discourage scavenging. The average individual carcass mass was 0.69 kg (±0.014 *SE*), and the total carcass mass added to each reach was equivalent (~27 kg). Carcasses were deployed on June 30, 2015.

In 2016, we wanted to examine the responses to greater additions of carcasses. We conducted this experiment in the same open and closed canopy reaches but selected different transects from those used in 2015 to accommodate a modified design. We delineated six transects in each reach. The upstream transect received no carcasses, and subsequent downstream transects were separated by 25 m, receiving 3, 6, 12, 24, or 48 carcasses in that order. Carcasses were bagged and secured to the stream as described above. The average individual carcass mass was 0.82 kg (±0.053 *SE*), and total carcass mass added to both reaches was equivalent (~73 kg). Carcasses were deployed in the stream on June 16, 2016.

The numbers of carcasses used among treatments represent ecologically realistic densities that may be deposited after spawning (Hogg, Coghlan, & Zydlewski, 2013; Nislow & Kynard, 2009). In a previous study, Weaver et al. (2016) demonstrated that carcass nutrients exhibit relatively localized effects on primary biomass accrual. Therefore, during both experiments, we assumed that carcass effects at one transect would not measurably impact subsequent downstream transects. We experimentally added carcasses after the period of natural spawning to avoid any potential confounding temporal effects.

In 2015, a light and temperature logger (Onset, Hobo Pendant UA-002-08, Cape Cod, Massachusetts, USA) was placed in the midchannel of each transect (n = 8), whereas in 2016, loggers were placed at each location receiving carcasses (n = 12). Loggers recorded temperature and relative light intensity (lux) at 10-min intervals.

2.3 | Algal and fungal biomass assays

During both experiments, we deployed substrate arrays to measure changes in algal and fungal biomass from the addition of sea lamprey carcasses as well as nutrient limitations to algal and fungal biomass accrual using the methods described in Tank and Webster (1998) and Tank et al. (2006). We glued 1.9-cm threaded polyvinyl chloride (PVC) caps to 24 × 10 cm PVC boards. To measure epilithic algal biomass (i.e., algae grown on inorganic substrates), we topped the threaded caps with 2.5-cm diameter, 0.7-μm glass microfiber filters (hereafter "filters"; GE Healthcare Life Sciences, Marlborough, Massachusetts, USA). We bored holes through 2.5-cm polypropylene screw-cap bottles, which were fastened over the filters and secured them to the PVC boards. To measure fungal biomass and epixylic algal biomass (i.e., algae grown on organic substrates), we cable-tied sterilized, untreated 15.0 × 1.6 cm birch (*Betula* sp.) popsicle sticks (hereafter "sticks") to 15 × 15 cm sheets of plastic mesh. Filter and stick arrays were cable-tied to cement blocks and deployed in riffle habitats of similar flow and depth immediately downstream of each carcass treatment. We measured epilithic algal and fungal biomass in 2015 and epilithic and epixylic algal and fungal biomass in 2016.

During both experiments, we deployed nutrient diffusing substrates upstream of all transects in each reach to assess nutrient limitations for algal and fungal biomass (see methods in Tank et al., 2006). Diffusers were topped with filters or birch veneer discs (hereafter "discs"). Nutrient diffusing substrates were deployed concurrently with carcass addition and filter and stick arrays.

We sampled three to five replicates of filters, sticks, and nutrient diffusing substrates from each array at 1, 2, and 3 weeks after carcass addition, bracketing the time when the majority of carcass tissue decomposes and liberates nutrients (Weaver et al., 2015). Filters and discs from nutrient diffusing substrates were removed from threaded caps with forceps, placed into polyethylene tubes, and transported on ice in the dark. Sticks were lifted out of the water, placed in labelled plastic bags filled with stream water, and kept on ice in the dark. In the laboratory, discs and sticks (4-cm long cut sections) were placed in 15-ml centrifuge tubes with 5.0 ml of high-performance liquid chromatography grade methanol. All filters, sticks, and discs were stored at −10 °C until processed.

2.4 | Laboratory analysis

Algal biomass was estimated from extracted chlorophyll *a* from sampled filters, sticks, and discs. Filters were homogenized by hand with

90% acetone solution and a mortar and pestle. Sticks and discs were agitated in 90% acetone by hand for 1 min. Extracted samples were analysed for chlorophyll *a*, corrected for pheophytin with spectrophotometry (Strickland & Parsons, 1972; APHA et al., 1999) via a Thermo Scientific Genesys 10S spectrophotometer (Thermo Fisher Scientific, Marietta, Ohio, USA).

Fungal biomass was estimated from ergosterol extracted from sampled sticks and discs as described by Tank and Webster (1998). We quantified ergosterol using a high-performance liquid chromatography system configured with a Kinetex (phenomenex) C18 4.6 × 250 mm column using a methanol solvent at a flow rate of 2 ml/min, 25-µl injection, 282-nm wavelength, and a 5-min retention time. Ergosterol standards (0, 1, 2.5, 5, 10, 20, 40, 80, 100, and 200 µg/ml) were run periodically throughout analyses. Absorbance values of samples were converted to ergosterol concentrations using multipoint standard calibration linear regression ($R^2 > 0.99$; $p < 0.05$). Fungal biomass was calculated using a conversion factor of 6 mg ergosterol/g fungal biomass (Tank & Webster, 1998).

2.5 | Larval sea lamprey nutrient assimilation

We identified adult carcass nutrient assimilation by larval conspecifics. Three 1 × 1 m sites were delineated in Kenduskeag Stream during 2015: one upstream reference and two downstream treatment sites across a 600-m reach. Sites were chosen based on suitable larval habitat characteristics, consisting of slow moving water and fine substrate (Hardisty & Potter, 1971). Larval sea lampreys were collected from adjacent habitats with a backpack electrofisher and dipnet. Fish were allowed to recover in an aerated bucket with frequent water changes before being assigned randomly to one of the three sites. A subset of individuals assigned to each site was sacrificed ($n = 4$) representing a sample taken before carcass addition, whereas the remaining individuals were released evenly among sites. We observed released individuals bury into the substrate immediately upon release. At the two treatment sites, we caged and staked five sea lamprey carcasses within the 1 × 1 m area. We sampled five to nine larvae at each site after 2, 4, and 10 weeks. Individuals were sacrificed according to approved Institutional Animal Care and Use Committee protocols.

Samples of adult sea lamprey tissue were taken prior to carcass addition for analysis. After sacrifice, a 1-cm^3 section of muscle tissue was removed from the left dorsolateral side of five individuals. All samples were stored at −80 °C until sample preparation and analysis.

2.6 | Stable isotopes analysis

Whole bodies of larval sea lamprey and adult sea lamprey tissue were prepared and analysed at the University of New Brunswick Stable Isotopes in Nature Laboratory. Samples were dried at 60 °C for 24–48 hr and then ground into a fine powder with a mortar and pestle. Approximately 0.5 mg of each sample was measured and placed into tin capsules and combusted using a Carlo-Erba NA1500 Elemental Analyzer. Measurements of $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta X = ([R_{sample}/R_{standard}] - 1) \times 1{,}000$, where X is ^{13}C or ^{15}N, and R is the ratio of the heavy isotope to the light isotope ($R = {}^{13}C{:}{}^{12}C$ or $^{15}N{:}{}^{14}N$ *sensu* Jardine, McGeachy, Paton, Savoie, & Cunjak, 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 samples and repeat samples were approximately 0.1‰ or less for $\delta^{13}C$ and $\delta^{15}N$.

2.7 | Statistical analyses

We characterized stream nutrient limitations during both experiments from filters and birch discs using a multifactor analysis of variance. We modelled epilithic and epixylic algal biomass and fungal biomass as a function of week and nutrient treatment (nitrogen, phosphorus, nitrogen and phosphorus, or none). Significant main effects or interactions among nutrients ($p < 0.05$) were interpreted as nutrient limitations or colimitation (Tank et al., 2006).

We constructed linear models to characterize the influence of subsidy quantity (i.e., the number of carcasses; Objective 1) and canopy shading (Objective 2) on algal and fungal biomass. We analysed algal and fungal biomass from sampled filters and sticks of our replicated carcass addition experiment (2015) using linear mixed-effects analysis of covariance models with carcass treatment and canopy cover as fixed effects, and the random effect of treatment replicates ($n = 4$) nested within sampling periods ($n = 3$). We analysed epilithic algal biomass from samples of our unreplicated carcass addition experiment (2016) using a linear mixed-effects analysis of covariance with carcass treatment and canopy cover as fixed effects and sampling week as the random effect. Epixylic algal biomass and fungal biomass were analysed similarly; however, because those organisms were derived from wood substrates and may potentially interact, we added the corresponding biomass response variable as a covariate to each of those models (e.g., fungi and epixylic algae). In all mixed-effects models, we included carcass treatment as a continuous variable and assumed a linear relationship between each response variable over increasing carcass treatment.

We conducted separate linear regression analyses to assess correlations between algal biomass and light intensity across both experiments. We modelled epilithic algal biomass (2015 and 2016) and epixylic algal biomass (2016 only) as a function of light intensity. Finally, we analysed stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) with multivariate analysis of variance and the Pillai's trace test to assess treatment (with or without carcasses) and temporal differences in isotopic values among larval lamprey (Objective 3). We modelled the isotope values as functions of site and week. We conducted post hoc tests on significant main effects of the univariate tests of $\delta^{13}C$ and $\delta^{15}N$ separately.

For all statistical tests, we applied log transformations to the response variables to satisfy assumptions of normality and multivariate normality, and statistical significance was gauged at $p < 0.05$. All mixed-effects model analyses were performed with the "lme" function in the "nlme" package (Pinheiro, Bates, DebRoy, Sarkar, & Core Team, 2017), and all other statistical analyses were performed with built-in functions using R (R Development Core Team, 2016).

3 | RESULTS

Stream temperatures and relative differences in light intensity among open and closed canopy reaches were consistent between experiments. Average stream temperatures ranged from 22.1–22.3 °C, and 21.7–21.9 °C over the course of the experiments in 2015 and 2016, respectively. The average daily (24 hr) light intensity in the closed canopy reach was 3,814 lux (±747 *SE*) and 4,560 lux (±944 *SE*), and the average light intensity in the open canopy reach was 18,848 lux (±3,000 *SE*) and 15,722 lux (±1,419 *SE*) for 2015 and 2016, respectively.

3.1 | Algal and fungal nutrient limitations

Stream nutrient limitation of algal biomass varied between open and closed canopy reaches, whereas nutrient limitation of fungal biomass was similar (Figure 2). In the closed canopy reach, epilithic and epixylic algal biomass was not limited by N or P during both experiments ($p > 0.05$, all factors). In the open canopy reach, however, algae on both substrates were either N-limited or N and P colimited ($p < 0.05$ for N, N + P factors). Among both reaches and experiments, fungi were N and P colimited ($p < 0.05$ among N, P, and N + P factors) or P limited with secondary N limitation ($p < 0.05$ among P and N + P factors). Between experiments, we generally observed higher biomass accrual among diffusers with added N, P, or N + P in the open canopy reach compared with the closed canopy reach (Figure 2).

3.2 | Algal and fungal biomass

Our linear mixed-effects models for the 2015 experiment revealed interactions between carcass treatment and canopy cover for algal and fungal biomass suggesting that carcass density was influencing algal and fungal biomass differently among open and closed canopies (Table 1; Figure 3). However, the regressions did not demonstrate a clear pattern of increased productivity with more light or higher numbers of carcasses. For example, we observed lower epilithic algal biomass at higher carcass densities (Week 1) and higher biomass at shaded sites (Week 2). For 2016, our mixed-effects models revealed no association between epilithic algae or fungi and either carcass treatment or canopy cover (Table 1; Figure 4).

Light intensity explained a very small portion of the variability in epilithic algal biomass (0.02% and 1.3% for 2015 and 2016, respectively; $p > 0.05$). However, there was a linear association between epixylic algae and canopy cover ($p < 0.05$). Light intensity explained 27.5% of the variability in epixylic algal biomass demonstrating a positive association and indicating higher productivity in areas with less

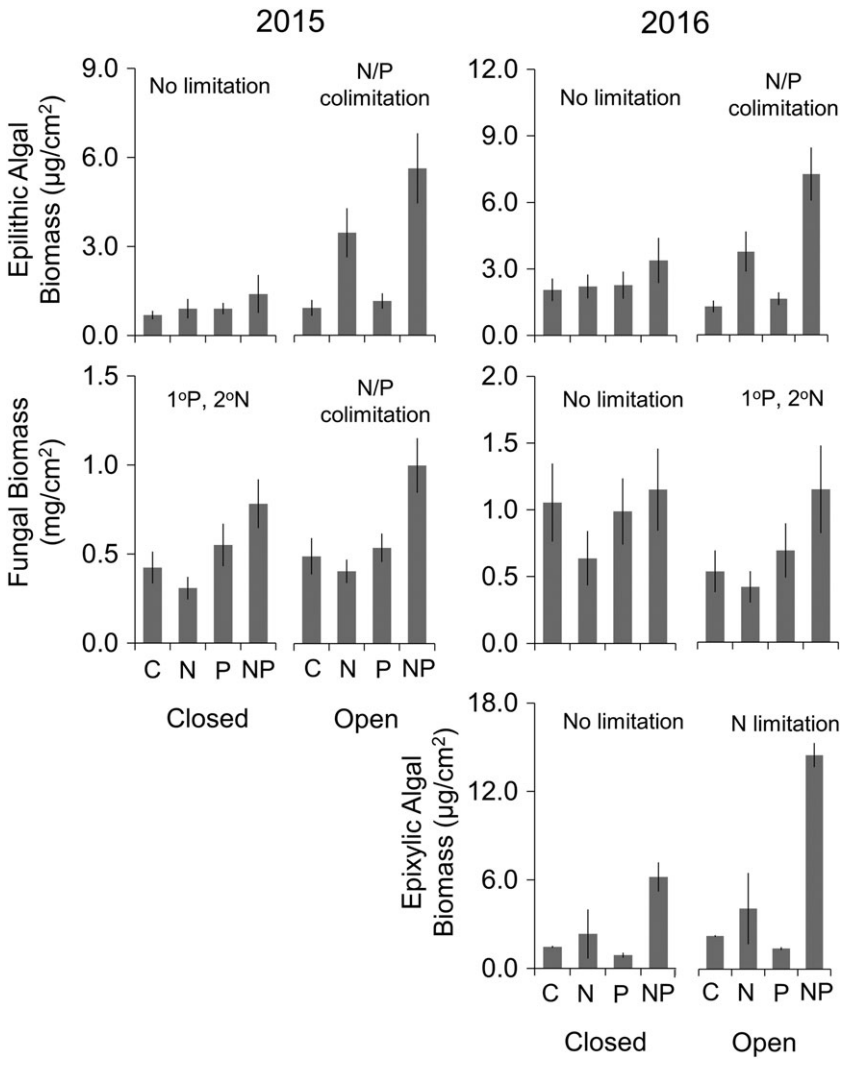

FIGURE 2 Algal and fungal biomass (mean ± *SE*) among nutrient diffusing substrate samples in closed and open canopy reaches averaged across a 3-week period during 2015 and 2016. Nutrient limitation or colimitation inferred from analysis of variance results are indicated above each plot. See Table S1 for *F* and *P* statistics

TABLE 1 *F* and *p* statistics from linear mixed-effects models testing algal and fungal biomass as a function of the number of carcasses and canopy cover (stream reach)

	Epilithic Algae		Fungi		Epixylic Algae	
Factor	***F***	***p***	***F***	***p***	***F***	***p***
2015						
Carcass treatment	0.013	0.908	4.052	**0.048**	—	—
Canopy cover	0.217	0.646	1.800	0.195	—	—
Interaction	8.361	**0.005**	6.205	**0.015**	—	—
2016						
Carcass treatment	0.451	0.507	0.192	0.664	1.857	0.184
Canopy cover	3.087	0.089	0.010	0.922	36.706	**<0.001**
Algae	—	—	5.624	**0.025**	—	—
Fungi	—	—	—	—	5.147	**0.031**
Interaction	0.071	0.792	3.281	0.081	2.782	0.106

Note. Bolded values indicate *p* values <0.05.

shading. Our results suggest a potential interaction of carcass treatment and riparian shading for epixylic algal biomass (e.g., Week 2; Figure 4); however, this was not statistically significant. For algae and fungi on wood substrates, we found linear associations with the other response variable added as a covariate in the model ($p > 0.05$; Table 1). Generally, increases in either the algal or fungal component of the biofilm indicated increases in the other component. Among algae and fungi, we found positive correlations and coefficients of determination ranged from 0.15–0.51 ($p < 0.05$).

3.3 | Larval lamprey stable isotopes analysis

Adult sea lamprey samples possessed an enriched stable isotopic signal for ^{15}N and ^{13}C (N = 8; mean ± *SE*, $\delta^{15}N$ = 12.15 ± 0.34; $\delta^{13}C$ = −18.02 ± 0.17) relative to the larval sea lamprey we sampled. Our multivariate analysis of variance revealed that ^{13}C and ^{15}N isotope values among larval sea lamprey differed among both sites (two with and one without carcasses) and weeks (Pillai test statistic = 0.337; $p < 0.005$; Figure 5). However, post hoc tests on univariate analyses revealed that larvae in the treatment site were more enriched in ^{13}C (−21.3‰) starting at Week 2, but not ^{15}N (8.4‰) when compared with larvae collected in the reference site (−25.3‰ and 8.3‰, respectively). Further enrichment in ^{13}C (−19.5‰) occurred at Week 4 among individuals in the treatment site, but by Week 10, the trajectory of ^{13}C (−22.4‰) in larvae exposed to carcasses had shifted away from the adult tissue signature. Stable isotope values from larvae collected between sites receiving carcasses did not differ.

4 | DISCUSSION

We provide empirical evidence that marine and freshwater ecosystems are connected through reciprocal lamprey subsidy exchanges via assimilation among larval conspecifics. We demonstrated that producers and decomposers exhibit varying nutrient limitations to contrasting light regimes controlled by riparian shading. We observed no effects of increasing resource quantity on decomposers; however, our work suggests that producers may be influenced by large quantities of carcasses mediated by riparian shading from adjacent forest ecosystems. More broadly, our study highlights several interacting biotic and abiotic processes that may alter the dynamics of nutrients and material transported by migrating fish.

We demonstrate a reciprocal exchange of nutrients between marine and freshwater ecosystems involving adult sea lamprey and larval conspecifics. Previous research demonstrating an analogous reciprocal dynamic among salmonids is replete. Nutrients from carcasses of Pacific salmon are assimilated by juvenile conspecifics and have been shown to increase growth rates (Kiernan et al., 2010; Lang et al., 2006; Rinella et al., 2012). Larval lampreys that consume these subsidies may experience enhanced growth during an otherwise nutrient limited period with life history consequences. The variability

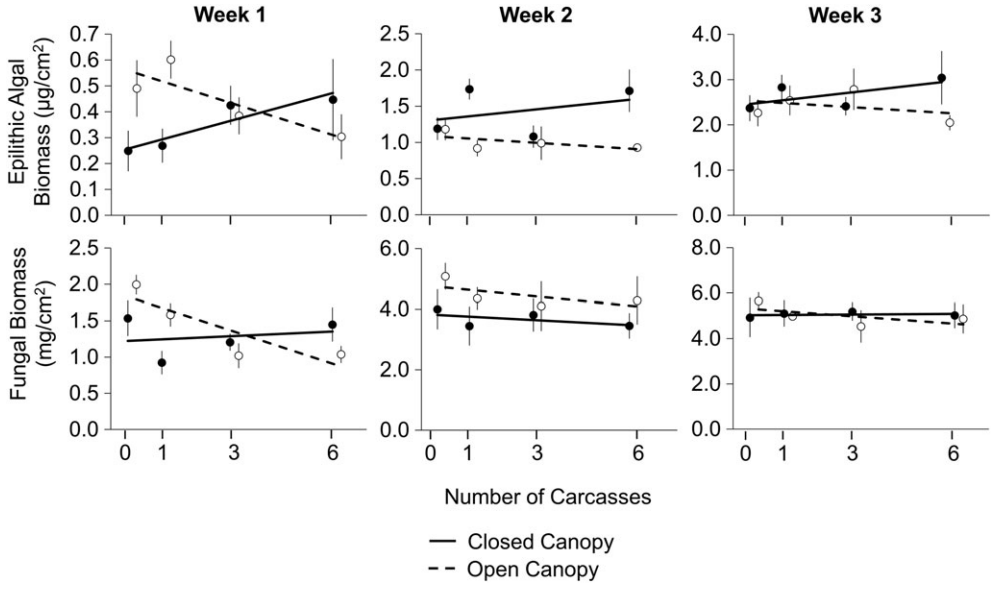

FIGURE 3 Algal and fungal biomass (mean ± *SE*) regressed among replicate carcass addition treatments in closed (solid line, filled markers) and open (dashed line, open markers) canopy reaches over a 3-week period during 2015. Standard error bars were calculated among replicated treatments. Lines do not indicate significance. See Table 1 for *F* and *P* statistics for terms in linear mixed-effects model

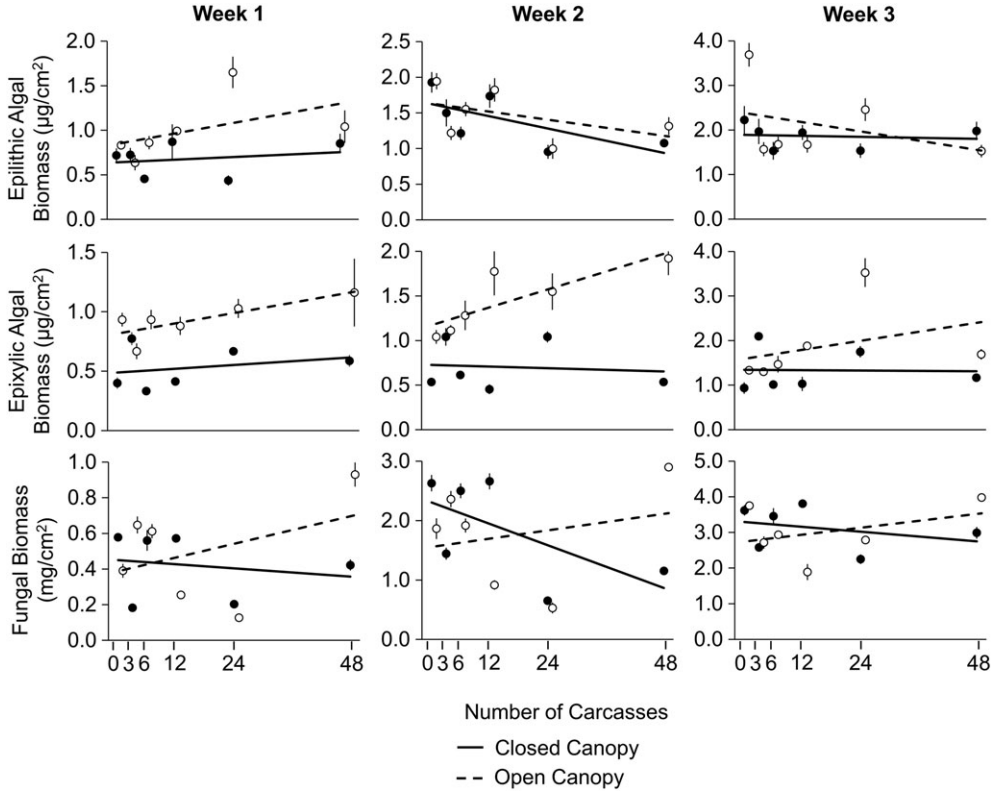

FIGURE 4 Algal and fungal biomass (mean ± *SE*) regressed among carcass addition treatments in closed (solid line, filled markers) and open (dashed line, open markers) canopy reaches over a 3-week period during 2016. Standard error bars were calculated among replicate samples taken from each treatment during each period. Lines do not indicate significance. See Table 1 for *F* and *P* statistics for terms in linear mixed-effects model

associated with the duration of the larval period (2–12 years) may be explained by the productivity of the inhabited waters (Dawson, Quintella, Almeida, Treble, & Jolley, 2015; Potter, 1980; Purvis, 1980). Weaver, Coghlan, and Zydlewski (2018) explored the hypothesis that larval sea lamprey growth and metamorphosis are manipulated by changes in productivity invoked by subsidies of adult carcass nutrients. Thus, the exchange of nutrients and material by sea lamprey may alter the reciprocation of the subsidy between marine and freshwater ecosystems through population level changes among larvae.

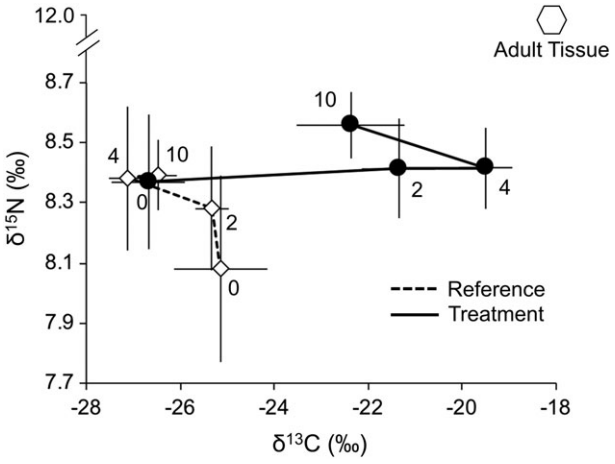

FIGURE 5 Mean (±*SE*) $\delta^{15}N$ and $\delta^{13}C$ isotope values among larval sea lamprey collected at a reference site that received no sea lamprey carcasses (diamonds) and the average of two treatment sites that received sea lamprey carcasses (circles) collected before carcass addition (Week 0), then 2, 4, and 10 weeks after carcass addition in Kenduskeag Stream, Maine, 2015. Dashed or solid lines refer to the trajectory of stable isotope enrichment or depletion among larvae sampled in the reference or treatment sites, respectively. The hexagon represents the stable isotope signature value of adult sea lamprey carcasses used in the experiment (mean ± *SE*, $\delta^{15}N$ = 12.15 ± 0.34; $\delta^{13}C$ = −18.02 ± 0.17)

We observed significant enrichment in the ^{13}C isotope among larval sea lamprey over time, but we did not observe a similar trend in the ^{15}N isotope. Other research has demonstrated that habitat and ontogenetic shifts in the diets of migrating fish create a lag between what is being consumed by the fish and what is indicated by their isotopic signatures (Buchheister & Latour, 2010; Hertz et al., 2016). Larval lampreys are a primary consumer and function as a deposit-feeding detritivore (Sutton & Bowen, 1994) and may consume adult carcass fragments or dead organisms enriched in carcass nutrients (i.e., biofilms). Our results indicate that the introduction of an additional detrital resource during spring may become an important component of their diet.

Subsidies transported by migratory fish from marine to freshwater ecosystems are influenced by the interacting terrestrial system (via the riparian shading). This novel dynamic among three ecosystems has rarely been characterized in the literature (although see Collins et al., 2016). Riparian vegetation creates a mosaic of varying segments that influence streams differently, which affects resource limitations and food-web responses to subsidies (Collins & Baxter, 2014; Hagen, McTammany, Webster, & Benfield, 2010). We demonstrated that producer and decomposer nutrient limitations varied between open and

closed canopy habitats. Nutrient limitation of algal biomass in the closed canopy reach was not detected, but N or N + P limitations were demonstrated in the open canopy reach with more than threefold greater incident light. Our results suggest that the strength of bottom-up trophic dynamics, influenced by nutrients from carcasses, are mediated by light limitations from adjacent terrestrial habitats. Conversely, fungal biomass was primarily P limited, and nutrient limitations between reaches were similar. Samways, Quinones-Rivera, Leavitt, and Cunjak (2015) demonstrated that increases of different magnitude of subsidized organisms (algae, fungi, and bacteria) to subsidies from several species of anadromous fish, including sea lamprey. Our results and the work of others indicate that resource fluxes from anadromous fish have varying influences on bottom-up processes of stream food webs. These processes coupled with abiotic patchiness, a common characteristic of many habitats, may further alter the dynamics of subsidies among freshwater and marine ecosystems.

Resource flows across space and time vary in quantity and have irregular consequences for recipient community structure and function. The quantity of resources delivered to recipient ecosystems is an important contributing factor to the strength of cascading trophic interactions (Leroux & Loreau, 2010), and studies have examined the effects of variable resource input to recipient ecosystems (Klemmer & Richardson, 2013; Leroux & Loreau, 2010; Marczak & Richardson, 2008; Samways et al., 2015). Results from the current study and those from Weaver et al. (2016) suggest that the relatively high quantities of nutrients, delivered through sea lamprey carcasses into streams, may be necessary to stimulate primary production. We were unable to detect an effect even at high densities of carcasses, and we note that variability among treatments and sites may have precluded the detection of significant effects. Cross-ecosystem subsidies may be expected to have greater effects when donor and recipient ecosystems are dissimilar in productivity (Gravel, Guichard, Loreau, & Mouquet, 2010; Marczak, Thompson, & Richardson, 2007; Polis et al., 1997). Sea lamprey subsidies arrive in freshwater during spring, a period characterized by increasing temperatures and nutrient limitations to producers and consumers. Therefore, it is not unexpected that organisms in recipient freshwaters (e.g., larval lamprey) would utilize these subsidies in the absence of other resources.

Many migratory animals translocate nutrients across ecosystem boundaries (Doughty et al., 2016). Climate change, over exploitation, habitat destruction, and anthropogenic barriers have caused population declines resulting in the loss of nutrient exchange among ecosystems (Wilcove, 2008). For many migratory fish species, dams have been particularly detrimental, leading to declines of many populations (Limburg & Waldman, 2009). For sea lamprey, which serve as vectors of nutrients to recipient freshwater systems, the consequence is predictable. Nutrient subsidies from anadromous fish that once flowed between connected marine and freshwater ecosystems are now reduced or eliminated (Saunders, Hachey, & Fay, 2006). Our work underscores the significance of detrital resource and consumer fluxes across marine and freshwater ecosystems and the role of organic matter in streams (Tank, Rosi-Marshall, Griffiths, Entrekin, & Stephen, 2010). This work improves our understanding of the mechanisms and consequences of reciprocal exchanges among multiple ecosystems as mediated by migrating fish.

ACKNOWLEDGEMENTS

We thank Janet Leese and Lori Carlos, and numerous others from the University of Maine for field and laboratory assistance. We also thank Jennifer Tank at Notre Dame for technical assistance with the methods of extracting and measuring ergosterol. Oliver Cox and Richard Dill from the Maine Department of Marine Resources provided technical assistance in collecting sea lamprey. We thank the Town of Bradley and Scott Richardson for land access. This research was supported by the U.S. Department of Agriculture National Institute of Food and Agriculture, Hatch project ME0-8367-0H (Maine Agriculture and Forest Experiment Station Publication Number 3589). Logistical support was provided by the U.S. Geological Survey Maine Cooperative Fish and Wildlife Research Unit. This research was performed under University of Maine approved Institutional Animal Care and Use Committee Protocol Number A2011-06-03. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. government.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Weaver DM, Coghlan Jr SM, Greig HS, Klemmer AJ, Perkins LB, Zydlewski J. Subsidies from anadromous sea lamprey (*Petromyzon marinus*) carcasses function as a reciprocal nutrient exchange between marine and freshwaters. *River Res Applic*. 2018;1–10. https://doi.org/10.1002/rra.3291