

# Bottom-up biodiversity effects increase resource subsidy flux between ecosystems

DANIEL C. ALLEN,<sup>1,4</sup> CARYN C. VAUGHN,<sup>1</sup> JEFFREY F. KELLY,<sup>1</sup> JOSHUA T. COOPER,<sup>2</sup> AND MICHAEL H. ENGEL<sup>3</sup>

<sup>1</sup>*Ecology and Evolutionary Biology Program, Oklahoma Biological Survey and Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019 USA*

<sup>2</sup>*Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019 USA*

<sup>3</sup>*School of Geology and Geophysics, University of Oklahoma, Norman, Oklahoma 73019 USA*

**Abstract.** Although biodiversity can increase ecosystem productivity and adjacent ecosystems are often linked by resource flows between them, the relationship between biodiversity and resource subsidies is not well understood. Here we test the influence of biodiversity on resource subsidy flux by manipulating freshwater mussel species richness and documenting the effects on a trophic cascade from aquatic to terrestrial ecosystems. In a mesocosm experiment, mussel effects on algae were linked through stable isotope analyses to mussel-derived nitrogen subsidies, but mussel biodiversity effects on algal accumulation were not significant. In contrast, mussel biodiversity significantly increased aquatic insect emergence rates, because aquatic insects were responding to mussel-induced changes in algal community structure instead of algal accumulation. In turn, mussel biodiversity also significantly increased terrestrial spider abundance as spiders tracked increases in aquatic insect prey after a reproduction event. In a comparative field study, we found that sites with greater mussel species richness had higher aquatic insect emergence rates. These results show that, because food webs in adjacent ecosystems are often linked, biodiversity effects in one ecosystem can influence adjacent ecosystems as well.

**Key words:** <sup>15</sup>N; aquatic–terrestrial linkages; biodiversity and ecosystem function (BEF); *Bivalvia*: *Unionidae*; resource subsidies; stable isotope.

## INTRODUCTION

Several decades of studies investigating the relationship between biodiversity and ecosystem function (BEF) have consistently shown that biodiversity can increase ecosystem productivity (Balvanera et al. 2006, Cardinale et al. 2006). Most BEF experiments have been conducted within a single, lower trophic level, but BEF studies need to include multiple trophic levels to understand the role of biodiversity in complex ecosystems (Duffy et al. 2007). Although some BEF studies have begun to incorporate trophic complexity (Douglass et al. 2008), these studies are often conducted within a single ecosystem or habitat type. Thus, little is known about how biodiversity influences the flow of resources between ecosystems.

An entirely separate field of research has shown that the flow of resources between ecosystems is common (Polis et al. 1997, Marczak et al. 2007). Streams and riparian forests are a model system for examining resource subsidies, as aquatic insects link aquatic and terrestrial ecosystems. Top-down effects in streams via predator addition can reduce aquatic insect subsidies to

riparian food webs (Baxter et al. 2004, Knight et al. 2005, Wesner 2010). However, investigations into whether bottom-up effects in streams enhance aquatic insect subsidies to riparian food webs are lacking. Further, most resource subsidy studies manipulating community structure add or remove entire trophic levels (Knight et al. 2005, Wesner 2010; but see Wesner 2012).

Here we examine how species diversity affects the flow of resource subsidies across aquatic and terrestrial ecosystems through a bottom-up trophic cascade. Freshwater mussels (*Bivalvia*: *Unionidae*, hereafter “mussels”) are a diverse guild of long-lived (6–100 yr), burrowing, filter-feeding bivalves that live in river sediments and are currently experiencing rapid biodiversity declines (Strayer et al. 2004). Mussels increase standing crops of benthic algae by providing nutrients that fertilize algae (Vaughn et al. 2007, Spooner and Vaughn 2008). Further, mussels are associated with increased abundances of grazing aquatic insect larvae (Spooner and Vaughn 2006, Vaughn and Spooner 2006, Spooner et al. 2012). Because mussel species vary in their effects on primary producers (Vaughn et al. 2007), and because grazing aquatic insects link aquatic and terrestrial ecosystems, mussel communities are a good system for testing hypotheses linking the concepts of BEF and resource subsidies.

We performed experiments examining effects of mussel species richness on a trophic cascade that

Manuscript received 23 August 2011; revised 5 April 2012; accepted 30 April 2012. Corresponding Editor: A. S. Flecker.

<sup>4</sup> Present address: School of Natural Resources and the Environment, University of Michigan, Ann Arbor, Michigan 48109 USA. E-mail: daniel.c.allen@gmail.com

increases the flux of aquatic insect subsidies to terrestrial predators. We hypothesized that: (1) because mussel species excreta vary in nutrient quantity and quality for algae fertilization, species effects may interact such that biodiversity increases algae growth; (2) grazing aquatic insect larvae will respond to increases in algae; thus mussel biodiversity will increase emergence rates of adult aquatic insects into terrestrial ecosystems; and (3) mussel biodiversity will increase the abundance of terrestrial spiders that prey on aquatic insects.

## METHODS

### *Mesocosm experiment*

*Design and sampling.*—In order to isolate mechanisms underlying mussel biodiversity effects on a trophic cascade crossing ecosystem boundaries, we conducted an eight-week experiment in 40 recirculating mesocosms in a greenhouse to simulate a natural stream under low-flow conditions (described in Allen and Vaughn [2009]; and Appendix A) during the summer of 2010. We filled mesocosms three weeks prior to the experiment with water (~100 L) from a nearby pond for aquatic insect and algae colonization, and conducted biweekly 50% water changes. Nutrient chemistry data from an experiment conducted in a previous summer that also simulated low flows indicate that starting nutrient levels in our mesocosm-pond water system were:  $1.280 \pm 0.130$  mg NO<sub>3</sub>/L (mean  $\pm$  SE),  $0.026 \pm 0.005$  mg NH<sub>3</sub>/L, and  $0.097 \pm 0.003$  mg PO<sub>4</sub>/L (Vaughn et al. 2008). We added a cultured algal mixture daily to mesocosms (dominated by *Scenedesmus* spp.; mean chlorophyll *a* = 0.212 mg/L); 200 mL per mesocosm in weeks 1–3 until algae established in the benthos, and 100 mL afterwards to sustain mussels. Nutrient concentrations of the algae culture were 54.71 mg NO<sub>3</sub>/L and 3.45 mg PO<sub>4</sub>/L.

We collected three mussel species (*Actinonaias ligamentina*, *Amblema plicata*, and *Quadrula pustulosa*) from a single site on the Kiamichi River, Oklahoma. These species are widespread and coexist throughout the central United States, but differ in physiological traits that influence nutrient excretion. *Actinonaias ligamentina* and *Q. pustulosa* are catabolic at summer temperatures (*A. ligamentina* especially so), while *A. plicata* is anabolic (Spooner and Vaughn 2008). These traits should lead to different effects on primary producers, because catabolic species are excreting endogenously, providing mussel-derived nutrients for algae to assimilate.

We used the three mussel species for eight treatments: 1 no-mussel control, 3 single-species monocultures, and 4 polycultures (3 two-species and 1 three-species). We recorded mussel length and tagged individuals, and mussels were randomly assigned to treatments to maintain constant densities of 6 mussels per mesocosm (~10 mussels/m<sup>2</sup>, a naturally occurring, low density for mussels in the region). The mussel species used vary in size (mean lengths  $\pm$  SD: *Actinonaias ligamentina*, 103  $\pm$  10.7 mm; *Amblema plicata*, 84  $\pm$  6.3 mm; *Quadrula*

*pustulosa*, 49  $\pm$  5.4 mm). We used a substitutive design at a constant density to maximize evenness and incorporate monocultures to test for nonadditive biodiversity effects in polycultures (Schmid et al. 2002). We chose a substitutive design based on density rather than biomass because species differences in mussel effects depend on species-specific physiological traits (such as thermal tolerance and N:P ratios of excreta) that are independent of biomass (Spooner and Vaughn 2008). Further, these species are similar in abundance in mussel communities (any one of these species can be the most abundant), and we wanted our experimental communities to reflect species compositions in nature. To prevent differences in species sizes from confounding mussel treatment effects, we estimated the mussel biomass of each treatment using length–dry mass regressions from Vaughn et al. (2007) for use as a covariate in statistical models. Mussel treatments were assigned to mesocosms in a randomized block design, with each of five blocks increasing in distance from an evaporative cooler in the greenhouse that causes a temperature gradient and is a source of aquatic insect colonists. The mean midday water temperature over the experiment was 29.7°C  $\pm$  1.0°C (mean  $\pm$  SD), similar to the Kiamichi River during the low-flow summer season (Vaughn et al. 2007).

We used three 2.5 cm diameter silica disks and one 232.3-cm<sup>2</sup> unglazed clay tile per mesocosm as colonization surfaces for benthic algae. Both substrates were sampled weekly and replaced. Chlorophyll *a* from algae on silica disks was extracted with acetone and analyzed spectrophotometrically with a correction for pheophytin (ASTM 1995). Biofilm on tiles was scrubbed in distilled water, forming a slurry that was passed through a 88- $\mu$ m sieve to remove macroinvertebrates and filtered through a precombusted 0.45- $\mu$ m pore glass-fiber filter. Filters were frozen and biofilm was analyzed for <sup>15</sup>N enrichment as described in the following paragraphs. Each mesocosm contained one floating trap to sample emergent aquatic insects, constructed following the design of Wesner (2010) with a collection area of 685 cm<sup>2</sup> and a mesh size of ~0.2 mm. We sampled traps twice per week beginning in week 2. Insects were preserved in ~75% EtOH, identified to family following Merritt et al. (2009), and enumerated and measured for length to estimate biomass using regressions in Sabo et al. (2002). Insect biomass was dominated by chironomids, primarily algivorous tube dwellers in the tribe Chironomini.

During week 3 we released 120 large adult tetragnathid spiders, 3 on each mesocosm, collected from a nearby stream (Byrd's Mill Creek, Fittstown, Oklahoma, USA). Tetragnathids are a common riparian predator that specialize on catching adult aquatic insects in webs spun near water. Beginning in week 4, we surveyed each mesocosm for spiders approximately two hours after sundown when spiders were most active (two surveys per week, 10 in total). If a web spanned multiple mesocosms,

its fraction was counted toward each mesocosm. At the end of the second week of the experiment we noticed visual differences in benthic algae communities in mesocosms. At the end of week 7, we sampled benthic algae from each mesocosm for identification by randomly selecting two pieces of gravel and taking a sample by scraping a small section of the wall of the west side of each mesocosm; samples were preserved in  $\sim 3.7\%$  formalin. Later, algae samples were homogenized and subsamples were identified to genus. We ranked each algae taxon on a 10-point log scale of biomass following methods described in Biggs and Kilroy (2000).

We used a stable isotope approach to track mussel-derived nutrients. Mussels were fed a cultured algal mixture enriched in  $^{15}\text{N}$  ( $\sim 100\%$  relative to atmospheric  $\text{N}_2$ ) for 13 weeks prior to the experiment in a separate greenhouse. Two weeks prior to the experiment, mussels were cleaned of biofilm and were moved to a holding tank in the experimental greenhouse. Mussels were starved to remove enriched algae from their digestive tract, water was changed daily, and shells were cleaned again before mussels were added to mesocosms. Stable isotope analysis of mussel hemolymph showed that  $^{15}\text{N}$  enrichment of mussels declined slightly throughout the experiment, but mussel species differed in  $\delta^{15}\text{N}$  values (two-way ANOVA: Time,  $F_{1,24} = 6.94$ ,  $P = 0.015$ ; Species,  $F_{2,24} = 14.84$ ,  $P < 0.001$ ; Interaction, NS). Mean hemolymph  $\delta^{15}\text{N}$  values were: pre-experiment =  $12.81\%$  and post-experiment =  $10.93\%$  (pooled by time including all species); *Actinonaias ligamentina* =  $10.08\%$ , *Amblema plicata* =  $9.62\%$  and *Quadrula pustulosa* =  $13.08\%$  (pooled by species including pre- and post-experiment samples). Background sources of nitrogen were organic matter in pond water used to fill mesocosms and cultured algae added to mesocosms during the experiment (mean  $\delta^{15}\text{N}$  values of  $7.73\%$  and  $0.43\%$ , respectively).

To isolate mussel-derived nitrogen isotopically from background sources, we standardized  $\delta^{15}\text{N}$  values to no-mussel controls that only received pond water and cultured algae, taking the difference in  $\delta^{15}\text{N}$  values of samples from mussel treatments from the mean  $\delta^{15}\text{N}$  value of the no-mussel controls. To account for mussel species differences in  $^{15}\text{N}$  enrichment, we estimated the  $\delta^{15}\text{N}$  value of the nitrogen pool that mussels could provide in a given mesocosm by using the mean  $\delta^{15}\text{N}$  value of hemolymph species contributions to polycultures weighted by relative biomass. Finally, we divided the difference in  $\delta^{15}\text{N}$  values from the sample and no-mussel controls by the  $\delta^{15}\text{N}$  value of the “mussel pool,” which gave us a metric describing the relative enrichment of the sample compared to the nitrogen pool available within the mussels themselves. We refer to this metric as “Mussel-derived Nitrogen Index (MDNI)”; a higher value indicates the sample has assimilated more mussel-derived nitrogen. We analyzed biofilm and emergent aquatic insect samples collected in weeks 2 and 8 for  $^{15}\text{N}$  enrichment. Freeze-dried biofilm and

chironomid samples were packed into tin capsules. Samples were analyzed for stable isotope ratios using a Costech elemental analyzer (Costech Analytical Technologies, Valencia, California, USA) interfaced through a Conflo III valve with a Thermo Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, West Palm Beach, Florida, USA).

*Statistical analysis.*—We analyzed the relationship between mussel species richness and algal accumulation rate, aquatic insect emergence rate (both  $\log [x + 1]$  transformed), and spider abundance (square-root  $[x + 1/2]$  transformed) with linear regressions on grand experimental means of mussel monocultures and polycultures (i.e., omitting no-mussel controls). We observed a spider reproduction event during the experiment (Appendix B), so we analyzed spider data separately as either pre-reproduction (weeks 4–5) or post-reproduction (weeks 6–8), using Bonferroni corrections on analyses of these data. This division of the data allowed us to make inferences on the abundance of adult spiders (pre-reproduction) and adult and juvenile spiders together (post-reproduction); juvenile spiders greatly outnumbered large adults. To test if mussel treatments differed in algal accumulation rates, aquatic insect emergence rates, and spider abundances, we used repeated measures ANOVAs with an ARH(1) variance-covariance structure including spatial block or mussel biomass as covariates if they were significant.

Following other BEF experiments (Douglass et al. 2008, Allen and Vaughn 2011), we conducted eight a priori planned linear contrasts to test for nonadditive biodiversity effects. The first set of “liberal” biodiversity contrasts ( $n = 4$ ) compared the observed polyculture mean against the expected mean based on additive monoculture performances (e.g., nontransgressive overyielding). The second set of “conservative” biodiversity contrasts ( $n = 4$ ) compared the observed polyculture performance against its monoculture with the strongest effect (e.g., transgressive overyielding). Following Douglass et al. (2008) and Allen and Vaughn (2011), we opted not to apply a table-wide adjustment of  $P$  values to our contrasts, but rather we report the effect size of each test along with exact  $P$  values, using both to interpret ecological significance (e.g., if the results of a contrast were statistically significant but had a small effect size, we would view the result with caution). Further, we interpret results of multiple statistical tests strictly within the context of our single broader hypothesis (i.e., whether or not nonadditive biodiversity effects are present). Effect sizes are reported as Cohen’s  $d$ , which measures the difference between group means relative to the standard deviation, with values above 0.5 considered “medium” effect sizes, and those  $>0.8$  considered “large” (Cohen 1988).

We collected 23 genera of benthic algae. Because we were interested in mussel influences on shifts in algae taxa dominance patterns, not the presence/absence of rare taxa, we reduced our data to the 10 most common

algae taxa (which were present in at least 80% of all samples). We then used nonmetric multi-dimensional scaling (NMDS) with the Bray-Curtis (Sørensen) distance measure and multiple response permutation procedure (MRPP) to compare algal communities between treatments. The MRPP determines the treatment effect size ( $A$ ), where  $A = 1$  indicates similarity and  $A = 0$  indicates heterogeneity among treatments. NMDS Axis 1 clearly separated algal communities along a gradient of diatom relative abundance, so we used this axis in further analyses to investigate how algal community dominance shifts toward diatoms were related to mussel-derived nutrients and aquatic insect emergence rates.

To test if mussel-derived nutrients influence algal accumulation rates and insect emergence, we ran linear regressions from samples collected in weeks 2 and 8 against the Mussel-derived Nitrogen Index (see *Mesocosm experiment: Design and sampling*). We ran linear regressions on NMDS axis 1 against Mussel-derived Nitrogen Index from biofilm in week 8 to see if mussel-derived nutrients influenced algal community composition. To determine if emergence rates were responding to changes in algal accumulation rates or algal community structure, we fit linear and quadratic regression models ( $n = 8$ ) using algal accumulation rates and NMDS axis 1 to predict emergence rates using block as a covariate. To see which model best explained emergence rates, we used a model selection approach using AIC (Burnham and Anderson 2002). Analyses were conducted using R (R Development Core Team 2011), except PC-ORD was used for NMDS and MRPP.

#### *Comparative field study*

*Design and sampling.*—In order to investigate mussel biodiversity effects on resource subsidy flux at a large spatial scale in a natural system, in the summer of 2009 we established nine study sites on two rivers in the south-central United States (five on the Little River, four on the Kiamichi River in southeast Oklahoma) that span a gradient of mussel species richness. At each site we established a 100-m study reach and deployed eight 0.33-m<sup>2</sup> emergence traps designed following Malison et al. (2010), with a mesh size of  $\sim 0.2$  mm. Traps were loosely secured to a rebar stake and haphazardly placed throughout the site, restricted to locations near mussels and not more than 50 cm in depth to accommodate a 45-cm rise in water level. Each trap had a mesh catch near the top to capture emerging insects that would have otherwise been lost to rain, wind, or mortality. Insects were removed with an aspirator and preserved with  $\sim 75\%$  EtOH. The study period was restricted to a single week because of an atypical high-flow event that flooded our sites. Insects were enumerated, identified to order or family, measured for length, and biomass estimated as described previously.

We sampled mussels by excavating one 0.25-m<sup>2</sup> quadrat at each emergence trap, and conducting a

120-minute timed search spanning the 100-m reach at each site. Mussels were brought to shore, identified, and returned alive to the streambed. We used these data to calculate mussel species richness and density (individuals per square meter), and species richness data were corroborated from data from other recent surveys at these sites. We measured physical habitat variables at each site to account for habitat differences. At each site we measured waterline slope with a surveyor's level, and at each trap we measured water depth ( $d$ ), flow velocity ( $u$ , measured at 0.6  $d$ ), qualitatively described substrate composition (% boulder, % cobble, % sand, and % silt), and estimated substrate roughness using a 30.5-cm chain (3-mm links), following Hardison and Layzer (2001). Using these measurements we estimated the hydraulic variables Reynolds number ( $Re$ ), boundary Reynolds number ( $Re^*$ ), Froude number ( $Fr$ ), and shear velocity ( $U^*$ ) using formulae in Stutzner et al. (1988; and Appendix C).

*Statistical analysis.*—We collected five orders of adult aquatic insects in our emergence traps: Diptera, Trichoptera, Ephemeroptera, Plecoptera, and Odonata. We removed odonates from our analysis since they are predators and we were interested in bottom-up effects of mussels on primary consumers. Additionally, we omitted ephemeropterans and plecopterans because they comprised a very small portion of overall biomass (1.6% and 1.7%, respectively) and were not present in all samples (60% and 48%, respectively). We wanted to test the hypothesis that insect emergence rates were higher at sites with greater mussel species richness, irrespective of habitat differences, so we standardized our emergence rates. We used a model-building information theoretic approach, developing a suite of multiple regression models using physical habitat variables on  $\log(x + 1)$  transformed emergence rates of each taxon (Diptera and Trichoptera) separately for each river. If variables were multicollinear ( $r > 0.85$ ), we kept the variable most correlated with the taxon of interest. We used AIC to determine the best set of candidate models, and used an averaged model (using all models with  $\Delta_i < 2$ ) to predict emergence rates based on physical habitat variables alone (Appendix D). We summed the residuals from taxon-specific averaged models to generate a single residual for aquatic insect emergence rates at each trap on each river. We refer to the residuals as “standardized aquatic insect emergence rates” because they are standardized for differences in physical habitat among rivers, and among sites within rivers. To test for a relationship between median standardized emergence rate and mussel species richness or abundance, we fit a mixed-effect linear regression model with the random effect of river on the intercept, using Bonferroni corrections to control for alpha. Mussel abundance data were square-root transformed prior to analysis (Zar 2010), and all analyses were conducted using R (R Development Core Team 2011).

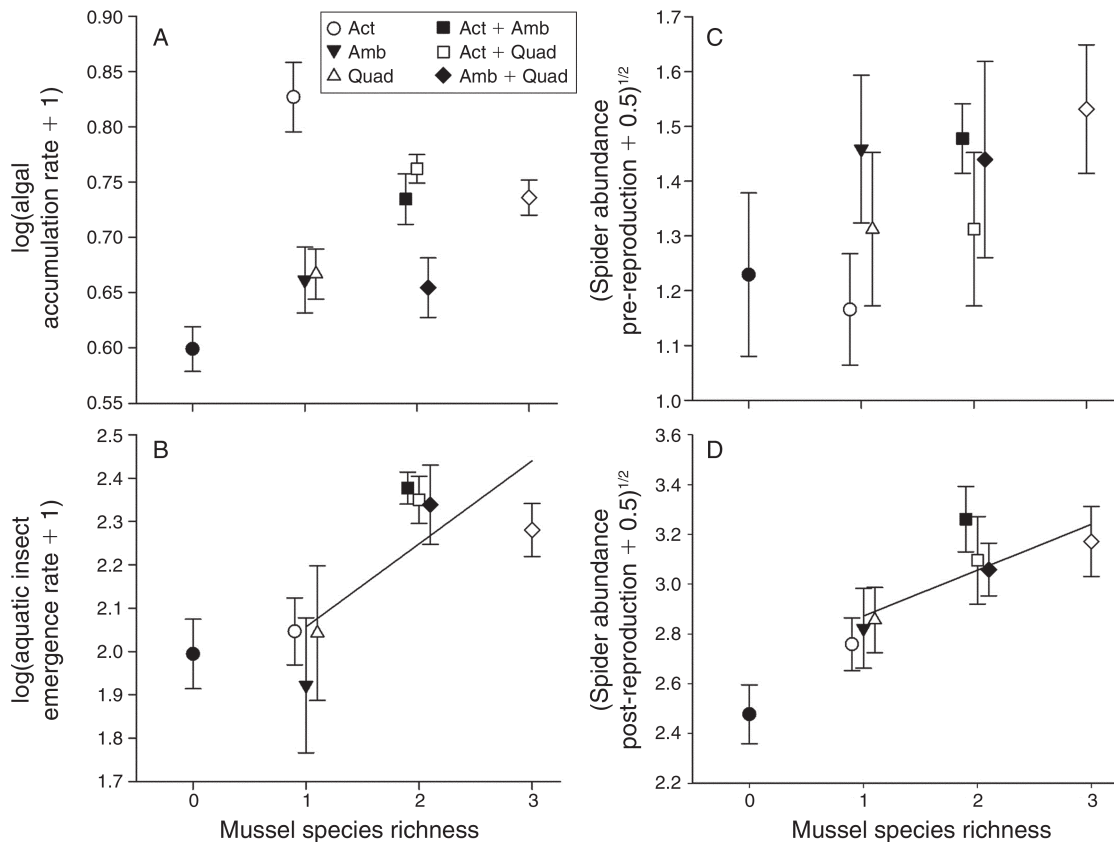


FIG. 1. Response variables from mesocosm experiment as a function of mussel species richness. (A) Algal accumulation rate ( $\text{mg Chl } a \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ); (B) aquatic insect emergence rate ( $\text{mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ),  $y = 1.8654 + 0.1915x$ ,  $P = 0.002$ ,  $R^2 = 0.24$ ; (C) pre-reproduction spider abundance; (D) post-reproduction spider abundance,  $y = 2.688 + 0.184x$ ,  $P = 0.032$ ,  $R^2 = 0.16$ . The solid circles refer to the 0-species treatment, and the open diamonds refer to the three-species treatment. Mussel treatment means and standard errors are shown. Act is *Actinonaias ligamentina*; Amb is *Amblyma plicata*; Quad is *Quadrula pustulosa*.

## RESULTS

### Mesocosm experiment

**Treatment effects.**—Linear regressions showed positive relationships between mussel species richness and aquatic insect emergence rates ( $P = 0.002$ ) and spider abundances post-reproduction ( $P = 0.032$ ), but were not significant for algal accumulation rates ( $P = 0.742$ ) or pre-reproduction spider abundances ( $P = 0.592$ ; Fig. 1). Repeated-measures ANCOVA models showed that mussel species treatments significantly differed in algal accumulation rate ( $F_{7,31} = 12.634$ ,  $P < 0.001$ ; mussel biomass covariate:  $F_{1,31} = 4.885$ ,  $P = 0.035$ ), aquatic insect emergence rate ( $F_{7,31} = 7.262$ ,  $P < 0.001$ ; block covariate:  $F_{1,31} = 38.687$ ,  $P < 0.001$ ), and post-reproduction spider abundance ( $F_{7,31} = 5.421$ ,  $P < 0.001$ ; block covariate:  $F_{1,31} = 6.657$ ,  $P = 0.015$ ); but mussel treatments did not differ in pre-reproduction spider abundance ( $F_{7,32} = 0.995$ ,  $P = 0.941$ ). Algae community structure differed significantly between mussel treatments (MRPP:  $A = 0.157$ ,  $P < 0.001$ ; Fig. 2A). NMDS axis 1 explained the most variation and broadly separated samples by association with diatoms,

with diatoms (*Gomphonema*, *Nitzschia*, and *Synedra*) positively related to axis 1 and green algae (*Cosmarium*, *Oedogonium*, *Pediastrum*, *Scenedesmus*, *Spirogyra*, and *Tetraedon*) and the synurid *Mallomonas* negatively related to axis 1.

All contrasts testing for nonadditive mussel biodiversity effects on algal accumulation rates were insignificant (Table 1). For aquatic insect emergence rates, all liberal biodiversity contrasts were significant, and three of four conservative biodiversity contrasts were significant (Table 1). Finally, for post-reproduction spider abundance, two of four liberal biodiversity contrasts and two of four conservative biodiversity contrasts were significant. Polyculture emergence rates were 90% higher, on average, than expected given additive performances of its monocultures; while post-reproduction spider abundance was 31% greater for the *Actinonaias ligamentina* and *Amblyma plicata* polyculture, and 26% greater for the *Actinonaias ligamentina* and *Quadrula pustulosa* polyculture, than additive expectations.

**Underlying mechanisms.**—Linear regressions showed positive relationships between algal accumulation rates and the Mussel-Derived Nitrogen Index of biofilm,

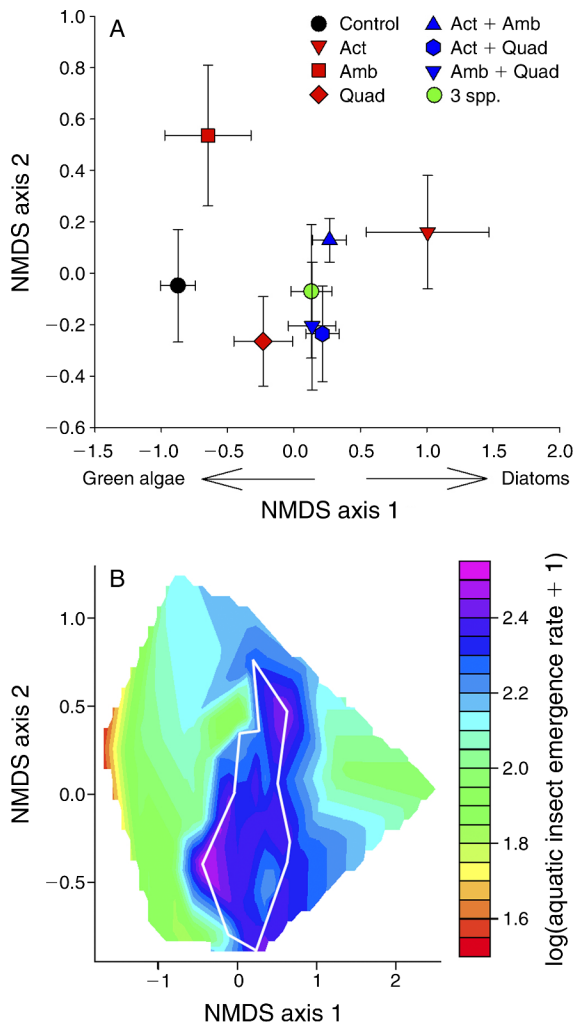


FIG. 2. Algae nonmetric multi-dimensional scaling (NMDS) axes plots from mesocosm experiment: axis 1 is positively correlated with diatoms and negatively correlated with nondiatom taxa. (A) The graph shows axes means and standard errors for mussel treatments; treatment abbreviations are as in Fig. 1. NMDS axis 1,  $R^2 = 0.61$ ; NMDS axis 2,  $R^2 = 0.19$ . (B) The graph shows the filled contour plot with log-transformed aquatic insect emergence rate ( $\text{mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ), shown as the color spectrum). The white polygon shows the extent of polyculture data points.

though this relationship was stronger in week 8 (week 2,  $P = 0.003$ ,  $R^2 = 0.24$ ; week 8,  $P < 0.001$ ,  $R^2 = 0.49$ ; Fig. 3A). Further, the Mussel-Derived Nitrogen Index of biofilm was positively related to NMDS axis 1 ( $P = 0.004$ ,  $R^2 = 0.22$ ; Fig. 3B). We did not find significant relationships between emergence rates and Mussel-Derived Nitrogen Index of emerged insects (linear regressions: week 2,  $P = 0.194$ ; week 8,  $P = 0.278$ ). However, a quadratic model describing a unimodal concave-down relationship between NMDS Axis 1 and emergence rates was the best-performing model, explaining 57% percent of the variation (Akaike weight,

$w_i = 0.99$ ; Fig. 2B), while models using algal accumulation rates performed poorly (Appendix E).

#### Comparative field study

Models using physical habitat variables to predict emergence rates explained 3–70% of the variation in aquatic insect emergence rates (Appendix D). The mixed-effect linear regression showed a significant relationship between mussel species richness and standardized emergence rate at a site ( $P = 0.012$ ,  $R^2 = 0.69$ ; Fig. 4A), but not for mussel density ( $P = 0.108$ ,  $R^2 = 0.27$ ; Fig. 4B). Thus, after accounting for habitat differences, emergence rates were more than five times greater at the site with highest mussel species richness relative to the least speciose site, and mussel species richness at a site could explain nearly 70% of variation in emergence rates.

#### DISCUSSION

##### Biodiversity effects cascade across ecosystem boundaries

The most striking result of this study is that the biodiversity of an aquatic community can increase the abundance of terrestrial organisms. Previous studies show that top-down biotic interactions in streams can affect terrestrial ecosystems (Baxter et al. 2004, Knight et al. 2005). Wesner (2012), in the only other study we are aware of that addresses biodiversity effects on resource subsidy flux, found that fish biodiversity can reduce emergence rates, presumably due to complementary interactions between fish predators on aquatic insect prey. However, here we show that bottom-up biodiversity effects can increase resource subsidy flux. By their very nature resource subsidies tend to produce bottom-up effects in food webs, and have their strongest effects when the quantity of resource subsidies from the donor habitat is greater than the quantity of equivalent resources in the recipient habitat (Marczak et al. 2007). Because biodiversity has been shown to increase both the production and consumption of resources (Balvanera et al. 2006, Cardinale et al. 2006), biodiversity should increase the strength of linkages between ecosystems that exist due to the flow of resources between them.

We found that freshwater mussel biodiversity affects a complex trophic cascade between streams and forests, but this principle should apply to other study systems as well. Fish biodiversity has been shown to affect nutrient cycling (McIntyre et al. 2007, 2008), which could create biogeochemical hotspots and initiate a bottom-up trophic cascade into riparian food webs, much as we observed in our mussel study system. Biodiversity increases terrestrial plant biomass production (Balvanera et al. 2006, Cardinale et al. 2006), and fallen leaves that wash into streams can drive stream productivity (Wallace et al. 1997). Biodiversity has been shown to increase seaweed cover in marine tidal zones (Stachowicz et al. 2008), and seaweed is an important detrital subsidy for terrestrial food webs (Spiller et al. 2010).

TABLE 1. Results from contrasts testing for nonadditive biodiversity effects.

Contrast	Liberal			Conservative		
	<i>t</i>	<i>d</i>	<i>P</i>	<i>t</i>	<i>d</i>	<i>P</i>
Algal accumulation rate						
Act + Amb	1.98	<i>0.71</i>	0.057	1.14	0.41	0.262
Act + Quad	1.58	<i>0.57</i>	0.124	1.16	0.42	0.253
Amb + Quad	0.26	0.09	0.794	-0.89	0.32	0.377
Three spp.	1.17	0.42	0.250	0.76	0.27	0.455
Aquatic insect emergence rate						
Act + Amb	3.53	<b>1.27</b>	<b>0.001</b>	2.54	<b>0.91</b>	<b>0.016</b>
Act + Quad	2.97	<b>1.07</b>	<b>0.006</b>	2.34	<b>0.84</b>	<b>0.026</b>
Amb + Quad	2.83	<b>1.02</b>	<b>0.008</b>	2.17	<i>0.78</i>	<b>0.038</b>
Three spp.	2.88	<b>1.03</b>	<b>0.007</b>	1.85	<i>0.66</i>	0.074
Post-reproduction spider abundance						
Act + Amb	2.82	<b>1.01</b>	<b>0.008</b>	2.35	<b>0.84</b>	<b>0.025</b>
Act + Quad	2.74	<b>0.98</b>	<b>0.010</b>	2.34	<b>0.84</b>	<b>0.026</b>
Amb + Quad	1.46	<i>0.52</i>	0.155	1.14	0.41	0.262
Three spp.	1.96	<i>0.70</i>	0.060	1.49	<i>0.53</i>	0.146

Notes: The Liberal column heading indicates contrasts that test polyculture performance against additive contributions from its monocultures. The Conservative column heading indicates contrasts that test polyculture against its monoculture with the strongest effect. Values in boldface type indicate  $P < 0.05$  and  $d > 0.8$  (large effect size). Values in italics indicate  $d > 0.5$  (medium effect size). Act is *Actinonaias ligamentina*; Amb is *Amblema plicata*; Quad is *Quadrula pustulosa*; and "three spp." refers to the three-species polyculture.

Thus, the potential for the importance of biodiversity in linking ecosystems via resource flows is high, and would provide a rich perspective complementing existing areas of biodiversity and resource subsidy research.

#### Underlying mechanisms

An interesting facet of this study is that mussel biodiversity effects differed across trophic levels. Mussel biodiversity itself had no effect on algal accumulation. However, mussel species treatments did differ in algal accumulation and in algal community composition. Our stable isotope approach showed that mussel effects on algae were due to mussel-derived nutrient subsidies. In particular, biofilm was more enriched in  $^{15}\text{N}$  when *A. ligamentina* was present, a species that is especially catabolic at summer temperatures and excretes ammonia at high rates (Spooner and Vaughn 2008, 2012). Algae communities also shifted toward the dominance of diatoms in response to mussel-derived nitrogen. Mussel-derived nitrogen subsidies likely increased N:P ratios, an important factor in determining competition outcomes in algae communities and thus algal community structure (Rhee and Gotham 1980). Interestingly, aquatic insects responded to changes in algal community composition, not changes in algal accumulation.

Mussel species richness was positively associated with aquatic insect emergence rates in our field study, and mussel polycultures had the greatest emergence rates in our mesocosm experiment. The dominant consumers in mesocosms were algivorous chironomids that create tube-shaped retreats from silk and small particles (sand, organic matter, algae, etc.). Diatoms are more nutritious for these grazers than filamentous green algae (Dodds

1991), and emergence rates increased as diatoms became more abundant. However we observed a unimodal relationship between insect emergence and NMDS axis 1, as an overabundance of diatoms was linked with a decline in emergence rates. This may reflect chironomid requirements for green algae filaments as tube-building material and for diatoms as food; Power et al. (2008) found that chironomids used green filamentous algae for physical habitat but primarily fed on diatoms. Thus, the nonadditive effects of mussel biodiversity on emergence rates may be due to the requirements for both high-quality food and tube-building materials. Finally, although the explanatory power of our regression between emergence rates and species richness in our mesocosm experiment was relatively low, this is partly due to not accounting for variation in space or time in this analysis. In our repeated-measures mixed model that accounted for these factors, seven of eight biodiversity contrasts were significant, and their effect sizes were generally high. Further, in our field study, mussel species richness explained a great deal of variation in emergence rates after accounting for habitat differences.

Spider abundance responded to aquatic insect emergence rates in our mesocosm experiment after a reproduction event, but not before. The area of our mesocosms could only accommodate webs of a few large adult spiders, but many webs of recently hatched, small juvenile spiders. Pre-reproduction, large spiders were likely space limited (supported by visual observations), but post-reproduction this spatial constraint was lifted and rapidly growing spiders then responded to a limited food source, emerging aquatic insects,

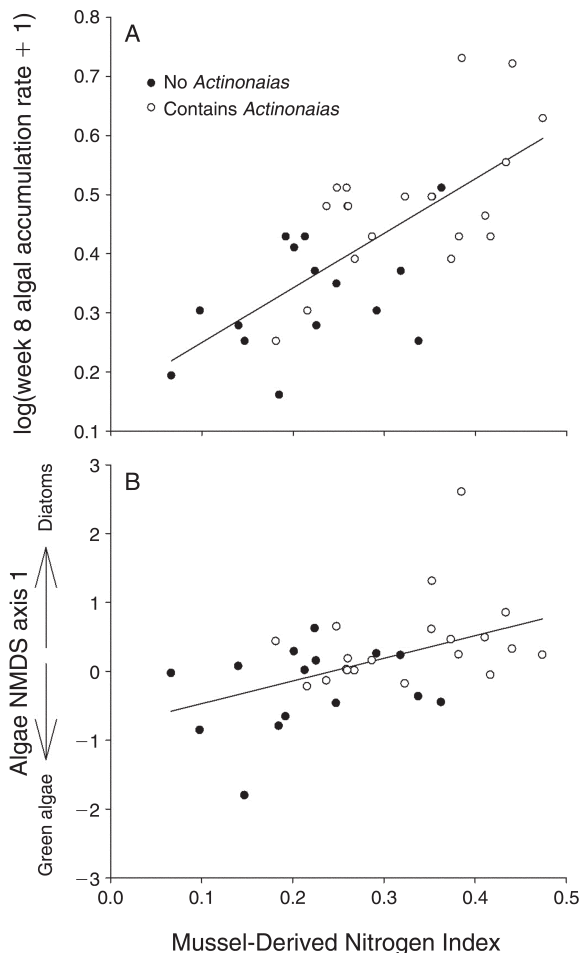


FIG. 3. Summary of stable isotope analyses investigating mechanisms for mussel effects on algae. (A) Linear regression using Mussel-Derived Nitrogen Index of biofilm enrichment to predict algal accumulation rate ( $\text{mg Chl } a \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ) in week 8;  $y = 0.157 + 0.924x$ ,  $P < 0.001$ ,  $R^2 = 0.49$ . (B) Linear regression using Mussel-Derived Nitrogen Index of biofilm sampled in week 8 to predict NMDS axis 1,  $y = -0.796 + 3.288x$ ,  $P = 0.004$ ,  $R^2 = 0.22$ . Points with open circles represent mesocosms with *Actinonaias ligamentina*, solid circles are those without.

which were more abundant in polycultures. This is probably because either juvenile spider migration or survival tracked increases in insect emergence rates. Finally, biodiversity effects on spider abundance were weaker than effects on insect emergence rates, as evidenced both by the lower  $R^2$  values of the linear regression, and that fewer biodiversity contrasts were significant with large effect sizes. This is presumably due to weakening of bottom-up effects as they extend into higher trophic levels, a result of energy losses or additional factors affecting the abundance of organisms in higher trophic levels.

Considerations of our study system

The results of this study are well supported by other investigations of mussel effects on ecosystems. We know

from previous field studies that nutrients limit algal growth in mussel beds during the summer low-flow season (Vaughn et al. 2007), that catabolic mussel species excrete more nitrogen at this time (Spooner and Vaughn 2008), and that these species facilitate benthic algae (Spooner and Vaughn 2012). In this study, we used  $^{15}\text{N}$ -labeled mussels to show that it was mussel-derived nitrogen that stimulated algal growth and altered algal community structure. While we seeded the mesocosms with pond biota, pond biota and river biota contain the same functional groups (i.e., bacteria, diatoms, unicellular and filamentous green algae, etc.), and previous mesocosm and field experiments by our laboratory have demonstrated that mussel effects are the same whether they are fed cultured, pond algae in the laboratory or are filtering a natural algal assemblage in the field (Vaughn et al. 2008).

Strong mussel effects on larval aquatic insects are also supported by previous studies. In a large-scale compar-

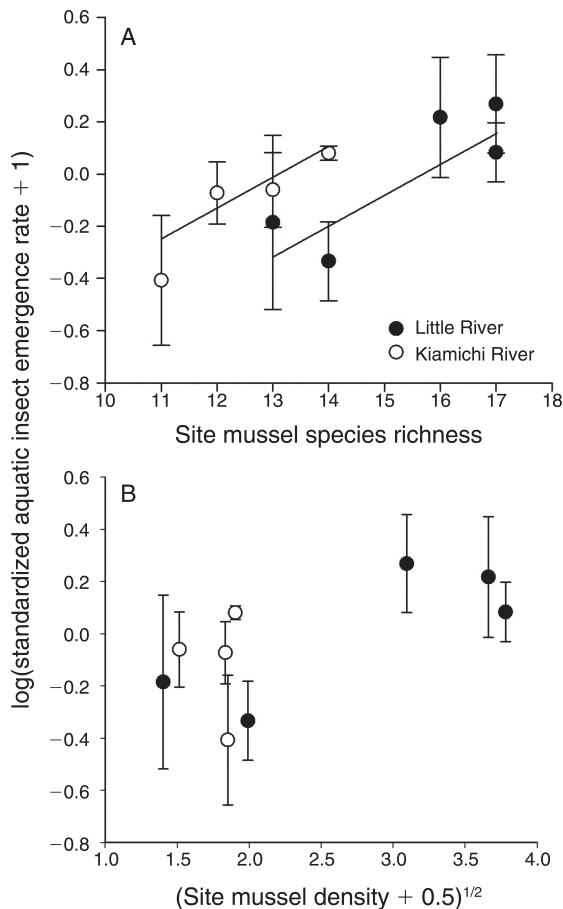


FIG. 4. Standardized aquatic insect emergence rates ( $\text{mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$  [median  $\pm$  SE]) using (A) site mussel species richness and (B) site mussel density as predicting variables in a mixed-effect linear regression for study sites on the Little and Kiamichi Rivers. The random river effect is shown as separate lines for each river (overall model in graph A:  $y = -1.70 + 0.118x$ ,  $P = 0.012$ ,  $R^2_{\text{adj}} = 0.69$ ).



ative field study of 30 sites across eight rivers, Vaughn and Spooner (2006) found that mussel assemblages alone explained >50% of the variation in aquatic insect community structure, even after accounting for differences in habitat and biogeography. Additionally, in a year-long field enclosure experiment manipulating the presence of mussels, Spooner and Vaughn (2006) found that larval insect abundance was higher in the presence of mussels, and that the macroinvertebrate community structure shifted toward the dominance of grazing aquatic insects in their presence (Vaughn et al. 2008). Finally, in an additional large-scale field enclosure experiment manipulating mussel biodiversity, Spooner et al. (2012) demonstrated that mussel biodiversity augments this effect, increasing the abundance of grazing chironomid larvae. Here we document that mussel biodiversity also affects the next step in the life stage of aquatic insects as they emerge as adults. Therefore, even though our field study was correlational by design, we know from manipulative approaches that mussels have strong effects on aquatic insects, and that they are not simply both responding to the same habitat gradients.

#### *Far-reaching consequences of biodiversity losses*

Although the influence of biodiversity on ecosystem processes has been well documented (Balvanera et al. 2006, Cardinale et al. 2006), implicit in many of these studies is that the consequences of biodiversity losses only extend as far as the ecosystem that actually contains the organism group of interest. An entirely separate area of research has shown that the flow of resources across ecosystem boundaries is ubiquitous (Polis et al. 1997, Marczak et al. 2007), but these studies rarely account for the nonadditive species interactions that biodiversity and ecosystem function studies have shown are so common in nature. Here we merge these two important areas of ecological research, and demonstrate that biodiversity effects in one ecosystem can reverberate across landscapes into adjacent ecosystems. Because biodiversity effects on productivity and linked ecosystems are both widespread, this suggests that effects of biodiversity losses on ecosystems may be far reaching.

#### ACKNOWLEDGMENTS

Whitney Allen, Carla Atkinson, Eric Bright, and Pascal Irmscher assisted in the lab and field, Rick Maynard assisted with stable isotope analysis, and the Little River National Wildlife Refuge and private landowners allowed access to field sites. Colden Baxter, Mary Power, Sherri Johnson, and Michael Patten provided advice on experimental design and statistics. Gary Wellborn, David Strayer, and anonymous reviewers provided comments on the manuscript. Funding was provided by the National Science Foundation (DEB-0910086, DEB-0211010) and the University of Oklahoma Graduate College. This work is submitted in partial fulfillment of the degree requirements for a Ph.D, Department of Zoology, University of Oklahoma, and is a contribution to the program of the Oklahoma Biological Survey.

#### LITERATURE CITED

- Allen, D. C., and C. C. Vaughn. 2009. Burrowing behavior of freshwater mussel species in experimentally manipulated communities. *Journal of the North American Benthological Society* 28:93–100.
- Allen, D. C., and C. C. Vaughn. 2011. Density-dependent biodiversity effects on physical habitat modification by freshwater bivalves. *Ecology* 92:1013–1019.
- ASTM (American Society for Testing and Materials). 1995. Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, D.C., USA.
- Balvanera, P., A. B. Pfisterer, N. Buchmann, J. S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* 9:1146–1156.
- Baxter, C. V., K. D. Fausch, M. Murakami, and P. L. Chapman. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* 85:2656–2663.
- Biggs, B. J., and C. Kilroy. 2000. Stream periphyton manual. New Zealand Ministry for the Environment, Christchurch, New Zealand.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference. Second edition. Springer, New York, New York, USA.
- Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443:989–992.
- Cohen, J. 1988. Statistical power analysis for the behavioral sciences. Second edition. Lawrence Erlbaum Associates, Hillsdale, New Jersey, USA.
- Dodds, W. K. 1991. Community interactions between the filamentous alga *Cladophora glomerata* (L.) Kuetzing, its epiphytes, and epiphyte grazers. *Oecologia* 85:572–580.
- Douglass, J. G., J. E. Duffy, and J. F. Bruno. 2008. Herbivore and predator diversity interactively affect ecosystem properties in an experimental marine community. *Ecology Letters* 11:598–608.
- Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thebault, and M. Loreau. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10:522–538.
- Hardison, B. S., and J. B. Layzer. 2001. Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers. *Regulated Rivers: Research and Management* 17:77–88.
- Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. *Nature* 437:880–883.
- Malison, R. L., J. R. Benjamin, and C. V. Baxter. 2010. Measuring adult insect emergence from streams: the influence of trap placement and a comparison with benthic sampling. *Journal of the North American Benthological Society* 29:647–656.
- Marczak, L. B., R. M. Thompson, and J. S. Richardson. 2007. Meta-analysis: Trophic level, habitat, and productivity shape the food web effects of resource subsidies. *Ecology* 88:140–148.
- McIntyre, P. B., A. S. Flecker, M. J. Vanni, J. M. Hood, B. W. Taylor, and S. A. Thomas. 2008. Fish distributions and nutrient cycling in streams: Can fish create biogeochemical hotspots? *Ecology* 89:2335–2346.
- McIntyre, P. B., L. E. Jones, A. S. Flecker, and M. J. Vanni. 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. *Proceedings of the National Academy of Sciences USA* 104:4461–4466.

- Merritt, R. W., K. W. Cummings, and M. B. Berg. 2009. An introduction to the aquatic insects of North America. Fourth edition. Kendall Hunt, Dubuque, Iowa, USA.
- Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28:289–316.
- Power, M. E., M. S. Parker, and W. E. Dietrich. 2008. Seasonal reassembly of a river food web: floods, droughts, and impacts of fish. *Ecological Monographs* 78:263–282.
- R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rhee, G. Y., and I. J. Gotham. 1980. Optimum N-P ratios and coexistence of planktonic algae. *Journal of Phycology* 16:486–489.
- Sabo, J. L., J. L. Bastow, and M. E. Power. 2002. Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Journal of the North American Benthological Society* 21:336–343.
- Schmid, B., A. Hector, M. Huston, P. Inchausti, I. Nijs, P. W. Leadley, and D. Tilman. 2002. The design and analysis of biodiversity experiments. Pages 61–75 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, UK.
- Spiller, D. A., J. Piovio-Scott, A. N. Wright, L. H. Yang, G. Takimoto, T. W. Schoener, and T. Iwata. 2010. Marine subsidies have multiple effects on coastal food webs. *Ecology* 91:1424–1434.
- Spooner, D. E., and C. C. Vaughn. 2006. Context-dependent effects of freshwater mussels on stream benthic communities. *Freshwater Biology* 51:1016–1024.
- Spooner, D. E., and C. C. Vaughn. 2008. A trait-based approach to species' roles in stream ecosystems: climate change, community structure, and material cycling. *Oecologia* 158:307–317.
- Spooner, D. E., and C. C. Vaughn. 2012. Species dominance and environmental gradients interact to govern primary production in freshwater mussel communities. *Oikos* 121:403–416.
- Spooner, D. E., C. C. Vaughn, and H. S. Galbraith. 2012. Species traits and environmental conditions govern the relationship between biodiversity effects across trophic levels. *Oecologia* 168:533–548.
- Stachowicz, J. J., M. Graham, M. E. S. Bracken, and A. I. Szoboszlai. 2008. Diversity enhances cover and stability of seaweed assemblages: the role of heterogeneity and time. *Ecology* 89:3008–3019.
- Statzner, B., J. A. Gore, and V. H. Resh. 1988. Hydraulic stream ecology: observed patterns and potential applications. *Journal of the North American Benthological Society* 7:307–360.
- Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* 54:429–439.
- Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society* 27:409–423.
- Vaughn, C. C., and D. E. Spooner. 2006. Unionid mussels influence macroinvertebrate assemblage structure in streams. *Journal of the North American Benthological Society* 25:691–700.
- Vaughn, C. C., D. E. Spooner, and H. S. Galbraith. 2007. Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology* 88:1654–1662.
- Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 277:102–104.
- Wesner, J. S. 2010. Aquatic predation alters a terrestrial prey subsidy. *Ecology* 91:1435–1444.
- Wesner, J. S. 2012. Predator diversity effects cascade across an ecosystem boundary. *Oikos* 121:53–60.
- Zar, J. H. 2010. *Biostatistical analysis*. Fifth edition. Prentice Hall, Upper Saddle River, New Jersey, USA.

#### SUPPLEMENTAL MATERIAL

##### Appendix A

Photograph of the mesocosms used in the experiment, showing the unglazed clay tile and three silica disks used for sampling biofilm, and the trap used for sampling emergent aquatic insects (*Ecological Archives* E093-205-A1).

##### Appendix B

Response variables from the mesocosm experiment over time (*Ecological Archives* E093-205-A2).

##### Appendix C

Summary of hydraulic variables estimated in the comparative field study (*Ecological Archives* E093-205-A3).

##### Appendix D

Summary of the models using physical habitat variables to predict the emergence rates of aquatic insects in the comparative field study (only models with  $\Delta_i < 2$  are shown) (*Ecological Archives* E093-205-A4).

##### Appendix E

Summary of the models using algal accumulation rates and algae NMDS axis 1 to predict the emergence rates of aquatic insects for each mesocosm (all models include treatment block as a covariate) (*Ecological Archives* E093-205-A5).