

Predator diversity effects cascade across an ecosystem boundary

Jeff Scott Wesner

Jeff Scott Wesner (jeffwesner@gmail.com), Biological Station and Dept of Zoology, Univ. of Oklahoma, Norman, OK, USA. Present address: Dept of Biology, Brigham Young Univ., 401 WIDB, Provo, UT 84602, USA

Food webs in different ecosystems are often connected through spatial resource subsidies. As a result, biodiversity effects in one ecosystem may cascade to adjacent ecosystems. I tested the hypothesis that aquatic predator diversity effects cascade to terrestrial food webs by altering a prey subsidy (biomass and trophic structure of emerging aquatic insects) entering terrestrial food webs, in turn altering the distribution of a terrestrial consumer (spider) that feeds on emerging aquatic insects. Fish presence, but not diversity, altered the trophic structure of emerging aquatic insects by strongly reducing the biomass of emerging predators (dragonflies) relative to non-feeding taxa (chironomid midges). Fish diversity reduced emerging insect biomass through enhanced effects on the most common prey taxa: predatory dragonflies *Pantala flavescens* and non-feeding chironomids. Terrestrial spiders (Tetragnathidae) primarily captured emerging chironomids, which were reduced in the high richness (3 spp.) treatment relative to the 1 and 2 species treatments. As a result, terrestrial spider abundance was lower above pools with high fish richness (3 species) than pools with 1 and 2 species. Synergistic predation effects were mostly limited to the high richness treatment, in which fish occupied each level of vertical microhabitat in the water-column (benthic, middle, surface). This study demonstrates that predator diversity effects are not limited to the habitat of the predator, but can propagate to adjacent ecosystems, and demonstrates the utility of using simple predator functional traits (foraging domain) to more accurately predict the direction of predator diversity effects.

Predator diversity loss may have greater consequences for ecosystem functioning than the more commonly studied effects of diversity loss at lower trophic levels (Duffy 2003). Accurately predicting the spatial extent and direction of the consequences of predator species loss is critical because predator species are potentially at greater risk of extinction than species at lower trophic levels due to overharvest and enhanced sensitivity to habitat fragmentation (Pauly et al. 1998, Duffy 2003). Changes in predator diversity can have cascading effects within an ecosystem (Bruno and Cardinale 2008), altering prey diversity (Nilsson et al. 2008), prey abundance (Soluk and Collins 1988), primary production (Hargrave 2009), elemental cycling and plant diversity (Schmitz 2009), and trophic cascades (Finke and Denno 2004). Such effects have been demonstrated in a variety of individual ecosystems: grassland (Schmitz 2009), freshwater (Nilsson et al. 2008, Hargrave 2009), and marine (Finke and Denno 2004, Griffin et al. 2008). However, ecosystems are rarely closed to external resource subsidies (Polis and Hurd 1996). The effects of predator diversity loss may therefore cascade across ecosystem boundaries by altering the emigration of mobile prey organisms that subsidize food webs in physically separate habitats. Knowledge of these effects in diversity studies is limited, but crucial for understanding, predicting and mitigating the spatial consequences of biodiversity loss.

Spatial prey subsidies are resources produced in donor habitats that cascade to adjacent habitats, elevating prey availability

in recipient food webs (Polis et al. 1997). Consumers that capitalize on prey subsidies can achieve higher densities than predicted based on in situ production alone, which can in turn alter food web dynamics in the recipient ecosystem (Polis and Hurd 1996, Nakano and Murakami 2001). For example, emerging aquatic insects subsidized insectivorous riparian birds along a Japanese stream. The resulting increase in bird abundance magnified top-down control of terrestrial winged insects in the riparian forest relative to the unsubsidized upland forests (Murakami and Nakano 2002). Thus, factors affecting biomass production in donor food webs can drive food web dynamics in recipient food webs by altering energy flux across ecosystem boundaries (Nakano et al. 1999; Baxter et al. 2004).

Nearly 40% of freshwater fish species in North America are at risk of extinction, or are already extinct (Jelks et al. 2008). Many of these are predators that consume larval insects and amphibians, which subsidize terrestrial food webs when they emerge as terrestrial adults (Baxter et al. 2004, Finlay and Vredenburg 2007). Fish predation effects on one stage of this life cycle can cascade across ecosystem boundaries, altering both trophic structure (Wesner 2010a) and prey availability in recipient terrestrial ecosystems (Baxter et al. 2004, Nakano and Murakami 2001). I tested the hypothesis that shifts in aquatic predator richness cascade to terrestrial food webs by altering the emergence of aquatic insects, which in turn alters the distribution of a terrestrial consumer (spiders) that exploits adult aquatic

insect prey. The fish in this experiment differed in their foraging domain (surface feeder *Gambusia affinis*, mid-water feeder *Cyprinella lutrensis*, benthic feeder *Etheostoma spectabile*). Theory suggests that predator foraging domain is a useful trait for predicting the direction of predator diversity effects (i.e. is predation under multiple predators linear, synergistic or antagonistic? Schmitz 2007). I predicted that combinations of predators with non-overlapping foraging domains would show linearity or synergism, but not antagonism, due to the reduced possibility of negative interspecific interactions between predators with complementary habitats.

Methods

Mesocosms

I manipulated fish species richness in a 57 day experiment using large outdoor stream mesocosms at the Univ. of Oklahoma Biological Station (UOBS), Kingston, OK, USA. Mesocosms consisted of a riffle flowing into and out of a single pool (riffle unit: 122 cm length, 43 cm depth; pool unit: 183 cm diameter, 80 cm depth) and are described in detail elsewhere (Matthews et al. 2006, Supplementary material Appendix A1 Fig. A1). All treatments were isolated by a waterproof divider in the center of the riffles, which prevented water mixing among units. Additionally, fishes were restricted to pool habitat of each mesocosm by plastic mesh at the outlet and inlet of each riffle. Partial shade was provided with shade cloths suspended approximately 150 cm above each mesocosm. Units were never completely covered during the experiment, allowing continuous oviposition and emergence by frogs and aerial insects, which were abundant. Substrate in the mesocosms was a mixture of cobble and gravel taken from Brier Creek (Marshall Co., OK, USA), and was homogenized among pools prior to filling with water. Forty units were filled with well-water from a public supply on 16 May 2008. On the same day each unit was inoculated with a 1-l slurry of algae scraped from rocks in Brier Creek. Filamentous algae (*Oedogonium* spp.) and macrophytes (*Chara* spp.) were abundant after several weeks and provided additional structural refuge; the volume of algae and macrophytes was estimated at the end of the experiment by multiplying the mean height of algae and macrophytes touching a meter stick at five random points by the visually estimated percent coverage on the substrate (Wesner 2010a). Algae and macrophyte volume was similar across treatments at the end of the experiment (one-way ANOVA, $F_{10,38} = 0.255$, $p = 0.987$). Units were maintained without fish for 19 days to allow colonization by tadpoles and aerial invertebrates. The primary route of colonization is oviposition by insects and amphibians, though small snails and other invertebrates were likely also introduced with the algal inoculation. Flow was initiated on 18 May with submersible pumps (2500 l h^{-1}). Temperature, dissolved oxygen, conductivity and flow in these mesocosms are commensurate with values of local streams (Matthews et al. 2006, Wesner 2010a).

Design

Treatments consisted of each possible fish combination (one, two, or three fish species) plus a fishless control (eight total treatments). Five replicates of each treatment were distributed randomly among 40 pools on 4 June. I used a substitutable design, holding total fish density ($n = 24$) constant among each predator treatment. Therefore, multiple predator treatments contained 12 or 8 individuals of each species in the 2 or 3 species treatments, respectively. Single species treatments contained 24 individuals. Total fish density (9.13 fish m^{-2}) is commensurate with the natural density of each species. One replicate of the *C. lutrensis* treatment was lost due to a fish kill on day 33 and these data are excluded from analyses.

I manipulated richness (0, 1, 2 or 3 species) of three predatory fish species with complementary habitat domains: benthic (orangethroat darter *Etheostoma spectabile*), water-column (red shiner *Cyprinella lutrensis*), and surface (western mosquitofish *Gambusia affinis*). Each is common and widespread throughout the central United States and they often co-occur. Species share a common prey guild (invertebrates), but differ in their habitat use. The darter *E. spectabile* feeds on the benthos, primarily on insects: chironomids, stoneflies, mayflies (Miller and Robison 2004). The minnow *C. lutrensis* feeds in the water-column, but occasionally feeds in benthic and surface habitats (Miller and Robison 2004). The mosquitofish *G. affinis* feeds on the surface and includes both aquatic and terrestrial insects in their diet (Miller and Robison 2004). Fish of similar size were collected from nearby streams by seining and transported to holding tanks at UOBS no more than 48 h before the start of the experiment.

I tested for effects of predator richness on insect emergence (biomass and trophic structure), benthic prey biomass, terrestrial spider abundance and aquatic algal biomass (chlorophyll a). Each response variable was measured several times during the experiment from each mesocosm (Supplementary material Appendix A1 Table A1 for experimental timeline). I measured insect emergence using a combination of emergence traps and exuviae counts. Floating emergence traps were deployed continuously for 2–4 days during each of four sampling periods (Supplementary material Appendix A1 Table A1). Traps with a galvanized steel frame and a collection area of 0.16 m^2 are described in detail in Wesner (2010a). Upon collection, insects were transferred to individually labeled vials and stored in 70% ethanol. All insects were identified to family (Triplehorn and Johnson 2005) and measured for length to the nearest 0.1 mm for regression estimation of biomass (Sabo et al. 2002). Adult insects were classified as predators or non-consumers (Triplehorn and Johnson 2005). Insects that crawl out of the water to emerge, such as odonates, can avoid emergence traps, but are easily estimated by counting discarded exuviae. I collected exuviae (all odonates) daily from the perimeter of each mesocosm. Odonate adult biomass was estimated based on published regression equations using exuviae head width as a proxy for adult head width measured to the nearest 0.1 mm (Benke et al. 1999).

I sampled benthic prey (macroinvertebrates and tadpoles) twice during the experiment using a steel cylinder core (0.031 m^2). The cylinder was driven approximately

5 cm into the substrate and the contents scooped into a sieve (500 μm), drained, and preserved in 70% ethanol. On each sample date three random samples were taken from each pool and combined into a single sample (total area sampled = 0.093 m^2). Due to the large number of macroinvertebrates in samples, 30% of each sample by weight was sorted to order or family (Merritt and Cummins 1996). Tadpole abundance was visually estimated on several days by counting tadpoles on mesocosm walls.

I measured the response of spiders to insect emergence by counting occupied spider webs directly above each pool on several nights. Spiders were categorized as horizontal (Tetragnathidae) or vertical orb-weavers (mostly Araneidae). These taxa are common near aquatic habitats and build webs at night on riparian vegetation to capture emerging aquatic and terrestrial insects (Kato et al. 2003). Spiders naturally colonized the mesocosms, and built webs at night on the emergence trap cages, edges of the mesocosm, and/or a pvc pipe approximately 30 cm above the water in each pool. Counts were limited to spiders that occurred directly above the mesocosm pools, and not the riffles, to ensure that spiders were capturing insects emerging directly from fish treatments.

I sampled periphyton using ten porous silica discs (2.5 cm \varnothing), placed randomly on the substrate in each pool 17 days before fish were introduced, to test for differences in algal biomass and evidence of trophic cascades. Three days before the end of the experiment I collected four discs from each mesocosm and froze them overnight to lyse cells. Discs were extracted in 90% acetone, and chlorophyll a was estimated spectrophotometrically with a correction for phaeophytin.

To ensure fish occupied their a priori assigned habitat domains, I observed fish from a single randomly selected replicate from each fish treatment three times over the course of the experiment (Supplementary material Appendix A1 Table A1). Observations lasted 5 min during early afternoon (Wesner 2010a). Notes were taken at 30-s intervals on the number of fish visible and their position in the pool: benthic (lower 1/3 of water column), water column (middle 1/3 of water column), or surface (upper 1/3 of water column). Feeding attempts and their location also were noted. At the end of the experiment fish wet weight was converted to dry weight assuming 70% water content (Hoar and Randal 1969).

Data analysis

I tested the null hypothesis of no differences among treatments using a linear mixed model (PROC MIXED in SAS 9.1), with treatment, time, and treatment \times time as fixed effects. The response variables were benthic insect biomass, insect emergence biomass, proportion of predators in emerging insect assemblage, emergence biomass of common prey taxa and spider abundance above mesocosms. Overall predator effects were tested by comparing response variables among fishless treatments and fish treatments (pooled across richness levels). Predator richness effects were tested using planned comparisons of the mean response among single, two, and three species treatments following the least square means procedure in SAS. Planned comparisons were adjusted for type I error using the sequential Bonferroni adjustment (adjusted p-values are denoted as p_{adj} in the results). I refer to

trends as marginally significant when results were significant before, but not after, the sequential Bonferroni procedure. Linear regression tested the relationship between fish species richness and mean insect emergence (biomass and trophic structure) and mean spider abundance. Chlorophyll a and mean counts of *Hyla* tadpoles on the mesocosm walls were analyzed using a one-way ANOVA with fish richness as a fixed factor in SPSS 16.0 for Macintosh (SPSS, Chicago, Ill). Multiple comparisons following one-way ANOVA's used the Tukey-Kramer post-hoc test. The Brown-Forsyth correction was applied when error variances of univariate ANOVA's were unequal according to Levene's test. All data were natural log transformed or arcsine square-root transformed (proportions) to improve normality.

Because of the substitutive design used here, intraspecific density declines as fish species richness increases. Thus, differences among treatments could be caused by release from intraspecific competition in addition to interactive effects of diversity. I assessed potential intraspecific effects by comparing habitat use, biomass, and diet of individual fish species across richness treatments. Shifts in either of these metrics could indicate differences in behavior or consumption due to differences in intraspecific density. First, I measured shifts in habitat use by fish in single versus multiple species treatments by comparing the proportion of fish feeding in, and occupying, a priori assigned fish habitats (benthic, middle, and surface) using a Kruskal-Wallis test. Second, I compared mean individual fish species biomass and the diet of individual fish species at the end of the experiment across treatments using one-way ANOVA followed by Tukey's post-hoc test. For diet analyses, I compared total prey abundance in fish stomachs and the relative abundance of surface prey (floating pupae and terrestrial insects) and aquatic prey (non-pupating benthic invertebrates) for individual fish species across treatments. Shifts in the relative abundance of these two prey categories among richness treatments would indicate whether microhabitat use and prey use differed across the intraspecific density gradient among richness treatments.

To test for linearity in multiple predator effects, I compared observed values of a response variable to values predicted from fish species performance in monoculture using a paired t-test (sensu Vaughn et al. 2007). This is a commonly used comparison in diversity studies that assumes that an additive model correctly predicts fish effects in polyculture from their effects in monoculture. Predicted values were calculated by first obtaining a mean value per mg of dry weight of fish for each response variable in each of the three single species treatments (e.g. mean emergence biomass $\text{m}^{-2} \text{day}^{-1} \text{mg}^{-1}$ dry weight of *E. spectabile*). This value was then multiplied by the dry mass of each species in the two and three species replicates and summed to obtain a predicted value for each mesocosm. A significant t-test indicates non-linearity where observed values are stronger (synergism) or weaker (antagonism) than expected based on individual fish species performances. To test for species identity effects, the relationship between response variables and the relative dry mass of individual fish species was examined using linear regression. A significant positive relationship between a response variable and relative dry mass of one fish species, but not others, would indicate that a single fish species is driving differences among treatments, and not species richness per se.

Results

Emerging insect biomass and trophic structure

Mean emergence biomass was 28% lower in pools with fish than the control treatment, though this was not significant ($F_{1,38} = 3.00$, $p = 0.0913$), due to wide variation among individual fish species treatments. The three species treatment caused a marginally significant 55% reduction in emergence biomass relative to the control (planned comparison $t = 2.36$, $p_{\text{adj}} = 0.0576$). Pools with one or two fish species reduced insect emergence 25% and 18%, respectively, on average relative to the control, but these reductions were not significant (Fig. 1a; planned comparison between control and single species: $t = 1.32$, $p_{\text{adj}} = 0.1954$; control and two species: $t = 1.47$, $p_{\text{adj}} = 0.1498$). There was no difference in mean insect emergence among fish richness treatments ($F_{2,32} = 1.16$, $p = 0.3260$).

Dragonflies (*Pantala flavescens*, *Tramea lacerata*, *Pachydiplax longipennis*, *Erythemis simplicicollis*) and chironomids were the most abundant of the eighteen insect taxa that emerged from the mesocosms. Fish reduced emergence biomass of the most common dragonfly species (*P. flavescens*) by an average of 67%

($F_{1,38} = 15.89$, $p = 0.0003$), which was significant relative to the control for all richness treatments ($p_{\text{adj}} \leq 0.0334$). Emergence of *P. flavescens* varied significantly among richness treatments ($F_{2,32} = 5.17$, $p = 0.0113$); emergence from the three species treatments was significantly lower than the mean of single species treatments ($t = 3.19$, $p_{\text{adj}} = 0.0367$) and marginally lower than the mean of the two species treatments ($t = 2.15$, $p_{\text{adj}} = 0.0647$), but did not differ between the single and two species treatments ($t = 1.49$, $p_{\text{adj}} = 0.1467$). Fish effects on chironomid emergence were marginally evident only in the three species treatment (Fig. 1b; $t = 2.07$, $p_{\text{adj}} = 0.0793$), which reduced chironomid emergence by 55% relative to the control. Single and two species treatments did not affect chironomid emergence relative to the control (Fig. 1b; control vs single species: $t = 0.64$, $p_{\text{adj}} = 0.5266$; control vs two species: $t = 0.57$, $p_{\text{adj}} = 0.6036$; overall MIXED model between subjects $F_{3,36} = 1.74$, $p = 0.1754$). Emergence of chironomids from the three species treatments was marginally lower than either the single ($t = 1.85$, $p_{\text{adj}} = 0.0731$) or two species treatment ($t = 1.98$, $p_{\text{adj}} = 0.0559$), but did not differ between the single and two species treatments ($t = 0.17$, $p_{\text{adj}} = 0.8670$).

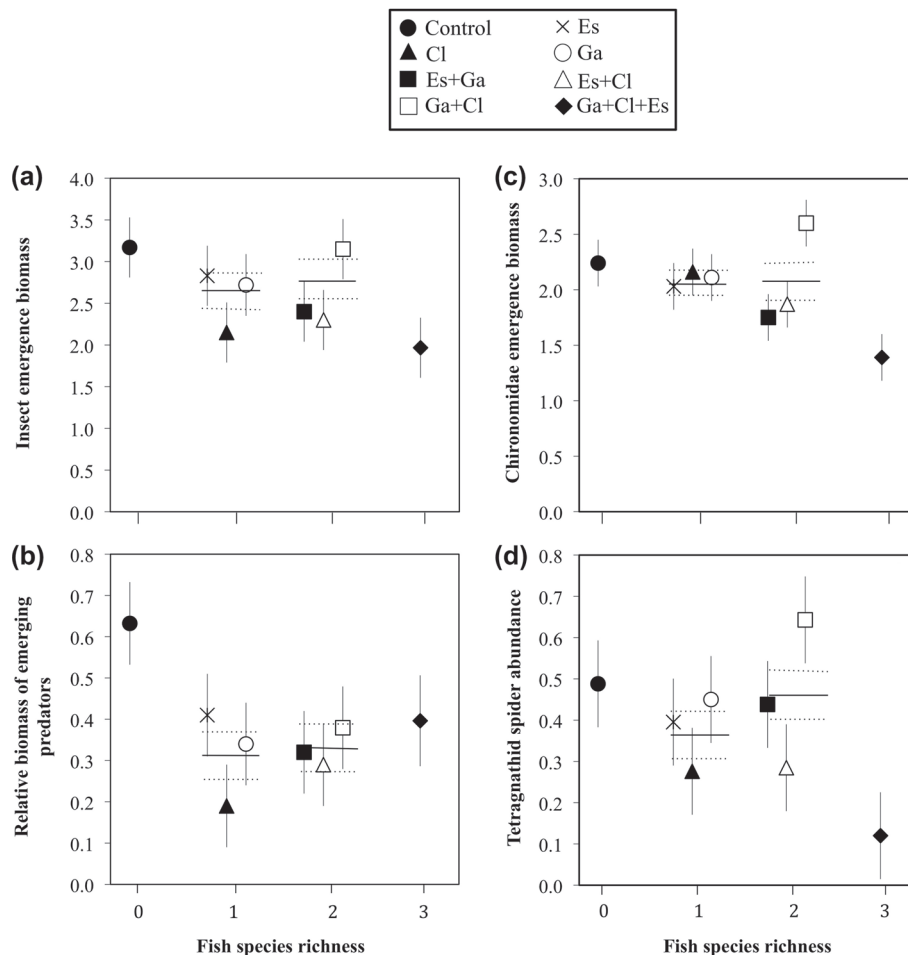


Figure 1. Effects of fish richness on (a) mean total insect emergence dry mass ($\text{mg m}^{-2} \text{day}^{-1}$), (b) mean trophic structure of the emerging insect assemblage (relative emergence of predatory insects; biomass $\text{m}^{-2} \text{day}^{-1}$), (c) mean emergence of Chironomidae, the main prey item in spider webs, and (d) mean density of tetragnathid spiders above mesocosms. Data are natural log transformed or arcsine square root transformed (proportions) least squares means \pm SE. Lines indicate single and two species treatment means \pm SE (dotted lines). Fish abbreviations are Es (*Etheostoma spectabile*), Cl (*Cyprinella lutrensis*), and Ga (*Gambusia affinis*). See Results for statistical comparisons.

Stronger predation effects on dragonflies relative to chironomids caused a shift in the trophic structure of the emerging insect assemblage. When fish were present, relative predator biomass in the emerging insect assemblages was lower than when fish were absent ($F_{1,38} = 8.61$, $p = 0.0056$). Among individual richness treatments this reduction was significant only for the single and two species treatments (Fig. 1c; control vs single species: $t = 2.83$, $p_{\text{adj}} = 0.0409$; control vs two species: $t = 2.64$, $p_{\text{adj}} = 0.0372$; control vs three species: $t = 1.61$, $p_{\text{adj}} = 0.1171$). Emerging insect trophic structure did not vary across fish richness treatments ($F_{2,32} = 0.22$, $p_{\text{adj}} = 0.8046$).

Terrestrial spiders

Spiders appeared to capture primarily adult aquatic insects (mostly chironomids), based on qualitative surveys of prey in spider webs. Linear regression revealed a positive relationship between mean insect emergence biomass and the abundance of spiders that build horizontal webs above the water (Tetragnathidae: $r^2 = 0.179$, $p = 0.007$; Fig. A2a). In contrast to horizontal web-weaving spiders, abundance of vertical web-weaving spiders (mostly Araneidae) was not linearly related to aquatic insect emergence ($r^2 < 0.001$, $p = 0.897$; Fig. A2b), suggesting that spider foraging strategy (horizontal vs vertical webs) affects their sensitivity to emerging aquatic insects. Mean tetragnathid abundance above pools with three fish species was 75% lower than control pools (Fig. 1d: planned comparison between control and high richness treatment was marginally significant: $t = 2.27$, $p_{\text{adj}} = 0.0695$; overall MIXED model between subjects: $F_{3,36} = 2.43$, $p = 0.0812$). Spider abundance above single and two species treatments did not differ relative to controls (control vs single species: $t = 0.86$, $p_{\text{adj}} = 0.4204$; control vs two species: $t = 0.25$, $p = 0.8071$). Comparisons among richness treatments revealed that tetragnathids were less abundant above the three species treatments than the two species treatment ($t = 2.55$, $p_{\text{adj}} = 0.0492$) and marginally less abundant than the single species treatment ($t = 1.94$, $p_{\text{adj}} = 0.0883$; overall MIXED anova: $F_{2,32} = 3.24$, $p = 0.0522$), but did not differ between the single and two species treatments ($t = -0.87$, $p_{\text{adj}} = 0.3886$).

Benthic prey and chlorophyll a

There was no variation among richness treatments for biomass of total benthic prey (invertebrates + tadpoles: $F_{2,32} = 0.37$, $p = 0.6938$), benthic insects ($F_{2,32} = 1.52$, $p = 0.2334$), or benthic dragonflies ($F_{2,32} = 0.09$, $p = 0.9178$). Visual counts of tadpoles on mesocosm walls revealed an 87% reduction when fish were present relative to controls ($F_{1,38} = 41.17$, $p < 0.0001$), which was consistent across richness treatments (all planned comparisons between richness treatments and control: $p < 0.0001$). Linear regression between the natural log of chlorophyll a and the natural log of fish dry mass was not significant ($r^2 = 0.011$, $p = 0.529$).

Fish habitat and diet

Fish observations confirmed their a priori assigned habitat domains. *Etheostoma spectabile* were rarely seen ($n = 10$

total observations) due to algal growth, but all observations of this species were on the substrate. *Cyprinella lutrensis* was observed in the water column 97% of the time, and *G. affinis* was observed near the surface 80% of the time. Fish also fed within habitats, with at least 100, 72 and 87% of observed feeding attempts occurring in the respective habitats for *E. spectabile*, *C. lutrensis* and *G. affinis*, respectively. These differences were consistent across fish richness treatments according to Kruskal-Wallis test (percent observed: $\chi^2 = 1.249$, $p = 0.535$; percent feeding: $\chi^2 = 0.348$, $p = 0.840$). Diets of individual fish species corroborated habitat observations. Only *G. affinis* and *C. lutrensis* had water-column or surface prey items (pupae + terrestrial insects; *G. affinis* – 25% of diet, *C. lutrensis* – 29% of diet). For individual fish species, there was no variation among treatments for total prey abundance, proportion of pupae, and proportion of terrestrial prey (all comparisons, $p \geq 0.063$). The mean weight of individual fish at the end of the experiment did not vary across treatments for any species (*C. lutrensis*, $F_{3,18} = 0.919$, $p = 0.456$; *G. affinis*, $F_{3,19} = 0.598$, $p = 0.626$; *E. spectabile*, $F_{3,19} = 0.528$, $p = 0.669$).

Tests for linearity

The relationship between a given response variable and fish richness was not a simple linear function of richness (linear relationship between response variables and fish species richness: insect emergence biomass: $r^2 = 0.079$, $p = 0.107$; insect trophic structure: $r^2 = 0.006$, $p = 0.651$; tetragnathid abundance: $r^2 = 0.019$, $p = 0.433$). Indeed, synergistic predation effects, in which predation under multiple fish species was stronger than predicted from individual fish performance, were mostly limited to the three species treatment, with the exception of a synergistic effect on tadpoles in the *C. lutrensis* + *G. affinis* treatment (Table 1). For the overall insect assemblage (total biomass and trophic structure), predation under multiple predators was linear. Overall, multiple predator effects were always linear or synergistic, but not antagonist. I found no evidence that patterns of richness effects were driven by species identity, as regressions of response variables across individual fish species biomass were similar in direction and strength (Supplementary material Appendix A1 Fig. A3).

Discussion

In this study, aquatic predation altered the trophic structure and biomass of insects emerging from aquatic to terrestrial habitats, causing a shift in the spatial distribution of a terrestrial consumer that was subsidized by this flux. Predation effects were usually strongest in the high predator richness treatment, reducing prey availability for terrestrial spiders by more than 50% relative to control treatments. In response, spider abundance above pools with high fish richness was lower on average than any other fish treatment, and could not be predicted from simple monoculture performance of fish species. These results demonstrate that predator diversity effects are not limited to the habitat of the predators, but can cascade across ecosystem boundaries to food webs in adjacent habitats.

Table 1. Results of a paired t-test comparing values of response variables observed in fish polyculture with values predicted from fish species performance in monoculture. Trophic structure is defined as the relative biomass of emerging predatory insects (mg dry mass m⁻² day⁻¹). Degrees of freedom = 4 for all comparisons. *Es* = *Etheostoma spectabile*, *Cl* = *Cyprinella lutrensis*, *Ga* = *Gambusia affinis*. Values in bold indicate synergism, in which emergence/abundance is significantly lower than expected based on species averaging ($\alpha = 0.05$). Non-significant values indicate redundancy.

Fish treatment	Insect emergence biomass		Emerging insect trophic structure		<i>P. flavescens</i> emergence biomass		<i>Hyla</i> spp. abundance on mesocosm walls		Chironomidae emergence biomass		Tetragnathid spider abundance	
	t	p	t	p	t	p	t	p	t	p	t	p
<i>Es</i> + <i>Ga</i>	-2.237	0.089	-0.105	0.354	-1.865	0.136	-1.848	0.138	-2.350	0.078	-0.332	0.756
<i>Es</i> + <i>Cl</i>	-1.879	0.133	0.243	0.820	-0.092	0.931	1.209	0.293	-1.889	0.132	-0.469	0.663
<i>Cl</i> + <i>Ga</i>	-0.448	0.677	0.712	0.546	-1.977	0.119	-7.325	0.002	-0.360	0.737	-0.332	0.266
<i>Es</i> + <i>Ga</i> + <i>Cl</i>	-1.681	0.168	-0.507	0.639	-3.838	0.018	-3.368	0.028	-1.511	0.205	-3.068	0.037

One promising trend in biodiversity studies is the potential to use species traits to make a priori predictions of the direction of diversity outcomes (Schmitz 2007). This is important, because previous diversity experiments demonstrate every possible response, from antagonism to synergism, making it difficult for resource personnel to make management decisions based on primary literature (Bruno and Cardinale 2008). In this study, I used a simple trait of predators (foraging domain) to predict that multiple predator effects would not be antagonistic. As predicted, multiple predator effects were never antagonistic, presumably because predators had complementary foraging domains, limiting the possibility for negative interspecific interactions. Additionally, two-species predator combinations generally did not differ fundamentally from single species treatments. Instead, strong predation effects were largely limited to habitats with high predator richness, in which predator species occupied each vertical level of microhabitat (surface, mid-water, and benthic feeders). Under this scenario, insects moved (actively or passively) through the water-column during emergence, experiencing predation in each microhabitat. In the low richness treatments, at least one of these levels was predator-free, potentially creating 'safe zones' for some emerging organisms. Soluk and Collins (1988) hypothesized a similar facilitation mechanism by which *Ephemera* mayflies escaped stonefly predators by moving to the tops of rocks, where they were then vulnerable to a benthic predatory sculpin. Because stoneflies and sculpins had different foraging domains, their combined effects were stronger than predicted based on monoculture performance (Soluk and Collins 1988).

One possible explanation for differences among prey taxa in response to multiple predator species is that predation was mediated by differences in prey escape behaviors. Larval dragonflies and tadpoles are active feeders during development and were visible throughout the water-column during the study. This mobility makes them vulnerable to predators, likely resulting in synergistic predation effects through facilitation. For example, dragonflies and tadpoles attempting to evade *E. spectabile* by moving higher in the water column were perhaps then vulnerable to *C. lutrensis* and *G. affinis*, and vice versa. In contrast, chironomids spend the majority of their larval life-cycle on the benthos, and often evade predation by burrowing in the substrate (Ball and Baker 1996), making them generally invulnerable to non-benthic predators until they enter the water column to emerge as adults. A

further complication is that chironomids typically emerge at night, which reduces their vulnerability to visual fish predators. Thus, facilitation effects are unlikely for chironomids, and linear effects seen in this study are more likely.

The multiple-predator effects observed in this study are consistent with theoretical predictions (Schmitz 2007), though it is possible that stronger predation effects in high richness treatments were driven by reduced intraspecific competition, which is inherent in the substitutive design used here. However, this mechanism seems unlikely for several reasons. First, the fish densities in this experiment are low relative to natural densities; *G. affinis* and *C. lutrensis* often occur in large groups with hundreds of individuals, and the major source stream for *E. spectabile* contains densities of greater than 20 fish m⁻², nearly twice that of the highest density in this experiment (unpubl.). Second, if lower intraspecific densities in high richness treatments caused higher consumption, then one would expect individual biomass in these treatments to increase as well. However, the mean biomass of individual fish at the end of the experiment did not vary across treatments for any species. Third, differences among species in foraging domains and diets were consistent across treatments, suggesting that fish foraging behavior did not change at reduced intraspecific densities. While these data do not conclusively rule out the effects of intraspecific density as a mechanism behind the patterns in the study, it seems unlikely to be an important factor.

The link between emerging aquatic insects and terrestrial food webs has been demonstrated in natural settings from temperate prairie streams (Wesner 2010b) to rainforests (Marczak and Richardson 2007) and for a number of terrestrial consumers that are subsidized by aquatic insect emergence: spiders (Baxter et al. 2004, Marczak and Richardson 2007), lizards (Sabo and Power 2002), birds (Nakano and Murakami 2001). Fish are often the top predators in aquatic habitats, and can reduce insect emergence substantially in natural streams (Baxter et al. 2004) and ponds (Knight et al. 2005). Further, the foraging traits used here are also common, often obligate traits in freshwater fish communities (Matthews 1998), and have been linked with predator performance of fishes (Baxter et al. 2004, Dahl and Greenberg 1998, Carey and Wahl 2010). Thus, the results presented here are especially important given the current biodiversity crisis for freshwater fishes (Jelks et al. 2008, Magurran 2009). They demonstrate that the consequences of continued fish species loss may extend to surrounding terrestrial

ecosystems, and that knowledge of simple traits like foraging domain may help forecast the food web effects of species loss in aquatic habitats (see also Carey and Wahl 2010).

While the ecological literature has established that both biodiversity and spatial subsidies are important determinants of food web structure and biomass production, these ideas have rarely been considered together. Most of our knowledge about the impacts of biodiversity loss is limited to a single habitat or ecosystem (e.g. diversity driven trophic cascades in grassland ecosystems (Schmitz 2009), coastal marshes (Fincke and Denno 2004), and marine kelp forests (Byrnes et al. 2006)). This study complements current biodiversity literature by showing that diversity effects are not necessarily limited to the habitat of interest, but can cascade across ecological boundaries. These effects were demonstrated across an aquatic-terrestrial boundary, an ecotone that has been the focus of subsidy research for several decades (Vannote et al. 1980, Polis and Hurd 1996, Nakano and Murakami 2001, Marczak and Richardson 2007). However, cross-ecosystem effects of biodiversity are likely to be common across other ecological boundaries. For example, high insect abundance in croplands can cascade ('spillover') to adjacent natural grasslands, elevating predation pressure on grassland insects (Rand et al. 2006), and insect abundance has been correlated, both positively and negatively, with plant species diversity (Haddad et al. 2001). However, the connection between plant diversity, insect production, and cross-habitat spillover has not been explicitly investigated to my knowledge. Because many animal species may exhibit complex life-histories (Werner 1988), in which organisms shift habitats during development (e.g. insects, amphibians, anadromous fish), the cross-boundary effects of biodiversity shown here may be common and should be investigated in other (i.e. non-aquatic) systems to fully understand the spatial consequences of biodiversity loss.

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Supplementary material (available online as Appendix O19413 at <www.oikosoffice.lu.se/appendices>). Appendix A1.