Fish predation alters benthic, but not emerging, insects across whole pools of an intermittent stream

Jeff S. Wesner[1]

Biological Station and Department of Zoology, University of Oklahoma, 730 Van Vleet Oval, Norman, Oklahoma 73019 USA

Abstract. Predation effects in streams can cascade to terrestrial food webs through the flux of organisms that develop in the stream and emerge as adults to the terrestrial system. This emergence subsidizes some terrestrial predators, an effect that generally varies based on the magnitude of the subsidy. Factors regulating this magnitude are relatively well known, but factors regulating the trophic structure of the subsidy are not. I tested the hypothesis that predatory fish in natural stream pools alter the biomass and trophic structure (proportion of predatory adults) of emerging aquatic insects. I created a 13× gradient of predatory fish biomass (4 species of *Lepomis* sunfish and the minnow *Notropis boops*; within the range of natural variation) across 10 pools in Brier Creek, Oklahoma (USA). Pool area and substrate composition varied naturally, so I also measured their effect on insects. At the end of the experiment after the stream became intermittent, fish reduced benthic insect biomass but not emergence to the terrestrial habitat. The proportion of predatory insects emerging from pools was positively associated with pool area, but was unaffected by fish density. The best predictor of emergence biomass among pools on any date was the standing crop of benthic insects before fish manipulation, a result suggesting a time lag between measured benthic standing crop in the stream and subsequent emergence. Fish manipulations occurred during the end of peak summer insect emergence, which may have limited my ability to detect fish effects on emergence. My study demonstrates that variation in the timing of predation may constrain the spatial scale of fish effects in aquatic and terrestrial food webs and suggests that pool size can influence the trophic structure of emerging aquatic insects.

Key words: predation, insect emergence, sunfish, *Lepomis*, aquatic–terrestrial linkages, subsidies, food webs, emergence, intermittence.

Changes in predator biomass can alter ecosystem functioning in the local food web (Brooks and Dodson 1965, Fauchald 2010) and may have cascading effects in adjacent food webs beyond the habitat or ecosystem of the predators (Polis et al. 1997, Nakano and Murakami 2001, Baxter et al. 2004, Knight et al. 2005, Wesner 2012b). Cross-boundary effects may be common when prey assemblages consist of organisms with complex life histories that cross habitat boundaries during development from larva to adult, thereby subsidizing food webs in recipient habitats (Nakano and Murakami 2001, McCoy et al. 2009). For example, aquatic insects are nearly ubiquitous in streams and lakes, and many taxa emerge to the terrestrial system as winged adults (Nakano and Murakami 2001). Before emergence, predation from fish often affects aquatic insects and prevents emergence. As a result, changes to fish communities can have effects that extend beyond the aquatic–terrestrial boundary (e.g., Baxter et al. 2004, Pope et al. 2009).

The importance of aquatic insects as subsidies to terrestrial consumers has been demonstrated in a variety of habitats: rainforest (Marczak and Richardson 2007), montane (Baxter et al. 2004), and prairie (Wesner 2010b). Some adult aquatic insects also feed in terrestrial food webs (e.g., dragonflies; Knight et al. 2005), but many do not feed at all in their terrestrial adult stages (e.g., mayflies). As a result, emerging aquatic insects may have both bottom-up (Nakano and Murakami 2001, Baxter et al. 2004, Epanchin et al. 2010) and top-down effects in terrestrial food webs (Knight et al. 2005). The potential for bottom-up effects depends largely on the biomass of the subsidy, whereas the potential for top-down effects depends on the adult trophic structure of emerging insects. Both of these can vary widely across streams. For

[1] Present address: Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523 USA. E-mail: jeffwesner@gmail.com

example, the biomass of adult aquatic insects in riparian forests varied ~10× across 3 streams in Oklahoma, USA (Wesner 2010b). Across 30 streams in North and South America, benthic insects with predatory adults made up 0 to 86% of benthic biomass, a result suggesting strong variation in potential adult trophic structure (Wesner 2012a).

Fish predation is one major contributor to variation in emerging aquatic insect communities, and fish populations are undergoing rapid global change (Magurran 2009). In North America alone, nearly 40% of freshwater fish species are threatened or already extinct (Jelks et al. 2008, Magurran 2009), and many of these species are insectivorous for at least part of their life. Fish species loss at the local scale has been combined with widespread introduction of nonnative species (Jelks et al. 2008). These changes to the size and composition of fish communities can alter the predation risk faced by benthic insects, thereby altering emerging insect biomass (Baxter et al. 2004, Epanchin et al. 2010).

The influence of fish predation on emerging insect biomass is relatively well documented, but less is known about the effects of fish predation on emerging insect trophic structure. Two factors suggest potential for fish to alter emerging trophic structure. First, adult feeding differs systematically among aquatic insects (e.g., all odonates feed as adults, all mayflies do not). Second, fish predation often affects only a subset of available prey taxa rather than all prey equally (Gilinsky 1984, Wesner 2010a, 2012b). Therefore, predation on benthic insects probably can alter both the biomass and trophic structure of emerging insects, especially if fish effects are limited exclusively to a subset of prey whose adult stages feed or do not. For example, fish in mesocosms reduced emergence of dragonflies more strongly than other prey taxa, presumably because of size-selective predation by fish on large dragonflies. This selectivity led to a change in the trophic structure of the emerging insect community because dragonflies are predatory as adults, and other prey in the experiments were not (e.g., chironomids; Wesner 2010a, 2012b). These studies demonstrated that fish can alter both the biomass and trophic structure of adult aquatic insect communities. However, whether these effects are evident in more complex and environmentally variable natural systems is unknown.

The goal of my experiment was to test the hypothesis that fish predation alters the biomass and trophic structure of aquatic insects emerging from a natural stream. I created a 13× gradient of fish density across stream pools that varied in area and substrate composition. I examined how models with combinations of fish density, pool area, and substrate composition explained variation in benthic and emerging insect communities during a 44-d experiment. Based on previous studies (Baxter et al. 2004, Wesner 2010a), I predicted that increasing fish density would reduce benthic insect biomass, thereby reducing emerging insect biomass. I also predicted that fish effects would be strongest on insects that are predatory as adults because they are typically larger than nonpredatory insects (Wesner 2012a) and that this would alter the proportion of predatory adults emerging.

Methods

Study site

I conducted this experiment during summer 2008 (June 18 to August 1) in Brier Creek, a clear, small tributary of the Red River arm of Lake Texoma in Marshall County, Oklahoma. During summer, adult aquatic insects make up ~45% of near-shore winged insect biomass (JSW, personal observation), a datum suggesting they are a potentially important subsidy in this system. Brier Creek is a flashy stream that can flood rapidly until late June (Wesner 2011). Therefore, I initiated the experiment in late June to avoid potential flooding and to coincide with expected peak aquatic insect emergence in summer. I manipulated fish density among 10 permanent pools in a 1-km reach described in detail by Power and Matthews (1983). Pools are separated by shallow riffles (~10 cm deep × 10 m long). The reach consists of a mixture of cobble, bedrock, and sand across a shallow gradient that produces minimal surface flow of clear water (Table 1; Matthews and Marsh-Matthews 2006). Pools with a maximum depth >1 m were present in the reach, but I excluded them from the study because I needed to select pools from which fish could be seined thoroughly. Riffles became dry during the last 2 wk of the experiment, creating intermittent flow. Intermittence is common during late summer in this reach, though extended drought is rare and most pools retain sufficient water for fish and invertebrates because of numerous spring seeps (Matthews and Marsh-Matthews 2006).

Experimental design

I erected plastic mesh fencing (5 mm) across the entire stream channel at the upstream and downstream ends of 10 pools on day 0 of the experiment to restrict among-pool movement of all but the smallest young-of-year fish (Table 2). Fences spanned the upstream and downstream outlet of each pool with

TABLE 1. Biotic and abiotic values across treatment pools in Brier Creek, Oklahoma, USA. Pool numbering is ordered downstream (1) to upstream (10). Fish biomass represents values for insectivorous fish after redistribution among pools on day 5. Biomass of individual fish taxa is given for the dominant fish (>90% biomass). Dissolved O₂ (DO) and temperature (Temp) are means (SD) of 2 measurements in each pool on day 19 (7 July). See Appendix S1 for full list of fishes.

Pool	Fish biomass (g/m²)			Substrate (proportion)			Length (m)	Mean width (m)	Mean depth (m)	DO (mg/L)	Temp (°C)
	Total insectivorous fish	Lepomis spp.	Notropis boops	Cobble	Sand	Bedrock					
1	1.13	1.13	0.00	0.50	0.50	0.00	27.7	5.3	0.2	7.2 (1.1)	26.6 (0.6)
2	0.90	0.82	0.00	0.38	0.25	0.38	33.2	4.8	0.4	4.8 (0.2)	27.0 (0.1)
3	2.97	2.66	0.19	0.44	0.11	0.44	45.7	4.2	0.3	6.2 (1.0)	26.4 (0.2)
4	3.75	3.34	0.22	0.50	0.17	0.33	22.9	7.5	0.4	4.5 (0.6)	25.9 (0.3)
5	1.97	1.75	0.18	0.50	0.00	0.50	34.5	5.0	0.3	5.5 (0.2)	25.9 (0.1)
6	2.76	2.57	0.13	0.50	0.50	0.00	36.0	5.2	0.3	4.7 (0.1)	26.0 (0.1)
7	0.51	0.49	0.00	1.00	0.00	0.00	37.8	8.7	0.2	9.6 (4.0)	26.3 (0.4)
8	0.98	0.84	0.14	0.75	0.00	0.25	25.0	6.2	0.2	5.9 (0.1)	26.9 (0.3)
9	0.32	0.26	0.03	0.25	0.00	0.75	25.0	8.3	0.2	7.4 (1.5)	27.4 (0.1)
10	4.19	4.01	0.14	0.29	0.00	0.71	40.9	5.7	0.4	9.0 (2.8)	27.8 (0.1)

the exception of one large pool, which I split in half by placing a fence across a natural narrowing.

I assessed fish assemblages in each pool by snorkeling on day 2. Snorkeling is a reliable technique for fish estimation in clear, shallow streams (Goldstein 1978) and has been used to document fish assemblages in this reach for 3 decades (Matthews and Marsh-Matthews 2006). Of the 10 fish species identified (see Appendix S1 for full list of fishes; available online from: http://dx.doi.org/10.1899/12-124.1.s1), sunfish (4 Lepomis spp.) and Bigeye Shiner (Notropis boops) were the most abundant, composing 92% of all fish biomass (sunfish: 87%, Bigeye Shiner: 5%). The 4 species of sunfish were dominated by Longear (Lepomis megalotis) and Green Sunfish (Lepomis cyanellus), but I grouped them as a single taxonomic unit (Lepomis spp.) because they are functionally similar and can be difficult to distinguish underwater, especially when small. Two species of bass were present in the reach (Micropterus salmoides and Micropterus punctulatum) at low abundance (<2% of total biomass) and were identified as Micropterus spp. I removed bass >~300 mm from experimental pools to limit potential confounding effects of intraguild predation on minnows and small sunfish.

On day 5, I randomly assigned 10 pools as either fish-addition pools (n = 5) or fish-removal pools (n = 5) using a random number generator. I seined as many fish as possible from the fish-removal pools and added them to the nearest addition pool. To minimize disturbance to the benthos during seining, 2 people held the seine just above the substrate (~1 cm) and walked as close to the shoreline as possible. This procedure was repeated once for a total of 2 seine hauls in each pool. I used the same seining procedure in fish-addition pools, but I did not remove any fish. Follow-up snorkel and bank count surveys revealed a consistent gradient from low to high insectivorous fish biomass across pools (Table 1). I did bank count surveys by slowly walking the bank and counting fish. This technique is reliable in this stream because the water is clear and the fish fauna is well known (Power and Matthews 1983). Based on follow-up surveys, dominant taxa (Lepomis spp. and N. boops) had similar relative abundances across the fish-biomass gradient (Lepomis relative biomass range: 69–96%; N. boops range: 0–12%).

Insect collection

I collected benthic invertebrates with a Hess sampler (0.087 m², 0.5-mm mesh collection net) once before fish allocation and once during the final week of the 44-d experiment (Table 2). I took 3 to 5 evenly

TABLE 2.   Experimental timeline of aquatic and terrestrial foodweb samples in whole pools of Brier Creek, Oklahoma, USA. Dates are given in month/day/year.

Date	Day of experiment	Event
6/18/2008	0	Erected fences across riffles; set emergence traps
6/19/2008	1	1st benthic sample
6/20/2008	2	Collected 1st emergence sample; counted fish (snorkel)
6/23/2008	5	Redistributed fish among pools to create gradient of fish biomass
7/1/2008	13	Set emergence traps
7/3/2008	15	Collected 2nd emergence sample
7/14/2008	26	Set emergence traps
7/16/2008	28	Collected 3rd emergence sample
7/24/2008	36	First pool became intermittent
7/29/2008	41	2nd benthic sample
7/30/2008	42	Set emergence traps
8/1/2008	44	Collected 4th emergence sample; end of experiment

spaced samples, depending on pool size, along the thalweg near the location of emergence traps and combined them into a single sample for each pool. For each sample, I disturbed the substrate and created flow by hand (10 strokes/sample) to force invertebrates into the collection bottle. During benthic collections, I took care not to disturb the substrate in a 1-m radius around emergence traps to limit effects on subsequent emergence collections.

I collected emergent insects 4 times with 3 to 5 floating emergence traps in each pool, depending on pool size (Table 2). I spaced traps with a collection area of 0.16 m^2 (Wesner 2010a) evenly along the thalweg of each pool and left them for 48 h. To collect insects that crawl on the shore prior to emergence, I scanned 1 m of shoreline on either side of each emergence trap for exuviae each morning that emergence traps were set. To ensure that exuviae represented insects that emerged only when emergence traps were set, I removed and discarded all exuviae from the shoreline before placing emergence traps. I estimated the area of shoreline emergence by multiplying the length of shoreline sampled in each segment (1 m) by ½ the stream width at each segment (sensu Benke and Benke 1975).

I identified insects to order or family in the laboratory and categorized them as having predatory or nonpredatory adults. I subsampled benthic collections by sorting 30% of each sample by mass, a fraction typically sufficient to capture most insect taxa (Petkovska and Urbanič 2010). I also categorized insects from the emergence traps as predatory or nonpredatory for analysis of trophic structure following Merritt and Cummins (1996). For all collections, I measured the length (to the nearest 0.1 mm) of up to 20 individuals/taxon and used published length–mass regressions to estimate biomass of larval (Benke et al. 1999) and adult (Sabo et al. 2002) insects.

Abiotic variables

I characterized substrate in each pool along transects that extended the width of the pool perpendicular to each emergence trap. At 1-m intervals along each transect, I visually estimated the dominant substrate as sand, cobble, or bedrock. I also measured water depth at these intervals with a meter stick, and pooled these measurements to estimate mean depth in each pool (Table 1). Cobble or bedrock were the dominant substrate in all pools and were negatively correlated ($r = -0.69$, $p = 0.03$). Therefore, I analyzed substrate as the proportion of cobble in each pool to avoid problems with collinearity among predictor variables in the linear regression models (see below). I estimated pool area by multiplying pool length by mean width averaged across all transects in each pool.

I measured dissolved O$_2$ (DO) and temperature on day 19 (before intermittence) by taking 2 readings in each pool. Temperature was relatively constant across pools (range: 25.9–27.8°C), but mean DO varied between 4.5 and 9.6 mg/L (Table 1). Manipulated fish density was not related to DO ($r^2_{unadjusted} = 0.08$, $p = 0.42$) or temperature ($r^2_{unadjusted} = 0.07$, $p = 0.47$). DO was positively related to pool area ($r^2_{unadjusted} = 0.80$, $p < 0.01$), perhaps reflecting more spring seeps in larger pools. These measurements should be viewed with caution because they were only spot measurements taken on 1 d to provide a rough description of the reach.

Fish diets

For diet analysis, I collected a subsample of fish from each pool ($n = 139$ fish) by seining on the last day of the experiment. I fixed fish in 10% formalin in the field, then washed them in water and stored them in 70% ethanol. I removed fish stomachs and counted

and identified to the lowest taxonomic category possible all items in the contents. I recorded the life-stage (larval, pupal, adult) and habitat (aquatic or terrestrial) of each prey item to assess whether fish fed primarily on the benthos (larval aquatic prey) or in the water column or surface habitats (pupal, adult, and terrestrial prey). Such foraging strategies can determine the strength of fish predation effects in the stream (Dahl and Greenberg 1996). To compare gut fullness across species, I first corrected for differences in body size by dividing the total number of prey items by the standard length (mm) of each fish.

Data analysis

I conducted all analyses in SAS (version 9.3; SAS Institute, Cary, North Carolina). I created 7 candidate models (analysis of variance [ANOVA], PROC MIXED) consisting of each additive combination of 3 predictor variables: fish density, pool area, and substrate. I considered adding 2 interaction terms: fish $\times$ area and fish $\times$ substrate. However, both interaction terms were strongly correlated with fish density (fish vs fish $\times$ area: $r = 0.97$, $p < 0.01$; fish vs fish $\times$ substrate: $r = 0.89$, $p < 0.01$), but not with area ($r = -0.13$, $p = 0.71$) or substrate ($r = -0.34$, $p = 0.33$). As a result, including the interactions terms would have been redundant, so I dropped them from consideration.

I used Akaike's Information Criterion with a correction for small sample size (AIC_c) to compare model performance in explaining variation in the following response variables: benthic insect biomass (mg/m^2), emerging insect biomass ($mg\ m^{-2}\ d^{-1}$), and emerging insect trophic structure (proportion of predatory adults). I ran separate model-selection routines on response variables measured before fish manipulation (days 0–5) and at the end of the experiment (days 41–44). In addition, I completed a complementary set of analyses based on the proportional change in benthic and emerging insect density ([density$_{t2}$ − density$_{t1}$]/density$_{t1}$) and the absolute difference in emerging insect trophic structure across all pools. I then added these variables as response variables to assess model performance in explaining variation in the magnitude of change of benthic and emerging insects over time across all pools. I regarded models within 2 AIC_c units of each other as having similar explanatory power (Burnham and Anderson 2002).

I also assessed model performance with adjusted r^2 (r^2_{adj}), effect size (ES), and Akaike weight (w_i), which estimates the probability that a given model is the best model among all competing models (Johnson and

Omland 2004). I calculated ES as $\sqrt{r^2_{adj}}$ and interpreted values as indicating small, medium, and large effects ($r = 0.1, 0.3, 0.5$, respectively; Nakagawa and Cuthill 2007). When r^2_{adj} was negative, I inferred an ES of 0. When multiple models received similar support, I used model averaging to compute parameter estimates and 95% confidence intervals (CIs) for each predictor variable (Johnson and Omland 2004). I considered predictor variables important if their 95% CI did not include 0.

I used linear regression to test the relationship between benthic insect biomass and emergence biomass. I further tested this relationship for common individual taxa and used the results to examine whether benthic and emergence data sets were uncoupled, which might reveal a lag between measured benthic standing crop and subsequent emergence.

I assessed residuals for normality using Shapiro–Wilk tests and residual plots. I transformed variables as needed: ln (emerging insect biomass density, pool area), arcsin$\sqrt{}$ (emerging insect trophic structure, relative change in emergence). All other variables were analyzed as raw data. I assessed outliers with scatterplots and Cook's D, which measures the influence of each observation on the estimate of regression slope (Quinn and Keough 2002). When I identified outliers (visually or with $D > 1$), I reran the model-selection routine with the outlier removed. I considered an outlier substantial if its removal changed the selection of a top model ($\Delta AIC_c > 2$). If it did not, I retained and reported the original model selection. If it did, then I reported the model selection results with and without the outlier.

Results

Fish gut contents

After correcting for differences in body length, *Lepomis* spp. (mean ± SD, 0.62 ± 0.74 insects/mm fish length, $n = 51$ stomachs) had >12× more insects in their diet, on average, than the next most common predatory fish, *N. boops* (0.05 ± 0.05 insects/mm fish length; $n = 25$ stomachs) (ANOVA PROC MIXED, $F_{1,74} = 17.46$, $p < 0.01$). Because *Lepomis* spp. also were the most abundant fish, this difference in gut fullness suggests they were the dominant insectivore in my study. Insect prey in *Lepomis* spp. stomachs were dominated by larval insects (90 ± 14% by abundance) with very few winged (0.07 ± 0.10%) or pupal insects (<1%), results suggesting a preference for benthic foraging. Larval dipterans made up 83% of *Lepomis* diets (Chironomidae: 63%, Ceratopogonidae: 19%), whereas Ephemeroptera made up 7%.

TABLE 3.   Model-selection results before and after the creation of a 13× gradient in fish biomass among pools. Results are given only for models within 2 AIC_c units of the top model. K = number of parameters in the model including the intercept and residual standard error, AIC_c = Akaike Information Criterion corrected for small sample size, ΔAIC_c = difference between a given model and the top model for each response variable, w_i = Akaike weight. ES = effect size based on r_{adj}, which is $\sqrt{r^2_{adj}}$. Zeros are given when r^2_{adj} is negative.

Response variable	Model	K	r^2_{adj}	AIC_c	ΔAIC_c	w_i	ES
Before fish manipulation							
Benthic insect biomass density	Fish	3	−0.05	125.0	0.0	0.38	0
	Cobble	3	−0.11	125.6	0.6	0.28	0
	Area	3	−0.12	125.7	0.7	0.27	0
Emerging insect biomass density	Area	3	0.02	−3.8	0.0	0.39	0.15
	Fish	3	−0.01	−3.4	0.4	0.33	0
	Cobble	3	−0.11	−2.5	1.3	0.21	0
Emerging insect trophic structure	Area	3	0.44	−28.7	0.0	0.76	0.66
After fish manipulation							
Benthic insect biomass density	Fish	3	0.39	86.9	0.0	0.74	0.63
Emerging insect biomass density	Cobble	3	0.03	−3.9	0.0	0.43	0.18
	Fish	3	−0.06	−3.0	1.0	0.27	0
	Area	3	−0.09	−2.7	1.2	0.24	0
Emerging insect trophic structure	Area	3	0.54	−37.7	0.0	0.86	0.74
Benthic chironomid biomass density	Area + cobble	4	0.46	1.1	0.0	0.41	0.68
	Area	3	0.10	1.6	0.6	0.31	0.31
Benthic chironomid biomass density (1 outlier removed)	Area	3	0.83	−4.3	0.0	0.82	0.91
Emerging chironomid biomass density	Cobble	3	0.06	−4.0	0.0	0.46	0.24
	Fish	3	−0.05	−2.9	1.1	0.27	0
	Area	3	−0.10	−2.4	1.6	0.21	0
Change from before to after fish manipulation							
Relative change in benthic density	Area	3	0.14	20.1	0.0	0.58	0.37
Relative change in benthic density (2 outliers removed)	Fish	3	0.43	−35.2	0.0	0.73	0.65
Relative change in emergence	Fish	3	0.01	−19.2	0.0	0.42	0.12
	Cobble	3	−0.08	−18.3	1.0	0.26	0
	Area	3	−0.09	−18.2	1.0	0.26	0
Absolute change in trophic structure	Fish	3	0.08	−38.7	0.0	0.44	0.29
	Area	3	−0.02	−37.6	1.1	0.26	0
	Cobble	3	−0.10	−36.9	1.8	0.18	0

Model selection

Benthic insects.—Before fish manipulation, 3 models were within 2 AIC_c units of each other but explained little variation in benthic insect biomass (Table 3). In contrast, in the final week of the experiment after fish manipulation, the univariate fish-density model was the best model and explained 39% of the variation in benthic insect biomass with a strong ES (0.63; Table 3). This model revealed a negative relationship between fish density and benthic insect biomass, a relationship that was not present before fish manipulation (Fig. 1A, B). Based on w_i, the chance that this model was the best among the candidate set was 74%. No other model was within 3 AIC_c units of the fish-density model.

Benthic insect biomass was substantially higher before fish manipulation than at the end of the experiment (Fig. 2A). This trend was consistent across 8 of 10 pools, in which benthic insect biomass declined 76 ± 11% (mean ± SD). In the remaining 2 pools, benthic insect biomass increased by 621% and 85% (Fig. 3A). I also assessed the effects of fish on benthic insect biomass and emergence to terrestrial habitats by calculating relative changes in benthic and emerging insect density. Variation in relative change in benthic insect biomass was best explained by the univariate pool-area model, which revealed a weak negative relationship with a medium ES (0.37; Table 3). However, this model was influenced by 2 outliers (the 2 pools with increases). When these pools were removed from the analysis, the univariate fish-density model was the top model and revealed a strong negative relationship with the relative change in benthic insect biomass (ES = 0.65; Table 3, Fig. 3B). This result suggested that, among those pools in
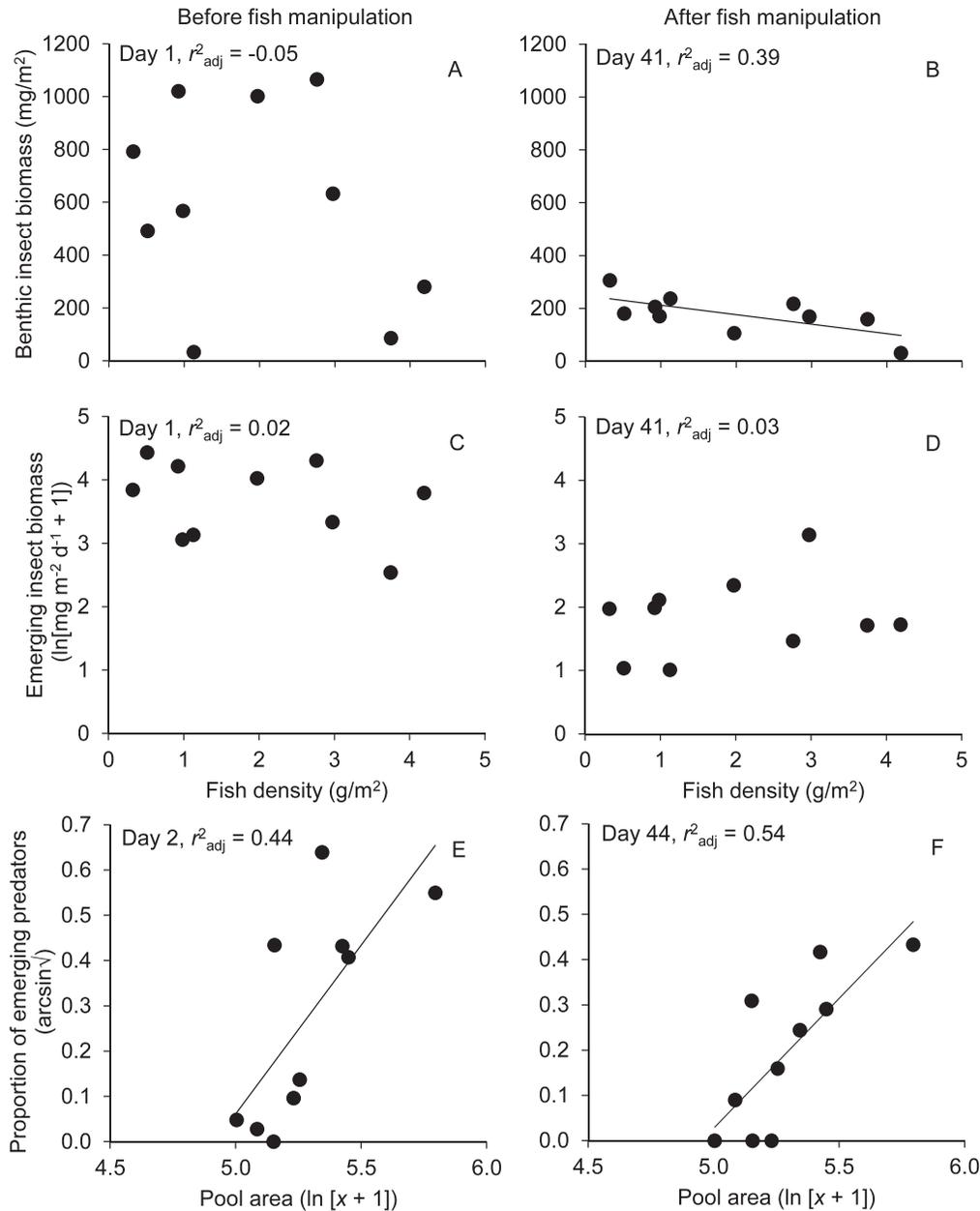
FIG. 1. Linear regressions between fish density and benthic insect biomass before (A) and after (B) fish manipulation, fish density and emerging insect biomass before (C) and after (D) fish manipulation, and pool area and emerging insect trophic structure before (E) and after (F) fish manipulation. Regression lines are given only for significant relationships.

which benthic insect biomass declined, the decrease was strongest in pools with high fish density (Fig. 3B). Chironomidae and Ephemeroptera made up >80% of benthic insect biomass at the beginning and end of the experiment, but their rank biomass differed on each sampling date (Chironomidae vs Ephemeroptera: June, 60 vs 29%; July, 37 vs 43%; see Appendix S2 for a full summary of benthic insect taxa; available online from: http://dx.doi.org/10.1899/12-124.1.s1).

Benthic insects that are predatory as adults were larger (2.22 ± 2.71 mg/individual) than benthic insects that are nonpredatory as adults (0.55 ± 0.29 mg/individual) (1-way ANOVA on ln-transformed means, $F_{1,17} = 4.38$, $p = 0.04$). However, the prediction that predation effects would be strongest on insects that are predatory as adults was not supported. Benthic insects that are predatory as adults were negatively associated with fish density
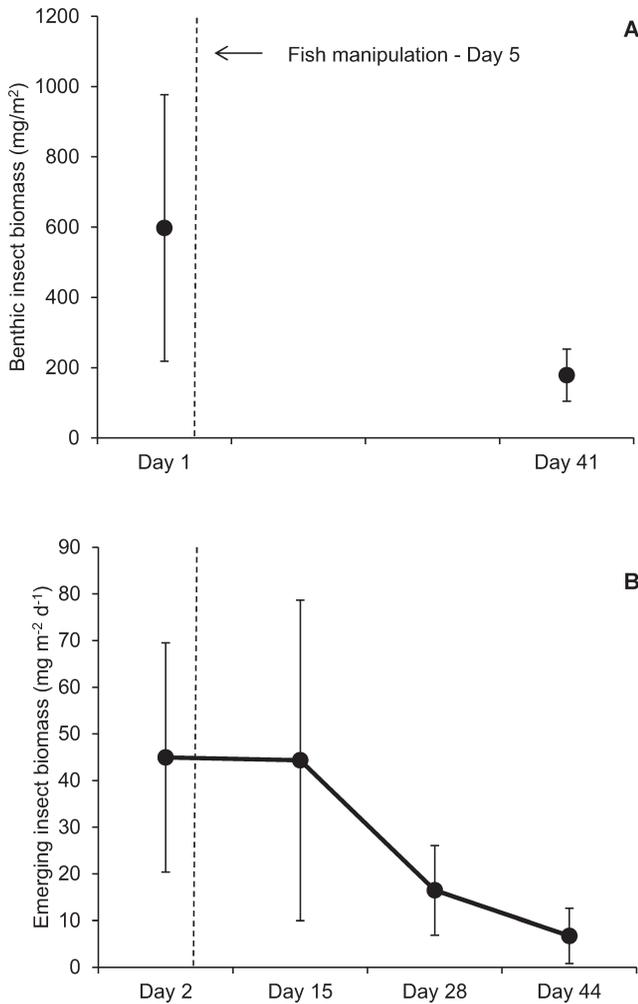
FIG. 2. Mean (±1 SD) aquatic insect biomass in benthic (A) and emergence (B) samples averaged across all pools on each date. Note differences in scale on the *y*-axes.
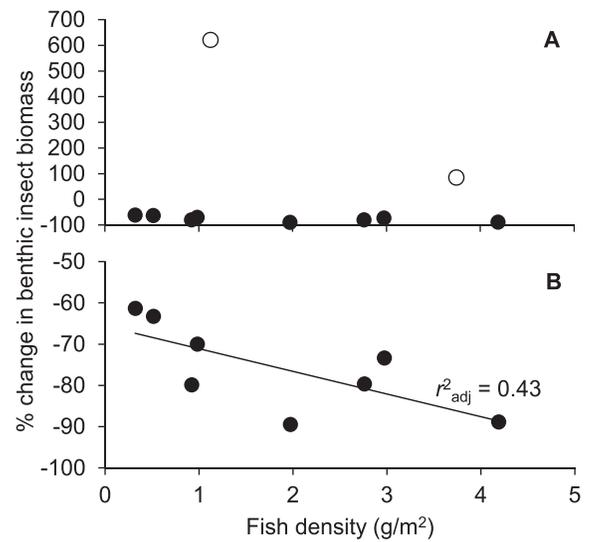

FIG. 3. Linear regressions between fish density and % change in benthic insect biomass from before fish manipulation to the final week of the experiment in all pools ($n =$ 10), including the 2 outliers (open circles) in which benthic insect density increased (A) and with outliers removed (B), leaving only those pools in which benthic insect biomass declined (closed circles, $n = 8$).

($r^2_{unadjusted} = -0.22$), but the relationship was not significant ($p = 0.17$). A nonsignificant negative relationship also existed between benthic insects that are nonpredatory as adults and fish density ($r^2_{unadjusted} = -0.29$, $p = 0.10$).

Emerging insects.—Three models were within 2 AIC$_c$ units of each other in explaining insect emergence biomass both before and after fish manipulation (Table 3), but none of these models, including the fish-density model (Fig. 1C, D), had good explanatory power (Table 3).

The trophic structure of emerging insects before and after fish manipulation was best explained by the univariate pool-area model (Table 3, Fig. 1E, F), which revealed a positive relationship between pool area and the proportion of emerging predatory insects on both dates (ES ≥ 0.66; Table 3, Fig. 1E, F). No other model was within 4 AIC$_c$ units of the pool-area

model on either date. This change in trophic structure was driven largely by a damselfly, *Argia* spp., which was the dominant predatory taxon (66% of predatory biomass; see Appendix S3 for a full summary of emerging insect taxa; available online from: http://dx.doi.org/10.1899/12-124.1.s1). Its emergence was positively related to pool area ($r^2_{unadjusted} = 0.78$, $p < 0.01$).

Mean emergence biomass across all pools was highest in the premanipulation collections on day 2 and declined over the course of the experiment (ANOVA with date as predictor variable: $F_{3,36} = 20.8$, $p < 0.01$; Fig. 2B). Unlike benthic insects, the decline in emergence was evident in all 10 pools. Emerging insect biomass was $50 \pm 21\%$ lower at the end of the experiment than before fish manipulation. The 3 univariate models had equivalent support in explaining variation in the magnitude of emergence decline, but the variation explained by each model was minimal (Table 3).

The proportion of predators emerging varied over time across pools: 7 declined (range: −0.2 to −30 percentage points) and 3 increased (0.7 to 9 percentage points). The 3 univariate models were within 2 AIC$_c$ units of each other in explaining variation in this decline, but the amount of variation explained was low with a small to medium ES for the top model, fish density (ES = 0.29; Table 3).

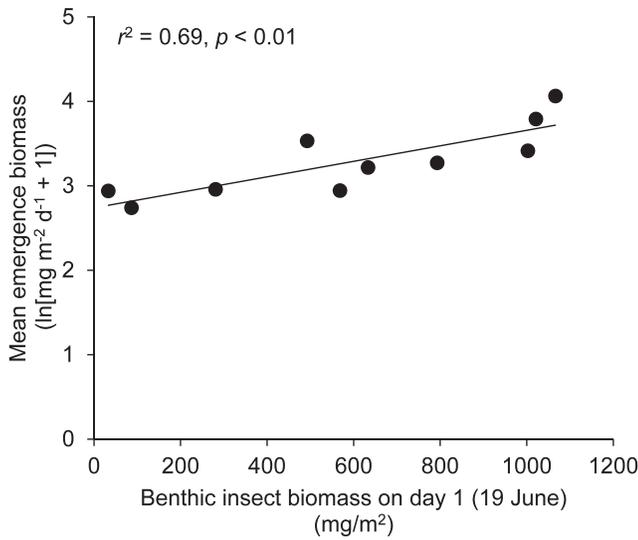Benthic and emerging biomass across pools were positively related at the beginning of the experiment

FIG. 4. Linear regression between benthic insect biomass at the beginning of the experiment (day 1) and emergence biomass averaged across the entire 44-d experiment. Data points are means from individual stream pools.

($r^2_{unadjusted} = 0.48$, $p < 0.03$) but not in the final week ($r^2_{unadjusted} = 0.03$, $p = 0.61$). When emergence was averaged across all collections, benthic insect biomass at the beginning of the experiment explained nearly 70% of emergence variation among pools ($r^2_{unadjusted} = 0.69$, $p < 0.01$, Fig. 4), whereas benthic biomass in the final week was unrelated to mean emergence ($r^2_{unadjusted} = 0.07$, $p = 0.46$).

Chironomids.—Because chironomids were the most common insect in fish diets, benthic samples, and emergence samples, I ran the model-selection routine on benthic and emerging chironomid biomass data

sets from the end of the experiment when overall fish effects were present. Limiting the test to chironomids minimized potential bias in emergence collections that may have uncoupled fish effects on benthic and emerging insects.

Two models were within 2 AIC$_c$ units of each other in explaining benthic chironomid biomass at the end of the experiment (Table 3). The best-ranked model was pool area + cobble, which was 0.6 AIC$_c$ units better than the univariate pool-area model (Table 3). This analysis was influenced by an outlier (Cook's $D > 1$). When the outlier was removed, pool area was by far the best model and revealed a strong negative relationship (r^2_{adj}) between benthic chironomid biomass and pool area (ES = 0.91; Table 3). The next best model was 3.8 AIC$_c$ units away (Table 3). For chironomid emergence at the end of the experiment, 3 models were within 2 AIC$_c$ units of each other, but none had good explanatory power (Table 3).

Model averaging

Model averaging was done on 7 response variables (Table 4). With one exception, all model averaged parameters had CIs that overlapped 0 (Table 4), results suggesting weak support for any predictor variable in explaining variation in benthic or emerging insects. The exception was a positive relationship between fish density and the magnitude of change in emergence trophic structure during the experiment (Table 4). Univariate regression between change in trophic structure and fish density suggested that the proportion of predatory adults emerging declined over time in pools with low fish density, but did not change in pools with high fish density. However, this relationship was weak ($r^2_{adj} = 0.08$).

TABLE 4. Model-averaged parameter values and unconditional 95% confidence intervals (parentheses) for each predictor variable. Results are given only for models where model selection did not identify a single best model at $\Delta AIC_c < 2$. AIC$_c$ = Akaike Information Criterion corrected for small sample size, ΔAIC_c = difference between a given model and the top model for each response variable.

	Predictor variables		
Response variable	Fish	Area	Cobble
Before fish manipulation			
Benthic insect biomass density	−73.82 (−166.02, 18.37)	−68.64 (−1249.91, 1112.63)	−213.91 (−1436.08, 1008.27)
Emerging insect biomass density	−0.14 (−0.28, 0)	0.99 (−0.82, 2.81)	0.18 (−1.88, 2.23)
After fish manipulation			
Emerging insect biomass density	0.1 (−0.01, 0.22)	−0.45 (−2.4, 1.5)	−1.08 (−2.95, 0.79)
Benthic chironomid biomass density	−0.11 (−0.26, 0.03)	−2.66 (−5.52, 0.2)	2.48 (−0.48, 5.44)
Emerging chironomid biomass density	0.12 (−0.27, 0.51)	0.14 (−2.19, 2.48)	−1.36 (−3.64, 0.93)
Change from before to after fish manipulation			
Relative change in emergence	0.07 (−0.001, 0.14)	−0.21 (−1.03, 0.61)	−0.23 (−1.16, 0.69)
Absolute change in trophic structure	0.04 (0.005, 0.07)	−0.18 (−0.55, 0.18)	0.15 (−0.27, 0.57)

Discussion

In my experiment, fish reduced benthic insect biomass in stream pools, but this reduction did not translate to reductions in adult insect emergence. My results contrast with those of other recent studies showing that fish predation on larval aquatic insects reduces the amount of energy emerging from the stream and entering the terrestrial food web (Baxter et al. 2004, Epanchin et al. 2010, Wesner 2010a). Trout reduced insect emergence by 50% from a montane Japanese stream (Baxter et al. 2004) and reduced mayfly emergence by similar or greater amounts in alpine lakes (Pope et al. 2009, Epanchin et al. 2010). A minnow species and a darter species reduced insect emergence from stream mesocosms by 50% and altered the trophic structure of emerging insects by reducing adult predatory insect biomass more strongly than nonpredatory insect biomass (Wesner 2010a). Fish in Florida ponds reduced adult dragonfly abundance by 50%, triggering a terrestrial trophic cascade (Knight et al. 2005). Despite a 13× gradient of fish biomass among pools, none of these effects on emergence were evident in my experiment.

Several factors may account for the lack of transfer between aquatic predation and aquatic–terrestrial insect flux. First, only 5 of the 18 aquatic insect taxa collected in benthic samples in the final week of the experiment (when predation effects were evident) were collected in emergence samples over the same time period. Of these 5, only chironomids were abundant in both benthic and emergence samples. Fish had no effect on chironomid biomass in the stream, so predation effects did not transfer to emergence collections.

Second, benthic insect biomass and emerging insect biomass declined during the experiment (3× and 7×, respectively). This result suggests that peak emergence for most pools occurred before or near the beginning of the experiment. In a mesocosm experiment, fish reduced insect emergence from aquatic habitats, but reductions were evident only at peak emergence, and disappeared when emergence declined (Wesner 2010a). Fish effects in my experiment may have been limited to the benthos because most insects had completed or were near completion of their aquatic life stages when I initiated fish treatments.

Third, the stream became intermittent over the final 2 wk of the experiment and most pools became isolated. Stream drying increased the density of predatory fish, which may have contributed to the fish effect on benthic insects in the final week. Removal of whole fish assemblages increased macro-invertebrate density in intermittent pools of an Arkansas (USA) stream, an effect that increased with time as the pools dried (Williams et al. 2003). Stream drying amplified the effects of an omnivorous minnow and predatory crayfish on chironomid density in a different Arkansas stream (Ludlam and Magoulick 2009) and the effects of *Lepomis* on benthic invertebrates in a Kentucky stream (Holomuzki and Stevenson 1992). Seasonal intermittency is common in many temperate prairie streams, and such temporal heterogeneity in habitat conditions may be a key factor regulating biotic control of stream food webs (Power et al. 2008).

Benthic insect biomass declined in 8 of 10 pools during the experiment. The magnitude of decline in these pools was positively associated with fish density, a result further supporting evidence of a predation effect. However, benthic insect biomass in the remaining 2 pools increased by as much as 621%. What caused these 2 pools to deviate so strongly from trends in most pools is unclear. One speculative hypothesis is that they were drift sinks, colonized by insects drifting to avoid drying riffles. This explanation seems unlikely because neither of these pools had exceptionally high benthic biomass at the end of the experiment (2nd and 8th highest biomass). In other words, the proportional change in these 2 pools was driven by low biomass at the beginning of the experiment and not by high biomass at the end.

My study lasted ~6 wk, the same length of time in which Baxter et al. (2004) demonstrated a strong reduction of emerging insects by predatory trout. However, the combination of manipulating fish at the end of peak emergence and ending the experiment during stream intermittence may have reduced my ability to detect fish effects on emergence. This argument is supported by the strong relationship between benthic biomass in June and emergence biomass across the experiment, which suggests a lag between measured benthic insect biomass and subsequent emergence. Thus, the effect of fish on benthic insects may have translated to an effect on emergence in a longer experiment. However, even if an emergence effect lagged behind a benthic predation effect, I found no evidence that benthic predation would have altered emerging trophic structure. Contrary to my prediction, the relationship between fish density and benthic insects at the end of the experiment was similar regardless of whether insects were predatory as adults. This similarity occurred despite the fact insects that are predatory as adults were larger as larvae than insects that are not predatory as adults, indicating that size-selective predation was not important.

Habitat size, and not fish predation, was positively related to emerging insect trophic structure. The mechanism behind this relationship is unclear, but it is consistent with results of previous studies showing that females with predatory larvae prefer to oviposit in larger ephemeral ponds (Wilcox 2001). Emerging predatory insect biomass was dominated by a damselfly (*Argia* sp.), which tended to increase in density with increasing pool area. *Argia* are predatory as both larvae and adults, so a similar mechanism might explain this result. Furthermore, odonate egg survival can be limited by low DO, and the positive relationship between pool area and DO in my experiment may have led to enhanced survival or oviposition of odonates in large pools (e.g., Tsubaki et al. 1994). However, this explanation is speculative because I measured DO once, and those values may not reflect the long-term O_2 conditions in each pool. Moreover, increased emergence of odonates from individual pools does not necessarily mean that the adults will feed around those pools. Rather, it indicates that the relative abundance of predatory adults in the subsidy probably is produced by a small number of habitat patches in the stream. As a result, modification of those patches may have a disproportionate effect on the trophic structure of the overall subsidy at the reach scale.

Fish predation can alter energy flux from aquatic to terrestrial habitats (Baxter et al. 2004, Knight et al. 2005, Pope et al. 2009, Wesner 2010a). However, in my experiment, flux to the terrestrial habitat was independent of fish biomass, despite a predation effect on benthic insect biomass. Instead, aquatic–terrestrial flux was determined largely by the distribution of benthic insects before fish manipulation. These patterns suggest a time lag between predation effects in the stream and emergence effects in the terrestrial system. In harsh, heterogeneous systems like Brier Creek, fish effects on aquatic–terrestrial subsidies may manifest only in short temporal windows that are dependent on the seasonally variable conditions of the stream.

Acknowledgements

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