

Effects of the herbivorous minnow, southern redbelly dace (*Phoxinus erythrogaster*), on stream productivity and ecosystem structure

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Received: 8 June 2006 / Accepted: 11 September 2006 / Published online: 10 October 2006
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Abstract We used field and mesocosm experiments to measure effects of southern redbelly dace (*Phoxinus erythrogaster*), a grazing minnow, on stream ecosystem structure and function. Ecosystem structure was quantified as algal filament length, algal biomass, size distribution of particulate organic matter (POM), algal assemblage structure, and invertebrate assemblage structure, whereas ecosystem function was based on gross and net primary productivity. Our experiments showed that moderate densities of *Phoxinus* temporarily reduced mean algal filament length and mean size of POM relative to fishless controls. However, there was no detectable effect on algal biomass or ecosystem primary productivity. Several factors could explain the lack of effect of *Phoxinus* on primary productivity including increased algal production efficiency in grazed treatments or increased grazing by other organisms in fishless treatments. The inability of *Phoxinus* to reduce algal biomass and system productivity contrasts with experimental results based on other grazing minnows, such as the central stoneroller (*Camptostoma anomalum*), and questions the generality of grazer effects in stream ecosystems. However,

environmental venue and the spatial and temporal scale of ecosystem measurements can greatly influence the outcome of these experiments.

Keywords Grazers · Prairie streams · Stream metabolism

Introduction

Accelerated rates of species extinctions (Lawton and May 1995; Pimm et al. 1995; Vitousek et al. 1997; Rosenzweig 1999; Sala et al. 2000) are forcing ecologists to consider the consequences of diversity losses on ecosystems. Whereas the loss of entire functional groups is likely to alter ecosystem processes (Ghilarov 2000; Schwartz et al. 2000; Rosenfeld 2002), recent studies indicate that even individual species can make unique contributions to an ecosystem (e.g., Cardinale et al. 2002). In particular, grazing animals are tightly coupled with primary production and can affect both structural (e.g., species composition, standing stock) and functional (e.g., productivity) components of ecosystems. Although grazers ingest producer biomass (Krebs 2001), they also remineralize nutrients, which may stimulate production and decrease turnover time of remaining cells (Cooper 1973; Hill et al. 1992). Grazing also can increase biomass-specific productivity by altering the availability of limiting resources such as light and nutrients (e.g., by decreasing shading and increasing the rate of delivery of nutrients across the boundary layer; Newbold et al. 1982; Power et al. 1988). The trade-off between biomass loss and increased photosynthetic efficiency of residual algae will dictate the rate of ecosystem primary productivity (Carpenter and Kitchell 1984).

Electronic supplementary material Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s00442-006-0569-y> and is accessible for authorized users.

Communicated by Joel Trexler.

The experiments described herein comply with the current laws of the country in which the experiments were performed.

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Effects of grazers on ecosystem structure and function in streams have been reported for a variety of organisms including insects (e.g., Wallace and Webster 1996), snails (e.g., Hill et al. 1992; Sarnelle et al. 1993; Vaughn et al. 1993; Turner 1997), crayfishes (e.g., Gelwick 2000; Evans-White and Dodds 2003), tadpoles (e.g., Nystrom and Abjornsson 2000), and fishes (e.g., Cooper 1973; Power 1990; Matthews 1998; Flecker et al. 2002). In prairie streams, which are typically more net autotrophic (i.e., production/respiration ≈ 1) relative to densely canopied streams (Webster et al. 2003), we might expect grazing organisms to have a stronger influence on ecosystem structure and function than in more heterotrophic systems because they interact directly with autotrophs (e.g., ingesting them) but only indirectly with heterotrophs. Studies of *Campostoma anomalum* (central stoneroller) demonstrate the potentially strong effects of grazing minnows in prairie streams (Matthews 1998; Evans-White and Dodds 2003), but it is not clear if these results can be generalized to other grazing fishes.

Our study tested for structural and functional effects of the herbivorous minnow, *Phoxinus erythrogaster*, which can occur in sympatry with *Campostoma* in prairie streams. Both of these species prefer streambeds dominated by pebble, gravel, or sand and avoid reaches with greater proportions of silt or clay (Lennon and Parker 1960; McKee and Parker 1982; Slack et al. 1997). *Phoxinus* are abundant in springfed headwater reaches, whereas *Campostoma* are typically found downstream from these habitats (Hill and Jenssen 1968; Settles and Hoyt 1976; Felley and Hill 1983). *Campostoma* appear to be selective feeders preferring diatoms to other forms of algae (Stewart 1987; Power et al. 1988; Napolitano et al. 1996), whereas *Phoxinus* are more generalist omnivores, feeding on algae and invertebrates when they are available (Phillips 1969; Settles and Hoyt 1976; Felley and Hill 1983). Whereas *Phoxinus* can only bite algae (Forbes and Richardson 1920), *Campostoma* use a cartilaginous ridge on the lower jaw (McKee and Parker 1982; Miller and Robinson 2004) to swipe, shovel, or bite attached algae from the substrate (Matthews et al. 1986). Although these fishes can use similar habitat and overlap in diet, it is unknown if the effects of *Phoxinus* are redundant (sensu Lawton 1994) with those of *Campostoma* in prairie streams.

To test the structural and functional effects of *Phoxinus* in prairie streams, we used field and mesocosm experiments. Mesocosm experiments allowed us to replicate treatments, control effects of heterogeneous discharge, and improve the precision of our whole stream productivity measurements. Measure-

ments of structural components from field experiments allowed us to extrapolate our experimental stream results to local natural streams. Based on studies of *Campostoma* (Gelwick and Matthews 1992), we predicted that structural effects of *Phoxinus* would include reduced algal filament length, reduced algal biomass, altered algal assemblage structure, and reduced mean particle size of particulate organic matter (POM). Moreover, these structural changes should result in functional changes (i.e., reduced primary productivity) and bottom-up effects on invertebrate assemblage structure.

Materials and methods

Field experiment

Study site

The field experiment was conducted in four pools in Kings Creek, Riley County, Kansas, from August to October 2002. Kings Creek drains 1,059 ha of tallgrass prairie on the Konza Prairie Biological Station (KPBS). Physicochemical and biological descriptions of this stream are in Gray et al. (1998) and Gray and Dodds (1998). The four study pools were located in a forested stream reach with perennial flow. Pool surface area ranged from 23 to 84 m² (mean = 59 m²), and substrate typically was cobble, pebble, and gravel, according to the Wentworth scale (Cummins 1962). Dominant fishes in the study reach included three minnows [*C. anomalum*, *P. erythrogaster*, and *Semotilus atromaculatus* (creek chub)] and the orangethroat darter (*Etheostoma spectabile*). Grazing invertebrates such as crayfish (*Orconectes* spp.) and snails (*Physa* and *Physella* spp.) also were present.

Experimental design

Wire screen (5-mm mesh) was used to block the upstream and downstream ends of four study pools (fishless enclosures and fish enclosures). Wire mesh was secured to steel poles and buried roughly 20 cm into the streambed to prevent the escape or entrance of fishes. Leaf litter was removed from the wire mesh as needed to maintain natural stream flow through the study pools. On 15 August 2002 (day 0) of the experiment, three-pass electrofishing depletion samples were conducted in each study pool. In two randomly selected enclosure pools, all captured *Phoxinus*, other fish species and crayfishes were removed. Fishes collected from the other two enclosure pools were

counted, measured (total length) and returned to the pools. Another depletion sample was conducted in each pool during the sixth week of the experiment to quantify the immigration of young-of-the-year fishes into exclosures. *Phoxinus* densities in each study pool were based on maximum-likelihood population estimates (Van Deventer and Platts 1989) from the three-pass electrofishing depletion samples. Because we did not initially remove all fish from the exclosures, we estimated the density of fish remaining as the difference between the maximum-likelihood population estimate and the total number of fish removed after three passes.

Mesocosm experiments

Study system

The second phase of this study was conducted in nine experimental streams at the KPBS. Experimental streams were similar to those used by Gido and Matthews (2001), and each stream consisted of a 2.54-m² pool connected to a 0.84-m² riffle. Recirculating flow was powered by an electric trolling motor with a mean discharge of 10.8 l/s, and water was supplied by a natural spring that also supplies nearby Kings Creek. Substrate was a mixture of gravel, pebble, and fines from a local quarry. Although algae and winged invertebrates (e.g., chironomids) readily colonized these systems, each stream was inoculated 1 week prior to the beginning of the experiment with an algal slurry obtained from Kings Creek to stimulate algal growth.

Experimental design

In fall 2002, two *Phoxinus* treatments {small *Phoxinus* [30–50 mm total length (TL), 28.3 fish/m²] and large *Phoxinus* (>60 mm TL, 5.7 fish/m²)} and a fishless control were randomly assigned to stream units with three replicates each. The different stocking densities were intended to equalize biomass and isolate the effect of body size; however, biomass in the small fish treatment (26.1 g/m²) was slightly greater than that in the large fish treatment (22.4 g/m²). The experiment began on 17 October 2002 (day 0) and concluded on 26 November 2002 (day 40). Mean water temperature was 7°C (range 2–13°C). Fish that were lost to natural mortality during this experiment were replaced within 1 week.

This experiment was repeated in the summer of 2003 with eight experimental stream units. However, we only compared a fishless control to a fish treatment [24

Phoxinus (mean TL = 56 mm, range TL 40–78 mm, 6.8 fish/m², 14.9 g/m²)] because we found no significant differences between large and small *Phoxinus* treatments in 2002 (see Results). This experiment began on 5 June 2003 (day 0) and concluded on 8 August 2003 (day 65). Mean water temperature was 22°C (range 13–31°C).

Data collection

Ecosystem function

Gross primary productivity (GPP) and net primary productivity (NPP) in experimental streams were based on diurnal changes in dissolved oxygen measurements from YSI 600XLM sondes (Yellow Springs Instruments). We used the open-system single-station approach to estimate productivity (Bott 1996). Water was recirculated at the same velocity and the bed-form was similar in all experimental units so turbulence-induced aeration was similar across experimental stream channels. Reaeration was estimated using the surface renewal model, which is calculated from velocity (V , in cm/s) and mean depth (H , in cm) using the formula

$$f_{(20^{\circ}\text{C})} = 50.8 \cdot V^{0.67} \times H^{-0.85} \quad (1)$$

(Owens 1974). The flow-through rates were the same for all experimental units leading to an approximate turnover time of 13 h (i.e., effective channel length ~1,700 m). The prolonged exposure to stream biota assured that diurnal changes in water oxygen concentration reflected biotic processes in these stream units. We estimated NPP as the mean rate of change per hour in oxygen concentration during daylight and darkness, whereas GPP was estimated by subtracting the mean hourly rate of oxygen uptake during darkness from the mean hourly rate of oxygen productivity during daylight. During the first experimental stream study (fall 2002), sondes were deployed in three streams for 24 h then transferred to another stream, such that metabolism in all nine experimental streams was measured over a period of 3 days. GPP was estimated for each stream twice: once between day 7 and 27 and again between day 31 and 40. On 14 of 25 sample days, we only recovered oxygen curves during the night and part of the day (before 1300 hours). However, on the other 11 days we recovered complete 24 h curves, and we found productivity between 0900 and 1300 hours to be a significant predictor of productivity between 0900 and 1700 hours ($r^2 = 0.48$, $P < 0.01$). Thus, for the 2002 experiment we used this relationship to predict daytime productivity rates for days without complete data.

In summer 2003, GPP was measured in each stream during eight, 4-day periods beginning on days 1, 8, 14, 20, 29, 38, 50, and 65.

Ecosystem structure

Algal biomass was estimated as the concentration of chlorophyll *a* extracted from pebbles taken from study pools or experimental streams. Pebbles were collected on site and frozen within 4 h of collection. Chlorophyll was extracted by submerging pebbles in a 78°C, 95% EtOH solution as described in Sartory and Grobbelaar (1984). Concentration of chlorophyll *a* was corrected for cross-sectional area of pebbles and algal biomass was reported per unit area. During the field experiment in Kings Creek 2002, we removed three pebbles along ten equally spaced transects perpendicular to the direction of flow from each pool on days 4 and 32. In fall 2002, chlorophyll *a* samples from pools were lost, but we present data from riffles that were collected across each of three equally spaced transects perpendicular to the direction of flow in the riffles of experimental streams on day 40; chlorophyll *a* in riffles was significantly correlated with that in pools during summer 2003 ($r = 0.65$, $P < 0.01$). In summer 2003, we collected four pebbles from the edges of the pools and one from the deep center of the pools on days 1, 6, 18, 29, 42, 54, and 65.

In the field experiment, algal height was measured on day 39 along the same ten transects used for collecting algal biomass samples. We measured the vertical height of the algae over the substratum at ten points along each transect (100 points per stream pool). The length of the longest filament (vertical or horizontal) was measured in experimental streams because filaments typically were much longer. In fall 2002, we measured three filaments along each of the same three transects used for collecting algal biomass (nine points per stream riffle) on day 45. In summer 2003, we measured filaments at the same sampling points used for collecting algal biomass samples (five points per pool) on days 12, 23, 35, and 47.

Invertebrate samples from Kings Creek were a composite of four replicate Hess samples (500- μ m-mesh bag) that were combined and subsampled (30–40% of total sample). Because these samples were only taken on day 4, these data only were used as a reference for assemblage structure comparison between experimental streams and the natural stream because the invertebrate assemblages likely did not have time to respond to treatments. In the experimental stream studies we used a modified core sampler that consisted of a 0.018-m² corer with an electric pump (0.1 l/s) to

collect invertebrates, POM, and algae from the substrate. Substrata inside the corer were agitated by hand for either 1 min (fall 2002) or 1.5 min (summer 2003) while materials were pumped through a 250- μ m sieve. In the fall 2002 experiment, we took one invertebrate sample from the approximate center of each riffle on day 16, and on day 40 we took two core samples from each riffle and each pool. The invertebrates were preserved in formalin and later identified to order or family. We took four additional replicate core samples on day 40 (two from the riffle and two from the pool) to estimate size distribution of POM. POM samples were preserved in formalin, and dry as well as ash-free dry mass (AFDM) was measured for six size classes: >1 mm, 1,000–500, 500–250, 250–180, 180–98, and 98–0.45 μ m. In summer 2003, we took separate core samples from both the riffle and the pool on days 1, 6, 18, 29, 42, 54, and 65. In summer 2003, the material pumped from each riffle or pool was homogenized in a bucket and subsampled for fine POM (FPOM; 500 ml) and algal assemblage structure (50 ml). The remaining invertebrates and detritus were concentrated on a 250- μ m sieve and preserved in formalin. Dry and AFDM of POM was measured for five size classes: >500, 499–250, 249–180, 179–100 and 99–1 μ m. Algal assemblage structure samples also were preserved in formalin and later categorized into four general taxonomic groups (unicellular green, filamentous green, diatom, or cyanobacteria). The first 100 algal cells that intersected the ocular transect were placed in these categories.

At the conclusion of the fall 2002 experiment and twice during the summer 2003 experimental stream study (days 54 and 78), we collected two *Phoxinus* from each experimental stream to characterize diet. Diet items in the foregut were identified and enumerated using a transect method similar to the procedure for quantifying algal assemblage structure; we categorized the first 100 algal cells as filamentous green algae or diatoms (unicellular green and cyanobacteria were absent or in very low abundance), and noted the occurrence of invertebrate animal matter.

Statistical analysis

Data from Kings Creek 2002 were not statistically analyzed because each treatment was only replicated twice. Thus, we were limited to qualitative comparisons between results from these experiments and those from experimental stream studies. In fall 2002, ANOVA was used to test for differences among treatments in the concentration of chlorophyll *a* on pebbles and algal filament length in the experimental streams. In 2003, we used repeated-measures ANOVA with

sample date (day) as the repeated factor to test for fish effects on ecosystem function and structure variables over time in the experimental streams. If the variance–covariance matrices of the repeated measures failed Mauchly’s sphericity test, we referred to the Huynh–Feldt adjusted P -value for tests of within-subjects effects. Because we found a significant correlation between GPP and mean daily solar irradiance (see [Results](#)), we used repeated-measures analysis of covariance with GPP as the response variable, day as the repeated factor, and irradiance as the covariate to test for differences in metabolism among treatments ([SAS 2003](#)). We used the value of Akaike’s information criterion (Akaike 1974) to select the most adequate covariance structure from those evaluated (Milliken and Johnson 2002). The covariance structure that best fit our data was first-order autoregressive. We then used backward model selection and χ^2 -tests, which compared reduced and full model -2 residual log likelihood values, to select the best model of our data (GPP = day + fish + day \times fish + irradiance \times day \times fish). In a repeated measures design such as this, with different sized experimental units, the denominator df must be computed from a linear combination of mean squares, and the denominator is not chi-squared. Thus, we used the Kenward–Rogers approximation to find approximate df for the F -test, which produced fractional denominator df . Where we found significant differences in main effects, we applied Tukey post hoc comparisons to test the relative differences between the fish treatment and the control. Oxygen sonde dysfunction in summer 2003 resulted in unequal replication between the fish treatment and the control on days 65–68 and analysis was limited to three replicates each for the fish treatment and the control on day 29–32. Thus, we excluded measurements from days 65–68 and used linear trend at point estimates (SPSS 2001) to replace the missing observations from days 29–32. Differences in proportional abundance of four major algal groups were tested with repeated-measures ANOVA. Proportions were arcsine square-root transformed prior to this analysis. In summer 2003, we used a paired t -test to evaluate differences in the ratios of filamentous green algae and diatoms between the diet and core samples. Ratios were square-root transformed prior to analysis to reduce inequality of variances among samples. We tested for differences in invertebrate assemblage structure in fall 2002 using a partial redundancy analysis (pRDA). This analysis tested the significance of the association between invertebrate assemblage structure and the presence of *Phoxinus* after controlling for effects of sample date. In summer 2003, we used principal response curve (PRC) analysis, which is an

extension of a pRDA that considers repeated measures designs (pRDA; ter Braak and Smilauer 2002), to test the effects of fish and a time \times fish interaction on invertebrate assemblage structure. Both pRDA and PRC used a Monte Carlo randomization procedure to test the significance of the first axis of the ordination. Monte Carlo simulations were based on 500 permutations and run using CANOCO (ter Braak and Smilauer 2002). Ordinations were based on square-root transformed densities of each taxa (i.e., number of individuals per core), but an analysis based on proportional abundance yielded similar results.

Results

Fish density and biomass in stream enclosures

Our removal efforts in field enclosures only initially affected total numbers and biomass of fishes. Immigration and rapid growth of juvenile fishes resulted in similar fish assemblage structure between enclosures and enclosures by the sixth week of the experiment. After the initial removal, *Phoxinus* densities were estimated at 0.4 and 0.5 fish/m² in enclosures as compared to densities of 3.1 and 1.4 fish/m² in enclosures. In the same study pools, the density of *Campostoma* was 1.5 and 1.0 fish/m² in enclosures and 1.6 and 0.5 fish/m² in enclosures. By the sixth week of the experiment, *Phoxinus* densities in the enclosures were 4.6 and 18.3 fish/m² (1.8 and 7.9 g/m²) compared to 5.5 and 5.6 fish/m² (8.0 and 10.3 g/m²) in enclosures. Although densities and biomass were similar, mean length of *Phoxinus* was much smaller in enclosures (31.2 and 32.6 mm TL) than in enclosures (44.5 and 47.3 mm TL). Density of *Campostoma* was similar between enclosures [8.2 and 10.5 fish/m² (10.3 and 10.9 g/m²)] and enclosures [9.4 and 9.2 fish/m² (16.7 and 12.4 g/m²)], but biomass was less in enclosures by the sixth week. Mean length of *Campostoma* was slightly smaller in enclosures (45.6 and 43.1 mm TL) than in enclosures (49.7 and 46.8 mm TL).

Ecosystem function

Primary productivity

There was no significant effect of the presence of *Phoxinus* on GPP in the experimental streams during fall 2002 (all $F \leq 0.44$ and all P -values ≥ 0.66 ; [Fig. 1a](#)). In 2003, we found a significant correlation between GPP and mean daily solar irradiance ($r = 0.57$,

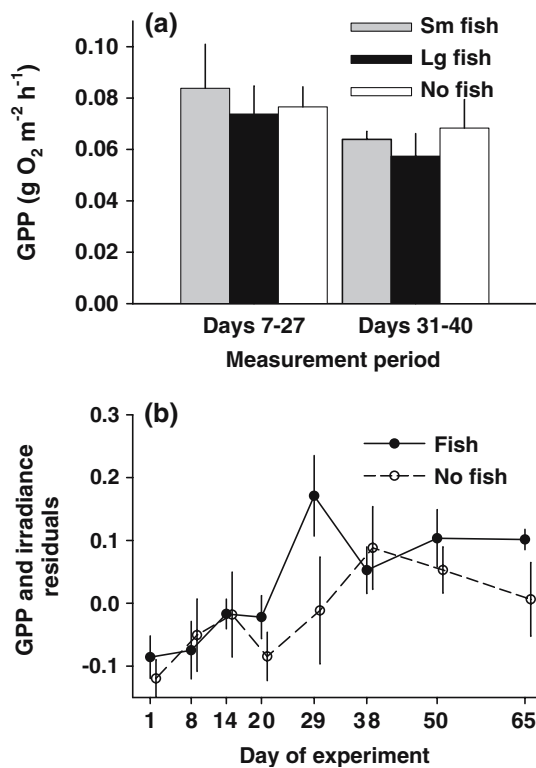


Fig. 1 Gross primary productivity (GPP; +1 SE) of streams with and without *Phoxinus erythrogaster* in **a** nine experimental streams in fall 2002 ($n = 3$), and **b** eight experimental streams in summer 2003 ($n = 4$). Data in **b** are corrected for irradiance. Control (no fish open circle) data points in **b** are offset 1 day later than data for streams with fish (filled circle) to prevent overlap. Sm Small, Lg large

$P < 0.01$; Fig. 2), but no effect of *Phoxinus* on GPP after controlling for the effects of irradiance ($F_{1,23.2} = 2.38$, $P = 0.14$; Fig. 1b). As with GPP, the presence of *Phoxinus* did not affect NPP in experimental streams (all $F \leq 2.62$ and all P -values ≥ 0.15).

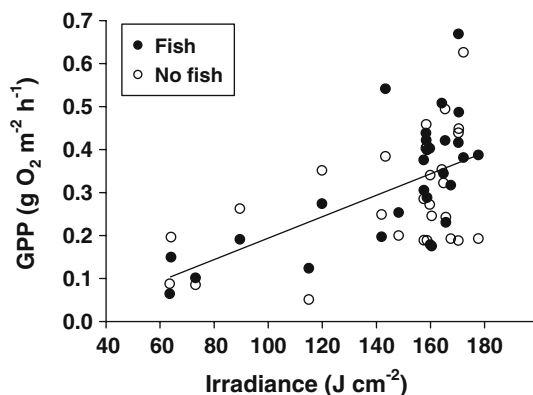


Fig. 2 GPP of streams with (filled circle) and without (open circle) fish as a function of mean daily solar irradiance in eight experimental streams in summer 2003

Ecosystem structure

Algal biomass

In Kings Creek, the concentration of chlorophyll *a* on pebbles was slightly higher (13–38%, on days 4 and 32, respectively) in the enclosures than enclosures on days 4 and 32, but there was high variability among pools (Fig. 3a). Similarly, in the experimental streams, algal biomass was not significantly affected by the presence of *Phoxinus* during fall 2002 ($F_{2,6} = 1.09$, $P = 0.40$; Fig. 3b) or summer 2003 ($F_{1,6} = 1.98$, $P = 0.21$; Fig. 3c).

Algal assemblage structure

In summer 2003, filamentous green algae dominated the assemblage (65%) followed by diatoms (17%), unicellular green algae (11%), and cyanobacteria (7%). We found a temporal pattern of increasing relative abundance of unicellular green algae ($F_{6,36} = 13.49$, $P < 0.01$) and a subsequent decline in filamentous green algae ($F_{6,36} = 6.99$, $P < 0.01$), but no effect of grazing by *Phoxinus* on the relative abundance of the four algal taxa (all $F \leq 4.43$ and all P -values ≥ 0.08).

Algal filament length

In Kings Creek, algal filaments were 0.2–1.6 cm shorter in enclosures than in the enclosures on day 39 (Fig. 4a). In the experimental streams during fall 2002, algal filaments were, on average, more than 2 orders of magnitude shorter in riffles with *Phoxinus* ($F_{2,6} = 104.87$, $P < 0.01$; Fig. 4b). Post hoc comparisons among treatment means revealed that the control had significantly (Tukey HSD $P < 0.01$) longer algal filaments than both the small *Phoxinus* and the large *Phoxinus* treatments, but filament lengths in the large *Phoxinus* treatments were not significantly different than in the large *Phoxinus* treatments (Tukey HSD $P = 1.00$). In summer 2003, using repeated-measures ANOVA, we found that the presence of *Phoxinus* significantly reduced mean algal filament length ($F_{1,6} = 6.24$, $P = 0.05$), but there also was a significant day \times fish interaction effect ($F_{4,24} = 6.12$, sphericity-assumed $P < 0.01$; Fig. 4c). The difference in mean algal filament length between treatments was greatest on day 9 and diminished by day 36.

Invertebrate assemblage structure

We did not observe an effect of *Phoxinus* on invertebrate assemblage structure in experimental streams

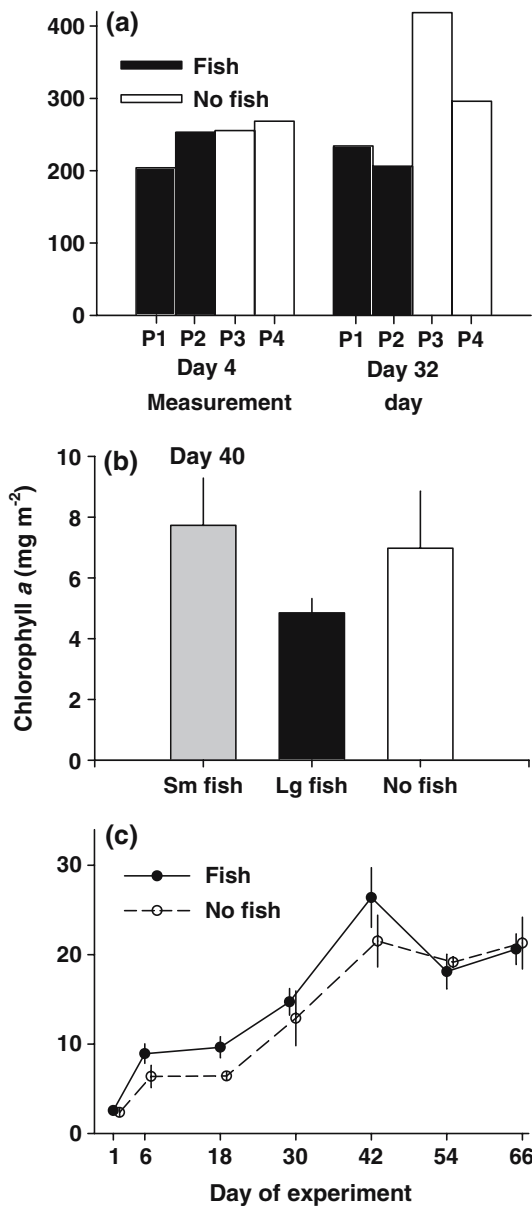


Fig. 3 Algal biomass (chlorophyll *a*, +1 SE) in stream pools and riffles with and without fish in **a** four Kings Creek pools in fall 2002 ($n = 2$), **b** nine experimental stream riffles in fall 2002 ($n = 3$), and **c** eight experimental stream pools in summer 2003 ($n = 4$). Each bar in **a** represents data recorded in one pool in Kings Creek (*P1*, *P2*, *P3*, or *P4*). Control (no fish open circle) data points in **c** are offset 1 day later to prevent overlap. For other abbreviations, see Fig. 1

(Appendix I). RDA showed that sampling date explained a significant fraction of the variability in invertebrate assemblage structure during fall 2002 (first axis eigenvalue 0.47, $F = 12.25$, $P = 0.01$; Fig. 5). However, when sample date was included as a covariate, the presence of *Phoxinus* did not explain the remaining variability among samples (first axis eigenvalue 0.08, $F = 2.24$, $P = 0.25$). Using PRC in summer

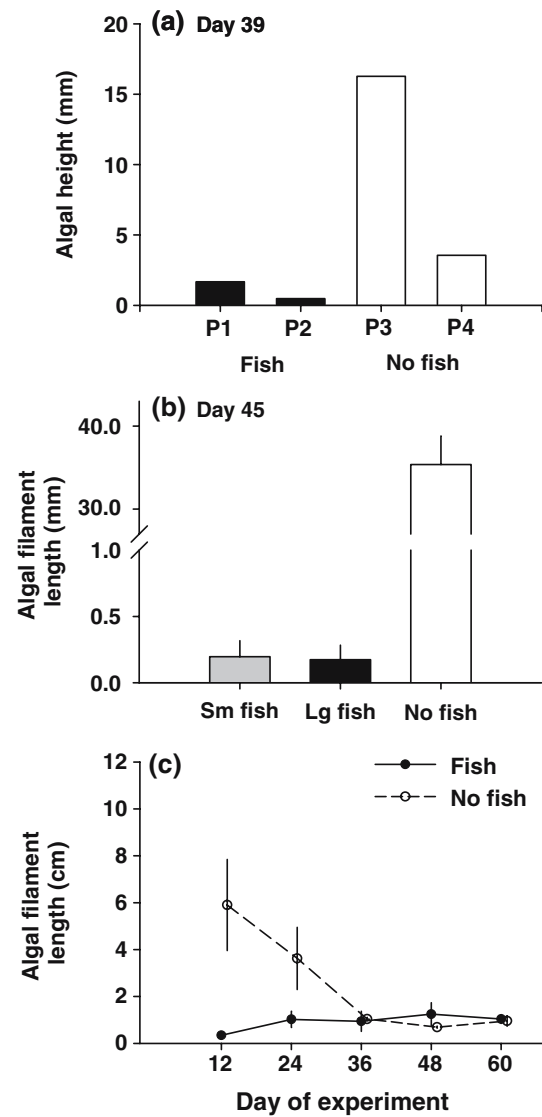


Fig. 4 **a** Algal height and **b**, **c** filament length (+1 SE) in **a** four Kings Creek pools in fall 2002 ($n = 2$) on day 39, **b** nine experimental stream riffles in fall 2002 ($n = 3$) on day 45, and **c** eight experimental stream pools in summer 2003 ($n = 4$) with (gray and solid bars and symbols) and without (open bars and symbols) fish. Each bar in **a** represents data recorded in one pool in Kings Creek (*P1*, *P2*, *P3*, or *P4*). Control (no fish open circle) data points in **c** are offset 1 day later to prevent overlap. For other abbreviations, see Fig. 1

2003, we also did not find a significant effect of fish or a day \times fish interaction on invertebrate assemblage structure (first axis eigenvalue 0.06, $F = 7.44$, $P = 0.26$; Fig. 6). In the four most abundant taxa, we found that *Bosmina* sp. and copepods decreased slightly where fish were present (*Bosmina* sp. density was 16% lower and copepod density was 90–95% lower in fish treatments but only on days 54 and 65), whereas oligochaetes and chironomids increased slightly (oligochaete density was 2% higher in the presence of

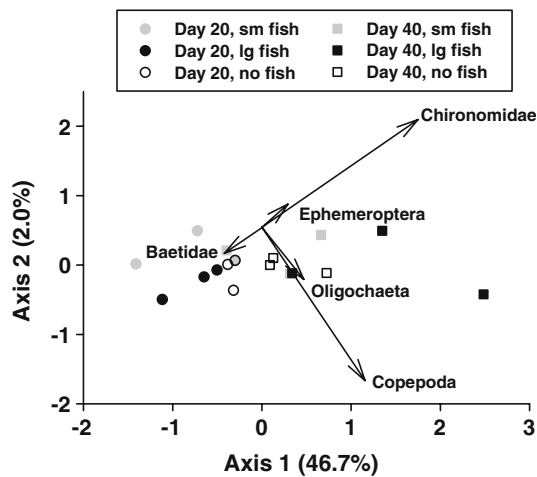


Fig. 5 Redundancy analysis (RDA) ordination of invertebrate assemblage structure data with and without fish on day 20 (circles) and 40 (squares) in nine experimental streams in fall 2002. Small fish are represented by gray symbols; large fish by solid symbols and controls (no fish) are represented by open symbols. Plotted vectors are dominant invertebrate taxa. Cumulative percent variation explained by each axis given in parentheses

fish and chironomid density was 7% higher in the presence of fish). Using repeated measures ANOVA, the only significant effect of *Phoxinus* on individual taxa densities was a day \times fish interaction on density of copepods ($F_{6,11.5} = 6.86$, $P < 0.01$).

Particulate organic matter

In the experimental streams in fall 2002, there was no significant difference in the relative mass of POM

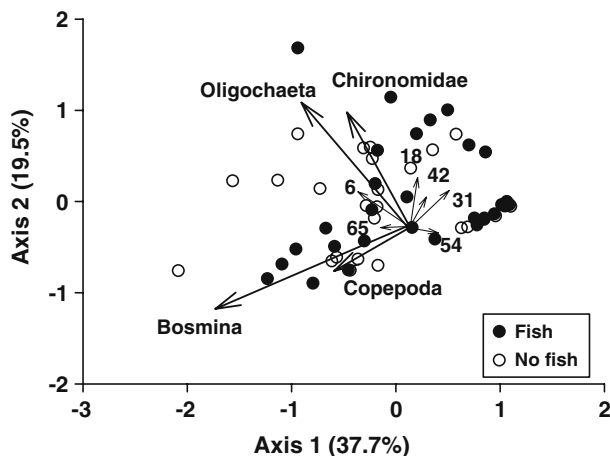


Fig. 6 RDA ordination of invertebrate assemblage structure data with (filled circle) and without (open circle) fish in eight experimental streams in summer 2003. Vectors plotted in bold are dominant invertebrate taxa, and numbered vectors indicate day of experiment. Cumulative percent variation explained by each axis given in parentheses

among the two fish treatments and the control for any size fraction. However, *Phoxinus* treatments had a greater proportion of the 98- to 1- μ m size fraction than no fish treatments across sample days in summer 2003 (Fig. 7). POM $>500 \mu$ m also was greater in the no fish treatments, but this effect diminished by day 42. Although there was a significant day \times fish interaction for POM 180–250 μ m, there was not a consistent temporal trend that would indicate an effect of the fish treatments.

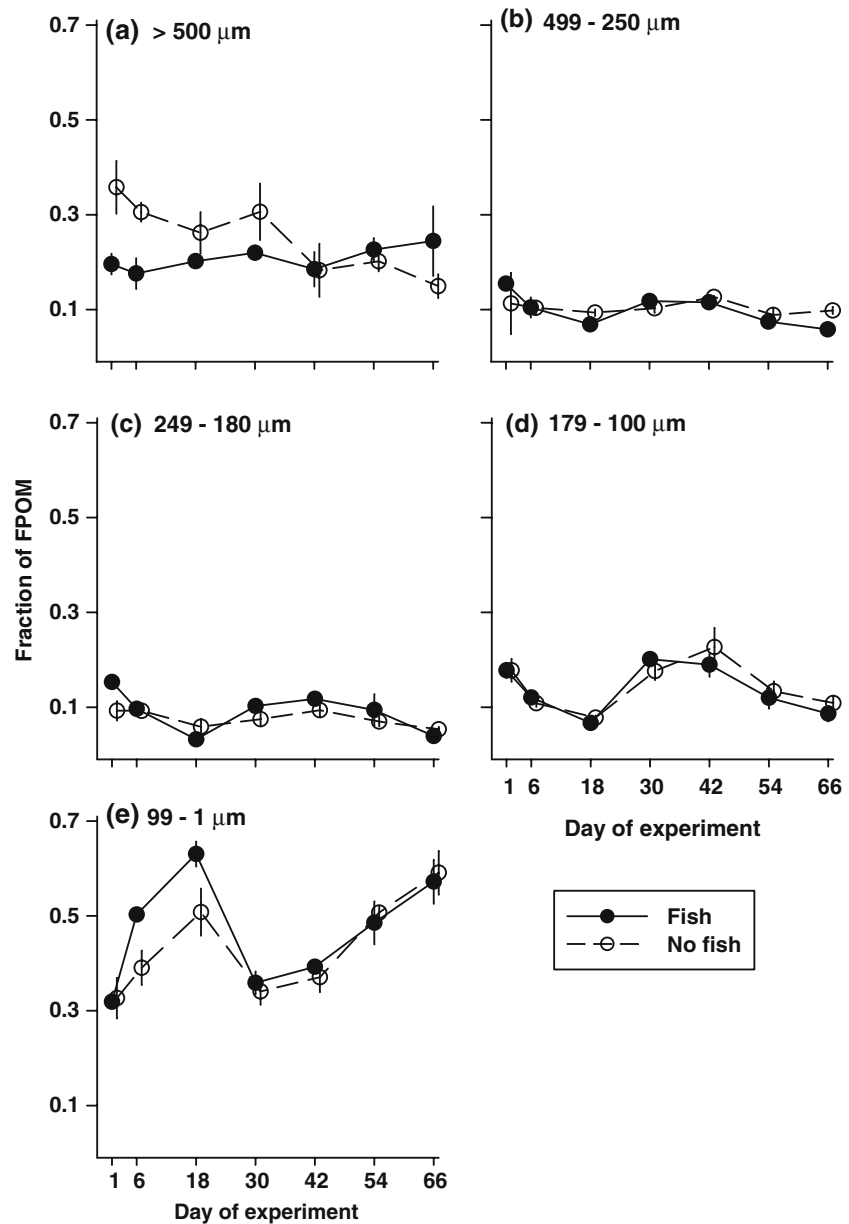
Diet

Phoxinus consumed diatoms and filamentous green algae in both the fall 2002 and summer 2003 experiments. We noted that one-third of the individuals examined had consumed some animal matter, but diatoms and filamentous green algae largely dominated gut contents. In fall 2002, there was no significant difference ($t_4 = 0.55$, $P = 0.30$) in the ratio of filamentous green algae to diatoms consumed by small and large *Phoxinus*; overall, diet consisted of approximately 2 times more filamentous green algae than diatoms. In summer 2003, *Phoxinus* ingested 5.4 times more filamentous green algae than diatoms. This was not significantly different ($t_7 = 0.26$, $P = 0.40$) from the ratio of available filamentous green algae to diatoms (i.e., 5.5 times more filamentous green algae than diatoms) measured from core samples.

Discussion

Results from our experiments suggest that *Phoxinus* affected algal filament length, but had negligible effects on other aspects of ecosystem structure and whole stream primary productivity. Specifically, grazing by *Phoxinus* did not change GPP or NPP in the mesocosm studies, even though significantly shorter mean algal filaments in all three experiments distinctly characterized grazing treatments. Whereas the relative abundance of major algal taxa was not significantly affected by the presence of *Phoxinus* in the summer 2003 experiment, the structural changes in the periphyton (i.e., decreased algal filament length and increased proportion of the smallest size fraction of POM) were apparent. In Kings Creek, algal filaments in exclosures grew markedly after the initial removal of *Phoxinus*, so much so that when juvenile *Phoxinus* and other grazing organisms (e.g., *Campostoma*) invaded, those fish were unable to crop the long filaments. Similarly, in summer 2003 the ability of *Phoxinus* to control long algal filaments disappeared by day 36, further suggesting that

Fig. 7 Mean fraction (± 1 SE) of total fine particulate organic matter (FPOM) in eight experimental stream pools with (filled circle) and without (open circle) fish in five size classes: **a** $>500 \mu\text{m}$ (day \times fish, $F = 2.74$, $P < 0.05$), **b** $499\text{--}250 \mu\text{m}$, **c** $249\text{--}180 \mu\text{m}$ (day \times fish, $F = 2.71$, $P < 0.05$), **d** $179\text{--}100 \mu\text{m}$ (day, $F = 7.09$, $P < 0.01$), and **e** $99\text{--}1 \mu\text{m}$ (day, $F = 16.37$, $P < 0.01$; fish: $F = 12.47$, $P < 0.05$) in summer 2003. Control (no fish open circle) data points are offset 1 day later to prevent overlap



moderate densities of *Phoxinus* may not be able to maintain short algal turfs once long filamentous forms become established. Concordantly, the proportion of organic matter in the smallest size fraction increased in the presence of *Phoxinus* until day 36, likely a function of *Phoxinus* maintaining short algal turfs. Grazers typically reduce mean algal filament length (Power and Matthews 1983; Gelwick and Matthews 1992; Liess and Hillebrand 2004), but this structural change is usually linked with an increase in the relative abundance of adnate diatoms and turf-forming cyanobacteria in the grazer treatments, as was reported for *Campostoma* (Power et al. 1985, 1988; Gelwick and Matthews 1992). In contrast, *Phoxinus* grazing replaced long algal fila-

ments that dominated the assemblage with more abundant, shorter filaments.

Although we were unable to measure GPP or NPP in Kings Creek, we assume rates of primary productivity in the experimental streams reflect processes that occur in a natural stream for several reasons. First, observations of reduced algal filament length and no change in algal biomass between fish treatments and controls in experimental streams were consistent with observations from field studies. Second, measurements of GPP and NPP in experimental streams are comparable with published estimates from Kings Creek. Finally, there was a significant positive association between irradiance and GPP ($r = 0.57$, $P < 0.01$),

which indicates our measurements of GPP in the experimental streams were sensitive to factors known to influence photosynthetic rates. However, ecosystem metabolism may typically be more heterotrophic ($P:R$ ratio = 0.75; Webster et al. 2003) and NPP slightly lower [-0.01 to -0.19 g O_2/m^2 h (O'Brien 2006)] in Kings Creek than in the experimental streams, because the experimental stream studies began at an early successional stage in the algal assemblage compared to that in Kings Creek. In the experimental streams, mean NPP was 0.12 g O_2/m^2 h in fall 2002 and -0.08 g O_2/m^2 h in summer 2003.

It is important to note differences in algal and invertebrate assemblage structure between Kings Creek and the experimental streams. The algal assemblage during the Kings Creek field study was in a late successional stage because the experiment was conducted during an interval with little precipitation and no flooding disturbances. In contrast, the experimental stream studies represented earlier successional stages of algal assemblage development. The temporal switch from net autotrophy to net heterotrophy during both mesocosm experiments illustrates this development of the algal community. Invertebrate grazers that could colonize by drift or over longer time scales were excluded from the experimental streams. Although lower invertebrate richness and nutrient limitation are potentially important components of ecosystem structure and function, the consistent effect of *Phoxinus* on structural components of the ecosystem in both the natural and experimental streams suggests our stream mesocosms reflect major processes occurring in the natural stream.

There are several explanations for the lack of a difference in productivity, algal biomass (chlorophyll *a*), and algal assemblage structure among treatments in the experimental streams. First, the densities at which we stocked *Phoxinus* may not have been high enough to significantly reduce biomass or productivity because the loss of algal cells by consumption may have been offset by increased production of remaining algal cells. Nevertheless we feel the densities at which we stocked *Phoxinus* in the experimental streams (6.8 fish/ m^2) was within the range of natural densities in Kings Creek (0.2 – 14.3 fish/ m^2), this was higher than densities in previous studies of *Camptostoma* that reported an effect on structure and function (mean density = 3.87 ; range density 0.4 – 10 fish/ m^2). Thus, the lack of effect on the measurements was likely not due to low densities of *Phoxinus* in our experiments.

Second, at moderate densities, mechanical removal of algae by grazers may stimulate algal growth by increasing basal regeneration or mucilage secretions of

algae (Power et al. 1988), reducing shading, and increasing the rate of material transport across the boundary layer (Mulholland et al. 1991). In addition, excretion of limiting nutrients can further stimulate algal growth; assuming nutrient turnover by fish is great relative to the nutrient loading to the system (Vanni 2002). In the experimental streams, low nutrient concentrations in our water supply limit algal growth (K. B. Guido, unpublished data), thus excretion by fish could increase availability of soluble nutrients. For example, in summer 2003 total N loading to experimental streams was estimated at 51.1 mg N/m^2 day, assuming an average inflow of $1,728$ l/day and mean total N concentration of 99.9 ± 17.5 $\mu g/l$ from inflow. Estimates of nutrient excretion by *Phoxinus* based on rates published for other stream fishes (Vanni 2002) range between 3 and 10 μmol N/g fish per h, which would yield a daily excretion rate between 13.4 and 44.8 mg N/m^2 per day, or 26 and 87% of the daily loading of N from spring water. These data suggest nutrient turnover by *Phoxinus* may play an important role in offsetting consumptive losses to grazing in these experiments.

Third, in the absence of *Phoxinus*, abundance of other grazers (i.e., fishes, crayfishes, snails, and insects) may have increased resulting in no difference between fishless controls and fish treatments. However, we found no measurable effect on the invertebrate assemblage among treatments in experimental streams, as the densities of invertebrates and species composition among grazing treatments were similar (e.g., between 50 and 70% of species were shared among treatments). This result was not surprising given the weak effect of *Phoxinus* on other measures of system function and structure. That is, if exploitative or interference competition was intense, we also would have expected a decrease in the abundance of grazing invertebrates in *Phoxinus* treatments. Nevertheless, the presence of *Phoxinus* could have altered the behavior of grazing insects such that their grazing rates were higher in the no fish treatments (Vaughn et al. 1993; Peckarsky et al. 2001; Alvarez and Peckarsky 2005).

The inability of *Phoxinus* to alter area-specific GPP or NPP across the mesocosm experiments stands in contrast to grazing effects reported for other aquatic grazers (e.g., Hillebrand 2002) and for *Camptostoma* (Stewart 1987; Gelwick and Matthews 1992), which decrease NPP/ m^2 and increase NPP/g of benthic algae. However, there were several factors limiting a comparison of our results to studies of *Camptostoma*. In particular, the spatial scale over which productivity was measured could strongly influence the effects of a

grazer, as measurements over larger scales (e.g., entire pools) are likely to be less sensitive to grazer effects, particularly if the system has a higher relative abundance of heterotrophs (e.g., the stream is net heterotrophic). Production estimates reported in studies of *Campostoma* were based on artificial (i.e., ceramic quarry tiles; Stewart 1987) or natural (i.e., limestone cobbles; Gelwick and Matthews 1992) substrates placed in enclosed containers, whereas our measurements reflected metabolism of an entire mesocosm. Enclosed chamber measurements may be more sensitive to grazing effects because uptake rate of nutrients by the attached algae from the water column is influenced by a smaller amount of water relative to the amount of substratum and a lack of water movement within the chamber (Uehlinger and Brock 1991; Carpenter 1996; Bott et al. 1997). Moreover, whole stream estimates of productivity encompass a broader range of habitats (e.g., deeper hyporheic zone) and might mask the effects of grazers, which are likely most intense at the substrate–water interface. Finally, surface heterogeneity of substrates placed in enclosures also could be an important source of bias, as Evans-White and Dodds (2001) found *Campostoma* prevented accumulation of algal biomass (chlorophyll *a* concentration) on artificial tile substrates but not on natural pebbles in experimental channels.

The ability of grazers to affect autochthonous primary productivity is particularly important for prairie streams in which allochthonous organic matter contributions are relatively low. Whereas, our experiments suggest that *Phoxinus* at moderate densities affected some aspects of ecosystem structure, they did not change algal biomass or primary productivity. Because these results contrast with studies of other grazing organisms, it will be important to evaluate if these differences were due to differences in interaction strengths among organisms or experimental design, as most tests of grazer effects were conducted at fine spatial and temporal scales. Whereas, finer scale measurements of productivity and algal biomass help identify the mechanisms through which grazers affect ecosystem properties, tests of species effects at the scale of natural stream pools or reaches will provide a more comprehensive assessment of the role of these organisms in ecosystems.

Acknowledgements Angie Lickteig, Nate Franssen, and Mike Peterson assisted in the field and laboratory on all three of the experiments. Jeff Falke, Layne Knight, and Nate Franssen helped maintain experimental streams and conduct experiments. This manuscript was greatly improved by the comments and suggestions of Walter Dodds, Nate Franssen, Joel Trexler, and two anonymous reviewers. Jackie Howe assisted with the sum-

mer 2003 experiment and data entry. Funding was provided by Konza Prairie Long-Term Ecological Research Program, Kansas Experimental Program to Stimulate Competitive Research, and the National Science Foundation, (DEB-0416126).

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