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Reach-scale manipulations show invertebrate grazers depress algal resources in streams

Abstract—Experimental tools that enable manipulations of organisms at larger scales allow for comparisons of processes across multiple spatial scales and expand our ability to make predictions about ecological processes. We performed reach-scale (i.e., 50 m^2) manipulations of invertebrate communities in streams using a modified electroshocking technique to non-destructively remove invertebrates. In addition, we conducted a microcosm experiment (i.e., 157 cm^2) with different grazer densities that enabled comparison of the strength of grazer–algal interactions at large and small spatial scales. In high-elevation headwater streams, electroshocking reduced total invertebrate abundance by 84% in a 50-m^2 reach of stream. Although mobile invertebrates recolonized the manipulated area rapidly, daily electroshocking maintained the density reduction. Electroshocking reduced the density of herbivorous invertebrates 86%, which resulted in a 57% increase in algal biomass, whereas in a stream that was not electroshocked, invertebrate density and algal biomass changed much less, only 16 and 8%, respectively. Comparison of grazer effect on periphyton between microcosm and reach-scale experiments revealed that the per capita interaction strength of grazers on primary producers was three times greater in the reach-scale manipulation than that observed in the microcosm experiments. Reach-scale manipulation of invertebrate grazers in streams provides a powerful method to experimentally test patterns observed in the field at a large spatial scale, with more realism than streamside microcosms or small cages in streams.

One of the major goals of ecology is to understand patterns and processes in complex, natural systems. This complexity usually requires simplifying the system to a few species and a small spatial scale to test a specific hypothesis. Experiments conducted at a smaller spatial scale and reduced complexity have been termed microcosm experiments, and their utility and interpretation have spawned considerable debate (Carpenter 1996; Drenner and Mazumder 1999). This healthy debate has generated a consensus that the scale of the experiment must be appropriate for the question of interest; the most informative approach often involves experiments at multiple scales (Cooper et al. 1998; Resetarits and Bernardo 1998; Huston 1999).

Large-scale biotic manipulations in streams are necessary to quantify how the biota affect ecological processes that operate over larger scales. Yet, the ability to perform field experiments at large scales remains a challenge, especially in streams where high immigration rates of invertebrates from upstream sections make maintaining treatments difficult (Allan 1982). This constraint often requires that entire streams be manipulated (Wallace et al. 1991). However, there may be ethical, logistical, and practical problems of manipulations at this scale, making them impossible at many study locales. As a result, much of what we know about interactions between stream invertebrate consumers and their resources come from experiments in streamside microcosms and in situ cages. We do not advocate abandoning the small-scale experimental approach but see small- and large-scale experiments as complementary approaches that increase the strength of inference and extrapolation across spatial scales.

Although these small-scale experiments have increased our understanding of causal mechanisms, they often lack relevance to natural systems (Schindler 1990). In other words, we do not know if these same patterns and processes are strong enough to operate within a natural background of abiotic and biotic factors. Existing techniques for manipulating invertebrates use enclosures or exclusions constructed of fine mesh that are size limited and often have associated artifacts (e.g., cage effects; Hulberg and Oliver 1980; Peckarsky and Penton 1990). In a recent review, only 6 of 100 studies used a large-scale experimental approach to examine algal–grazer interactions (Feminella and Hawkins 1995). Therefore, much of the information on algal–grazer interactions in actual streams at large scales is correlative. To advance our understanding of how invertebrates and ecosystem structure and function are linked, we need a method that enables us to experimentally manipulate invertebrates at large scales, in natural streams, with minimal experimental artifacts.

Here, we present a new approach for performing reach-scale manipulations of stream invertebrates that enabled non-destructive removal of invertebrates by electroshocking a 50-m-long section of stream. Other recent studies have used electricity to exclude decapods (Phillips and Scolaro 1980; Pringle and Blake 1994), invertebrates (Brown et al. 2000), and to sample stream invertebrates (Fièvet et al. 1996; Rabeni et al. 1997; Taylor et al. 2001). This study expands on previous work by asking the following questions. (1) Can invertebrate densities be reduced at a large spatial scale (42–55 m^2) using electroshocking? (2) How long will reduced invertebrate densities persist? (3) If a reduction of invertebrate grazers can be maintained, will there be a subsequent increase in primary producer biomass? (4) How do grazer effects on periphyton vary with spatial scale?

Materials and methods—Study site: The experiments were performed in streams near the Rocky Mountain Biological Laboratory (RMBL), a high-altitude field station located in the East River watershed 13 km north of Crested Butte in western Colorado. The two study streams, Avery and Marmot, were selected based on their proximity to one another, similar size, aspect (western slope), substrate, and elevation (Table 1). Invertebrate assemblages of both streams were characterized by Ephemeroptera (mayflies), Plecoptera (stoneflies), Diptera (true flies), and Trichoptera (caddisflies).

Electroshocking and sampling techniques: We manipulated invertebrate density by inducing invertebrate drift (entry into the water column), using electrical current (500–700 V DC; 6 ms pulse width; 60–80 pulses s^{-1}) applied over a large area (42–55 m^2) with a generator-powered backpack electroshocker equipped with a circular 10-cm anode (Model 15-

Table 1. Physical and biological characteristics of the treatment (Avery Creek) and reference (Marmot) streams during July–September, 2000.

Variable	Treatment stream	Reference stream
Elevation (m)	2,940	2,950
Discharge (L s^{-1})	120	109
Velocity (cm s^{-1})	71.2	62.1
Depth (cm)	15.5	17.0
Width (m)	1.40	1.00
Conductivity (μS cm^{-1})	177	286
PO_4–P (μg L^{-1})	4.40	1.20
NO_3–N (μg L^{-1})	19.3	67.4
NH_4–N (μg L^{-1})	1.60	0.20

C, Smith-Root). To minimize physical disturbance of the stream bottom, we electroshocked from the stream bank and did not disturb the substrate with the anode. To prevent drift into the treatment area from upstream while electroshocking, we placed a 280-μm mesh blocknet at the upstream end of the reach. After three consecutive passes were made with the electroshocker, the upstream blocknet was removed to allow natural movement of invertebrates within the stream.

Invertebrates and periphyton were sampled using a stratified random sampling design. Each reach was divided into 5-m-long sections with flagging, and four to six benthic samples were taken randomly with a Hess sampler (0.09 m^2), mesh size 202 μm. Hess samples were taken from the streambank to ensure minimal disturbance to the study reach. Invertebrates were preserved in 95% ethanol, stained with Rose Bengal, and identified to genus and species in the laboratory. Chlorophyll *a* (Chl *a*) was measured by extracting 10 rocks in 90% ethanol for 24 h and measuring absorbance on a narrow bandwidth spectrophotometer using the equation for 90% ethanol extractions (Nusch 1980). Rock area was estimated using a leaf area meter to estimate the two-dimensional surface area from paper tracings.

Pulsed treatment with electricity: On 9 July 1999 we determined whether electricity reduced invertebrate abundance by comparing the benthic density of a 30-m section of Avery Creek (mean width = 1.4 m, total area = 42 m^2) to the benthic density of an upstream 30-m reference reach (mean width = 1.3 m, total area = 39 m^2). Before shocking, the initial abundance of invertebrates was measured using four Hess samples taken (as above) from each reach (mean width = 1.4 m, total area = 42 m^2). Then we placed a blocknet at the upstream end of the treatment site and made three consecutive passes downstream with the electroshocking unit (mean time $pass^{-1}$ = 20 min). To verify that invertebrates were drifting while the electricity was on, another 280-μm mesh blocknet was placed at the downstream end of the reach. Invertebrates caught in the net were returned to the stream alive, downstream of the study area. Immediately following electroshocking, we took four additional invertebrate samples to measure the effect of electricity on benthic density. To examine recolonization, we sampled invertebrates 1 and 5 d after electroshocking. In the reference reach, we controlled for the possibility that our activity, and not electricity, induced drift by passing the anode over the stream bottom with the electricity off for a similar amount of time.

Pressed treatment with electricity: To determine whether reach-scale removal of consumers affected the biomass of primary producers (estimated as Chl *a*), we repeatedly electroshocked a 50-m reach (mean width = 1.1 m, total area = 55 m^2) of Avery Creek to maintain low densities of grazers for 12 d in 1999. We repeated the treatment daily because of the quick recolonization time we observed in the pulsed treatment. We compared the temporal patterns of abundance of benthic invertebrates and algal biomass in the treatment reach to those of a reference section in Marmot Creek (mean width = 0.8 m, total area = 47 m^2). Density of invertebrates was measured three times before (August) and three times during (September) the manipulation, and algal biomass was measured two times before and two times during the treatment period in both streams. We treated the reference stream similarly, but with the electroshocking unit off.

Algal–grazer interactions in microcosms: We compared the effect of invertebrate grazing on periphyton in our reach-scale manipulation to effects of grazers under simplified conditions at a small spatial scale, using data from an experiment conducted in streamside flow-through microcosms (157 cm^2) (illustrated in Peckarsky and Cowan 1991). In July 1998 we added four densities (0, 319, 637, 1,275 m^{-2}) of the dominant mayfly grazer, *Baetis bicaudatus* (Ephemeroptera), to microcosms with 10 rocks with a mean size of 33.7 $\pm$ 0.59 (mean cm^2 $\pm$ 1 SE), from the East River to microcosms (N = 10 per density treatment). Rocks were scrubbed with toothbrushes before the experiment to standardize initial levels of periphyton. One entire rock was extracted for Chl *a* analysis from each microcosm at 2, 4, 9, 14, and 16 d.

Per capita interaction strength: To compare the magnitude of invertebrate grazer influence on periphyton in the microcosm experiment and the reach-scale manipulation, we calculated per capita interaction strength as follows.

$$\mathrm{DI} = [\ln(G/R)]/Dt \quad (1)$$

DI is the dynamic index of per capita interaction strength (e.g., Wootton 1997; Berlow et al. 1999), G is chlorophyll with grazers present, R is chlorophyll with no (or reduced) grazers, D is density of grazers, and t is duration of the experiment. As expressed here, the dynamic index measures the effect of grazers on chlorophyll as a function of invertebrate density. The units of DI are fractional loss of chlorophyll per unit change in invertebrate density (i.e., slope of line relating grazer effect to density). For the reach-scale manipulation, we used the reference stream chlorophyll values for R, which were similar to the chlorophyll levels in the no grazer treatment of the microcosm experiment. In addition, to calculate the reach scale per capita interaction strength, we used the density of invertebrate grazers only and excluded predatory and filter-feeding taxa. Per capita interaction strength standardizes for invertebrate abundance and chlorophyll so that the per capita effect of grazers on periphyton could be compared between the two scales and experiments. Measures of interaction strength are appealing

because they are expressed as a common metric, so the relative importance of an interaction can be compared among species or experiments.

Because replication was not feasible for the reach-scale experiment, inferential statistics were not used (Hurlbert 1984). Although a recent response to Hurlbert's comments on the use of inferential statistics in unreplicated designs advocates reporting *P*-values out of courtesy to the reader (Oksanen 2001), temporal autocorrelation inherent in such designs can artificially inflate the probability of finding significant results (Stewart-Oaten et al. 1992) and thereby mislead the reader. Variability around the means for each sample date are reported as standard deviations, not standard errors, to emphasize that this variation cannot be used to assess treatment effects. Effects of the electroshocking manipulation were assessed by comparing the magnitude of temporal change in the treatment stream compared to the temporal change in the reference stream, not by comparing differences between the reference and treatment streams. For the microcosm experiment, we natural log–transformed the Chl *a* values to linearize the relationship with time, tested for homogeneity of slopes (density by time interaction: $F_{12,39} = 1.23$, $P = 0.29$) and then tested for main effects of density and time as continuous variables in a multiple regression model.

Results—Pulsed treatment with electricity: Total invertebrate abundance decreased by an order of magnitude immediately following electroshocking in our initial test (Fig. 1). The fine mesh blocknet placed at the downstream end of the treated section contained few invertebrates prior to shocking but had thousands of invertebrates following shocking, indicating invertebrates were drifting out of the study area. The striking decline in total invertebrates was driven by a pronounced decrease in the most abundant group, Ephemeroptera (*Baetis bicaudatus, Cinygmula* sp., and *Rhithrogena robusta*). Plecoptera, Diptera, Trichoptera, and Oligochaeta combined into the group ''other taxa'' also decreased, but the magnitude of their decline was much lower. The highly mobile mayfly, *Baetis*, recolonized the treated area rapidly (~180 individuals m^{-2} d^{-1}), and densities returned to initial levels in <5 d.

Press treatment with electricity: Total invertebrate abundance decreased by 84% in the treatment stream immediately following our initial electroshocking and remained at this level during the 10-d treatment period (Fig. 2). The decrease in abundance varied in magnitude among taxonomic groups, with the most abundant taxa, mayflies, declining the most. Stoneflies, dipterans, trichopterans, and oligochaeta combined into the group ''other taxa'' also declined but the magnitude of response was much lower. Invertebrate assemblages in the reference stream changed less and in the opposite direction, increasing 16% (Fig. 2).

Periphyton response to pressed treatment: Chlorophyll displayed different temporal trends in the treatment and reference streams. In the treatment stream, chlorophyll levels were low (mean = 9.5 mg Chl *a* m^{-2}) prior to invertebrate removal but increased substantially following invertebrate removal (mean = 22.2 mg Chl *a* m^{-2}). In contrast, in the adjacent reference stream premanipulation chlorophyll levels (mean = 52.2 mg Chl *a* m^{-2}) and postmanipulation levels (mean = 48.0 mg Chl *a* m^{-2}) changed much less over the study period. As a result, Chl *a* in the treatment stream increased 57% during the treatment period, whereas Chl *a* in the reference stream decreased slightly 8.0% (Fig. 3).

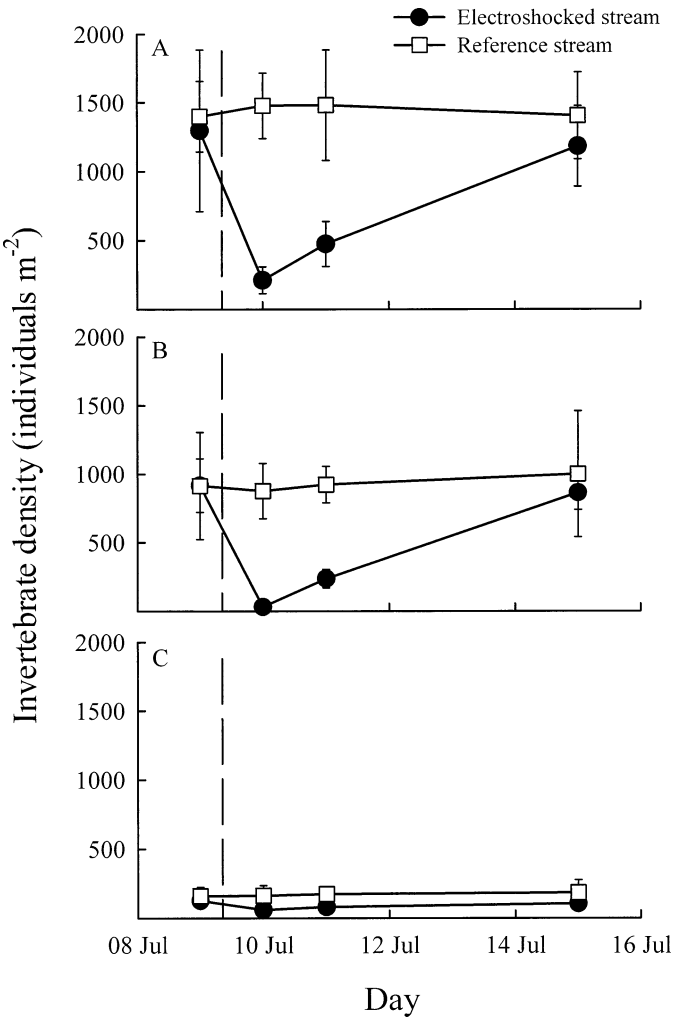

Fig. 1. After electroshocking a 30-m reach on 10 July 1999, total invertebrate density (A) decreased one order of magnitude, mainly because of a decrease of the most abundant group, Ephemeroptera (B). Less abundant invertebrates included in the group ''other taxa'' (C)—composed of Plecoptera (11%), Diptera (13%), Oligochaeta (4%), and Coleoptera (1% of total invertebrate abundance)—declined, but the relative magnitude was less. Temporal invertebrate abundance did not change in the reference reach upstream that was not electroshocked. The dashed vertical line indicates the day of electroshocking. Values are means ± 1 SD of four Hess samples.

Periphyton response to grazing in microcosms: Chlorophyll *a* stayed very low for the first week in microcosms containing all densities of *Baetis*. After day 9, Chl *a* diverged, with twofold higher levels in microcosms with no *Baetis* than with *Baetis* present (Fig. 4). This experiment

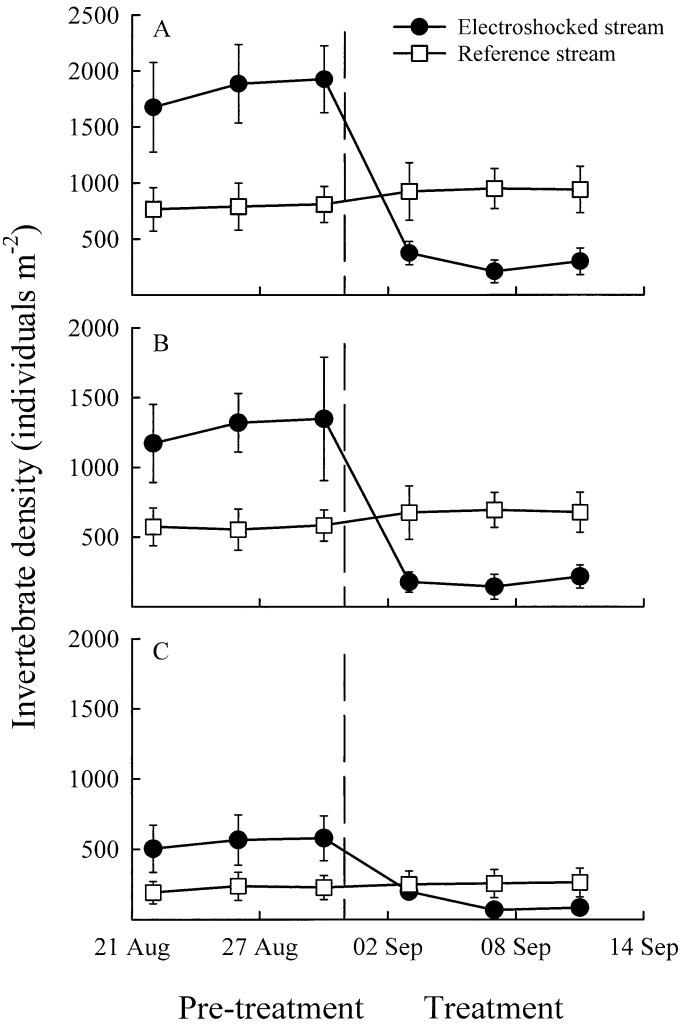

Fig. 2. Total invertebrate abundance (A) decreased 84% and was maintained following daily electroshocking in the treatment stream (50 m long). The magnitude of the decrease in abundance was primarily due to Ephemeroptera (B). There was little decline in the less abundant taxa included in the group ''other taxa'' (C). Electroshocking began on 31 August 1999 (indicated by the dashed vertical line) and was repeated each day until 13 September 1999. There was relatively little change in abundance in the reference stream reach that was not electroshocked. Values are means ± 1 SD of six Hess samples.

demonstrated that as *Baetis* density increased, Chl *a* decreased ($F_{3,51} = 7.85$, $P = 0.0002$).

Comparing interaction strength across scales: Per capita interaction strength of invertebrates on chlorophyll was significantly greater for the reach-scale manipulation than for the microcosm experiment (Fig. 5) ($F_{1,4} = 40.13$, $P = 0.0032$ for the density × experiment interaction term). Because the two experiments were conducted for approximately the same number of days, time-dependent effects on interaction strength were unlikely (Berlow et al. 1999).

Discussion—Invertebrate abundance decreased following electroshocking of a large area of stream. A single pulse perturbation with electricity reduced invertebrate abundance by an order of magnitude, but mobile invertebrates recolonized the 42-m² area rapidly (within 5 d). However, by electroshocking daily, a 10-fold reduction of invertebrate abundance was maintained for 14 d. This reduction in mostly herbivorous consumers was followed by a subsequent increase in periphyton, which we attribute to a release from herbivory.

Given that we sacrificed inferential statistics and replication for realism and experimentation at a large spatial scale, we must address the possibility that changes in invertebrate and periphyton abundance occurred by chance or by some other mechanism. We are confident in attributing the 84% decrease in invertebrate abundance to the treatment, because we have never observed comparable temporal variation in invertebrate

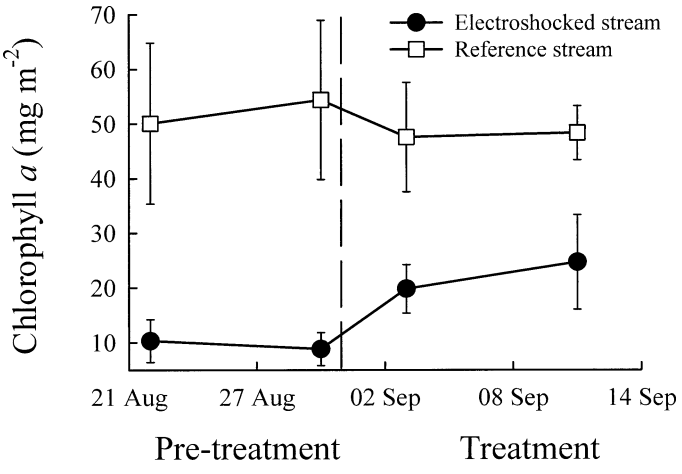

Fig. 3. Following the reduction of invertebrates, Chl *a* increased 57% in the treatment stream compared to an 8% decrease in the reference stream. Values are means ± 1 SD of 10 rocks for each sample date.

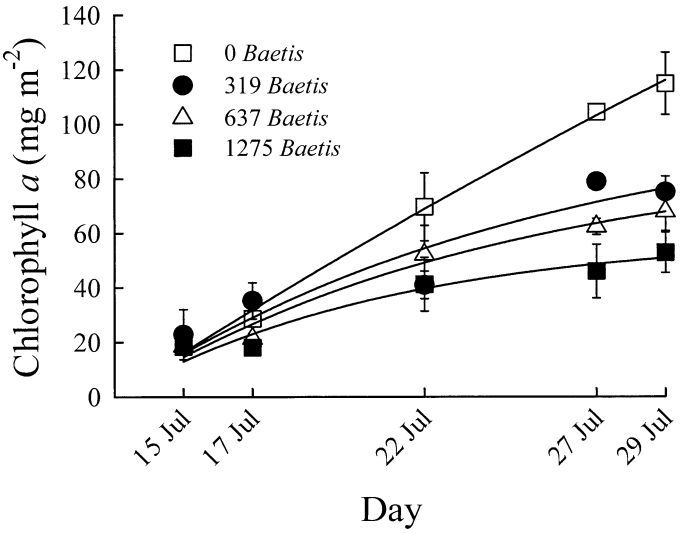

Fig. 4. The effect of four *Baetis bicaudatus* densities (i.e., 0, 319, 637, and 1,275 individuals m^{-2}) on periphyton levels measured from small flow-through microcosms receiving natural stream water. Values are means ± 1 SE of 10 rocks from replicate microcosms.

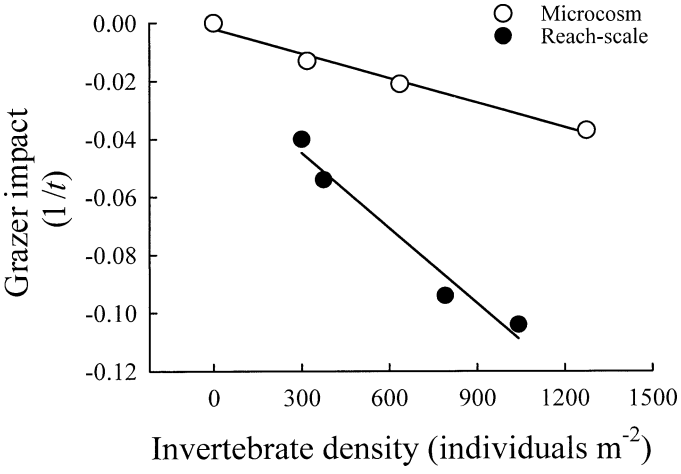

Fig. 5. Grazer effect on periphyton as a function of invertebrate density was stronger in the reach-scale experiment than in the microcosm experiment. The per capita interaction strength (or dynamic index) is the slope of the line ($m = -0.00009$ and $m = -0.00003$ for reach-scale and microcosm experiments, respectively) regressing $\ln(G/R)$ against invertebrate density (D). Thus, the fractional loss of chlorophyll per unit time is $1/t$. Reach-scale invertebrate densities include grazing invertebrates only (i.e., predatory and filter-feeding taxa were not included).

abundance, even after high discharge events (Peckarsky 1991; Peckarsky et al. 2001). Moreover, using a smaller scale but replicated design, we have shown previously that electroshocking effectively removed 90% of invertebrates from the substrate (Taylor et al. 2001). Previous studies have shown that short-term electricity does not influence Chl *a* (Pringle and Blake 1994; Brown et al. 2000). Thus, we can think of no alternative explanations for the observed 57% increase in periphyton other than reduction of grazer densities.

The effects of invertebrates on periphyton in both the small- and large-scale experiments were consistent with the strong herbivore–algal link observed in many other systems (Lamberti et al. 1989; Feminella and Hawkins 1995; Steinman 1996). At both scales, periphyton increased when grazing invertebrate density was reduced. Interestingly, the per capita effect of grazers was greater at the reach scale in the natural system. This difference may be explained in part because the small-scale experiments were performed with only one species of grazer, whereas the reach-scale manipulation affected the entire invertebrate assemblage. The grazer we used in the microcosms, *Baetis bicaudatus,* constituted 90% of the grazer abundance in the reach-scale experiment but were smaller in size and were also in the presence of competitors. However, interaction strength calculated as per capita or per unit biomass showed that the larger effect observed in the reach-scale experiment was not simply a function of higher invertebrate biomass. In fact, the disproportionately stronger effect in the reach-scale experiment suggests that grazer traits, interactive effects, or differences in algal communities, singly or in combination, may be important determinates of algal–grazer interactions.

Kohler and Wiley (1997) reported similar variations in effect size with experimental scale in their comparison of small- and large-scale effects of *Glossosoma* density on periphyton. They found the direction of response for small- and large-scale experiments was similar, but the grazer effect was underestimated by small-scale experiments. Although a few other studies have demonstrated reach-scale removal of invertebrates and subsequent increases in periphyton, these required application of pesticides (Yasuno et al. 1982) or serendipitous events such as pathogen outbreaks (Kohler and Wiley 1997) to reduce grazing invertebrates. Our electroshocking technique is unique because it allows deliberate manipulation of invertebrates in a natural stream reach.

Most studies that have demonstrated a reduction in algal biomass due to grazers have used taxa that are large bodied and slow moving and that drift little (e.g., caddisflies or snails), because they are relatively easy to manipulate (e.g., McAuliffe 1984; Lamberti et al. 1989). In streams near RMBL, there are no snails, and grazing caddisflies (i.e., Glossomatidae) are rare (Peckarsky 1991). Mayflies are the numerically dominant grazing invertebrates and are highly mobile, making them difficult to manipulate. This electroshocking technique provides an effective method for field manipulation of mobile grazers at the reach scale. More importantly, this reach-scale manipulation reveals the importance of herbivores in regulating periphyton, even in the presence of physical factors that also influence periphyton distributions and abundance (Stevenson 1996).

Recovery of invertebrates to pretreatment abundance varied among taxa as a function of taxon behavior and effectiveness of removal. The fast recolonization rate of mayflies (*Baetis, Cinygmula,* and *Rhithrogena*) is related to their high mobility and their ability to actively enter the drift to colonize new food patches (Kohler 1985). Likewise, mayflies are easily induced to drift by electroshocking (Mesick and Tash 1980; Taylor et al. 2001). In contrast, stoneflies (included in ''other taxa'') were also reduced in abundance by electroshocking but had a low rate of recovery, probably because their natural drift rate is low (Rader 1997). Thus, this reach-scale removal suggests the importance of drift for invertebrate recolonization and movement, which agrees with results from other studies (Hershey et al. 1993; Matthaei et al. 1997). Furthermore, our measured recolonization rates support the hypothesis that fast exchange rates are one reason why effects of predators are often obscured in stream studies, even at large scales (Allan 1982; Cooper et al. 1990; Sih and Wooster 1994).

Several factors may affect the efficacy of the electroshocking technique, including water velocity, substrate type, conductivity, and invertebrate assemblage (*see* Taylor et al. 2001). For instance, this technique may not work well in slow-flowing streams because flow is required to transport electroshocked invertebrates downstream and because both high ($>$1,000 μS cm^{-1}) and low (10 μS cm^{-1}) conductivity affects the passage of electricity through water to organisms. Finally, the electroshocking method may be taxon-specific in some streams. Some invertebrates are not induced to drift or do not drift easily (e.g., snails or cased caddisflies). These taxa will not be reduced in abundance by electroshocking. However, this inefficiency creates an opportunity to reduce the densities of certain groups (e.g., grazing mayflies) without affecting others.

A major challenge confronting ecological research is to

experimentally test hypotheses in a setting that resembles a natural system and at a relevant spatial scale (Cooper et al. 1998; Resetarits and Bernardo 1998). Many field manipulations have employed physical methods to exclude or enclose consumers, but these structures usually alter other factors (e.g., flow, detritus, and sedimentation), creating undesirable experimental artifacts (Cooper et al. 1990; Peckarsky and Penton 1990). Recent studies have shown that electrified hoops can be used for small-scale exclusions with no apparent artifacts (Pringle and Blake 1994; Brown et al. 2000). Our study shows that the use of electricity can also be extended for manipulations at much larger scales.

Finally, there is a growing interest in ecology to understand the linkage between species and ecosystem function. However, the processes governing ecosystem structure and function are best observed at large scales. Using electroshocking techniques to manipulate invertebrates in natural streams provides a powerful approach for examining the links between stream invertebrates and ecosystem function.

Brad W. Taylor[1]

Rocky Mountain Biological Laboratory
P.O. Box 519
Crested Butte, Colorado 81224
Department of Zoology and Physiology
University of Wyoming
Laramie, Wyoming 82071

Angus R. McIntosh

Rocky Mountain Biological Laboratory
P.O. Box 519
Crested Butte, Colorado 81224
Department of Zoology
University of Canterbury
Private Bag 4800
Christchurch, New Zealand

Barbara L. Peckarsky

Rocky Mountain Biological Laboratory
P.O. Box 519
Crested Butte, Colorado 81224
Department of Entomology
Cornell University
Ithaca, New York 14853

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[1] Corresponding author (btaylor@uwyo.edu).

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Quantification, base composition, and fate of extracellular DNA in marine sediments

Abstract—The discovery of high concentrations of DNA in marine sediments unaccounted for by living biomass suggests the presence of a large fraction of extracellular DNA, which might play an important role in gene transfer via natural transformation as well as in phosphorous biogeochemical cycling. But a universally accepted procedure for extracellular DNA extraction is not available yet. In this study, we developed a new nuclease-based procedure to extract extracellular DNA from marine sediments. Coastal sand and deep-sea mud samples were collected to test the efficiency of extracellular DNA removal from different sediment types. Extracellular DNA concentrations were quantified at six sediment depths, and changes in base composition were investigated to gather information on extracellular DNA fate. The extraction procedure was highly specific and only extracellular DNA was hydrolyzed after nuclease treatment. Hydrolyzable DNA accounted for <10 to $>70\%$ of the total DNA pool, suggesting that extracellular DNA can only be partially degraded. Base composition changed vertically with depth in the sediment as deoxycytidine content increased and deoxyadenosine decreased with increasing depth. Integrating our results for the top 15 cm of the sediment, we calculate that more than 50% of extracellular DNA was recalcitrant to enzymatic degradation. This finding might explain why DNA accumulates in surface sediments and suggests that DNA might play a nonnegligible role in P biogeochemical cycle.

Extracellular DNA is a ubiquitous component of both dissolved and particulate organic matter pools of freshwater, seawater, and benthic environments (Lorenz and Wackernagel 1994). Recent studies have shown that among aquatic systems, marine sediments from shallow depths down to the abyssal floor are characterized by high concentrations of extracellular DNA (Novitsky and Karl 1985; Danovaro et al. 1999; Dell'Anno et. al. 1998). The pool size of extracellular DNA in marine sediments is the result of complex interactions, including DNA inputs from the photic layer through particle sedimentation, autochthonous DNA production, and degradation or utilization or both by heterotrophic organisms (Dell'Anno et al. 1999). Extracellular DNA diagenesis in marine sediments is also influenced by DNA binding to complex refractory organic molecules, to inorganic particles, or to both, which might strongly reduce its enzymatic degradation (Nielsen et al. 1998). In this regard, Romanowski et al. (1991) showed that DNA adsorbed on sand and clay par-