Ecology, 68(6), 1987, pp. 1955–1965

EXPERIMENTAL ANALYSIS OF THE GRAZING INTERACTION BETWEEN A MAYFLY AND STREAM ALGAE[1]

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Abstract. The interaction between the grazing mayfly *Ameletus validus* and periphyton in a small, northern California stream was examined by manipulating the density of the mayfly in flow-through plexiglass channels. Containing natural cobble substrate and located in situ, the channels established an initial gradient of *A. validus* at 0, 0.5, 1, and 4 times the average density of the mayfly in Barnwell Creek. After 23 d, *A. validus* significantly depressed periphyton standing crop: ash-free dry mass (AFDM) at the 0, 0.5, 1, and 4 N grazer densities was 5.067 ± 1.389 (SE), 1.829 ± 0.173, 1.741 ± 0.325, and 1.009 ± 0.199 g/m^2 (ANOVA: $P < .01$). The mayfly also influenced two structural attributes of the periphyton, increasing the amount of chlorophyll *a* per unit biomass and decreasing the relative contribution of the loose, upper layer to total periphyton biomass.

Principal component analysis of algal relative abundances contrasted the effect of grazing on two groups of diatoms. A group of species found primarily in the loose layer of periphyton (*Nitzschia* spp., *Surirella spiralis, Cymatopleura elliptica,* and *Navicula cryptocephala*) was disproportionately reduced in abundance, while an adnate group (*Gomphonema clevei, Achnanthes minutissima, Synedra ulna, Rhoicosphenia curvata,* and an undescribed species of *Epithemia*) increased its relative abundance with increasing grazing pressure. The decline in relative abundance of the loose layer diatoms did not appear to result from selective consumption by *A. validus,* but may have been mediated by a reduction of inorganic sediment in the periphyton by *A. validus.* Inorganic sediment was highly correlated with the relative abundances of the loose layer group of diatoms, a group of species that are adapted for locomotion on sediment substrates.

A. validus growth in the experimental channels was strongly density dependent. Growth in length over 23 d for the 0.5, 1, and 4 N treatments was 2.24 ± 0.17, 1.80 ± 0.23, and 1.15 ± 0.25 mm (ANOVA: $P < .01$). The significantly greater growth of *A. validus* at subnormal densities in the experimental channels suggested that the *A. validus* population in Barnwell Creek was food-limited.

Key words: algae; Ameletus; *assemblage structure; competition; diatoms; grazing; indirect effects; mayfly; periphyton; standing crop; streams.*

INTRODUCTION

Almost all streams contain insects adapted for grazing periphyton. Despite their common occurrence, little is known about the impact of these grazers on their food resource (Lamberti and Moore 1984). Invertebrate grazers have the potential for profound effects on algal populations, as demonstrated repeatedly in lentic and marine intertidal ecosystems (Paine and Vadas 1969, Porter 1977, Lubchenco 1978, 1980, Robles and Cubit 1981). Streams, however, are harsh environments where extreme fluctuations in discharge can significantly reduce insect abundance (Hynes 1970, Siegfried and Knight 1977) and may prevent grazers from attaining densities at which they influence their food resource. Streams are also characterized by marked spatial heterogeneity; local variations in current, light, and substrate may mask grazing effects even when grazer densities are relatively high. Consequently, although grazers in streams obviously affect the individual algae they consume, their overall impact on populations and assemblages is less predictable.

Field experiments on the interaction between insect grazers and periphyton in streams have been restricted primarily to caddisfly manipulations (Hart 1981, 1985*a, b,* Lamberti and Resh 1983, McAuliffe 1984). These studies demonstrated that natural densities of insects were capable of cropping substantial portions of algal biomass and that competition for food between grazers was likely. Quantitative analysis of the effect of stream insects on in situ algal assemblages has been limited to Hart's (1985*a*) report that the sedentary caddisfly *Leucotrichia* creates diatom-*Schizothrix* patches within *Microcoleus* mats. Considerably more literature exists about the impact of freshwater snails on algal assemblages. Larger algal cells are reported to be susceptible to grazing by freshwater snails, leaving small adnate species dominant on grazed substrates (Patrick 1970, Sumner and McIntire 1982, Cuker 1983). Marine limpets appear to have a similar effect on mi-

[1] Manuscript received 18 September 1986; revised 17 February 1987; accepted 20 March 1987.

croalgal assemblages: large overstory diatoms are disproportionately reduced by grazing (Nicotri 1977).

In addition to affecting algal populations directly through consumption, aquatic herbivores can exert indirect influences. Regeneration of limiting nutrients has received much attention in phytoplankton studies (Porter 1977, Lynch and Shapiro 1981, Sterner 1986), but physical disturbance of algal habitat may be more important as an indirect effect of periphyton grazers. Epilithic algae live within a complex matrix of silt, detritus, biogenic mucilage, and heterotrophic microorganisms. Disruption of this matrix by the activities of substrate-grazing insects could reduce or eliminate niches for specific algae, altering assemblage structure.

In this study we examined the interaction between a grazing mayfly, *Ameletus validus* McDunnough, and periphyton in a small, northern California stream. We hypothesized that natural densities of the mayfly influenced both standing crop and community structure of the periphyton, and proposed the secondary hypothesis that the growth of *A. validus* was food-limited. To test these hypotheses, we established a gradient of *A. validus* density in flow-through plexiglass channels, and assessed the effect of grazer density on periphyton and grazer growth.

Study Area

This study was conducted in Barnwell Creek, a permanent second-order tributary of the South Fork of the Eel River in Mendocino County, California. Located in the Nature Conservancy's Northern California Coast Range Preserve (39°45′ N, 123°40′ W), Barnwell Creek flows from an altitude of 670 to 370 m over a stream bed composed primarily of hard sandstone cobbles and gravel. Like other unregulated streams in mediterranean climates, Barnwell Creek's discharge is high and variable in winter, but relatively low and stable in the dry season. Discharge was ≈4 L/s in this study. The water in Barnwell Creek is clear and cool; water temperature ranged between 12° and 16°C during the study period.

The old-growth redwood (*Sequoia sempervirens*) forest surrounding Barnwell Creek was clearcut during the early 1950's. Since then, the watershed has not been disturbed by human activity. Vegetation is now composed of a mixed assemblage of regenerating redwood, Douglas-fir (*Pseudotsuga menziesii*), tan oak (*Lithocarpus densiflora*), *Ceanothus* spp., madrone (*Arbutus menziesii*), and big-leaf maple (*Acer macrophyllum*). The development of riparian vegetation has been minimal because of logging-induced sediment instability, and the regrowth of overstory trees has been slow. Consequently, much of Barnwell Creek is relatively unshaded. Full sunlight strikes the stretch of stream used in this experiment for ≈5–6 h/d during September and October.

Numerous insect species graze periphyton in Barnwell Creek. Mayfly grazers include *A. validus, Baetis* sp., *Leptophlebia* sp., *Parameletus* sp., and *Cinygma* sp. *A. validus* nymphs are present most of the year, but reach their greatest densities in spring through autumn. At this time *A. validus* is the most conspicuous grazing mayfly in Barnwell Creek. Caddisfly grazers include *Neophylax rickeri, Neophylax spendens, Glossosoma* sp., *Agapetus* sp., and *Lepidostoma* sp. All attain relatively high biomass levels seasonally, but only *Glossosoma* was found in the riffle-run sections of the stream used during this study. Dipterans (Tipulidae and Chironomidae) are occasionally common on upper rock surfaces, while the water penny (*Eubrianix edwardsi*) is common on lower surfaces. Grazing snails have not been found in Barnwell Creek.

Methods

We used flow-through plexiglass channels to manipulate *A. validus* densities in situ. Each channel was 45 × 15 × 15 cm, with removable screens of 500-μm Nitex mesh at both ends. Channels were constructed in parallel blocks of four, and four of these blocks (16 total channels) were placed in an upstream-downstream series on the stream bed in a 20-m stretch of Barnwell Creek. The upper portions of the channels projected 3–4 cm above the water surface, forming a barrier to aquatic insect movement. Because the screens slowed current within the channels by ≈30%, the channels were placed in areas of locally fast flow. Water velocity in the channels averaged 5.0 ± 0.5 (SE) cm/s. Water velocity in the 20-m stretch of stream surrounding the channels ranged from <1 to 15 cm/s.

Dry gravel from the stream bank was spread to a depth of ≈1 cm over the bottom of each channel, then cobbles from which all macroinvertebrates were removed were taken from the surrounding stream bed and placed in the channels. Cobbles were placed in the channels so as to resemble the natural stream bed. Care was taken to minimize the time the cobbles were exposed to the air in order to disturb the established periphyton as little as possible.

A. validus densities were surveyed before the experiment in a 100-m stretch of stream that included the study site. A 196-cm^2 quadrat and underwater viewer were used to count *A. validus* nymphs; *A. validus* density was estimated as 192 ± 19 nymphs/m^2 (mean ± standard error, $n = 17$). *A. validus* nymphs were then collected from the stream with a net and distributed into the channels. A random sample of the nymphs collected for the channels was frozen on dry ice for measurement of initial length and mass; initial length was 6.24 ± 0.15 mm ($n = 32$), and initial mass (ash-free dry mass) was 0.419 ± 0.046 mg ($n = 24$). Each channel in a block received 0, 6, 12, or 48 nymphs, approximating 0, 0.5, 1, or 4 times the mean surveyed density (N). Blocks were replicated four times in the stream, resulting in a 4 × 4 randomized complete block design.

The duration of the grazing experiment was 23 d,

from 12 September to 5 October 1984. During this time, channel screens were replaced every other day to prevent clogging.

Periphyton analysis

Two separate layers of periphyton were sampled in each channel at the end of the experiment: an upper, loose layer and a lower, adnate layer. A neoprene ring (inside area = 5.31 cm^2) was pressed onto the upper surface of a submerged rock randomly chosen for sampling, and held firmly as the rock was removed from the water. Water remaining in the neoprene ring prevented any loose periphyton in the ring from washing away as the rock broke the water surface. The loose layer of the periphyton enclosed in the ring was dislodged with several strong jets of water, collected with a syringe, and placed in an opaque vial. The adnate layer was then loosened with a brush and plastic syringe tip, collected with the syringe, and placed in a separate opaque vial. Three randomly chosen rocks in each channel were sampled in this manner. The sampled material from these three rocks was combined, so that a single loose-layer sample and a single adnate-layer sample resulted from each channel. Samples of loose and adnate periphyton from six cobbles in the adjacent stream bed were also collected at the end of the experiment.

Periphyton samples were refrigerated overnight, adjusted to equal volume, then each sample was divided volumetrically with a syringe into three equal subsamples. These subsamples were analyzed separately for ash-free dry mass (AFDM), chlorophyll *a,* and algal assemblage structure.

AFDM subsamples were filtered onto preweighed Whatman GFF filters (pore size = ≈0.7 μm) and dried overnight at 60°C. The filters were then weighed on a Cahn electrobalance, placed in a 500° muffle furnace for four hours, and reweighed. Representing all organic matter in the periphyton (detritus, algae, bacteria, protozoans, fungi, etc.), AFDM was the difference in mass before and after incineration. AFDM was calculated as grams per square metre of original substrate.

Chlorophyll *a* subsamples were diluted with sufficient 100% acetone to make a 90% acetone solution. Chlorophyll *a* was extracted for 24 h at 6° and then measured fluorometrically. A correction for pheopigments was made following Holm-Hansen et al. (1965). Chlorophyll *a* was calculated as milligrams per square metre of original substrate.

The subsample collected for analysis of algal assemblage structure was preserved as a 2% glutaraldehyde solution. A measured aliquot (depending on the amount of particulate matter) of the subsample was filtered onto a Millipore AA membrane filter (pore size = 0.8 μm), which was then cleared with immersion oil and mounted under a coverslip with Permount (Wetzel and Likens 1979). Algal cells containing chloroplasts were identified and counted using a compound microscope. More than 100 cells, representing at least three complete transects of the filter, were counted at 450×. The combined counts of both loose and adnate samples equaled 200–300 total cells counted per channel. Rare, large diatoms such as *Cymatopleura elliptica* and *Surirella spiralis* were counted by scanning entire filters at 40×. A proportion of the subsample equivalent to 1 cm^2 of the original substrate was examined in this manner for large cells. Algal enumeration data were calculated as both density (cells per square centimetre) and relative abundance (percent).

Diatoms with chloroplasts were identified to the species level by associating their sizes and shapes with those of acid-cleared diatoms from Barnwell Creek, which were mounted in cumar and identified at 1000×. Principal taxonomic sources were Hustedt (1930) and Patrick and Reimer (1966, 1975).

A species of *Epithemia,* which was the largest contributor to algal biovolume, could not be identified. Rectangular in girdle view, this diatom had an average valve length of 45 μm, an average valve breadth of 10 μm, and 4–5 capitate costae per 10 μm. It possessed some of the characteristics of both *Epithemia argus* var. *alpestris* Grun. and *Epithemia adnata* var. *minor* (Perag. & Herib). Patr., and was probably an undescribed species (R. Patrick, *personal communication*). In this paper it will be designated as "*Epithemia* sp. 1." Two other diatoms could not be identified to species: a small (15–20 μm length) *Navicula* species and a small (15–20 μm length) *Nitzschia* species. These two diatoms will be referred to as "*Navicula* sp. 1" and "*Nitzschia* sp. 1" in this paper. Blue-green and green algae were uncommon (<1% of numbers and biomass) and are not listed in the results.

Algal cell densities (cells per square centimetre) were converted to algal biovolume (cubic micrometres per square centimetre) by multiplying the cell density of each species by an estimated volume per cell. Cell volume for each species was computed by applying average cell dimensions to the volume formula of the geometric shape (or combination of shapes) that best approximated the shape of the cell. At least 20 individuals of each species were measured with an ocular micrometer at 1000× to determine average cell dimensions. Cell volume was not corrected for the volume occupied by the vacuole or cell wall.

The results from the separate analyses of loose and adnate periphyton layers were summed for each channel to obtain data for total periphyton. Unless noted otherwise, periphyton results refer to total periphyton.

Insect analysis

All insects >3 mm were collected with a handnet (500-μm mesh) after the rocks were removed from each channel. The insects were frozen on dry ice immediately after collection. In the laboratory, 6–11 *A. validus* from each channel were measured at 10× with a dissecting microscope equipped with an ocular microm-

TABLE 1. Efficacy of grazer enclosures. Values are means ± standard errors. AFDM = ash-free dry mass.

	Densities (channels)			
	0 N*	0.5 N	1 N	4 N
Initial number of *Ameletus validus*	0	6	12	48
Final number of *A. validus*	0.3 ± 0.3	6.5 ± 0.6	13.5 ± 1.7	35.8 ± 3.5
Final *A. validus* AFDM (mg)	0.2 ± 0.2	10.3 ± 1.6	19.3 ± 2.9	29.6 ± 3.6
Non–*A. validus* grazer AFDM (mg)	6.4 ± 1.8	9.0 ± 2.3	10.0 ± 5.0	4.1 ± 3.0
Total grazer AFDM (mg)	6.6 ± 1.8	19.3 ± 3.5	29.3 ± 7.3	33.7 ± 3.0

* N = mean density (192 ± 19 nymphs/m^2) of *A. validus* in Barnwell Creek, surveyed at the beginning of the experiment.

eter. AFDM was determined for *A. validus* and all of the other insects collected from each channel.

A. validus was identified to species by rearing nymphs to adults in a laboratory stream. Adult males were identified using the descriptions of McDunnough (1923).

Statistics

Each time analysis of variance (ANOVA) was employed, the assumptions of treatment additivity and variance homogeneity were tested with Tukey's test of nonadditivity and Barlett's chi-square test, respectively. Logarithmic, arcsine, or χ^2 transformations were applied when necessary. Nonparametric statistics were used when transformations could not satisfy the assumptions of parametric procedures.

The effect of grazing on algal assemblage structure was tested by ANOVA of principal components of the relative abundance data. Principal components were calculated using standardized percentage data from all channels. An arcsine transformation applied before standardization had no qualitative effect on the principal components or subsequent ANOVAs, so the analysis based on standardized raw percentages is presented in this paper.

RESULTS

Efficacy of experimental channels

The screened channel design limited insect movement to a large degree. Minor *A. validus* immigration occurred at the 0, 0.5, and 1 N density treatments, while emigration or mortality reduced the high-density treatment 25% (Table 1). A number of non–*A. validus* grazers (mostly small *Baetis* sp., but also *Parameletus* sp., *Leptophlebia* sp., *Cinygma* sp., and *Eubrianix edwardsi*) were collected in the channels at the end of the experiment. Immigration and emigration of *A. validus* and other larger grazers probably occurred when the channel screens were being replaced. The small *Baetis* sp. and *Parameletus* sp. may have passed through the screen mesh and into the channels while in early instars. Overall, the screened channel design maintained a gradient of grazer density consistent with the original *A. validus* application.

Periphyton effects

Standing crop.—All measures of periphyton biomass declined with increasing grazer density. When expressed as a function of the total biomass of grazing insects collected from the channels at the end of the experiment, periphyton AFDM and algal biovolume decreased in a nonlinear fashion, while the decrease in chlorophyll *a* appeared somewhat more linear (Fig. 1). Logarithmic transformations were applied to all three periphyton biomass measures to straighten nonlinear relationships and to reduce the greater variance associated with the large values of periphyton biomass. Multiple regression of the log-transformed data, using the biomass of *A. validus* and the biomass of other grazers collected from the channels as the two independent variables, resulted in significant F values for the overall regressions (Table 2). Partial regression coefficients for *A. validus* biomass were highly significant for each measure of periphyton biomass, while the coefficients for the biomass of non–*A. validus* grazers were insignificant, indicating that *A. validus* was the only important grazer variable in this study.

AFDM and algal biovolume again appeared to decline nonlinearly as functions of applied *A. validus* density (i.e., grazing), with the greatest reduction occurring between the 0 and 0.5 N densities (Fig. 2). Multiple comparisons between mean values from the four channel treatments confirm that even subnormal densities of *A. validus* significantly depleted periphyton standing crop. Periphyton exposed to ambient densities of grazers on the stream bed outside the experimental channels was similar to that in the grazed channels: 95% confidence intervals included the 0.5, 1, and 4 N treatments.

A. validus biomass was associated with changes in the relationships between several standing-crop parameters. Ratios of chlorophyll *a* to periphyton AFDM and chlorophyll *a* to algal biovolume both increased with increasing *A. validus* AFDM (Fig. 3), even though chlorophyll *a* decreased in absolute terms. The relative contribution of the loose layer to total periphyton AFDM and chlorophyll *a* also changed, decreasing as a function of *A. validus* biomass (Fig. 4).

Diatom assemblage structure.—Fourteen species of diatoms occurred consistently in all blocks and in-

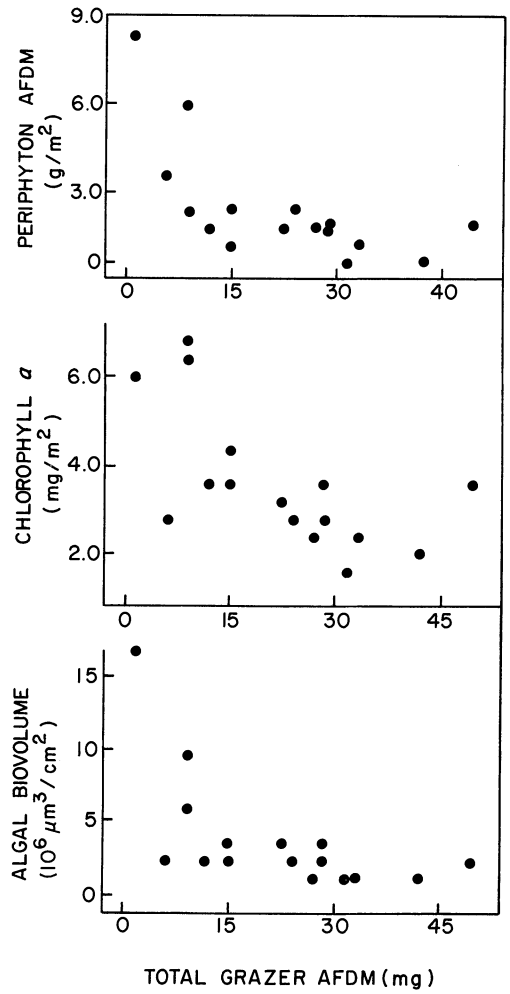

FIG. 1. Three periphyton biomass measures vs. the ash-free dry biomass (AFDM) of all grazers collected from each experimental channel.

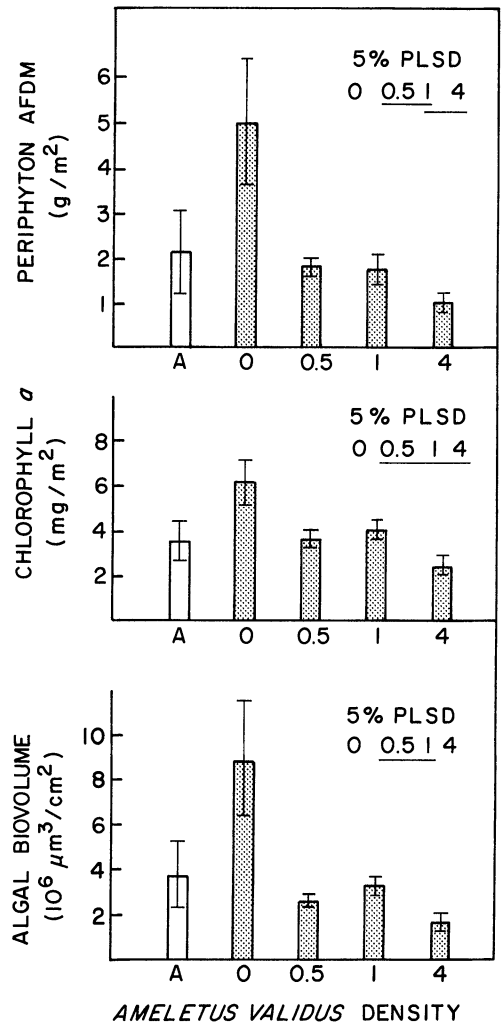

FIG. 2. The effect of *Ameletus validus* on periphyton standing crop. Open bars represent samples taken from outside the experimental channels and exposed to ambient (A) grazer densities. Stippled bars represent samples taken from the experimental channels, which contained 0, 0.5, 1, or 4 times the mean (±SE) surveyed density (192 ± 19 nymphs/m²) of *A. validus* in Barnwell Creek at the beginning of the experiment. Analyses of variance performed on log-transformed channel data: AFDM, $F = 12.43$, $P < .01$; chlorophyll *a*, $F = 5.28$, $P < .05$; algal biovolume, $F = 10.97$, $P < .01$. Fisher's protected least significant difference (PLSD) test was used to compare the means of log-transformed channel data; underlined treatments are not significantly different. Error bars are ±1 SE.

cluded >90% of total algal biovolume and numbers. The biovolume of almost all species decreased with increasing grazing intensity, although the decrease was statistically significant only for *Epithemia* sp. 1, *Surirella spiralis, Nitzschia palea, Nitzschia linearis,* and *Nitzschia* sp. 1 (Table 3). The general pattern of biovolume decrease was the same as with the total periphyton measures: the greatest decrease occurred between 0 and

TABLE 2. Regression statistics for the equation $Y = B_0 + B_{am}X_1 + B_{other}X_2$.†

Y	Units of measurement	B_0	B_{am}	B_{other}	F	r^2
Log AFDM	(g/m²)	0.587	−0.020***	−0.001	16.179***	0.71
Log chlorophyll *a*	(mg/m²)	0.668	−0.010**	0.001	5.355*	0.45
Log biovolume	(10^3 μm³/cm²)	3.778	−0.020**	−0.002	8.378**	0.56

* $P < .05$, ** $P < .01$, *** $P < .001$.

† Y = periphyton biomass parameter. B_0 = regression constant, B_{am} = *Ameletus validus* regression coefficient, X_1 = total *A. validus* ash-free dry mass (AFDM), B_{other} = other grazers regression coefficient, X_2 = total AFDM of grazers other than *A. validus*.

0.5 N *A. validus* density. No taxa (including blue-green and green algae not listed in Table 2) consistently increased in absolute abundance with increasing *A. validus* density. The biovolumes of individual species on the stream bed outside the channels resembled those inside the channels: the means of most species from outside the channels fell between the 0 and 0.5 N treatment means, and 95% confidence intervals included either all treatment means or all three grazed treatment means.

When considered as individual entities, only three species significantly changed in relative abundance as a function of *A. validus* density in the channels (Table 4). *Nitzschia palea, Nitzschia linearis,* and *Surirella spiralis* all declined with increasing mayfly density. The binomial probability of three significant differences by chance alone in 14 separate ANOVAs was 0.03. The relative abundances of most diatoms from outside the channels fell within the range of channel means, and only *Nitzschia linearis* had a 95% confidence interval that did not include any of the channel means (Table 4). The probability that one or more of the 14 confidence intervals would not include the channel means as a result of chance alone was 0.51.

Principal component analysis of the relative abundance data revealed broader effects of grazing. Two groups of diatoms were contrasted by the first principal component. *Surirella spiralis, Nitzschia* sp. 1, *Nitzschia palea, Nitzschia linearis, Cymatopleura elliptica,* and *Navicula cryptocephala* constituted a group that had moderate to highly positive coefficients; while *Gomphonema clevei, Achnanthes minutissima, Synedra ulna, Rhoicosphenia curvata* and *Epithemia* sp. 1 made up a group possessing moderately negative coefficients (Table 5). The former group was composed entirely of motile biraphid species, while most of the latter group were species that form strong attachments to the substrate. The coefficients assigned to each species were positively associated (Spearman's rho = 0.74, $P < .01$) with the degree to which the species segregated into the loose vs. the adnate layers (Table 5). *Cocconeis placentula, Navicula* sp. 1, and *Achnanthes lanceolata* contributed little to the first principal component and were found almost equally in the loose and adnate layers. Principal components beyond the first were difficult to interpret and therefore were not included in this paper.

A significant shift in the value of the first principal component from positive to negative demonstrated that *A. validus* altered algal assemblage structure (Fig. 5). Species with positive coefficients contributed less to assemblage structure at higher *A. validus* densities than species with negative coefficients, indicating increased dominance by adnate diatoms. As with the biomass parameters and the individual relative abundances, the greatest change in the first principal component occurred between the 0 and 0.5 N treatments. Multiple

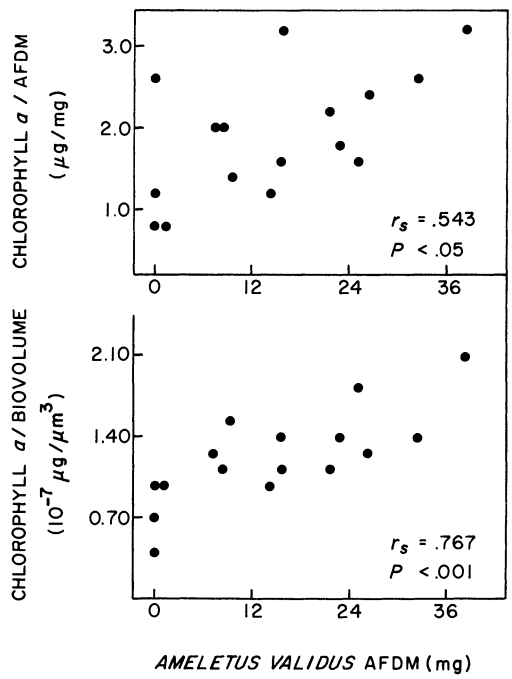

FIG. 3. Chlorophyll *a*/biomass ratios in the periphyton vs. total *Ameletus validus* ash-free dry biomass (AFDM) in each channel. Spearman's rank correlation coefficient = r_s.

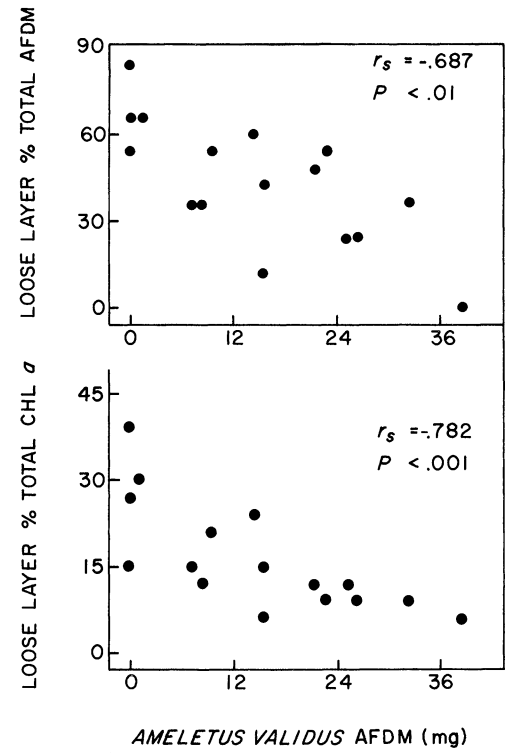

FIG. 4. Relative reduction of the loose periphyton layer as a function of total *Ameletus validus* ash-free dry biomass (AFDM) in each channel. Spearman's rank correlation coefficient = r_s.

TABLE 3. Diatom biovolumes (10^3 $\mu m^3 \cdot cm^{-2}$) in unenclosed stream and in experimental channels. Values are means ± standard errors.

Diatom taxon	Unenclosed stream	Channels†				
		0 N	0.5 N	1 N	4 N	*F*
Epithemia sp. 1	1500 ± 378	4140 ± 1050	1600 ± 233	2190 ± 264	867 ± 209	6.33*
Cymatopleura elliptica (Breb.) W. Sm.	1190 ± 1130	2590 ± 2170	206 ± 75	264 ± 155	86 ± 35	3.07
Nitzschia linearis W. Sm.	196 ± 95	253 ± 106	51 ± 21	26 ± 9	12 ± 4	4.78*
Nitzschia palea (Kutz.) W. Sm.	191 ± 131	360 ± 114	69 ± 26	65 ± 22	23 ± 7	8.24**
Surirella spiralis Kutz.	128 ± 99	512 ± 157	126 ± 54	111 ± 45	34 ± 7	13.08**
Cocconeis placentula Ehr.	58 ± 59	63 ± 16	41 ± 12	48 ± 14	26 ± 6	1.51
Gomphonema clevei Fricke	56 ± 11	107 ± 23	100 ± 27	94 ± 22	79 ± 14	0.33
Navicula sp. 1	43 ± 24	22 ± 9	12 ± 4	6 ± 2	4 ± 1	3.29
Synedra ulna (Nitz.) Ehr.	33 ± 18	11 ± 9	18 ± 8	13 ± 11	3 ± 2	0.72
Rhoicosphenia curvata (Kutz.) Grun. *ex* Rabh.	20 ± 7	35 ± 12	12 ± 1	19 ± 7	15 ± 5	0.72
Navicula cryptocephala Kutz.	19 ± 16	12 ± 6	5 ± 2	4 ± 1	4 ± 1	2.47
Achnanthes lanceolata Breb.	13 ± 10	19 ± 4	6 ± 2	8 ± 3	6 ± 2	3.43
Nitzschia sp. 1	10 ± 6	26 ± 8	7 ± 4	9 ± 4	2 ± 0	6.84*
Achnanthes minutissima Kutz.	3 ± 1	4 ± 1	4 ± 1	12 ± 5	2 ± 1	2.32

* $P < .05$, ** $P > .01$ (ANOVAs).
† Analyses of variance performed on log-transformed channel data; unenclosed stream data were not included in the analysis. Abbreviations as in Table 1.

comparisons between the means at each density confirmed that even subnormal densities of *A. validus* significantly affected assemblage structure. ANOVA of the second principal component showed no significant effect of *A. validus* density.

The first principal components of the diatom relative abundances outside the channels were calculated with the scaled coefficients in Table 5 and relative abundances standardized against the means and standard deviations of the channel data. The mean of the resulting scores fell between the 0 and 0.5 N treatment means (Fig. 5). A 95% confidence interval around this mean included all channel treatment means, indicating that diatom assemblage structure was little affected by channel conditions.

Silt effects.—Silt (particulate inorganic matter) in the loose layer was strongly associated with diatom assemblage structure. The first principal component was more closely related to the amount of silt in the loose layer ($r = 0.78$, $P < .001$) than with *A. validus* AFDM ($r = -0.68$, $P < .01$). Taxa with high positive coefficients in the first principal component were correlated individually with silt, and except for *Nitzschia linearis,* had lower correlation coefficients for *A. validus* AFDM (Table 6). However, silt itself was significantly related to *A. validus* AFDM ($r = -0.55$, $P < .05$), so it was not possible to separate the causal effect of silt on algal assemblage structure from the effect of *A. validus.*

A. validus *growth*

The growth of *A. validus* in the channels over 23 d was calculated by subtracting the mean length and mass

TABLE 4. Relative abundances (%) of diatoms in unenclosed stream and in channels. Values are means ± standard errors.

Diatom taxon	Unenclosed stream	Channels†				
		0 N	0.5 N	1 N	4 N	*F*
Epithemia sp. 1	21.9 ± 8.3	21.1 ± 6.8	21.7 ± 4.0	23.9 ± 4.8	17.6 ± 4.2	0.26
Nitzschia palea	13.5 ± 5.0	22.9 ± 3.4	12.0 ± 4.1	8.8 ± 1.6	6.8 ± 2.2	6.41*
Cocconeis placentula	11.5 ± 2.8	6.4 ± 1.8	8.8 ± 2.2	8.0 ± 0.9	8.9 ± 2.1	0.48
Gomphonema clevei	10.1 ± 2.6	9.2 ± 0.6	24.2 ± 6.2	18.1 ± 5.2	29.0 ± 5.2	3.82
Navicula sp. 1	8.9 ± 2.7	2.4 ± 1.2	4.3 ± 1.2	1.5 ± 0.3	2.1 ± 0.6	1.53
Nitzschia sp. 1	5.4 ± 1.8	8.5 ± 1.9	4.5 ± 2.6	4.6 ± 0.6	2.4 ± 0.7	3.10
Achnanthes lanceolata	4.4 ± 1.3	5.9 ± 1.0	4.9 ± 1.0	4.9 ± 1.0	7.1 ± 2.8	0.38
Achnanthes minutissima	3.1 ± 1.4	2.4 ± 0.7	5.8 ± 1.4	13.4 ± 4.8	5.1 ± 1.8	3.31
Rhoicosphenia curvata	3.0 ± 0.6	3.6 ± 1.3	3.0 ± 0.6	3.1 ± 0.8	5.2 ± 1.3	0.71
Nitzschia linearis	2.1 ± 0.1‡	1.6 ± 0.4	0.9 ± 0.3	0.3 ± 0.2	0.4 ± 0.1	5.07*
Navicula cryptocephala	2.0 ± 1.2	1.5 ± 0.4	1.9 ± 0.4	1.1 ± 0.2	2.2 ± 0.9	0.91
Synedra ulna	0.25 ± 0.10	0.04 ± 0.02	0.18 ± 0.07	0.54 ± 0.33	0.04 ± 0.03	2.25
Cymatopleura elliptica	0.15 ± 0.11	0.36 ± 0.24	0.09 ± 0.03	0.07 ± 0.06	0.07 ± 0.03	2.64
Surirella spiralis	0.05 ± 0.02	0.17 ± 0.03	0.12 ± 0.04	0.07 ± 0.02	0.05 ± 0.01	9.81**

* $P < .05$, ** $P < .01$ (ANOVAs).
† Analyses of variance performed on arcsine-transformed channel percentages; unenclosed stream data were not included in the analyses. Abbreviations as in Table 1.
‡ 95% CI does not include any channel mean.

of the initial sample of *A. validus* nymphs collected at the beginning of the experiment from the length and mass of the nymphs collected from the channels at the end. Immigrants could not be distinguished from the nymphs originally applied to the channels, and were included in the calculation of growth. Because immigrants were indistinguishable from the original nymphs, and because they constituted a small portion of the nymphs in the channels, their influence on the calculated growth rate was probably small.

A. validus growth was clearly a negative function of density (Fig. 6). Multiple comparisons confirmed significant differences between all densities for growth in both length and mass. Although *A. validus* growth outside the channels was not monitored for comparison, nymphs appeared unstressed and grazed normally on the cobbles in the 0.5 and 1 N density channels. At the 4 N density, a number of nymphs always congregated at the upstream screen, perhaps in response to low levels of periphyton at this density.

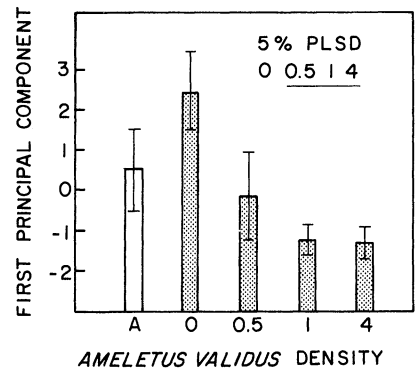

FIG. 5. The effect of *Ameletus validus* on the first principal component of diatom relative abundances. Symbolism and data presentation as in Fig. 2. Analysis of variance: $F = 5.80$, $P < .05$. Error bars are ±1 SE.

DISCUSSION

Experimental conditions

The ecological relevance of a manipulative field experiment depends to a great extent on how well natural conditions are maintained within the experiment. In this study, natural conditions were preserved as much as possible while simultaneously restricting mayfly movement. The use of natural substrate and an in situ location undoubtedly accounted for the close resemblance of the periphyton in the channels to that on the surrounding stream bed. The only major deviations were high biomass values from the 0 N channels. These channels clearly represented artificial conditions compared with Barnwell Creek, where at least moderate densities of grazers were always present. The lack of an obvious cage effect, coupled with the natural variability associated with cobbles and in-stream conditions, would appear to make the grazing effects measured in this experiment realistic.

Periphyton effects

Standing crop.—The results of this study indicate that grazers in Barnwell Creek played a significant role in limiting periphyton standing crop. Lightly grazed channels produced about twice the periphyton that either the normal density channels or the adjacent stream bed produced. These results are in general agreement with grazing experiments in several other western United States streams. Using empirically derived rates of grazing and algal renewal, Hart (1981) calculated that the caddisfly *Dicosmoecus gilvipes* substantially depleted periphyton standing crop in a California stream. In the same stream, Lamberti and Resh (1983) found that *Helicopsyche borealis* was capable of at least a five-fold reduction of periphyton biomass on ceramic tiles. *Glossosoma* sp. exclusions by McAuliffe (1984) in a Montana stream resulted in a large increase

TABLE 5. First principal component coefficients of standardized relative abundances (e_{1i}) and the proportion of each species found in the loose periphyton layer (loose/total).

Diatom taxon	e_{1i}*	Loose/total†
Surirella spiralis	0.427	0.73^{+}
Nitzschia sp. 1	0.415	0.69^{++}
Nizschia palea	0.407	0.79^{++}
Nitzschia linearis	0.338	0.80^{++}
Cymatopleura elliptica	0.338	0.88^{++}
Navicula cryptocephala	0.160	0.88^{++}
Achnanthes lanceolata	0.056	0.57
Navicula sp. 1	−0.050	0.48
Cocconeis placentula	−0.070	0.43^{-}
Epithemia sp. 1	−0.149	0.13^{--}
Rhoicosphenia curvata	−0.159	0.35
Synedra ulna	−0.198	0.64
Achnanthes minutissima	−0.227	0.29^{-}
Gomphonema clevei	−0.278	0.35^{-}

* The first principal component accounted for 32% of the variation in the relative abundance data.

† Wilcoxon test of H_0: loose/total = 0.50: $^{+}$ loose/total > 0.50, $P < .05$; $^{++}$ loose/total > 0.50, $P < .01$; $^{-}$ loose/total < 0.50, $P < .05$; $^{--}$ loose/total < 0.50, $P < .05$.

TABLE 6. Correlation coefficients for relative abundance vs. log silt mass (silt mass measured as mg/m²) and for relative abundance vs. log *Ameletus validus* AFDM (measured as mg/channel).

Diatom taxon	Correlation coefficient: Silt mass	Correlation coefficient: *A. validus* AFDM
Nitzschia palea	0.825	−0.771
Nitzschia sp. 1	0.779	−0.563
Nitzschia linearis	0.561	−0.746
Cymatopleura elliptica	0.591	−0.488
Surirella spiralis	0.708	−0.656

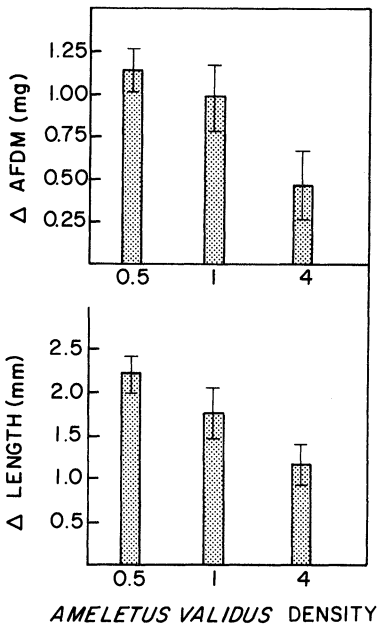

FIG. 6. Growth of *Ameletus validus* over 23 d in experimental channels. Analysis of variance of AFDM growth performed after χ^2 transformation of data: $F = 180$, $P \ll .001$. Analysis of variance of growth in length: $F = 38.4$, $P < .001$. All individual means of each parameter were significantly different from each other with 5% Fisher's protected least significant difference (PLSD) multiple comparisons. Error bars are ±1 SE.

in the number of algal cells on ungrazed bricks compared with bricks experiencing natural grazing pressure. Only Murphy (1984) presents dissenting evidence: he concluded that exclusions of all insects from cobbles in a freshwater portion of an Alaskan stream produced no significant effect on standing crop. However, his experiment lacked an adequate number of replicates and was situated in a highly shaded stream where light limitation would be expected to slow the accumulation of periphyton.

The pattern of *A. validus* grazing showed decreasing periphyton reduction as the number of *A. validus* increased. Several explanations for this pattern are possible. *A. validus* nymphs may have physically interfered with each other's grazing activity at higher densities, reducing the time spent grazing per individual. This seems an unlikely explanation, since no agonistic behavior was observed in the channels or in the unmodified stream. The efficiency of primary production may have increased with grazing pressure, so that periphyton biomass was replaced faster at high *A. validus* densities. Grazing-induced increases in primary production have been reported for a wide variety of herbivores (Flint and Goldman 1975, McNaughton 1976, Lamberti and Resh 1983), and the increase of chlorophyll *a* per unit algal biomass with increasing *A. validus* biomass suggests that the efficiency of primary production may have been increased by grazing. However, a large harvestable increase in primary production in the grazed channels seems unlikely in light of the significant density-dependent growth depression of individual *A. validus*. Another explanation for the nonlinear depletion of periphyton could be that it became more resistant to grazing as its biomass was diminished. A greater proportion of periphyton biomass was located in the adnate layer at higher *A. validus* densities, perhaps because the nymphs were less effective in harvesting this layer than the loose layer. *A. validus* possesses rakelike mouthparts that appear better suited to collecting and gathering loose particles than scraping adherent organic matter from mineral substrates (Merritt and Cummins 1984). *A. validus* may have harvested much of the loose layer at low densities, leaving mostly adherent organic matter that was progressively more difficult to harvest as the mayfly's density increased.

Assemblage structure.—*A. validus*'s effect on algal assemblage structure was related to its disproportionate reduction of the loose periphyton layer. Taxa associated with this layer declined in relative abundance while adnate taxa increased. Sumner and McIntire (1982) observed that grazing by the snail *Juga plicifera* decreased the relative abundance of some of the same taxa (*Nitzschia palea, N. linearis,* and *Surirella*) that dominated the loose layer in our study. In the snail study, these taxa were associated with an overstory matrix formed by the filaments of *Melosira varians,* a large diatom particularly susceptible to the snail's scraping radula. Marine limpets appear to have the same effect on overstory diatoms, selectively reducing taxa such as *Melosira* and *Fragilaria* (Nicotri 1977).

The specific cause of the disproportionate decline of loose layer species with increasing *A. validus* density was unclear. Relatively few frustules of these species were found in microscopic examination of the mayfly's gut contents, suggesting that selective consumption was not responsible for their decline. A more likely explanation for the decline of loose-layer species may involve the impact of *A. validus* on the diatoms' physical habitat. The mayfly significantly reduced the amount of silt in the loose layer of periphyton, and silt was strongly correlated with the relative abundances of *Nitzschia* spp., *Cymatopleura elliptica,* and *Surirella spiralis.* These diatoms possess keeled raphes adapted for movement through fine-particle substrates (Belanger et al. 1985) and have an obvious advantage over immobile species when accumulating sediment blocks light. Their low abundances under grazed conditions could have been due to an inability to colonize cobbles that lack sediment, a requirement for sediment-associated organic nutrients, or a competitive inferiority to adnate species. Growth of large, filamentous algae (e.g., *Melosira*) into an overstory can also provide an organic matrix suitable for biraphid, motile diatoms, and may explain the general similarity between *A. validus,* freshwater snail, and marine limpet grazing.

A. validus *growth*

No territorial or aggressive behavior between individual *A. validus* nymphs was observed in the channels. The apparent absence of such agonistic behavior, combined with the evidence of resource depression, indicated that the density-dependent growth of *A. validus* in the experimental channels was due to food limitation. Extrapolation of food limitation to the *A. validus* population in the surrounding stream is problematic, however. Because the growth of the unenclosed mayflies was not measured, the potential effect of the channels themselves on *A. validus* growth could not be estimated. The presence of other grazing species in Barnwell Creek also complicates the extrapolation of the channel results, as these species may have reduced the periphyton resource and competed exploitatively with the *A. validus* population in the unenclosed stream. The similarity of the periphyton in the grazed channels to that in the unenclosed stream argued against a large reduction in periphyton by non–*A. validus* grazers, but an interaction where these grazers share a limited amount of the periphyton resource cannot be dismissed.

Density-dependent growth of mayfly nymphs has implications for population limitation. Fecundity in many species appears to be directly related to adult size (Clifford 1970, Clifford and Boerger 1974, Harvey et al. 1979), and because adults do not feed during their brief existence, adult size is determined by nymphal growth. If the effect of density on the growth of *A. validus* outside the channels was similar to that inside, then the depression of nymphal growth at natural densities in the channels suggested that the reproductive output of *A. validus* in Barnwell Creek was constrained by intraspecific competition.

Considerable interest exists in the role catastrophic flooding plays in stream ecology (Fisher et al. 1982, Grossman et al. 1982, Reice 1985). In California streams, winter floods produce profound reductions in insect densities (Siegfried and Knight 1977) and mitigate biotic interactions such as competition (Hemphill and Cooper 1983). Catastrophic flooding clearly did not prevent *A. validus* from reaching densities in Barnwell Creek at which food depletion appeared likely during the period of this study. However, winter floods were notably mild in the northern California coast region during the 1983–1984 wet season. Continuous discharge records at Elder Creek, a tributary of the South Fork of the Eel River located 1.6 km upstream of Barnwell Creek, showed lower peak flows during 1983–1984 than during the previous 4 yr (Anderson et al. 1984). If peak flows in winter are important in determining summer grazer densities, competition for food was probably more pronounced in 1984 than in other years.

The seasonal nature of discharge in Western streams facilitates competition among periphyton grazers during late summer and early fall, the period in which this study took place. Due to the lack of precipitation in the extended dry season, floods do not occur after April. Instead, stream flow slowly diminishes over the course of the dry season, reducing the submerged portion of the stream bed. Periphyton grazers become concentrated over progressively smaller areas of periphyton, and can have a significant impact on their food resource. Competition for food may be common at this time, even if grazer populations are limited by other factors during the rest of the year.

Acknowledgments

The Nature Conservancy generously allowed us to use Barnwell Creek for this manipulative study. We thank Ruth Patrick for examining our diatoms and for giving us the benefit of her expert taxonomic opinion. This paper was improved with critical reviews by Norma Lang, Peter Richerson, and two anonymous reviewers. The research was supported in part by a Jastro-Shields Graduate Fellowship to Walter Hill and by the United States Department of the Interior, Geological Survey, through the State Water Resources Research Institute, Project No. CA-05-1984, and by the University of California Water Resources Center, Project UCAL-WRC-W-651. Contents of this publication do not necessarily reflect the views and policies of the U.S. Department of the Interior, nor does mention of trade names or commercial products constitute their endorsement or recommendation for use by the U.S. Government.

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