

The influence of crevice size on the protection of epilithic algae from grazers

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SUMMARY

1. This study investigated how the size of crevices might affect their effectiveness as refuges for diatom-dominated algal assemblages from the grazing minnow *Campostoma anomalum*.
2. Crevice size was manipulated by making fired-clay substrates, using moulds to produce eight substrates with pits from 1.17 to 22.0 mm diameter. Non-pitted clay-stones and limestone were also tested. Cages were used to control the access of *Campostoma* to arrays of the 10 different algal-colonised substrates. The grazing treatments were: open and grazed, caged and ungrazed, and a grazed cage control. The experiment was replicated in eight large outdoor tanks. After 3 weeks, substrates were brushed and chlorophyll *a* concentrations of the removed algae and the algae remaining in pits were measured.
3. The experiment was field-validated by exposing arrays of substrates to grazing *Campostoma* in five pools of a limestone stream.
4. The clay-stone and limestone substrates accrued similar algal biomass and assemblages.
5. Smaller crevices provided more protection against grazing than larger crevices. Specifically, pits with openings smaller than 2 mm protected the enclosed algal assemblages in both the tank and field experiments. Larger pits provided less protection and pits over 7 mm in diameter were heavily grazed and may even be preferentially grazed by *Campostoma*.
6. None of the tested pit sizes were protective against larval chironomid grazers in the tank experiment, demonstrating that differences in the grazer size influence the effectiveness of crevice refuges.

Keywords: disturbance ecology, refuges, refugia, spatial heterogeneity, surface texture

Introduction

Crevices and other refuges are used by organisms to shelter from a variety of unfavourable conditions, including predation and grazing (Lubchenco, 1983; Dudley & D'Antonio, 1991; Gosselin & Chia, 1995; Catesby & McKillup, 1998). Mobile species may actively select refuges and the distribution of crevices can affect the distribution of these species, particularly in disturbance-prone habitats (Emson & Faller-Fritsch,

1976; Harper & Williams, 2001; Minton & Gochfeld, 2001). Species with low mobility, such as stream algae, also benefit from protection in crevice refuges (Dudley & D'Antonio, 1991; Bergey & Resh, 1994; Bergey, 1999), where they are protected from grazer and abrasive disturbances.

Despite the potential importance of refuges to organisms in streams, there is little understanding of the attributes that influence the protectiveness of refuges. Natural crevices are highly variable in size and the physical characterisation of crevices is helpful in quantifying and assessing the effectiveness of refuges (Hildrew, 1998). For crevices to be effective refuges, organisms must not only fit within crevices, but also be located in disturbance-protected parts of

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crevices. Understanding the spatial scales of organisms, crevices and disturbances will improve our understanding of how crevices function as refuges and enhance the recognition of effective refuges.

The objective of this study was to assess the influence of crevice size on refuge effectiveness in protecting algal assemblages from disturbance. Specifically, substrates were constructed with different pit sizes and their effectiveness in protecting diatom-dominated algal assemblages from grazing by a minnow, *Campostoma anomalum* (Rafinesque) (the central stoneroller), was tested using a mesocosm experiment with field validation. Grazing of epilithic algae is a biological disturbance (*sensu* White & Pickett, 1985) because it alters the architecture of the algal assemblage and opens patches to algal colonisation.

Methods

Substrates and grazers

Substrates with different crevice sizes were made using moulds of closed-cell Styrofoam and glued-on spherical or hemispherical items. Eight sets of items ranged from small glass balls and plastic beads to relatively large wooden furniture buttons. White stoneware clay was pressed onto the moulds and impressions of the items formed the crevices on the upper flat surface of the resulting clay-stones. Clay-stones that lacked crevices were made using a flat mould. Clay-stones were air dried, fired once and not glazed. Clay-stones averaged 6.8 cm in diameter. Crevice diameters and depths were measured after firing with digital calipers and pit density was measured as the number of pits falling within a grid of known size. Eight replicates of each clay-stone substrate type were measured for pit diameter and density. Mean pit diameters (SE) were 1.2 (0.1), 1.3 (0.0), 2.3 (0.1), 3.6 (0.1), 5.5 (0.1), 7.6 (0.1), 11.5 (0.1), and 22.0 (0.3) mm.

Limestone rocks provided the 10th substrate type. Using limestone allowed comparison of algal growth on smooth clay-stones with a natural stone. Limestone rocks were collected from the dry banks of the Illinois River (Cherokee County; northeast Oklahoma). Selected rocks were relatively flat and averaged 6.4 cm in diameter. Any limestone rocks with a greenish tinge were discarded; the remaining rocks were scrubbed,

soaked in 90% EtOH and dried in the sun for 2 weeks to destroy any residual chlorophyll.

Campostoma anomalum is a widespread and abundant minnow (Cyprinidae) that is common in small stony streams (Tomelleri & Eberle, 1990) and is an abundant species in the study stream, Brier Creek (Matthews, Harvey & Power, 1994). These fish are algal grazers, and their grazing habits and effects on stream algae have been well studied (e.g. Matthews, Power & Stewart, 1986; Power & Stewart, 1987).

Tank experiment

The influence of crevice size in protecting algae from grazing fish was tested in a controlled, replicated outdoor experiment at the University of Oklahoma (Cleveland County). The experiment was housed in eight, 1.8 m-diameter, 0.6 m-deep plastic aquaculture tanks. Tanks had flow-through well-water systems that included an overflow pipe that maintained an average water depth of 0.38 m (SE = 0.02 m).

Variables were grazing level and substrate type (constructed clay-stones with different crevice sizes and limestone). Grazing level was controlled using cages. Cage frames of 1.3 cm diameter PVC pipe measured 0.5 × 0.5 m on the bottom with taller sides of 0.6 m that extended above the water. Fine nylon seine mesh (0.4 cm) was initially used, but was augmented with 0.3 cm polyester mesh 2 days into the experiment, when it became evident that some of the smaller fish could enter the grazer-exclusion cages. Grazer-exclusion cages ('cage') were covered with mesh on the bottom and all four sides; control cages ('control') were similar but had two open sides, and fully open cages ('open') had a bottom but no sides. Control cages allowed fish access but had some of the environmental effects of enclosed cages. One open cage, one closed cage and one control cage were placed in random locations in each of the eight tanks.

Because the well-water system did not provide a source of algae to colonise substrates, substrates were precolonised prior to the experiment. Algae were brushed from stones in Honey Creek, a spring-fed limestone and travertine stream in south-central Oklahoma (Murray County). The resulting slurry was poured onto the aggregated substrates in two non-experimental tanks and substrates were incubated for 2 weeks, during which a thick mat developed.

Camptostoma anomalum were collected from the Blue River, a limestone river in Johnston County (south-central Oklahoma) by seining, and held for 2 days prior to stocking into tanks. Although common in Brier Creek (the site of the field study), fish were collected elsewhere because of an ongoing long-term fish survey programme in the stream. Collected fish averaged 5.7 cm in length (SE = 0.2 cm, range = 4.2–7.2 cm; $n = 50$) and represented mixed age classes, minus the smaller young-of-the-year. *Camptostoma* grow to a length of about 8 cm (Tomelleri & Eberle, 1990).

At the start of the experiment on 1 November, the precolonised substrates were carefully transferred in water to each of the cages and arranged in a predefined random order. Two replicates of each of the 10 substrates were placed in each of the cage, control and open treatments (=60 substrates per tank). No other substrate was added to the tanks. Twenty-five fish were stocked into each tank; this number was considered adequate for typical schooling behaviour (William Matthews, personal communication) and, at a density of 10 fish m^{-2} , was within the limit of natural densities in Brier Creek (2–10 fish m^{-2} ; Power, Matthews & Stewart, 1985; Matthews *et al.*, 1994). The experiment lasted 26 days.

Initial samples were taken to assess possible differences in algal assemblages among the substrate types at the start of the experiment. Eight replicates of each substrate type were sampled after transfer to another tank, which exposed these substrates to the same handling as the substrates used in the experiment. At the end of the experiment, one of the two replicate substrates for each substrate type was selected randomly for sampling from each treatment-tank combination (=30 sampled substrates per tank).

Sampling involved carefully brushing the entire upper surface of substrates with a wiping motion. The brush (mesh scouring brush, Leonardo's Kitchen Hardware, Phoenix, Arizona) had a flat surface of compressed mesh (430 ends cm^{-2}), which effectively removed algae from limestone and the upper surface of the clay-stones without removing algae from pits. Brushes were rinsed often and the washings saved as the 'removed' algal sample. After brushing, substrates were kept for chlorophyll analysis. Substrates and removed algal samples were initially iced, then stored frozen.

Chlorophyll *a* concentration was used to quantify algal biomass (Stevenson, 1996). Removed algal samples were diluted with water to a constant volume, homogenised with a hand-held blender, and subsamples were filtered onto glass fibre filters (Gelman A/E, pore size = 1 μm). Chlorophyll *a* concentrations were determined using ethanol extraction (Sartory & Grobbelaar, 1984). Substrates were bagged with a premeasured volume of ethanol in Seal-a-meal heat-sealed pouches (Rival, Kansas City, MO, U.S.A.) and processed similarly to the removed algal samples.

The algal assemblage composition of the algae removed in brushing substrates was described using a relative abundance technique (Biggs, 1995) on wet mounts of subsamples remaining after chlorophyll analysis. Initial samples were pooled by substrate type ($n = 8$ per pool); final samples were pooled across substrates within tank-grazer treatment ($n = 10$ per pool). In scoring relative abundance, each species in a sample was ranked on a scale of 1 (=small, rare, and low relative biomass) to 5 (=abundant and highest relative biomass), based on a visual assessment of the assemblage at 400 \times . Both species density and cell biovolume are used in scoring; hence relative abundance scores reflect the relative total biovolumes of the taxa. Additional diatom subsamples were cleaned, slide-mounted in Naphrax (NPS, Ipswich, England) and viewed at 1000 \times for species identification of diatoms.

Field experiment

A field experiment was used to check the results of the tank experiment under natural conditions. The field site was Brier Creek (Marshall County, Oklahoma), a limestone first-order stream with large populations of *Camptostoma*. An array of single replicates of the 10 substrates was placed in each of five pools on 8 August. Substrates were randomly placed within each array. All substrates were exposed to ambient densities of grazers. Substrates were colonised and grazed for a 3-week period prior to final sampling. During final sampling, an additional stream limestone similar in size to the experimental stones was sampled (=ambient limestone; mean diameter = 6.8 cm).

There were two rainstorms during the experiment. The first storm was 4 days after the start of the experiment and substrates were placed into submerged tubs, which were moved to the shore prior

to the rain-associated increase in discharge to avoid damage to the experiment. Substrates were replaced in the stream after 2 days. The second, larger storm occurred unexpectedly a few days before harvest and substrates were subjected to spate conditions. Substrates were sampled using the same brushing and laboratory procedures used in the tank experiment.

Data processing

In order to calculate the chlorophyll *a* concentration of algae in the pits of clay-stones, the chlorophyll *a* concentration of the non-pit algae of these substrates was determined and subtracted from the total chlorophyll of the substrate. This non-pit chlorophyll included both the algae removed by brushing and algae remaining behind on brushed substrate surfaces. It was assumed that the chlorophyll concentration left behind on brushed surfaces was the same among clay-stones in the same tank-grazer or stream pool treatment. Three types of chlorophyll *a* data were calculated for clay-stone substrates (Fig. 1):

removed = chlorophyll removed by brushing;
area = total upper surface area – pit area; (measured for all substrates)

remaining = chlorophyll remaining on non-pitted substrates after brushing these substrates; area = total upper surface area; (measured for only limestone and

non-pitted clay-stones; this chlorophyll concentration was used to calculate pit algae in pitted substrates)

pit algae = chlorophyll remaining on a pitted clay-stone after brushing – remaining chlorophyll from non-pitted clay-stone in the same tank-grazer or stream pool treatment; area = pit area.

The upper surface area of clay-stones was calculated as the area of a circle, using the mean of three diameter measurements. Because limestone substrates were more irregular in shape than clay-stones, their surface area was measured as the planar area of individual stones. Limestone substrates and a scale were scanned with a computer scanner and the images printed. Images were cut out, weighed and the surface areas calculated from the density of paper, using the scale to determine paper area (this corrected for changes in image size during scanning and printing). Pit areas and densities were used to calculate the percents of pit and non-pit areas for each of the eight pitted substrate types.

Three-factor, blocked ANOVAs were used to test chlorophyll differences among substrate types and grazing treatments (both fixed effects); tanks were used as the blocking variable (a random effect). Calculations of *F* followed Zar (1996). Because there was only one replicate per cell (i.e. one substrate per substrate type-grazer treatment-tank combination = 240 substrates) the three-way interaction term mean square was used as the error mean square (Zar, 1996), and only main effects and 2-way interactions were tested.

Algal composition data were used to assess the similarity of algal assemblages among substrates at the beginning of the tank experiment and to test for variation in the composition of the algal samples at the end of the tank and field experiments. Algal composition data were limited to algae removed by brushing the upper substrate surfaces and the analysed datasets were restricted to the more common algal taxa (15–18 species, depending on the set of samples) because the relative abundance technique may miss rare taxa. Relative abundance scores were standardised (to adjust for differences in the spread of values among samples) and square root transformed prior to constructing Curtis-Bray similarity matrices. Similarity data were analysed with non-metric multidimensional scaling (MDS), followed by either CLUSTER (using group-average) for one-factor data or ANOSIM2 (using Spearman rank correlation) for

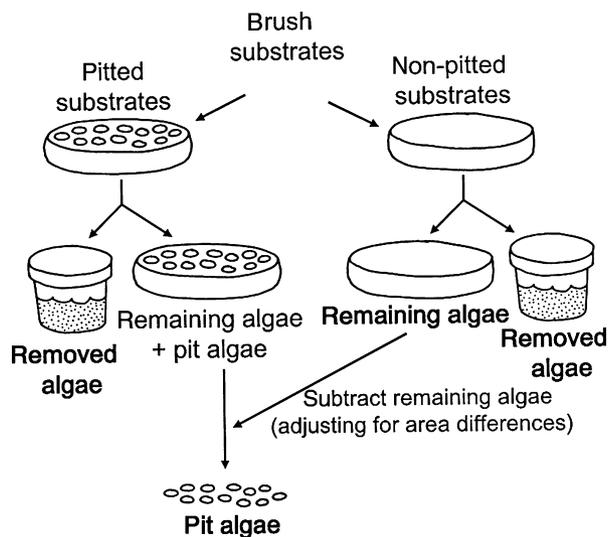


Fig. 1 Method of quantifying chlorophyll *a* concentrations from clay-stones.

two-factor data. SIMPER was used to identify taxa contributing to differences among sample groups. These analyses were done using PRIMER, Version 5 (Primer-E Ltd, Plymouth Marine Laboratory, U.K.).

Results

Tank experiment: initial substrates

After inoculation, all substrates developed a loosely adherent diatom-dominated mat that included considerable silt. Chironomid larvae (Insecta: Diptera) had colonised the mat by the start of the experiment. There was no significant difference in chlorophyll concentration among the eight different clay-stone substrates at the beginning of the experiment (chlorophyll removed by brushing: ANOVA, $F_{7,56} = 2.00$, $P = 0.071$; chlorophyll in pits: ANOVA, $F_{7,56} = 1.92$, $P = 0.084$). Limestone had higher chlorophyll concentrations than the artificial clay-stones (total chlorophyll: ANOVA, $F_{1,14} = 8.24$, $P = 0.010$), with means (SE) of 43.5 (4.5) and 28.1 (2.6) mg m^{-2} , respectively.

In addition to inhabiting relatively large surface pits, small algae may occur in small pore spaces in stones. Algal biomass in such pores can be estimated by determining the percentage of total algae that is left on non-pitted substrates after brushing. Limestone retained 17% of total chlorophyll with brushing, whereas clay-stones retained only 5% of total chlorophyll.

Algal composition was similar among substrates at the beginning of the tank experiment (mean Curtis-Bray similarity, $S = 89.0\%$; range: 80.7–94.3%), although similarity was higher among clay-stone substrates (mean $S = 90.5\%$) than between clay-stone and limestone (mean $S = 84.1\%$) (Fig. 2). Divergence of substrate compositions in the MDS plot (Fig. 2) resulted from differences in rarer species; for example, the segregation of limestone from the clay-stone substrates resulted primarily from a higher abundance of *Nitzschia sigmaidea* (Nitzsch) W. Sm. on this substrate (limestone rank = 3 of 5; mean of all other substrates = 1.22, range = 0–2). Composition analysis does not include the substrate set with pit size = 11.5 mm because these samples were accidentally discarded after chlorophyll analysis; however, it is unlikely that algal composition of these substrates differed substantially from the other substrates, given the close similarity among all other substrates.

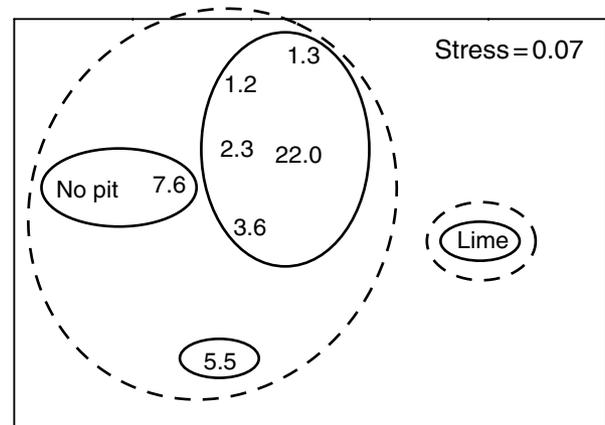


Fig. 2 Non-metric multidimensional scaling (MDS) and CLUSTER plots of algal compositions for each substrate at the start of the tank experiment. Numbers show the diameter (in millimetre) of pits for pitted clay-stones; 'no pit' = non-pitted substrates; and 'lime' = limestone. Data for the 11.5 mm pit substrates are missing. Solid circles enclose samples with 90% Curtis-Bray similarity; dashed circles show 85% similarity. Stress indicates how well the viewed ordination represents the multiple-dimensional ordination (stress <0.10 indicates a good representation).

Dominant algal species were all diatoms: *Nitzschia palea* (Kütz.) W. Sm., *Encyonema minuta* (Hilse ex Rabh.) M. Mann, *Navicula arvensis* Hust., *Gomphonema parvulum* (Kütz.) Kütz., and *Achnantheidium minutissimum* (Kütz.) Czarn.

Tank experiment: harvest

Differences among tanks. Chlorophyll concentration differed among the eight replicate tanks (ANOVA, $F_{7,230} = 12.59$, $P < 0.0001$; Fig. 3), with mean chlorophyll concentrations ranging from 15.5 to 70.7 mg m^{-2} . Differences among tanks corresponded to relative light levels, as half of the tanks were partly shaded by a roof overhang (ANOVA, $F_{1,237} = 49.8$, $P < 0.0001$). Chlorophyll concentrations of shaded tanks were approximately half of chlorophyll concentrations of sunny tanks [means (1 SE) of 26.8 (1.9) and 53.7 (3.3) mg m^{-2} , respectively]. Additionally, chlorophyll differences among tanks corresponded to relative density of grazing chironomids (ANOVA, $F_{2,236} = 20.9$, $P < 0.0001$; Tukey's test, $P < 0.05$). Tanks were visually scored for chironomid density prior to harvest. Tanks with low and high densities of chironomids had less chlorophyll than tanks with no evident chironomids [means (SE) of 35.9 (2.6), 30.2

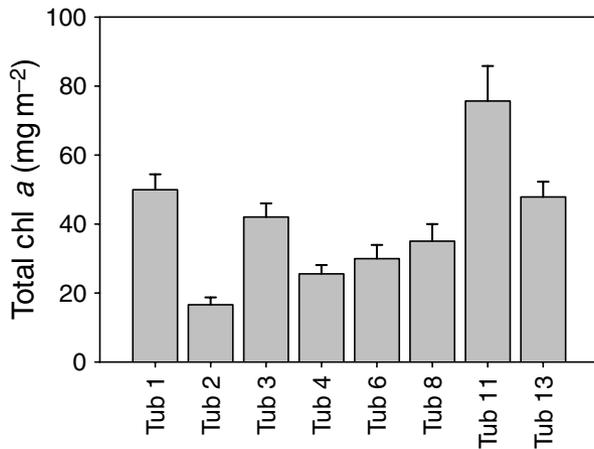


Fig. 3 Total chlorophyll *a* concentration of substrates in the eight tanks at the end of the grazing experiment. Odd numbered tanks were located in the sun; even numbered tanks were in the shade. Tanks 4, 6, and 8 had high chironomid densities in cage treatments; tanks 1, 2, and 3 had low densities. Chironomids were not apparent in tanks 11 and 13. $n = 30$; Bars show 1 SE.

(2.3), and 61.2 (5.7) mg m^{-2} and $n = 3, 3,$ and 2, respectively]. Light and chironomids were confounding variables because chironomids were generally associated with shaded tanks. During the experiment chironomids were apparent only in cage treatments, where they occurred on all substrate types.

Algae in pits. Chlorophyll concentration in the pits of the clay-stones differed among the substrates (Table 1; Fig. 4). Chlorophyll concentration peaked at 140 mg m^{-2} in pits of 1.3 mm diameter. As pit size increased, chlorophyll concentrations decreased. Pit diameters between 2 and 6 mm contained 81–97 mg m^{-2} chlorophyll, a reduction of 35% from the peak. As pit size was further increased to 7–22 mm, chlorophyll levels dropped by 59% to 34–43 mg m^{-2} . These results were consistent with observations that indicated heavy grazing in large pits, grazing in the bottoms but not sides of medium pits, and either thick growth of algae or, sometimes, algae mostly absent in small pits. The apparent reduction in pit algae as pit size decreased slightly from 1.3 to 1.2 mm was likely an artefact resulting from the shallowness of the smallest pits (1.1 mm versus 1.4 mm deep). In these smallest-pit substrates, pit algae were susceptible to loss where the algae formed a thin mat (the pit algae were apparently pulled out of some shallow pits with the loss of the surrounding mat).

Table 1 ANOVA analysis of chlorophyll *a* concentrations in pit crevices and on exposed surfaces (=algae removed by brushing) in the tank experiment. Grazing treatments are open to grazers, caged from grazers and a cage control. Substrates are pitted clay-stones for pit chlorophyll *a* or all clay-stone substrates for removed algae

	MS	F	v_1, v_2	P
Pit chlorophyll <i>a</i>				
Grazing treatment	7764.9	0.49	2, 12	NS
Substrate	30718.4	12.08	7, 42	<0.001
Tank (block)	35136.7	22.33	6, 84	<0.001
Treatment \times substrate	2828.5	1.80	14, 84	NS
Treatment \times tank	15807.4	10.04	12, 84	<0.001
Substrate \times tank	2543.9	1.62	42, 84	NS
Treatment \times substrate \times tank	1573.6			
Pit chlorophyll <i>a</i> (without tank 11)				
Grazing treatment	25115.3	9.50	2, 10	<0.01
Substrate	24403.3	9.97	7, 35	<0.001
Tank (block)	14012.9	8.98	5, 70	<0.001
Treatment \times substrate	2562.0	1.64	14, 70	NS
Treatment \times tank	2643.2	1.69	10, 70	NS
Substrate \times tank	2447.7	1.57	35, 70	NS
Treatment \times substrate \times tank	1560.1			
Removed chlorophyll <i>a</i>				
Grazing treatment	37.4	0.01	2, 14	NS
Substrate	434.8	1.78	8, 56	NS
Tank (block)	5037.5	16.11	7, 112	<0.001
Treatment \times substrate	163.8	0.52	16, 112	NS
Treatment \times tank	3837.4	12.27	14, 112	<0.001
Substrate \times tank	244.8	0.78	56, 112	NS
Treatment \times substrate \times tank	312.7			

Grazer effects on pit algae. The relationship of crevice size and algal concentration in pits varied among the grazer treatments (Fig. 5). The two treatments with fish grazers (open and cage-control) followed the general pattern of higher algal levels in smaller pits relative to larger pits (Fig. 5a,b). In contrast, there was no relationship between pit size and algal level in the fish-exclusion treatment (Fig. 5c). Although this effect of fish grazers on pit algae was apparent with regression, ANOVA result for grazer treatment was non-significant (Table 1). Instead, ANOVA showed a significant grazer treatment \times tank interaction. This interaction was largely the result of tank 11, which was not colonised by chironomids and had very high chlorophyll concentrations in the cage treatment (Fig. 5c). Indeed, if tank 11 was removed from the ANOVA analysis, all three main effects (grazer treatment, substrate, and tank) became significant, and no interactions were significant (Table 1). Open treatments had significantly more chlorophyll than control and cage treatments (Tukey's test, $P < 0.05$;

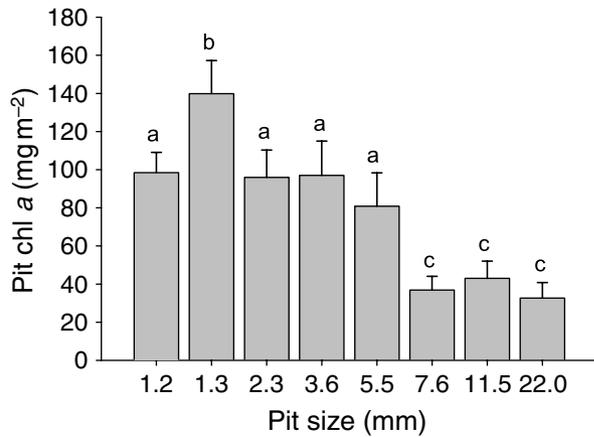


Fig. 4 Pit chlorophyll concentrations in clay-stone substrates at the end of the tank experiment. Significant differences among substrates are indicated by different letters above bars ($P \leq 0.05$); error bars are 1 SE.

Fig. 6), suggesting firstly a cage effect and secondly that chironomids are more efficient than *Campostoma* at grazing pit algae.

Removed and remaining algae. Algae removed by brushing were algae exposed on the upper surfaces of the substrates. Chlorophyll concentrations of removed algae did not differ among the grazer treatments or among substrates (Table 1). The significant tank effect and treatment \times tank interactions are consistent with results in pit chlorophyll. Chlorophyll concentrations of removed algae were significantly smaller than concentrations of algae in pits (paired t -test: $t_{183} = 10.86$, $P < 0.0001$). Removed algae averaged $27.0 \text{ mg chlorophyll m}^{-2}$ (SE = 5.3), whereas algae in pits averaged $77.9 \text{ mg chlorophyll m}^{-2}$ (SE = 2.0).

The amounts of algae removed by brushing or remaining with the substrate did not differ between non-pitted clay-stones and limestone (MANOVA: Wilks' Lambda - $F_{2,43} = 2.53$, $P = 0.092$). The initial differences in chlorophyll concentrations between the artificial clay-stones and limestone had vanished by the end of the experiment. Additionally, the difference in algal retention by substrates that was apparent at the start of the experiment had also vanished, producing mean chlorophyll retentions of 20.2% for non-pitted clay-stones and 18.6% for limestone.

Assemblage composition. Removed algal assemblages were strongly diatom-dominated. *Gomphonema parvulum* (Kütz.) Kütz. was by far the most abundant

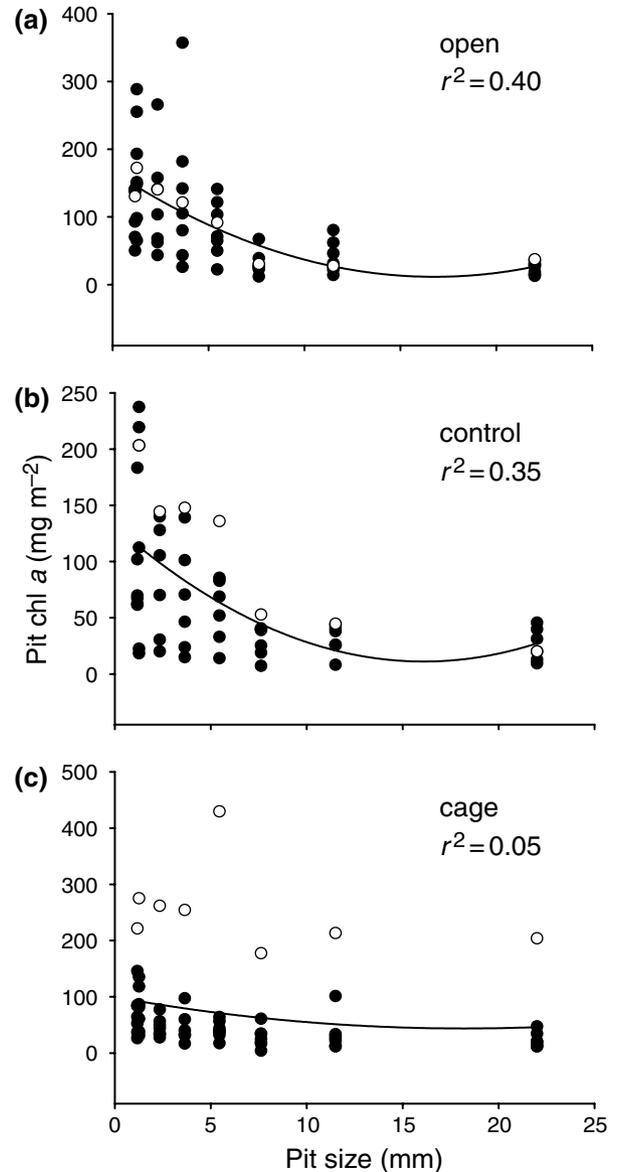


Fig. 5 Relationship between pit size and chlorophyll *a* concentrations within pits for the different grazer treatments: (a) open and grazed by *Campostoma*; (b) grazed cage control; (c) caged and ungrazed by *Campostoma* (chironomid grazers are usually present). Second-order polynomial regression lines and their corresponding r^2 values are shown. White circles are data from the tank that was not grazed by chironomids (tank 11).

species; *Gomphonema subclavatum* (Grun.) Grun., *A. minutissimum*, and *N. palea* were also common. Algal composition was significantly different between tanks (ANOSIM2: $\rho = 0.255$, $P = 0.028$) but not among grazer treatments ($\rho = 0.119$, $P = 0.235$). Tank differences corresponded to sun/shade exposure (Fig. 7) and four uncommon diatom species accounted for

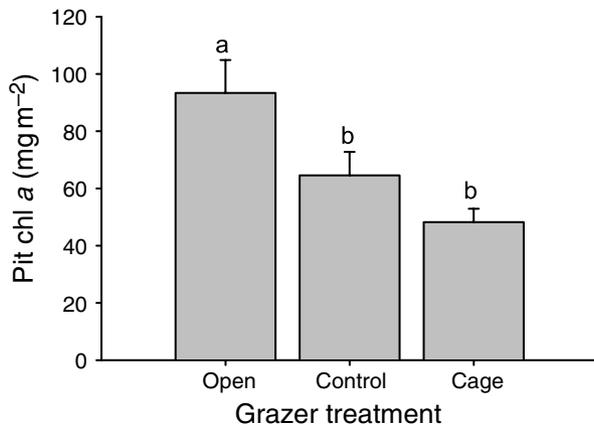


Fig. 6 Mean pit chlorophyll *a* concentrations of clay-stones in the three grazer treatments.

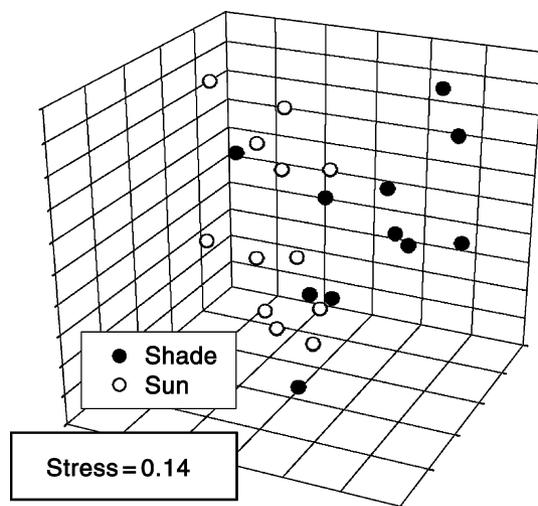


Fig. 7 The MDS plot of the algal composition of substrates at the end of the tank experiment. Samples are coded by shade or sun location on tanks. As stress increases from 0.10 to 0.20, the ordination corresponds less well to the multi-dimensional ordination and a stress >0.20 indicates a poor representation.

50% of the dissimilarity between sun and shade exposed tanks (SIMPER). Three of these species were found more frequently in sun-exposed tanks (*Achnanthydium levanderi* (Hust.) Czarn, *N. sigmoidea*, and *Surirella angusta* Kütz.) and one species was more frequent in shaded tanks (*Amphora veneta* Kütz.).

Field experiment

Although the field experiment was set-up with a balanced design, a severe spate near the end of the

experiment resulted in the loss of 36% of the original substrates and data were consequently analysed as a regression with crevice size as the independent variable rather than by ANOVA. Lost substrates were either not found or were upside down, and nearly all substrates had moved. The five pool sites differed in water depth and canopy shading (and relative damage by the unplanned spate), and the corresponding differences in chlorophyll *a* values among pool sites could not be removed by including pool as an ANOVA variable (as planned). Instead, chlorophyll data were standardised by proportionally adjusting values at each site so that all sites had the same mean chlorophyll concentration. The physical disturbance caused by the spate was a pulse disturbance superimposed on the press disturbance of grazing; hence algae remaining in crevices were protected from two disturbances.

As in the tank experiment, chlorophyll concentration was highest in pits with diameters smaller than 2.0 mm (Fig. 8a; nonlinear regression: $r^2 = 0.17$, $P = 0.02$). As pit size increased from 2 to 7 mm, chlorophyll concentrations decreased and then levelled off beyond diameters of 7 mm.

Chlorophyll concentrations of removed algae were significantly smaller than concentrations in pits (paired *t*-test: $t_{69} = 6.45$, $P < 0.0001$). Removed algae averaged 3.7 mg chlorophyll m⁻² (SE = 0.4), whereas algae in pits averaged 18.5 mg chlorophyll m⁻² (SE = 2.3). As pit diameter increased, chlorophyll *a* removed by brushing increased (Fig. 8b; $r^2 = 0.22$, $P = 0.04$), indicating a possible shift in grazing activity from areas between pits to the pits, themselves, as pit size increased.

After 3 weeks of stream incubation, clay-stones and precleaned limestone had accrued similar amounts of algae (means = 6.4 and 9.4 mg chlorophyll m⁻², respectively). Chlorophyll concentrations on these two substrates were significantly less than concentrations on ambient limestone in Brier Creek (ANOVA: $F_{2,9} = 38.23$, $P < 0.0001$; Tukey's test <0.05), which had an average concentration of 71.9 mg chlorophyll m⁻².

The amounts of algae removed by brushing and remaining with the substrate differed among substrate types (MANOVA: Wilks' Lambda: $F_{4,16} = 12.97$, $P < 0.0001$). For both algae removed and remaining on substrates, there were no significant differences between clay-stones and precleaned limestone, but

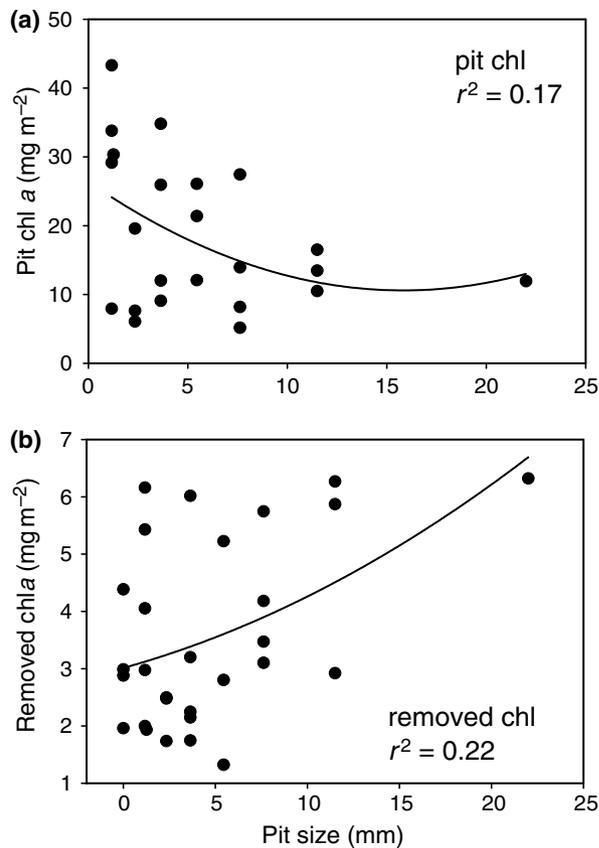


Fig. 8 Chlorophyll *a* concentrations of clay-stone substrates at the end of the field experiment in Brier Creek. Chlorophyll *a* concentrations are shown for (a) pits and (b) algae removed by brushing. Third-order polynomial regressions and their corresponding r^2 values are shown.

there were large differences between these substrates and ambient stream stones (ANOVAs: removed chlorophyll: $F_{2,9} = 10.18$, $P = 0.0049$; remaining chlorophyll: $F_{2,9} = 19.43$, $P = 0.0005$). Among the substrates, mean retention of chlorophyll *a* was highest in ambient limestone, intermediate in precleaned limestone and lowest in clay stones (means of 76.5, 67.3, and 53.1%, respectively; ANOVA: $F_{2,8} = 4.31$, $P = 0.0536$, $n = 4$; Tukey's test: $P < 0.05$). A green colour on the stones was sometimes apparent after brushing the ambient limestone.

At the end of the field experiment, algal assemblages of clay-stone substrates, precleaned limestone and ambient limestone were not significantly different (ANOSIM2: $\rho = -0.063$, $P = 0.664$). Likewise, there was no significant difference among assemblages from different pools ($\rho = 0.042$, $P = 0.331$). Assemblage biomass was dominated by the diatoms

Nitzschia spp., *Reimeria sinuata* (Gregory) Kociolek & Stoermer, *Navicula cryptocephala* Kütz., *Navicula menisculus* (Grun.) Grun., and *Gyrosigma spencerii* (Quek.) Griff. & Henfr.

Discussion

A variety of artificial substrates is commonly used in studying stream algae (Aloi, 1990). Algal assemblages on such artificial substrates often differ from assemblages on natural substrates during early colonisation (Blinn, Fredericksen & Korte, 1980) but resemble stream assemblages after a few weeks (Blinn *et al.*, 1980; Lamberti & Resh, 1985; Lowe *et al.*, 1996). This pattern was seen in the colonisation of clay-stones and limestone, in that initial differences in chlorophyll concentration had vanished by the end of the tank experiment. In the field experiment, algal colonisation of clay-stones and precleaned limestone was similar, but both of these substrates had less algal biomass and less algal retention with brushing than did ambient stream limestone. Endolithic growth of cyanobacteria in the ambient limestone contributed to the higher algal biomass on this substrate. Algal assemblage composition was also similar between clay-stones and limestone in the tank and field experiments. These similarities in algal assemblages indicate that results from this experiment can be generalised beyond the artificial substrates to streambeds of limestone and possibly other rocks.

This study tested aspects of a crevice size hypothesis (Bergey, 1999) that integrates the relative sizes of crevices and organisms with protection from disturbance. Specifically, for crevice refuges that are small relative to the organism size, crevices protect relatively few individuals of small species or small stages. Larger crevices can protect a wider range of organism size and larger numbers of individuals and, for small species, a wider range of species. As crevices increase further in size, disturbance effects enter crevices and crevices are no longer refuges.

This study supported the crevice size hypothesis by demonstrating the transition from protective to non-protective crevices as pits exceeded 2 mm in diameter and *Campostoma* were able to graze within these larger crevices. This study complements an earlier study that demonstrated protection of only the very small diatom *A. minutissimum* in the small crevices of sanded glass surfaces and the protection of a larger, more diverse

diatom assemblage in larger crevices (Bergey, 1999). Together, these studies support the close relationships between crevice size and diatom size for small crevices (Bergey, 1999) and the decoupling of crevices as refuges for larger crevices (this study).

This size hypothesis apparently also applies to refuges among stable stream stones. Holomuzki & Biggs (1999) described how the New Zealand snail *Potamopyrgus antipodarum* was better protected in the inter-particle spaces of stabilised finer gravels and pebbles than among coarser cobbles. Flow 'skims' over the finer gravels and pebbles, but turbulently enters the larger spaces among cobbles (Morris, 1955; Davis & Barmuta, 1989), thereby dislodging snails among cobbles (Holomuzki & Biggs, 1999). Similarly, invertebrate colonisation of rock baskets was greater for gravel and pebbles than in cobble (Khalaf & Tachet, 1980). Cobble baskets were preferentially colonised by filter-feeding hydropsychid larvae (Khalaf & Tachet, 1980), which may indicate higher water flow through the cobble baskets than through baskets with finer substrates. Mobilisation of substrates by spates can reverse this relationship, with the more stable cobbles becoming more protective than less stable gravels and pebbles (Gurtz & Wallace, 1984).

Campostoma grazing showed a stair-step relationship of protectiveness with crevice size. Small crevices effectively excluded *Campostoma* from grazing, intermediate crevices were partially protective and large crevices were not protective. Intermediate-sized crevices were grazed primarily on the bottoms and had a ring of brown algal growth at the top of the pits. This pattern of grazing indicates that the angle of 'attack' by grazing *Campostoma* may have been limited by the inflexibility of their bodies and they were not able to graze effectively the nearly vertical surface just below the rim of the crevice. Larger pits provided more room to manoeuvre and all surfaces within the pit were grazed. Invertebrate grazers also show zonation by feeding primarily in the bottoms of crevices and not on the sides (Robson & Barmuta, 1998). Clearly, fine-scale grazing patterns in crevices vary with crevice size and the size, morphology and behaviour of the grazer.

Campostoma form schools that feed in a limited area and then move as groups. As a result, grazing effort is localised. If the preference or the ability for grazing in pits changes as pit size increases, a trade-off between grazing in pits and grazing on exposed surfaces may

be evident. Indeed, there was an inverse relationship between the algal biomass of pits and of clay-stone surfaces in the field experiment but not in the tank experiment. As pit size increased in the field experiment, algal biomass within pits decreased and algal biomass outside pits increased, possibly indicating a preference for grazing on the curved inner surface of large, accessible pits relative to the grazing on the adjacent flat, exposed surfaces. In contrast, this grazing behaviour was not noted in the tanks. Instead, the limited grazing area in tanks may promote more evenly grazed exposed surfaces and preferences may be masked.

Results of the field experiment in Brier Creek corroborated those of the *Campostoma*-grazed substrates in the tank experiment, in that the smaller pits were the most effective refuges and refuge effectiveness declined with pit size. Brier Creek contained a suite of grazers, including snails and mayflies, but the high density of *Campostoma* in this stream and previous studies of its grazing effects (including several at the study site on Brier Creek) suggest that this fish was the dominant grazer (e.g. Power *et al.*, 1985; Power & Stewart, 1987; Gelwick & Matthews, 1997), and that other grazers had limited effect. In addition to *Campostoma* grazing, experimental substrates in Brier Creek were disturbed by a large spate. That the combination of spate and grazer disturbance in the field experiment produced the same pit-size relationship as the *Campostoma* grazing in the tank experiment, indicates that disturbance by spates may affect the effectiveness of pits as refuges in a similar manner as does grazing by *Campostoma* (i.e. these disturbances may operate at the same scale).

Campostoma was not the only grazer in the tank experiment. Chironomids had colonised experimental substrates prior to *Campostoma* stocking and chironomids remained in the cages that excluded *Campostoma*. *Campostoma* evidently consumed chironomids in the tanks and, indeed, this typically algivorous fish also consumes invertebrates (Robison & Buchanan, 1988; Evans-White *et al.*, 2001). The remaining chironomids were effective grazers and algal biomass in chironomid-grazed cages was lower than in *Campostoma*-grazed treatments.

The relationship between pit size and algal protection differed between *Campostoma* and chironomids. In contrast to the crevice-size relationship associated with *Campostoma* grazing, no crevice size effects were

found for chironomid grazers. Chironomids are much smaller than *Campostoma* and evidently are able to graze in smaller pits. The pit sizes tested were apparently too large to show a crevice-size effect for chironomid grazers; however, a size relationship would be expected if smaller pit sizes had been included in the experiments. This grazer-specific difference in crevice protection is an example of different disturbances operating at different spatial scales (e.g. Townsend & Hildrew, 1994).

This study demonstrated the importance of crevice size as a determinant of refuge protection and the results indicated that different disturbances may operate on the same spatial scale with respect to refuge protection (*Campostoma* grazing and spates), or on different scales (*Campostoma* and chironomid grazing). Crevice refuges are not restricted to streams, but are common on hard marine substrates and in terrestrial habitats. Greater understanding the three-way interaction among characteristics of refuges, the size of contained organism(s) and disturbance would benefit our knowledge of processes of colonisation and resistance to disturbance in streams and other disturbance-prone habitats.

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