Journal of Animal Ecology 2003
72, 1015–1026

Caddisfly diapause aggregations facilitate benthic invertebrate colonization

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Summary

1. We used natural and manipulative field experiments to examine the effects of caddisfly (Trichoptera) diapause aggregations on benthic macroinvertebrates communities in a Vermont river.
2. Natural substrates with aggregations of *Neophylax* and *Brachycentrus* (Trichoptera: Uenoidae and Brachycentridae) had higher species richness than did substrates lacking aggregations. Aggregations of caddisfly cases added to artificial substrates (bricks) also accumulated greater abundance, species density (number of species per unit area), and species richness (number of species per standard number of individuals) than did control bricks.
3. Low-density, uniformly spaced, *Brachycentrus* cases accumulated higher species density and species richness than did an equivalent density of clumped cases. Similarly, empty *Neophylax* cases accumulated higher diversity than did cases still occupied by *Neophylax* pupae.
4. Although natural substrates had higher species richness than artificial substrates, substrate type did not change qualitatively the effect of caddisfly aggregations on species richness.
5. We subsampled individuals randomly from aggregations and control surfaces to provide an estimate of species richness unbiased by abundance. Expected species richness was higher in aggregations than on control surfaces. These results suggest that caddisfly aggregations increase species density by altering the shape of the species–abundance distribution as well as by accumulating individuals and species passively.
6. We conclude that caddisfly diapause aggregations increase habitat complexity and facilitate colonization of other benthic species.

Key-words: biogenic structures, community, ecosystem engineering, positive interactions, rarefaction.

Journal of Animal Ecology (2003) **72**, 1015–1026

Introduction

After decades of neglect (Boucher, James & Keeler 1982; Hunter & Aarssen 1988; Bertness & Callaway 1994; Stachowicz 2001), the study of positive interactions in ecological communities has recently enjoyed a resurgence of interest (Poulin & Grutter 1996; Hacker & Gaines 1997; Kareiva & Bertness 1997; Holzapfel & Mahall 1999; Cardinale, Palmer & Collins 2002). Although studies of competition and predation continue to dominate community ecology, positive interactions are important in communities of plants (Andersen & MacMahon 1985; Chapin *et al.* 1994; Tardiff & Stanford 1998; Holzapfel & Mahall 1999), marine invertebrates (Duggins 1981; Gallagher, Jumars & Trueblood 1983; Wieczorek & Todd 1997; Wieczorek & Todd 1998) and freshwater invertebrates (Pringle 1985; Diamond 1986; Englund & Evander 1999; Stewart *et al.* 1999).

Positive interactions may be pervasive because some species act as 'ecosystem engineers', creating or modifying habitats that are used by other species (Jones, Lawton & Shachak 1994). A large-scale example of ecosystem engineering is a beaver dam, which alters stream flow and sedimentation (Pollock *et al.* 1995). On a smaller scale, the shells, cases and tubes of sessile invertebrate species may stabilize substrates and/or increase area and complexity of habitats that are

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available to other colonizing species (Gallagher *et al*. 1983; Pawlik, Butman & Starczak 1991). Organisms that facilitate community-level colonization may also be considered 'keystone species' if the magnitude of their influence far exceeds expectations based on their biomass (Power *et al*. 1996).

In this paper, we tested the role of case-bearing caddisfly larvae as facilitators of colonization in benthic stream assemblages. In streams of North America, larvae of *Brachycentrus* (Curtis) and *Neophylax* (McLachlan) form dense aggregations on rock surfaces, and their cases can persist for weeks (in the instance of *Brachycentrus*) to months (for *Neophylax*). In a natural experiment, we measured the species richness and species density of macroinvertebrates on rock surfaces with and without caddisfly cases. In a field experiment, we manipulated the density and spatial arrangement of empty pupal cases and living prepupae, and established appropriate controls for the manipulations. Our results suggest that caddisfly aggregations influence both passive accumulation of individuals and species, and alter the nature of interspecific interactions in benthic communities.

Materials and methods

STUDY SITE

The study site was in a third-order section of the LaPlatte River in Northern Vermont. The LaPlatte is a productive stream draining a mixture of agricultural, forested and suburban areas. The stream bordered an old field and a woodlot with overhanging canopy. Stream depth ranged from 5 to 60 cm and substrates consisted of sand, silt, pebbles, cobbles and boulders. Small, geologically diverse boulders (20–40 cm diameter) occupied most of the stream bed.

CADDISFLY LIFE HISTORY

Caddisflies in the genera *Neophylax* (Trichoptera: Uenoidae) and *Brachycentrus* (Trichoptera: Brachycentridae) are common and widespread in North America (Flint 1984; Wiggins 1998). During spring, larvae of both genera form aggregations on the undersides and vertical surfaces of boulders and cobbles. Once aggregated, *Brachycentrus* larvae immediately pupate, and adult caddisflies emerge in early spring (Flint 1984). In contrast, most *Neophylax* species enter a prepupal diapause beginning in mid spring to mid summer and lasting up to six months. Pupation occurs in late summer, and adults emerge in early autumn (Beam & Wiggins 1987; Martin & Barton 1987). Empty *Neophylax* cases persist in the environment for weeks (Martin & Barton 1987) to months (personal observation) after adult emergence. Empty *Brachycentrus* cases do not persist for more than a few weeks and are relatively rare by comparison to *Neophylax* cases (Fig. 1). *Neophylax fuscus* (Banks) and *Brachycentrus apalachia* (Flint) were the dominant aggregation-forming species encountered at our field site.

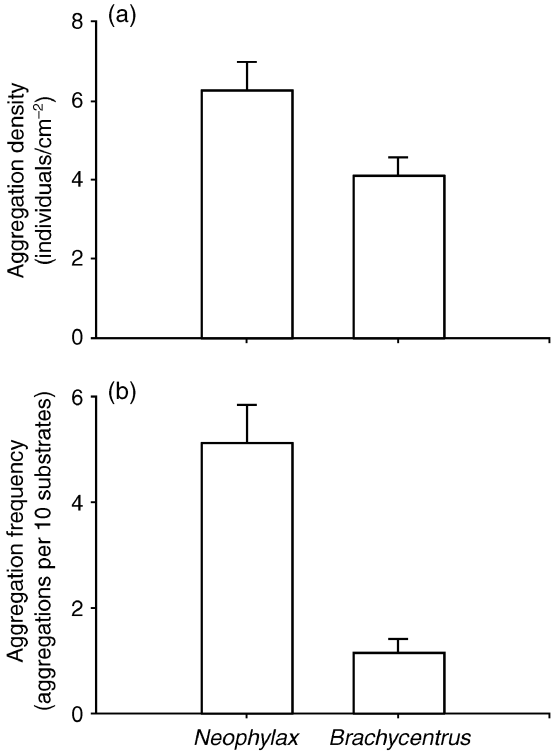

Fig. 1. (a) Density of aggregated caddisflies based on 52 mm by 23 mm samples (n = 10 for each species) taken from the centres of large aggregations. Error bars represent standard error. High densities are due in part to multiple layers of individuals. (b) Frequency of occurrence of aggregations of caddisflies on substrates (cobbles and boulders) in random-walk transects consisting of 10 substrates (n = 7 transects). Error bars represent standard error.

FIELD EXPERIMENTS

Separate case-addition experiments for *Brachycentrus* and *Neophylax* were carried out. To minimize variance caused by non-treatment factors, ceramic fire bricks ($52 \times 90 \times 23$ mm) were used as experimental substrates. Fire bricks are chemically inert and similar in texture to many of the natural boulders and cobbles at the site. For each case-addition experiment, 10 replicates of the following five treatments were established: (1) plain-brick control; brick without cases or glue; (2) glue control; spots of glue were applied to the brick end (to measure effects of glue); (3) low density aggregation; caddisfly cases distributed uniformly across the brick surface; (4) low-density clumped treatment; the same number of cases were used as in treatment 3, but attached in two high-density clumps (to separate effects of total case number from case density); and (5) high-density aggregation; caddisfly cases were attached to the entire end-surface of the brick (Fig. 2). For the *Neophylax* experiment only, a sixth treatment was added; live caddisfly prepupae were attached at high density to the brick end. The densities used were within

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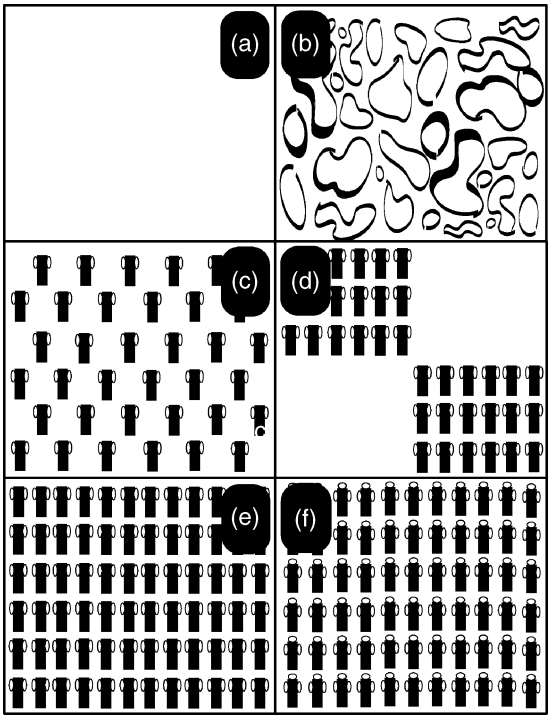

Fig. 2. Aggregation treatments applied to the small surfaces of bricks: (a) control, (b) glue control, (c) low total density, (d) low total density clumped, (e) high total density, (f) high total density live prepupae (*Neophylax* experiment only).

the natural range observed at the site. Throughout the paper the treatments are referred to as follows: (1) control; (2) glue control; (3) low density; (4) clumped; (5) high density; and (6) live.

For both experiments, caddisflies from prepupal aggregations were collected from the experimental site 2 weeks prior to the experiment. All larvae and pupae were removed from the cases used for the manipulations. Each case was examined under a dissecting microscope, and any attached macroinvertebrates were removed. The cases were then dried for 48 h and attached with silicone glue to brick surfaces. Live pupae were collected 1 day before the *Neophylax* experiment. We blotted dry the ventral exterior surfaces of the prepupal cases, removed attached macroinvertebrates, and glued the prepupal cases to the brick surfaces. The pupae were draped immediately with wet paper towels and refrigerated until they were transported back to the field site, 12 h later. This treatment was not practical for *Brachycentrus* because of their short pupation period.

Substrates in each experiment were arrayed in a single stream run in a randomized-block design. The treated end of each substrate was orientated downstream, which coincides with the orientation of most aggregations at the site (personal observations). Each block in our experimental array spanned the width of the stream with treatment position randomly assigned within blocks. Treatments were not replicated within blocks. The distance between substrates within blocks was on average 0·5 m. Blocks were separated by approximately 1 m. The *Neophylax* experiment began on 24 July 1996 and ended on 14 September 1996. The *Brachycentrus* experiment ran from 21 May 1997 until 23 June 1997. The timing of the experiments coincided with occurrence of natural aggregations of each caddisfly species.

All macroinvertebrates were collected from the treated ends of the bricks following the experiment. Beginning at the downstream end of the experimental array, samples were collected in an upstream direction to minimize disturbance of substrates prior to collection. A downstream net was not used because it would have sampled invertebrates from the untreated surfaces of the bricks (McAuliffe 1984). Loss of mobile invertebrates was minimized by stretching a latex glove over the treated surface of each brick before it was lifted from the streambed. All material from the treated brick surfaces was removed using razor blades (to cut the glue) and scrubbing brushes. Each sample was placed in a 145-μm sieve and rinsed to remove fine sediment before adding 95% EtOH.

Immediately following the experiments, macroinvertebrate samples were collected from 20 haphazardly selected boulders that contained aggregations of the target trichopteran species. A wooden template was used to ensure that the area sampled was equal to the area of a brick end and therefore comparable to our experimental samples. To ensure a valid comparison to experimental aggregations, natural aggregations smaller than the template were rejected. Natural aggregation densities were calculated from these samples (Fig. 1a).

Following the *Neophylax* experiment, we counted *Neophylax* prepupae, pupae, and empty cases as well as empty *Brachycentrus* cases on 70 cobbles to assess the frequency of aggregation occurrence in the stream (Fig. 1b). The substrates were selected haphazardly from seven random-walk transects. We defined aggregations as containing groups of five or more cases. Average aggregation size (sum of prepupae, pupae and empty cases) was 30 for *Neophylax* (n = 36) and 18 for *Brachycentrus* (n = 17; only empty *Brachycentrus* cases existed; the next generation of active larvae was not included in this analysis).

In addition, we sampled invertebrates from the entire surfaces of 20 haphazardly selected boulders that lacked trichopteran aggregations.

SAMPLE PROCESSING

Caddisfly pupae and cases added to the treatments were counted to confirm that the treatments had persisted through the experiments, but these counts were excluded from our analysis. With the exception of the live treatment in the *Neophylax* experiment, there was negligible loss of caddisfly cases during the experiments (< 5%). An average of 73% of the cases from the live pupae treatment persisted through the experiment and remained sealed. Of these, 70% contained live pupae.

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Table 1. Hypothetical community responses to experimental addition of a putative facilitator

Response of species density	Response of species richness	Interpretation
0	0	Null hypothesis
+	0	Passive sampling (PS)
0	+	Shifts in relative abundance distribution (RAD)
+	+	RAD with or without PS

All invertebrates contained in the experimental samples and natural aggregation samples were identified and counted. With the exception of chironomids, invertebrates were identified to the lowest possible taxonomic level. We have therefore measured taxon richness and taxon density but will refer to these metrics as species richness and species density throughout the paper. Analysis of our data at the family level yielded qualitatively similar results to the finer-level taxonomic analysis presented here. The majority of the hydroptilids sampled was at the free-living, early-instar stage and could not be identified using existing keys. Later-instar hydroptilids were identified to genus.

Samples from stream boulders and cobbles lacking aggregations were split using a Folsom plankton splitter (Wildco, model number 1831-f10 0298). We identified a minimum of 300 randomly selected invertebrates to adequately represent the communities on these substrates (Vinson & Hawkins 1996).

SPECIES DENSITY VS. SPECIES RICHNESS

We quantified species counts as *species density*, the number of species per replicate in each sample. To control for the effects of abundance on species density, we also quantified species richness, the number of species per standard number of individuals counted. The distinction between species richness and species density is important in studies of biodiversity, because species density is very sensitive to total abundance, whereas species richness standardizes samples that differ in their abundance (Gotelli & Colwell 2001). Separate analyses of species richness and species density reveal whether facilitation occurs through passive accumulation of individuals, or through changes in the relative abundance distribution of assemblages with and without caddisfly aggregations (Table 1).

A Monte Carlo simulation similar to rarefaction (Hurlbert 1971; Simberloff 1978) was used to estimate the expected species richness for a given number of randomly drawn individuals from each sample. The smallest samples in the *Neophylax* and *Brachycentrus* data sets contained 32 and 35 individuals, respectively. For all but the smallest sample in each experiment, we used ECOSIM software (Gotelli & Entsminger 2001) to sample 32 individuals randomly from each *Neophylax* sample and 35 individuals from each *Brachycentrus* sample. We repeated this procedure 100 times for each sample and used the average number of species as the expected species richness for each sample. For the smallest sample in each experiment the total number of species in the sample represented species richness and species density.

The nine most abundant taxa (summed over the two experiments) were selected for individual analysis. These taxa accounted for more than 90% of the organisms collected from each experiment. Although the rank abundance of organisms differed between experiments, the same list of organisms was used in each for consistency. To stabilize variances, the total abundance and individual taxon abundance data were $\log_{10}$ transformed.

ANALYSIS OF FIELD EXPERIMENT DATA

In all analyses, the response variables were total abundance ($\log_{10}$ transformed), species density and species richness. Data sets were analysed first using a MANOVA, the results of which were highly significant (Table 2). We therefore proceeded with individual ANOVAs for $\log_{10}$ (total abundance), species density and species richness.

For the community-level response variables, a randomized-block one-way ANOVA was used to compare all six (in the case of *Neophylax*) or five (*Brachycentrus*) treatments. This model assumes that there is no interaction between blocks and treatments (Underwood 1997). We used a set of orthogonal a priori linear contrasts to test the following null hypotheses for each response variable: (1) that the glue control did not differ from the plain control; (2) that the low-density treatment did not differ from the low-density clumped treatment; (3) that aggregation treatments did not differ from control treatments; and (4) that aggregations of live *Neophylax* pupae did not differ from aggregations of empty cases of similar density.

COMPARISON BETWEEN ARTIFICIAL AND NATURAL SUBSTRATES

Because the samples from natural substrates lacking aggregations were not collected from a standardized area, and because surface area of these substrates was not recorded, comparisons of species density and total abundance were not appropriate. Analysis of expected species richness, however, was appropriate because collections were standardized to an equivalent number of individuals. A two-way factorial ANOVA was used to

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Table 2. MANOVA of effects of (a) *Neophylax* and (b) *Brachycentrus* aggregations on abundance, species density, and species richness. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}005$

Effect		d.f.	SS	MS	*F* ratio
(a) *Neophylax*					
Block	Abundance	9	0·8	0·09	3·4***
	Species density	9	471·3	52·4	6·6***
	Species richness	9	36·4	4·0	3·3***
Treatment	Abundance	5	2·1	0·43	16·1***
	Species density	5	864·4	172·9	21·8***
	Species richness	5	42·3	8·5	6·8***
Error	Abundance	44	1·2	0·027	
	Species density	44	348·9	7·9	
	Species richness	44	54·5	1·2	
(b) *Brachycentrus*					
Block	Abundance	9	0·569	0·06	0·998
	Species density	9	130·3	14·5	0·868
	Species richness	9	7·0	0·774	0·461
Treatment	Abundance	4	3·6	0·9	14·2***
	Species density	4	793·4	198·4	11·9***
	Species richness	4	21·2	5·3	3·2*
Error	Abundance	35	2·2	0·06	
	Species density	35	583	16·7	
	Species richness	35	59	1·7	

evaluate the main effects and interactions of substrate type (artificial or natural) and trichopteran aggregations (present or absent). Separate analyses were carried out for *Neophylax* and *Brachycentrus*, with four treatment groups in each analysis: (1) aggregations on natural substrates, (2) aggregations on bricks, (3) natural substrates lacking aggregations and (4) bricks lacking aggregations. 'Bricks lacking aggregations' included both control treatments. For the 'aggregations on bricks' treatment, all aggregation densities and spatial arrangements were pooled. Pooling was justified because natural aggregations span a wide range of densities, and pooled samples fell within the natural range.

EXAMINATION OF INDIVIDUAL SPECIES RESPONSES

A MANOVA followed by a series of nine one-way analyses of variance was used to examine the responses of the nine most common taxa to the treatments. The taxa examined were: Chironomidae, early instar Hydroptilidae, *Oecetis* avara (Banks), *Aturus carolinensis* (Habeeb), *Torrenticola rufoalba* (Habeeb), *Antocha* (Osten Sacken) sp., Crustaceans, *Torrenticola* (Piersig) sp. Nymphs and *Hydropsyche* (Pictet) sp.

Results

FIELD EXPERIMENT

Abundance

There was a significant treatment effect on abundance in both the *Neophylax* and *Brachycentrus* experiments (Table 3). In both experiments, abundance in aggregation treatments was higher than total abundance in the two control treatments (a priori linear contrasts; *Neophylax*: $P < 0{\cdot}001$, Fig. 3a; *Brachycentrus*: $P < 0{\cdot}001$, Fig. 4a). In the *Neophylax* manipulation, the glue control had higher abundance than the plain brick control ($P = 0{\cdot}007$; a priori linear contrast; Fig. 3a). There was a significant block effect in the *Neophylax* experiment only: downstream blocks tended to have higher abundance than upstream blocks ($P = 0{\cdot}005$).

Table 3. ANOVA of effects of (a) *Neophylax* and (b) *Brachycentrus* aggregations on abundance ($\log_{10}$-transformed). 'Glue control vs. control', 'Low density vs. clumped', and 'Live vs. high density' are a priori linear contrasts comparing specific treatments described in the text. 'Aggregation vs. control' is an a priori linear contrast comparing the mean of the aggregation treatments to the mean of the control treatments. Symbols as in Table 2

Effect	d.f.	SS	MS	*F* ratio
(a) *Neophylax*				
Block	9	0·78	0·086	3·2**
Treatment	5	2·1	0·42	15·6***
Error	44	1·19	0·027	
Glue control vs. control	1	0·23	0·23	7·9**
Low density vs. clumped	1	0·006	0·006	0·21
Live vs. high density	1	0·056	0·056	2·1
Aggregations vs. controls	1	1·84	1·84	67·9***
(b) *Brachycentrus*				
Block	9	2·9	0·33	0·69
Treatment	4	22·7	5·7	12·0***
Error	36	17·1	0·48	
Glue control vs. control	1	0·093	0·093	0·20
Low density vs. clumped	1	0·69	0·69	1·5
Aggregations vs. controls	1	20·2	20·2	42·4***

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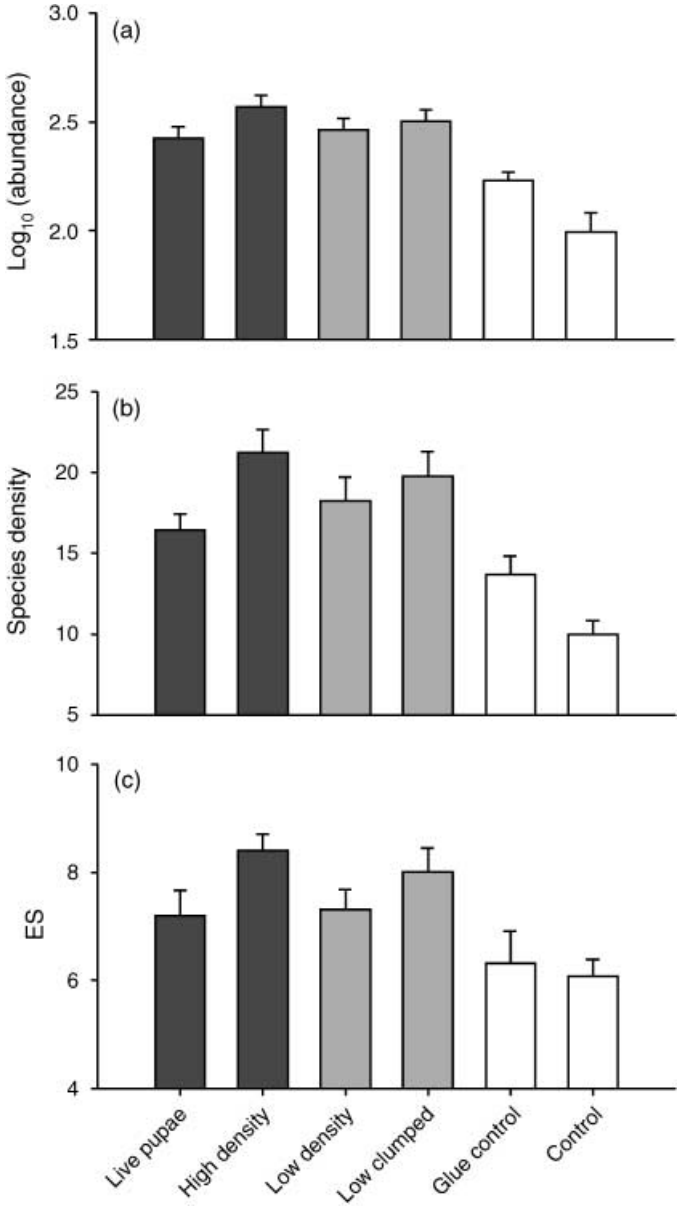

Fig. 3. Community responses to *Neophylax* aggregation treatments and controls. (a) Total abundance of macroinvertebrates. (b) Species density or the total number of macroinvertebrate species found on each substrate. (c) Expected species richness (ES) standardized to a standard number of individuals. Bars of identical shading represent treatments with identical total caddisfly case densities. Black bars represent total coverage of the substrate by caddisfly cases. Grey bars represent 50% coverage. White bars represent zero cases. Error bars represent standard error.

Species density

The effect of treatment on species density was significant in both experiments (Table 4). As with total abundance, species density in aggregation treatments was higher than on control substrates (a priori linear contrasts; *Neophylax*: $P < 0{\cdot}001$, Fig. 3b; *Brachycentrus*: $P < 0{\cdot}001$, Fig. 4b). Species density was significantly higher on glue controls than on plain brick controls in the *Neophylax* experiment ($P = 0{\cdot}01$; a priori linear contrast; Fig. 3b). In the *Brachycentrus* experiment, species density was significantly higher in the low-density treatment in which cases were uniformly distributed, than in the low density clumped treatment ($P = 0{\cdot}011$; a priori linear contrast; Fig. 4b). Species density on aggregations of live *Neophylax* pupae was lower than on aggregations of empty *Neophylax* cases ($P = 0{\cdot}011$; a priori linear contrast; Fig. 3b). There was also a significant block effect on species density in the *Neophylax* experiment ($P < 0{\cdot}001$).

SPECIES RICHNESS

Aggregation treatments had highly significant effects on species richness in both the *Brachycentrus* and *Neophylax* experiments (Table 5). Species richness in aggregation treatments was higher than on control substrates (a priori linear contrasts; *Neophylax*: $P < 0{\cdot}001$; Fig. 3c, *Brachycentrus*: $P = 0{\cdot}014$; Fig. 4c). Species richness was higher on treatments with high-density *Neophylax*

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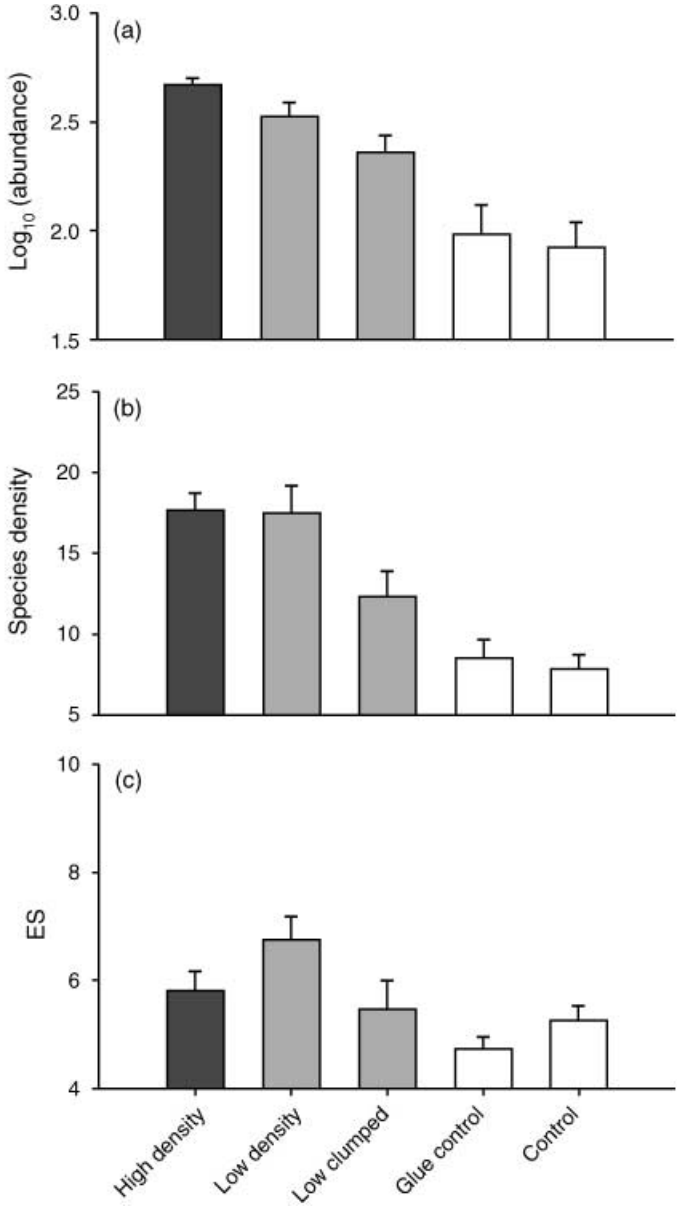

Fig. 4. Community responses to *Brachycentrus* aggregations and controls. Response variables and symbols are defined in Fig. 3.

cases than on treatments with live pupae attached (a priori linear contrast; $P = 0{\cdot}01$; Fig. 3c). Species richness was higher in treatments the low-density clumped *Brachycentrus* cases than in the low density treatment without clumping (a priori linear contrast; $P = 0{\cdot}03$; Fig. 4c).

INDIVIDUAL SPECIES RESPONSES

Of the nine most common taxa on the artificial substrates, five responded significantly to the experimental treatments in both experiments (Chironomidae, *Oecetis avara*, *Aturus carolinensis*, *Hydropsyche* sp.; one-way ANOVA; Table 6). There were significant treatment effects on *Antocha* sp. and *Torrenticola* sp. nymphs in the *Neophylax* experiment and on free-living hydroptilids in the *Brachycentrus* experiment. Most taxa were more abundant on aggregation treatments than on controls (Figs 5 and 6). Interestingly, several taxa of smaller size (free-living hydroptilids, *Oecetis avara*, *Torrenticola rufoalba*, *Torrenticola* sp. nymphs) were more abundant on high-density empty cases than on live pupae (Fig. 5).

Artificial vs. natural substrates

Both substrate type (brick or natural) and aggregation presence or absence had significant main effects on expected species richness (two-way factorial ANOVA; Table 7). For both trichopteran taxa, species richness was higher in the presence of aggregations than in their absence regardless of substrate type (*Neophylax*: $P < 0{\cdot}001$, Fig. 7a; *Brachycentrus*: $P < 0{\cdot}001$, Fig. 7b). In both the presence and absence of aggregations, natural substrates had higher species richness than did artificial substrates (*Neophylax*: $P = 0{\cdot}02$, Fig. 7a; *Brachycentrus*: $P < 0{\cdot}001$,

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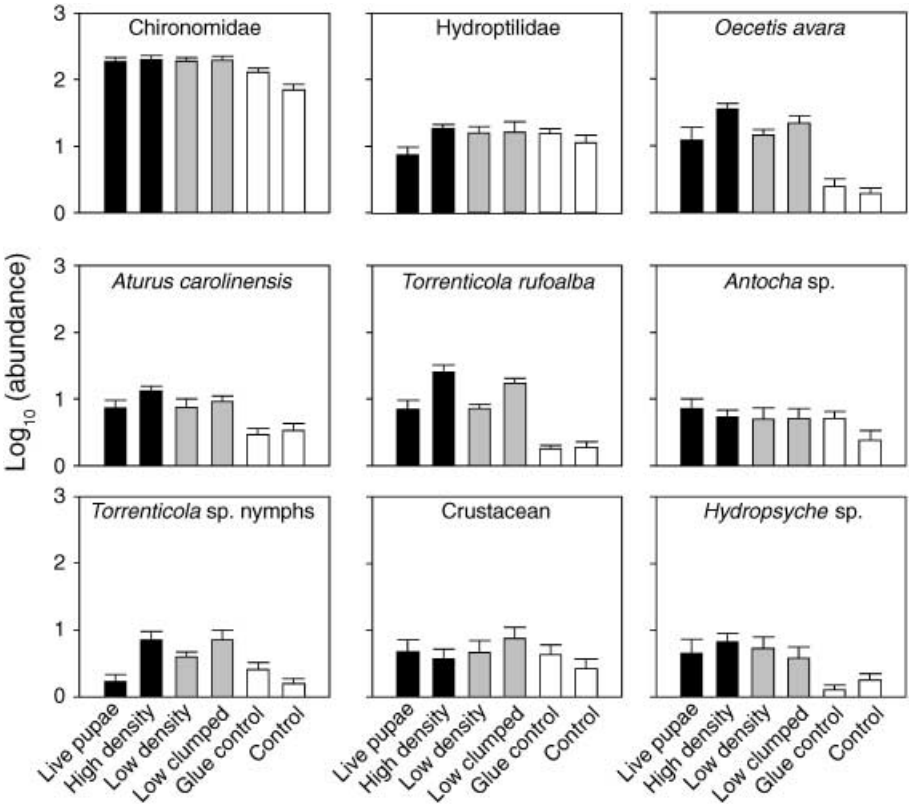

Fig. 5. Effects of *Neophylax* aggregations on abundance of the numerically dominant taxa. Symbols are defined in Fig. 3.

Table 4. ANOVA of effects of (a) *Neophylax* and (b) *Brachycentrus* aggregations on species density. 'Glue control vs. control' and 'Low density vs. clumped' are a priori linear contrasts comparing specific treatments. 'Aggregation vs. control' is an a priori linear contrast comparing the mean of the aggregation treatments to the mean of the control treatments. Symbols as in Table 2

Effect	d.f.	SS	MS	*F* ratio
(a) *Neophylax*				
Block	9	457·7	50·9	6·4***
Treatment	5	861·9	172·4	21·6***
Error	44	351·5	8·0	
Glue control vs. control	1	58·2	58·2	7·3*
Low density vs. clumped	1	13·1	13·1	1·6
Live vs. high density	1	120·1	120·1	15·0***
Aggregations vs. controls	1	665·9	665·9	83·4***
(b) *Brachycentrus*				
Block	9	118·6	13·2	0·74
Treatment	4	879·1	219·8	12·3***
Error	36	643·7	17·9	
Glue control vs. control	1	2·45	2·45	0·14
Low density vs. clumped	1	130·1	130·1	7·3*
Aggregations vs. controls	1	696·2	696·2	38·9***

Table 5. ANOVA of effects of (a) *Neophylax* and (b) *Brachycentrus* aggregations on expected species richness. 'Glue control vs. control' and 'Low density vs. clumped' are a priori linear contrasts comparing specific treatments. 'Aggregation vs. control' is an a priori linear contrast comparing the mean of the aggregation treatments to the mean of the control treatments. Symbols as in Table 2

Effect	d.f.	SS	MS	*F* ratio
(a) *Neophylax*				
Block	9	36·3	4·0	3·3***
Treatment	5	42·6	8·5	6·9***
Error	44	54·2	1·2	
Glue control vs. control	1	0·54	0·54	0·44
Low density vs. clumped	1	2·3	2·3	1·9
Live vs. high density	1	9·0	9·0	7·3*
Aggregations vs. controls	1	30·3	30·3	24·6***
(b) *Brachycentrus*				
Block	9	6·9	0·77	0·46
Treatment	4	21·2	5·3	3·16*
Error	35	58·8	1·7	
Glue control vs. control	1	1·2	1·2	0·69
Low density vs. clumped	1	8·6	8·6	5·1*
Aggregations vs. controls	1	11·2	11·2	6·7*

Fig. 7b). There was no significant interaction between substrate type and aggregation treatment (*Neophylax*: $P = 0{\cdot}54$; *Brachycentrus*: $P = 0{\cdot}49$): aggregations increased species richness in a similar manner on both natural and artificial substrates (Fig. 7).

Discussion

FACILITATION IN FRESHWATER

Our field and natural experiments demonstrated that both *Neophylax* and *Brachycentrus* facilitate invertebrate

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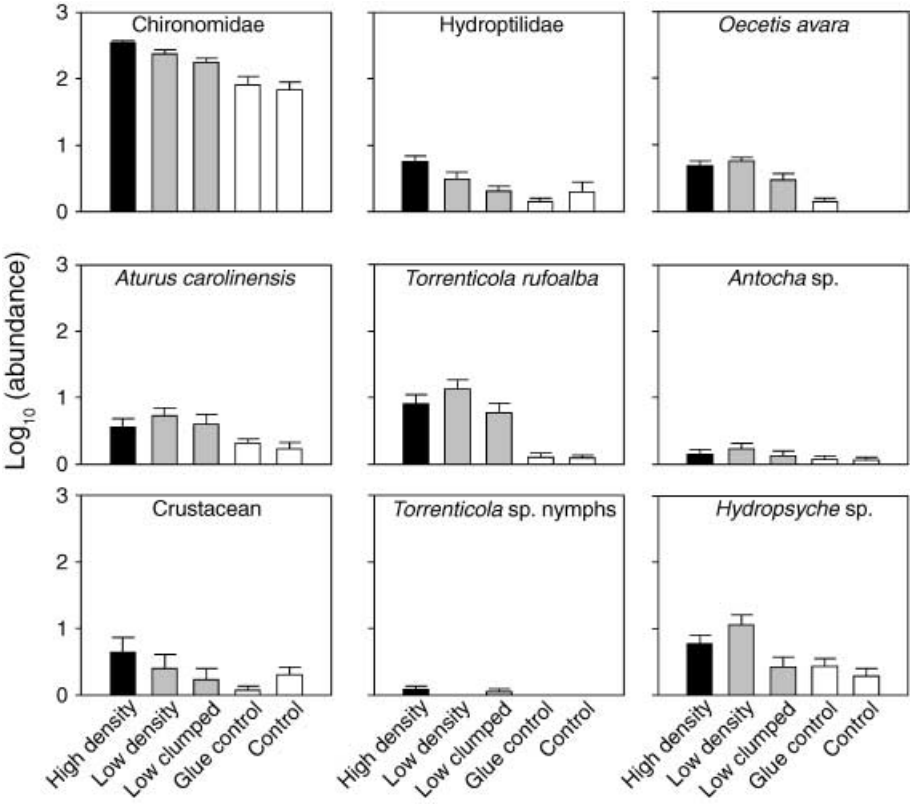

Fig. 6. Effects of *Brachycentrus* aggregations on abundance of the numerically dominant taxa. Symbols are defined in Fig. 3.

Table 6. *P*-values of the MANOVA and from the individual one-way ANOVAs testing for treatment effects on the log-transformed abundance of the nine most common taxa on experimental substrates. Taxa are listed in order of decreasing abundance

	P-values	
Taxon	*Neophylax* experiment	*Brachycentrus* experiment
MANOVA	< 0·001	< 0·001
Chironomidae	< 0·001	< 0·001
Free-living *Hydroptilidae*	0·079	0·001
Oecetis avara	< 0·001	< 0·001
Aturus carolinensis	< 0·001	0·012
Torrenticola rufoalba	< 0·001	< 0·001
Antocha sp.	0·029	0·386
Torrenticola sp. nymphs	< 0·001	0·195
Crustaceans	0·365	0·323
Hydropsyche sp.	< 0·001	< 0·001

colonization. Whereas previous studies focused on the proximate and ultimate mechanisms of trichopteran aggregation (Otto & Svensson 1981; Gotceitas 1985; Martin & Barton 1987), no study that we are aware of has examined the effects of trichopteran diapause aggregations on the benthic invertebrate community. Fixed retreats (as distinct from mobile cases of many trichopterans) of hyropsychid caddisflies have been demonstrated to increase local mayfly abundance (O'Connor 1993) and invertebrate diversity (Diamond 1986). Cardinale *et al*. (2002) demonstrated that interspecific facilitation among caddisfly species enhanced ecosystem

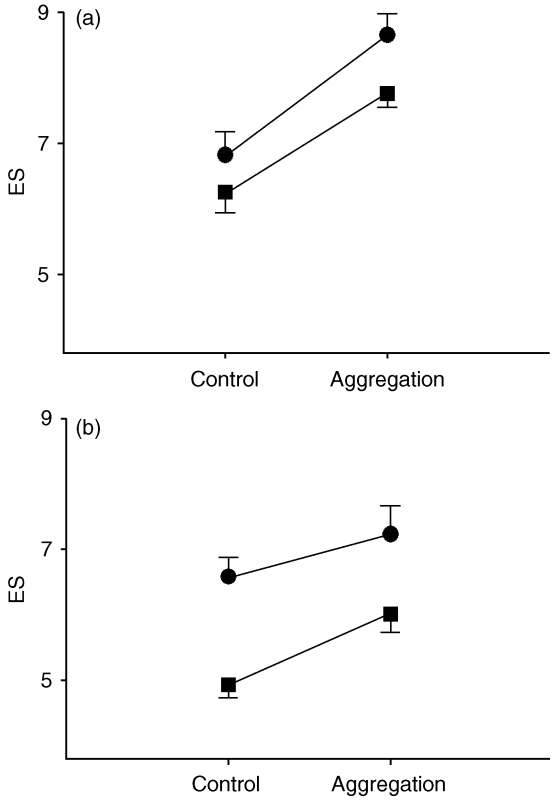

Fig. 7. Effects of aggregations on expected species richness (ES) on both natural and artificial substrates. (a) Effects of *Neophylax* aggregations. (b) Effects of *Brachycentrus* aggregations. Squares represent artificial substrates (bricks); circles represent natural substrates (cobbles and small boulders; error bars represent standard error.).

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Table 7. Two-way factorial ANOVA testing for main effects of substrate type and presence or absence of aggregations. The two levels of the substrate factor are artificial and natural. The second factor is presence or absence of aggregations. 'Controls' are natural and artificial substrates lacking caddisfly aggregations. Both glue controls and bare brick controls are included in the artificial substrates. 'Aggregations' represent all artificial aggregation treatments as well as samples taken from natural aggregations. Symbols as in Table 2

Effect	d.f.	SS	MS	*F* ratio
(a) *Neophylax*				
Aggregation	1	62·1	62·1	32·3***
Substrate	1	10·7	10·7	5·6*
Aggregation × substrate	1	0·7	0·71	0·31
Error	92	176·7	1·9	
(b) *Brachycentrus*				
Aggregation	1	16·5	16·5	7·6**
Substrate	1	44·9	44·9	20·8***
Aggregation × substrate	1	1·0	1·0	0·47
Error	86	185·2	2·2	

functioning. Englund (1993) and Englund & Evander (1999) found that caddisfly nets facilitated chironomid colonization, and Poff & Ward (1988) reported that baetid mayflies colonized still-occupied mobile cases of *Glossosoma* (Trichoptera: Glossosomatidae). These studies demonstrate that caddisfly retreats and cases have important habitat value, even when active, feeding and sometimes predacious (Englund & Evander 1999) caddisfly larvae are present. Diapause aggregations may have even greater habitat value because the immobile caddisflies are neither predacious nor territorial.

Facilitation of benthic invertebrate colonization has also been well documented in marine (Fager 1964; Gallagher *et al*. 1983; Gosselin & Chia 1995) and freshwater environments (Diamond 1986; Englund 1993; Stewart *et al*. 1999). A series of studies on zebra mussels (*Dreissena polymorpha*) has revealed their importance as facilitators of benthic invertebrates (Botts, Patterson & Schloesser 1996; González & Downing 1999; Horvath, Martin & Lamberti 1999; Bially & MacIsaac 2000). These observations and the results of our experiments suggest that positive interactions among freshwater species may be ubiquitous.

Caddisfly larvae fit the definition of an ecosystem engineer because case construction assembles particles from the environment into a new form (Lawton & Jones 1995) and alters their habitat value for periphyton (Bergey & Resh 1994). When caddisfly cases are aggregated they alter stream substrate texture, which affects species richness (Downes *et al*. 1998; Downes *et al*. 2000) and species density (Hart 1978) of stream invertebrate communities. When cases of the species we examined are aggregated, macroinvertebrate abundance (Figs 3a and 4a; Table 3) and diversity (Figs 3b,c, 4b,c) increase significantly (Tables 4 and 5). Facilitation as demonstrated in our experiments is likely to increase intersubstrate variance in the community metrics measured. To determine whether aggregations affect system-wide diversity would require experiments in multiple streams as well as natural comparisons among streams.

Whether caddisflies are important facilitators in a given stream depends on a number of factors including: the taxa present, the densities achieved during diapause, and the disturbance regime. Not all caddisflies aggregate during pupation although many widespread taxa do (examples: *Neophylax*, *Brachycentrus*, *Glossossoma* and *Agapetus*). Natural disturbance would determine how long the aggregations persisted in a particular stream. Thus facilitation by caddisflies is to some degree context-dependent, but aggregation-forming species are sufficiently common that their impacts on community dynamics should be considered in most freshwater communities.

MECHANISMS OF FACILITATION?

The general mechanisms of facilitation discussed commonly in the literature include resource enhancement (O'Connor 1993; Power 1990), habitat amelioration and associational defences (Bertness & Callaway 1994; Stachowicz 2001). The specific mechanisms of facilitation are diverse and vary by study system. Previous studies of facilitation in aquatic communities have implicated substrate stabilization (Fager 1964; O'Connor 1993), increased habitat heterogeneity or complexity (Botts *et al*. 1996; González & Downing 1999; Horvath *et al*. 1999), removal of sediment (Power 1990), accumulation of benthic organic matter (Horvath *et al*. 1999), provision of prey refuges (Gallagher *et al*. 1983) and hydrodynamic changes (Cardinale *et al*. 2002) as the direct mechanisms of facilitation. With the likely exception of sediment removal, all the factors listed above have potential as mechanisms for facilitation in caddisfly diapause aggregations. However, without additional data, it would be difficult and speculative to narrow the list further. In this case we can say, based on the positive response of species richness to caddisfly cases (Figs 3 and 4), that the facilitative effect is not simply due to passive sampling of a larger number of individuals from the community. The observed response is non-random and could result from differential settling, mortality, emigration, species interactions or a combination of these factors.

USE OF ARTIFICIAL SUBSTRATES

An obvious criticism of artificial substrates is that they are 'unrealistic' and cannot be compared to natural substrates (Rosenberg & Resh 1982; Casey & Kendall 1996). Although our data demonstrate that bricks do not accumulate as many species as natural stream substrates (Table 7; Fig. 7), our interest is not in the effects of the substrate type, but rather in the effects of caddisfly aggregations. The lack of a significant interaction between substrate type and presence of aggregations (Table 7) confirms that the direction and magnitude of

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the species richness response to aggregations is consistent between substrate types.

SPECIES DENSITY AND SPECIES RICHNESS

Because abundance increased in response to our aggregation treatments (Figs 3a and 4a), increased species density would be predicted based on passive accumulation alone. However, our analyses of species richness suggest that passive or random accumulation of individuals alone cannot account for the increased species density in the presence of caddisfly aggregations. An increase in a measure of species richness that is standardized to a fixed number of individuals is possible only with a shift in the relative abundances of the organisms in the community. It is necessary to invoke a non-random mechanism during or after colonization to achieve this result. Potential mechanisms include interspecific interactions in the aggregations (including predation, competition and facilitation) and selective sampling (caddisfly cases may preferentially accumulate colonists of certain species). Whether the relative abundances changed during colonization, or after, it is clear that caddisfly cases result in higher species richness and total abundance of organisms in this system.

Low-density *Brachycentrus* aggregations had significantly higher species density and species richness than did clumped treatments (same number of cases placed in high density clumps). High-density *Neophylax* cases had higher species density and richness than live pupae of similar density. In each instance the combination of significant species density and species richness responses, and the lack of significant differences in total abundance between the same treatments imply that the diversity differences are not simply passive sampling effects, but reflect changes in the relative abundance distribution of species (Table 1). There were no other significant community-level differences detected between aggregation types in either experiment. In general, the presence of an aggregation seems to matter more than case density or spatial arrangement.

Conclusions

This study demonstrates that two broadly distributed, taxonomically distant caddisflies facilitate colonization and increase abundance and diversity of stream macroinvertebrate communities and may function as ecosystem engineers (Jones *et al*. 1994). Caddisfly cases lead to increased invertebrate abundance (Figs 3a and 4a), and this factor is partly responsible for the increase in species density (Figs 3b and 4b). However, even after controlling statistically for differences in abundance, species richness is still greater in the presence than the absence of caddisfly cases (Figs 3c and 4c). Consequently, the relative abundance distribution and community structure must be altered by facilitation. How responses to ecosystem engineers are affected by the interplay of total abundance, species density and species richness merits additional attention (James & Wamer 1982; Gotelli & Colwell 2001). Examination of this interplay is particularly important because facilitators have positive effects on other populations (e.g. Pringle 1985; Pawlik *et al*. 1991). Positive interactions may be more common in freshwater habitats than was assumed previously and merit a closer examination by aquatic ecologists.

Acknowledgements

This research was funded by Vermont EPSCoR and a Theodore Roosevelt grant from The American Museum of Natural History. We gratefully acknowledge Dr J.C. Conroy for identifying a series of mites from this study and Dr J.S. Sykora for his examination of adult *Neophylax* specimens. This project could not have been completed without the help of several University of Vermont undergraduate students: K. Baltus, M. Toomey, A. Pitt, J. Wheeler and K. Marvin.

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Received 5 August 2002; revision received 27 June 2003

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