8-11-2000

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INFLUENCE OF SUBSTRATE SIZE, CLADOPHORA, AND CADDISFLY PUPAL CASES ON COLONIZATION OF MACROINVERTEBRATES IN SAGEHEN CREEK, CALIFORNIA

Steven D. Ellsworth

ABSTRACT.—Colonization of stream macroinvertebrates onto artificial substrates was examined in Sagehen Creek, California, during 1993. Substrate size and presence or absence of pupal cases of the caddisfly Anagapetus (Glossosomatidae) were examined as possible influences on macroinvertebrate colonization. Macroinvertebrates were collected from substrates after 60 d of incubation and identified to the lowest feasible taxonomic unit. Dry weight of the filamentous green alga Cladophora also was determined for each substrate.

A general linear model indicated a significant positive relationship between both substrate size and presence of pupal cases on richness ($P < 0.001$). There was no significant effect of cases on the $E_5$ evenness index, which supports the passive sampling model as a mechanism giving rise to the effect of cases on richness. A multivariate general linear model indicated a significant positive effect of Anagapetus cases on Micrasema bactro, Epeorus, and Rhynchotheca sp. 1, and of substrate size on Hydropsyche occidentalis and Epeorus. Neither cases nor substrate size had a significant effect on Cladophora density ($P > 0.05$). There were significant positive relationships between Cladophora density and both Shannon $H'$ and $J'$ indices. There were significant negative correlations between Cladophora density and both Shannon $H'$ and $J'$ indices. There were significant positive relationships between Cladophora density with Baetis, Simulium, and Chironomidae density. Combined main effects of substrate size, Anagapetus pupal cases, and Cladophora have dynamic influences on this macroinvertebrate fauna. This study demonstrates the complex interplay of biotic and abiotic factors that determine macroinvertebrate distribution and abundance in this system.

Key words: colonization, Anagapetus, Cladophora, substrate size, macroinvertebrates, habitat heterogeneity.

Determining relative influences of biotic and abiotic factors on distribution and abundance of organisms has been a central theme in ecology (Strong et al. 1984, Diamond and Case 1986, Schoener 1987); lotic ecology is no exception (e.g., Peckarsky and Dodson 1980, Grossman et al. 1982, McAuliffe 1984a, Minshall and Peterson 1985, Power et al. 1988, Resh et al. 1988, Death and Winterbourne 1994, Robertson et al. 1997). Examination of factors that influence colonization onto substrates has been a means of determining relative importance of abiotic and biotic factors in stream macroinvertebrate communities (Mackay 1992). Substrate size, colonization surface texture, and algal abundance all affect stream macroinvertebrate colonization (Fuller et al. 1986, O’Conner 1991, Mackay 1992, Douglas and Lake 1994). The present study was designed to examine influences of substrate size, the filamentous green alga Cladophora, and pupal cases of the caddisfly Anagapetus on macroinvertebrate colonization onto substrates in Sagehen Creek, California.

Substrate size significantly influences stream macroinvertebrate distribution and abundance on natural (Clements 1987, Douglas and Lake 1994) and artificial substrates (Clements 1987, Giller and Cambell 1989, Love and Bailey 1992, Douglas and Lake 1994) in streams. Species richness usually increases with substrate size in streams (Douglas and Lake 1994, Ellsworth 1997). Mechanisms generating this “species-area relationship” are not well understood for substrates in streams, although habitat heterogeneity, disturbance, and fragmentation effects all have been hypothesized (Douglas and Lake 1994, Ellsworth 1997). Substrate size also can influence distribution and abundance of individual taxa. The relationship between number of individuals and stone size has been shown to vary among taxa (Shelley 1979, McElhone and Davies 1983, Clements 1987).

Algae on substrate surfaces also can influence macroinvertebrate colonization (Hart 1979, Lamberti and Resh 1983, Fuller et al. 1986, Robinson et al. 1990, Mackay 1992, Death and Winterbourne 1995) and overall

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community structure in streams (Power et al. 1985, Dudley et al. 1986, Hart 1992). Relatively simple interactions between algae and invertebrates have been reported when algae are microscopic (McAuliffe 1984b, Fuller et al. 1986, Morin and Peters 1988). In contrast, macroalgae may affect macroinvertebrate colonization in more complex ways. Dudley et al. (1986) suggested that macroalgae may influence stream macroinvertebrate community structure by providing food to herbivores, altering physical conditions such as flow patterns, and competing with sessile macroinvertebrates for attachment space. Cladophora, a filamentous green alga, can influence macroinvertebrates by reducing current velocities over substrate surfaces (Hart 1992), providing attachment sites for invertebrates (Dudley et al. 1986), and shading substrate surfaces (Feminella and Resh 1991, Hart 1992). Orth and van Montfrans (1984), Duffy (1990), and Dodds (1991) suggested mutualisms exist between grazer-resistant macroalgae and grazers that consume their epiphytes. Cladophora may benefit epiphyte grazers by providing structural habitat for epiphytes and grazers that consume their epiphytes. Cladophora may benefit Cladophora by removing epiphytes that compete for resources with Cladophora or increase drag, thereby increasing the probability of dislodgment (Dodds 1991).

Substrate surface heterogeneity also may affect macroinvertebrate distribution and abundance in streams (Hart 1979, Diamond 1986, O’Connor 1991, Douglas and Lake 1994). The focus of many studies has been to determine effects of grooves and pits in substrate surfaces on algal and macroinvertebrate colonization (e.g., Hart 1979, Douglas and Lake 1994). Structures made by macroinvertebrates also can alter the physical complexity of substrate surfaces. Caddisfly retreats and cases, black fly pupal cases, and chironomid larvae tubes all can facilitate the colonization of other taxa (Diamond 1986, Malmqvist and Otto 1987, Bergey and Resh 1994). In the present study pupal cases of the caddisfly Anagapetus were used to alter surface complexity of stream substrates. Anagapetus, an abundant taxa in Sagehen Creek (Erman 1989, Ellsworth 1997), is in the family Glossosomatidae, a group of aquatic insects that build larval cases unique to the family (Merritt and Cummins 1996). After pupation and emergence, empty Anagapetus cases remain firmly attached to the stone surfaces in Sagehen Creek (Ellsworth personal observation). Cases may influence colonization of other macroinvertebrates by altering flows over substrate surfaces, providing refuge from predators, providing attachment sites, and promoting accrual of algae. This study provided an opportunity to test the resource availability hypothesis and passive sampling hypothesis (described in Dean and Connell 1987a, 1987b) as mechanisms affecting richness patterns on substrates.

**Materials and Methods**

The study was located in Sagehen Creek, California, near the University of California Sagehen Creek Biological Station. Sagehen Creek is on the eastern slope of the Sierra Nevada and is part of the Truckee River watershed, the terminus of which is Pyramid Lake, Nevada. The creek is largely spring fed and ranges in elevation from approximately 1800 to 2250 m.

Artificial substrates rather than natural substrates were used as colonizing surfaces to control for confounding effects of factors such as substrate shape and surface heterogeneity that differ among natural substrates. Use of artificial substrates can reduce variability of samples (Rosenberg and Resh 1982) and accurately represent the composition of natural substrates with a sufficiently long exposure period of 1–2 months (Lamberti and Resh 1985). Substrates used were 2 sizes of unglazed clay tiles (small, 8 × 8 × 0.5 cm, and large, 16 × 16 × 0.5 cm). Substrates were soaked in water and scrubbed thoroughly prior to placement in the creek. Anagapetus pupal cases were attached with a trace of epoxy glue and were evenly placed in a 3 × 3 arrangement on small substrates and a 6 × 6 arrangement on large substrates so that case densities were the same on both substrates. Densities were chosen to be representative of what was described from natural substrates by Ellsworth (1997). Eight separate riffles over an approximately 85-m stretch of the creek that had similar depths, widths, current velocity, and canopy cover were selected. Four treatments were used in the study: small substrates without Anagapetus cases (SWO), small with cases (SW), large without cases (LWO), and large with cases (LW). Substrates were placed in longitudinal
fashion in the center of the creek. Treatments were randomized within 8 clusters of each of the 4 treatments and incubated in the creek for 60 d from August to October 1993. This period of time was selected to ensure development of epilithic texture and interstitial deposition, and to provide enough time to reach equilibrium in macroinvertebrate richness and densities.

Substrates were collected by placing a Surber sampler (net mesh size 250 µm) immediately downstream from substrates and lifting them into the sampler. Macroinvertebrates and Cladophora filaments that accrued during the study were removed from substrates by a combination of manual agitation, scrubbing, manual picking with forceps, and rinsing with 95% ethanol. In the laboratory macroinvertebrates were identified to the lowest feasible taxon, and Cladophora filaments were separated using forceps. Cladophora was then placed in a drying oven, dried, and weighed to obtain dry weight for each substrate.

Richness, diversity, and evenness were used as response variables to compare macroinvertebrate assemblages on experimental substrates. Richness was analyzed using a general linear model with substrate size and presence or absence of cases as independent factors. Diversity was measured with the Shannon index \( H' \), and evenness with the \( J' \) and E5 index as described in Ludwig and Reynolds (1988). The possible effect of Anagapetus cases on macroinvertebrate assemblage richness of substrates in Sagehen Creek was examined by a method used by Dean and Connell (1987a, 1987b). The E5 index, also called the “modified Hill’s ratio,” is expressed:

\[
(\frac{1}{\lambda}) - 1 = \frac{1}{e^{H'} - 1}
\]

where \( \lambda \) is Simpson’s diversity index and \( H' \) is the Shannon index. Dean and Connell (1987a, 1987b) suggested that a resource availability hypothesis is supported if the positive relationship between richness and habitat complexity is explained by an increase in evenness. This hypothesis states that richness is increased by increasing the number of potential resources. Alternatively, the sampling phenomenon hypothesis states that species evenness is the same between simple and complex habitats and that an increase in richness on complex habitats is due solely to increase in the number of individuals. A Kruskal-Wallis test was performed to determine if there was an effect of Anagapetus cases on the E5 index.

To examine the possible influence of substrate size and Anagapetus cases on densities of individual taxa, a multivariate general linear model (MANOVA) was utilized. The 10 most abundant taxa on the substrates were chosen for analyses. A natural logarithmic transformation \( \ln(n + 1) \) of macroinvertebrate density data was used to stabilize variances. For individual univariate tests, the sequential Bonferroni technique was used to adjust probability values to take into account an increased probability of a type I error when multiple tests are done with the same model. A 2-way ANOVA was used to determine if there was an effect of substrate size and cases on Cladophora dry weight density. Dry weight density \( \ln \) transformed was used in regression analyses as predictor variable with individual macroinvertebrate taxa densities \( \ln(n + 1) \) transformed as response variables.

**RESULTS**

Two substrates overturned during the 60-d period and were not sampled or considered in analyses. Of 23 taxa identified over all samples, Chironomidae, Simulium, and Baetis were the dominant taxa in terms of abundance. Also identified were the caddisflies Brachycentrus americanus, Micrasema bactro, Anagapetus, Hydropsychie occidentalis, Ochotrichia, Neophylax, Rhacocaphila sp. 1, and Rhacocaphila sp. 2; the mayflies Cinygmula, Serratella, Epeorus, Drunella doddsi, and Drunella grandis; the dipterans Simulium, Cricotopus, and Antocha monticola; and the stoneflies Calineuria californica, Yoraperla, Nenoura, and Zapada.

Size and case treatments had significant positive effects on richness of individual substrate, with no significant interaction (Table 1, Fig. 1). There was no significant effect of case treatment on the E5 index (Kruskal-Wallis test, \( P = 0.454 \)). There was a significant negative correlation between Cladophora dry weight density and the Shannon diversity index (Fig. 2) and \( J' \) equitability index (Fig. 3). There was a significant effect of substrate size on the \( J' \) equitability index (Kruskal-Wallis test, \( P = 0.001 \)).
A multivariate general linear model with substrate size and presence or absence of *Anagapetus* cases as factors and individual macroinvertebrate taxa densities as response variables revealed several significant main effects (Table 2). Statistics for the multivariate test all showed a significant main effect of case treatment ($P < 0.05$) and substrate size ($P < 0.05$) on macroinvertebrate densities. There was no significant interaction between treatments ($P > 0.05$). In univariate 2-way analyses, case treatment had a significant effect after a sequential Bonferroni adjustment ($\alpha = 0.05$) on densities of *Micrasema bactro, Epeorus,* and *Rhyacophila* sp. 1 (Table 2, Fig. 4); and substrate size had a significant effect on *Epeorus* and *Hydropsyche occidentalis.* Neither case nor size treatments had a significant effect on *Cladophora* dry weight density ($P > 0.05$). Linear regression analyses of macroinvertebrate densities on dry weight density of *Cladophora* (ln transformed) revealed significant positive relationships with *Chironomidae, Baetis,* and *Simulium* (Fig. 5).

**DISCUSSION**

Ecologists have long sought to determine the relative importance of biotic and abiotic factors on populations and communities. A central theme developed in lotic ecology over the last several decades is that biotic interactions are linked closely to abiotic influences (Power et al. 1988, Mackay 1992). Referring to biotic and abiotic factors, Power et al. (1988) state “the relative importance and effect of such factors or ‘controls’ shift with dynamic changes in density of organisms and environmental conditions.” This study examined effects of 3 factors—presence of caddisfly pupal cases, the filamentous alga *Cladophora,* and substrate size—on distribution, abundance, and assemblage structure of stream macroinvertebrates. Results show significant effects of each factor, indicating that there are dynamic multilateral controls on the macroinvertebrate community in Sagehen Creek.

**Effects of *Anagapetus* Pupal Cases**

This study determined that pupal cases made by the caddisfly *Anagapetus* had an effect on densities of several macroinvertebrate taxa and overall richness on artificial substrates, although mechanisms involved have not yet been determined. *Anagapetus* cases are likely to accrue algae that are utilized by herbivores such as *Epeorus* and *Micrasema,* similar to the facilitation of grazers by *Gumaga* cases documented by Bergey and Resh (1994). Cases also may provide refuge from predators such as the stoneflies *Calineuria californica* and *Dorineuria,* the caddisfly *Rhyacophila,* trout, and sculpin, all of which are common in Sagehen Creek (Erman 1989, Ellsworth 1997). Results provide further evidence that structures made by macroinvertebrates are an important component of structural complexity of substrates and have significant influences on macroinvertebrate distributions and abundances. Bergey and Resh (1994) found that empty cases of the caddisfly *Gumaga nigricula* promoted accrual of algae on which other taxa grazed. Diamond (1986) found that larval retreats of the caddisfly *Cheumatopschye* facilitated colonization of other macroinvertebrate taxa onto stones through the presence of larval retreat and not the larvae themselves. Poff and Ward (1988) found that early instars of *Baetis* occupy *Glossosoma* cases to a greater extent than surrounding rock surfaces.
An examination of the mechanism causing the significant effect of pupal cases on richness suggests the sampling phenomenon hypothesis is supported in this study. This means the structural complexity created by *Anagapetus* cases acted to support more individuals per unit area, thereby increasing species richness, rather than increasing available resources (as stated in the resource availability hypothesis.) Dean and Connell (1987b) suggested that increased habitat complexity may increase the number of individuals through decreased levels of disturbance, or through active selection of complex habitats by colonists and passive transport and sorting of individuals into habitats. O’Conner (1991) proposed that decreased predation rates, increased food levels, and increased living space as mechanisms give rise to the passive sampling phenomena. In contrast to the present study, O’Conner (1991) demonstrated that the resource availability hypothesis was supported as an explanation for an increase in stream macroinvertebrate richness on wood with higher surface complexity. Douglas and Lake (1994) also supported the resource availability hypothesis as an explanation of increased richness on stones with higher degrees of surface heterogeneity. The relative influence of sampling phenomenon and resource availability mechanisms needs further attention to better understand how habitat heterogeneity influences stream macroinvertebrate distribution and abundance.

**Effects of the Green Alga Cladophora**

Dudley et al. (1986), Dodds (1991), and Hart (1992) all found that *Cladophora* affect abiotic and biotic characteristics of stream microhabitats. *Cladophora* has been shown to have positive and negative effects on individual macroinvertebrate taxa (Dudley et al. 1986, Hart 1992). The present study found influences of *Cladophora* on individual macroinvertebrate taxa and measures of community structure. The positive relationship between *Cladophora* and *Simulium* documented in this study is especially noteworthy as the 2 have been
reported to have strong negative relationships and to compete for attachment space (Dudley et al. 1986). Dudley et al. (1986) and Downes and Lake (1991) both suggested that black fly larvae avoid stones with heavy algal coats or attached filamentous algae. *Cladophora* may have significantly lowered light penetration to substrates, which in turn lowered periphyton levels on substrates (Feminella and Resh 1991). This effect may have enabled *Simulium* to better attach to substrate surfaces, assuming that attachment space is not limiting for *Simulium* and *Cladophora*. *Simulium* may also attach to *Cladophora* filaments, which would provide more attachment area on substrates with *Cladophora*. Alternatively, *Cladophora* and *Simulium* may have responded in the same manner to a variable that was not measured in the study. Hart (1992) suggested that mature *Cladophora* may deter grazing by chemical defenses but lack defenses during initial growth phases. The significant positive correlations of *Cladophora* abundance to *Baetis* and chironomid densities may be explained by the ability of these grazers to consume *Cladophora* in early growth stages. *Baetis* and chironomids also may utilize epiphytes growing on *Cladophora* and hence have a mutualism with *Cladophora* as described by Dodds (1991). Dodds (1991) suggested that when grazers remove epiphytes from *Cladophora*, the alga benefits from decreased competition for light and nutrients, and lower levels of drag. Clearly, manipulative experiments similar to that of Dudley et al. (1986), Dudley and D’Antonio (1991), and Hart (1992) are needed to sort out the mechanisms of *Cladophora* influence on these individual taxa in Sagehen Creek.

*Cladophora* had significant effects on measures of macroinvertebrate assemblage structure on individual substrates. The negative correlation between *Cladophora* and the Shannon diversity index on substrates can be explained by the negative relationship between *Cladophora* and evenness (as determined by $J'$) and not by an effect on richness. The decline in evenness at high levels of *Cladophora* density can be explained by the alga’s significant positive relationship to densities of the 3 most abundant taxa (*Baetis*, *Simulium*, and Chironomidae). This study provides evidence that *Cladophora* exerts a strong influence on macroinvertebrate community structure by modifying distribution and abundance of these 3 abundant taxa.

### Effects of Substrate Size

The positive relationship between substrate size and richness is consistent with the classic species-area relationship (McGuinness 1984, Hart and Horowitz 1991). Hart (1979), Clements (1987), Douglas and Lake (1994), and Ellsworth (1997) all have documented a species-area relationship for macroinvertebrates on stream stones. Mechanisms giving rise to the species-area relationship have not been well understood (McGuinness 1984, Hart and Horowitz 1991). However, recent studies have suggested that habitat heterogeneity, fragmentation effects, and disturbance may generate the species-area relationship for macroinvertebrates on stream stones.

### Table 2. Results of MANOVA showing the effect of substrate size and presence/absence of *Anagapetus* cases on density of individual macroinvertebrates. An asterisk indicates $P$ values that are significant ($\alpha = 0.05$) after a sequential Bonferroni adjustment.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Presence/absence of pupal cases</th>
<th>Substrate size</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micrasema bactro</em></td>
<td>0.001*</td>
<td>0.251</td>
</tr>
<tr>
<td><em>Anagapetus</em></td>
<td>0.337</td>
<td>0.013</td>
</tr>
<tr>
<td><em>Hydropsyche occidentalis</em></td>
<td>0.441</td>
<td>0.005*</td>
</tr>
<tr>
<td><em>Baetis</em></td>
<td>0.100</td>
<td>0.534</td>
</tr>
<tr>
<td><em>Cinygmula</em></td>
<td>0.265</td>
<td>0.027</td>
</tr>
<tr>
<td><em>Epeorus</em></td>
<td>0.004*</td>
<td>0.001*</td>
</tr>
<tr>
<td><em>Simulium</em></td>
<td>0.708</td>
<td>0.713</td>
</tr>
<tr>
<td><em>Rhyacophila</em> sp. 1</td>
<td>0.005*</td>
<td>0.077</td>
</tr>
<tr>
<td><em>Rhyacophila</em> sp. 2</td>
<td>0.019</td>
<td>0.340</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>0.271</td>
<td>0.931</td>
</tr>
</tbody>
</table>
Because artificial substrates used were not disturbed (i.e., overturned) during the study period, habitat heterogeneity and disturbance can be ruled out as mechanisms generating the species-area relationship. However, passive sampling, whereby large substrates collect more individuals and hence more species (Coleman 1981), and fragmentation effects cannot be ruled out.

Conclusions

By documenting significant effects of substrate size, Anagapetus pupal cases, and Cladophora on individual macroinvertebrate taxa densities and community structure, this study emphasizes that multiple biotic and abiotic factors determine the organization of stream communities. Probable influences of the factors are not clearly delineated into “biotic” or “abiotic.” The direct effect of pupal cases may be abiotic, such as altering flow conditions over substrate surfaces, but their structure and placement are a product of activities of an organism. Cladophora may have biotic influences, such as providing food to macroinvertebrates either directly or indirectly (through epiphytes), but it also may do this by altering abiotic variables on substrates such as current velocity and light penetration to substrate surfaces.

Artificial substrates were chosen for this study to control for variability in factors such as size and surface texture of natural substrates. There may be limitations to conclusions that can be reached in this study because artificial substrates and their associated macroinvertebrate assemblages may not reflect those of natural substrates. Anagapetus cases were artificially held in place for 60 d during this study, a time that may not reflect natural persistence. Persistence of empty Anagapetus cases should be determined in part to positioning on substrates and flow, 2 variables that were controlled in this study. Further investigation should be conducted to determine factors that influence persistence of cases and how this in turn affects seasonal dynamics of macroinvertebrate distribution and abundance.

ACKNOWLEDGMENTS

I thank J. Dunham, J. Ellsworth, A.W. Knight, D. Messer, P.B. Moyle, M. Parker, H. Phipps, and G. Vinyard for providing valuable input at various stages in this research. M. Reynolds and V. Boucher provided valuable support in the use of facilities at the University of California Sagehen Creek Field Station. This research was supported in part by a Jastro-Shields grant from the University of California at Davis.

LITERATURE CITED


Received 1 July 1999
Accepted 6 December 1999