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Substrate characteristics affect colonization by the bloom-forming diatom *Didymosphenia geminata*

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Abstract The long-stalked *Didymosphenia* is capable of forming large blooms and is expanding its range. To better understand the colonization dynamics of this species, we investigated the role of substrate characteristics—rock roughness and biofilm condition—on *Didymosphenia* colonization in a montane Colorado stream. Rocks differing in roughness (shale and sandstone) were treated to manipulate the diatom-dominated biofilm by scrubbing or submersion in 30% hydrogen peroxide. Initial chlorophyll concentration differed among rock types (sandstone > shale) and biofilm treatments (untreated > scrubbed > hydrogen peroxide-treated). Rocks were placed in a *Didymosphenia* bloom area for 8 days. More *Didymosphenia* colonized the rougher sandstone than the smoother shale, and more colonized stones with intact biofilms than stones with reduced biofilms (intact > scrubbed > hydrogen peroxide). These results suggest that rougher stones may be targeted for surveillance for new populations and that the colonization of intact biofilms is consistent with *Didymosphenia*'s habitat in regulated rivers, where biofilm-scouring spates may be suppressed.

Keywords Invasive species · Diatom colonization · Substrate texture · Periphyton · Rock substrata

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Introduction

Didymosphenia geminata (Lyngbye) Schmidt has the notoriety of being the only invasive diatom species with potentially large ecological (Larned et al. 2007) and economic (Branson 2006) effects. This species was historically reported in low numbers in several rivers in the northern hemisphere. In the last several years, however, reports of nuisance blooms have become regionally common, and populations have appeared at new sites within this range (reviewed by Spaulding and Elwell 2007) and have occurred under more varied environmental conditions (Kawecka and Sanecki 2003; Bhatt et al. 2008). Additionally, *Didymosphenia* has established large populations in several rivers in the South Island of New Zealand since its discovery in 2004 (Kilroy 2004), despite containment strategies.

Didymosphenia is a relatively large diatom that occurs as colonies of cells on long branching stalks. Colonies are initially small tufts, and as the

population grows, these tufts can grow and coalesce into mats. In some blooms, mats cover much of the streambed and, in New Zealand, mats may be several centimeters thick (Kilroy et al. 2005). Mats, composed primarily of stalks (Larned et al. 2007), may accumulate fine sediments and persist longer than the diatoms that made them (Kirkwood et al. 2007; Spaulding and Elwell 2007). Ecological studies of *Didymosphenia* blooms are ongoing, with the majority of research centering on the extensive, invasive growths in New Zealand (e.g., Kilroy et al. 2005; Larned et al. 2007). Such studies have examined associations with discharge and water chemistry, effects of floods, nutrient limitation, effects on invertebrate and fish assemblages and on water chemistry. Using information on habitat preferences and river characteristics, Kilroy et al. (2008) predicted the potential geographical range of *Didymosphenia* within New Zealand rivers.

There has been a paucity of research on early colonization dynamics of diatoms, including *Didymosphenia*. Specifically, the role of substrate characteristics in initial settlement of diatoms and other streambed algae has been little explored, although crevices have been implicated as selective settlement sites by motile zoospores of the filamentous green alga *Cladophora glomerata* (Dudley and D'Antonio 1991). In contrast, surface roughness is recognized as an important determinant in the settlement of many species of marine invertebrates and macroalgae on hard surfaces.

Settling diatoms are only one component of benthic stream biofilms, which also include organic compounds and detritus, and bacteria and other microbial organisms. Developing biofilms may obscure substrate characteristics, including textural features, of underlying surfaces. For example, Blinn et al. (1980) found differences in algal colonization among three rock types after the first week of colonization, but this difference disappeared after the second week because of changes in surface characteristics as biofilms and organic matter accumulated.

Our objective was to test whether substrate characteristics (i.e., rock roughness and biofilm presence) affected settlement and early establishment by *Didymosphenia geminata*. This was accomplished by means of a field experiment in which rough sandstone and smooth shale rocks with intact or modified biofilms were introduced into a section of the East River during a nuisance bloom of *Didymosphenia*, and colonization of *Didymosphenia* on these rocks was evaluated after a colonization period of 8 days.

Materials and methods

Research sites were located in the upper East River (Gunnison County, Colorado), where it is a second-order unimpounded montane stream within the Gunnison National Forest. The stream meanders through a glacial valley and has a wide riparian zone of short willows. The field experiment was conducted within the town of Gothic (location of the Rocky Mountain Biological Laboratory) at an elevation of 2,900 m. Here, the stream remains meandering, but the higher banks support riparian conifers (Fig. 1).

The geological diversity of the watershed (Gaskill et al. 1991) results in a diversity of igneous, sedimentary, and meta-sedimentary rocks in the streambed, which contains at least ten types of rock throughout the areas used in this study. The smoothest rock type in the streambed is Mancos shale and the roughest is red-sandstone of the Maroon formation (Bergey, unpublished data).

The diatom *Didymosphenia geminata* formed dense small mats in parts of the East River during summer 2007. Mats resembled coalescing tufts of dirty cotton and were easily visible in the stream. *Didymosphenia*

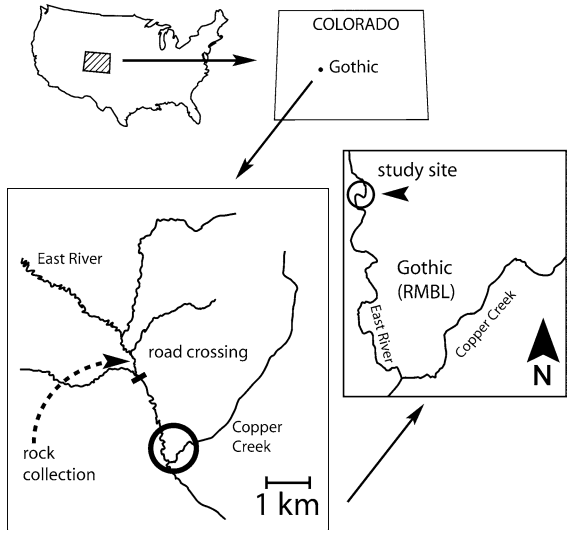

Fig. 1 Map of locations of the East River study sites—both the upstream rock collection area and the downstream field experiment site

mats were common in the section of the East River that passes through Gothic, but had not been found much further upstream. Ongoing surveys had failed to find *Didymosphenia* above the Gothic Road crossing on the East River, 2 km upstream of Gothic (Brad Taylor, unpublished data). Fifty-four river-smoothed shale and 54 river-smoothed sandstone rocks were collected from two adjacent riffles about a half kilometer upstream of the Gothic Road crossing, where *Didymosphenia* did not yet occur. The underside of rocks was marked using a wax crayon to show orientation, and rocks were stacked in ice chests and taken to the downstream experimental site.

Two-thirds of the rocks were processed to alter the biofilm. Eighteen shale and eighteen sandstone rocks were vigorously scrubbed with a brush and an equal number of rocks were placed in 30% hydrogen peroxide for 5 min. Processed rocks were soaked in stream water, bagged, and put in coolers.

Directly after biofilm treatment, rocks were placed in shallow water along the outer bend of a meander of the East River within Gothic, where most streambed rocks had numerous visible tufts of *Didymosphenia* colonies. The upper set was in an area of coarse sand; the lower set was in an area of mixed coarse sand and cobbles, some of which were emergent. Six replicates of each rock type (shale and sandstone) and biofilm treatment (untreated biofilm, scrubbed, and H_2O_2-treated) were placed in the upstream set and six replicates were placed in the downstream set; the marked undersides were used to prevent rocks from being placed upside-down. Treatments were assigned randomly within each set. Six replicates of each stone type-biofilm treatment were returned to the lab and refrigerated prior to chlorophyll analysis, which was started a couple of hours later.

Rocks were placed in the East River on 1 August 2007 and were retrieved and placed in labeled bags 8 days later on 9 August. During retrieval, rocks were lifted carefully to minimize loss of loosely adhered material. Rocks were stored frozen. During processing, rocks were placed in open-top vacuum-seal bags with 25–50 ml of 30% hydrogen peroxide and heated in a 78°C water bath until effervescence stopped in 15–20 min. Bags were shaken and the liquid poured into glass settling chambers. Samples were rinsed by settling and decanting to remove hydrogen peroxide and the final liquid volume was adjusted to 15 ml. Subsamples of 0.15 ml were dried onto coverslips and mounted on microscope slides with Naphrax mounting medium (Northern Biological Supply, Ipswich, UK). Slides were labeled only by their sample number, on the basis of the random location in the field experiment; hence, the rock type and treatment were not known during diatom counting. Slides were viewed at 200× with DIC, using a Leica DMLB microscope (Leica Microsystems, Wetzlar, Germany). All *Didymosphenia* valves were counted on the slide and numbers were converted to number of valves per square centimeter of rock using each rock's surface area. The planar area of each rock was obtained by weighing prints of scanned rocks and using the printer paper's mass–area relationship (as in Bergey and Getty 2006).

Rocks reserved at the start of the experiment were analyzed for chlorophyll *a* concentration, as an indicator of biofilm removal by the scrubbing and hydrogen peroxide treatments. An ethanol extraction technique was used (Sartory and Grobbelaar 1984). To prevent loss of ethanol during whole-rock extraction, individual rocks and 50 ml of ethanol were sealed in vacuum-seal bags. Absorbances were measured with a Beckman Coulter DU530 spectrophotometer (Beckman Coulter, Inc., Fullerton, California, USA).

Rock roughness was measured for the sandstone and shale rocks used in the experiment, using the method of Bergey (2006). Briefly, this method entailed finding the ratio of two surface area measurements—an idealized surface area based on the length, width, and height measurements and a more accurate surface area based on the weight gain when the rock was wetted with a soap solution. The resulting roughness value is dimensionless. Twenty rocks of each type were measured.

Data analysis

Because rock size was variable, potential relationships of (1) rock area and chlorophyll *a* concentration of the initial rocks and (2) rock area and *Didymosphenia* density of the experimental rocks were tested using linear regression. Rock roughness was compared between sandstone and shale using an unpaired *t*-test.

Potential differences in chlorophyll concentration and in *Didymosphenia* density among rock types and biofilm treatments were each tested with three-way ANOVAs (rock type or chlorophyll concentration,

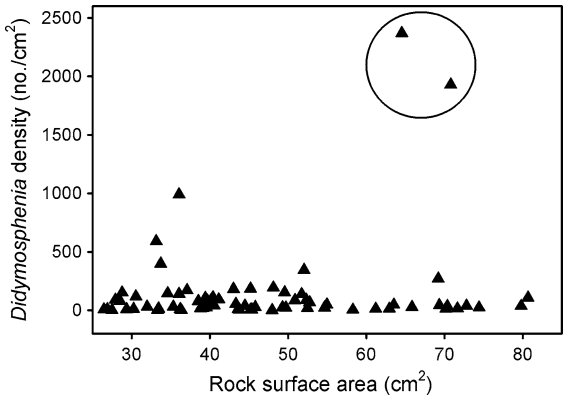

Fig. 2 Regression between the rock size of hydrogen peroxide treated rocks and *Didymosphenia geminata* density after 8 days colonization in the East River. Two outlying data points are *circled*

biofilm treatment, and experiment location), followed by Tukey's multiple comparison tests to distinguish differing means. Experiment location was included in the ANOVAs because differing local hydraulic conditions, indicated by different stream substrates, may affect settlement of *Didymosphenia*. To better meet the assumptions of ANOVA, data were square-root transformed prior to analyses. For clarity, all graphs show non-transformed data.

The *Didymosphenia* density dataset included two outliers with counts of 4.8 and 6.0 standard deviations from the mean (Fig. 2). Because of the large effect of these outliers on the ANOVA, these two data points were removed from the formal statistical analysis but were then discussed relative to the ANOVA results.

Results

Because rock size can affect chlorophyll concentration and possibly *Didymosphenia* settlement, rock size was compared among sets of rocks. Rock size ranged between 24.9 and 80.7 cm^2. There was no significant difference in rock size between shale and sandstone [mean (SE) 44.7 (12.4) and 45.5 (14.5) cm^2, respectively; $t = -0.37$, $P = 0.71$; $N = 54$], nor between initial and experimental stones [mean (SE) = 42.3 (1.9) and 46.6 (1.7), $N = 36$ and 72, respectively; $t = -1.50$, $P = 0.14$]. Among the experimental stones, rock size did not differ between the two locations or between shale and sandstone, but differences occurred among the biofilm treatments (ANOVA; $P = 0.25$, 0.21, and 0.002; respectively). Hydrogen peroxide stones were larger than scrubbed stones [mean (SE) = 53.9 (2.9) and 40.7 (2.1]; Tukey's test $P < 0.05$). To test whether stone size affected *Didymosphenia* settlement, *Didymosphenia* density was regressed against the rock size of hydrogen peroxide-treated rocks, which had the highest size range (26.5–72.8 cm^2; Fig. 2). Rock size was not related to *Didymosphenia* colonization density (regression $R^2 = 0.094$; ANOVA, $F = 2.27$, $P = 0.15$).

Sandstone rocks used in the experiment were much rougher than the shale rocks (*t*-test: $t = 6.895$, $P < 0.0001$). Sandstone roughness averaged 3.25 (SE = 0.34), whereas shale averaged 0.77 (SE = 0.05).

Chlorophyll *a* analysis showed that the biofilm treatments at the beginning of the experiment did, indeed, affect the biofilm (Fig. 3). Overall, sandstone had higher chlorophyll concentration than did shale [mean (SE) = 29.5 (4.0) and 7.4 (1.7) mg m^{-2}, respectively; ANOVA, $F = 29.76$, $P < 0.0001$] and this difference was consistent across biofilm treatments. The intact biofilm (no treatment) had the highest chlorophyll biomass, which was significantly different from the hydrogen peroxide-treated rocks, which had the lowest biomass (Tukey's test, $P < 0.05$). Scrubbing the rocks produced an intermediate chlorophyll biomass. Field observations were consistent with these results—both scrubbing and hydrogen peroxide treatment removed the slimy

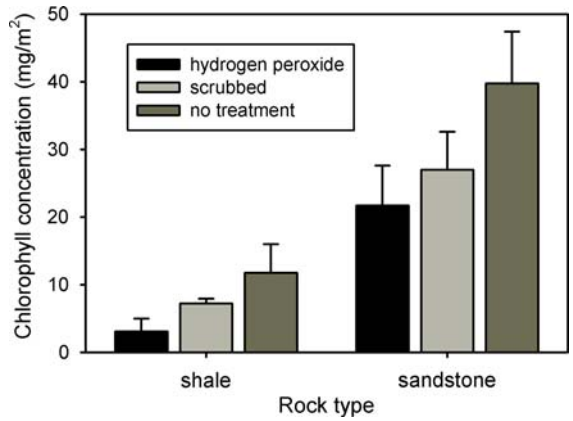

Fig. 3 Chlorophyll *a* concentration (used to indicate biofilm quantity) of transplanted streambed rocks at the start at the colonization experiment. Hydrogen peroxide and scrubbing were used to reduce biofilm. *Bars* are +1 SE

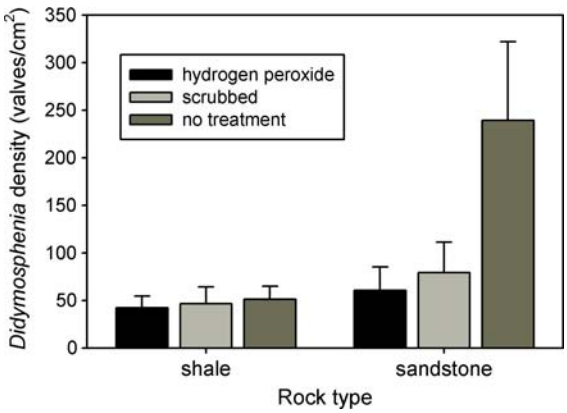

Fig. 4 *Didymosphenia* colonization, as density of valves, on rocks after 8 days exposure in a section of river with a moderate *Didymosphenia* bloom. Two types of rock (shale and sandstone) and three biofilms treatments (no treatment, scrubbed rocks, and rocks treated with hydrogen peroxide) were tested. *Bars* are +1 SE

brownish coating remaining on rocks with untreated biofilms.

Substrate colonization by *Didymosphenia* differed between shale and sandstone, and among the biofilm treatments (Fig. 4). Sandstone accrued more *Didymosphenia* than did shale [means (SE) = 130.3 (34.2) and 47.8 (8.3) cells cm^{-2}, respectively; $F = 5.93$, $P = 0.018$]. The intact biofilm accrued more *Didymosphenia* than did either of the treatments that reduced the biofilm [mean (SE) = 145.4 (45.3), 63.0 (18.3), 50.7 (13.0]; respectively, for no treatment, scrubbed, and hydrogen peroxide treatments; $F = 3.30$, $P = 0.044$). *Didymosphenia* colonization did not differ between the upstream and downstream sets of rocks ($F = 0.61$, $P = 0.44$).

The two outlier data points occurred in the hydrogen peroxide-treated sandstone. *Didymosphenia* densities were 2,369 and 1,929 per cm^2, compared with a mean of 87.4 valves per cm^2 in the other 70 rocks. Small tufts of *Didymosphenia* were noticed on a couple of sandstone rocks at the end of the experiment, and it was likely in the high-density rocks.

Discussion

Didymosphenia colonization differed between the rougher sandstone and the smoother shale, with many more colonizers on sandstone, the rougher surface. This pattern was also noted in streams near Gothic, where visible colonies were first seen on sandstone. Likewise Blinn et al. (1980) found more diatom biomass on sandstone than on basalt or limestone after a comparable colonization period and attributed the difference to stone roughness and/or solubility. A subsequent experiment on the role of rock chemistry on algal colonization (Bergey 2008) showed that rock chemistry had little effect. Unfortunately, rock type is seldom reported in habitat descriptions, making larger scale evaluation of the importance of rock type and rock roughness difficult.

Because *Didymosphenia* forms colonies, colonization may occur by settlement of individual cells or by colony fragments. The density of colonizing *Didymosphenia* on our rock substrates was highly variable. Two of the 72 rocks had about 2,000 valves cm^{-2}, in contrast to the rest of the rocks, which averaged about 90 valves cm^{-2}, which may indicate colonization by colony fragments on the two high-density rocks. Although in situ production contributes to biomass increase after settlement starts (Peterson 1996), it is unlikely that the short 8-day colonization period would produce such high densities on only two rocks if colonization occurred as single cells. Indeed, the two high-density rocks were the roughest combination (sandstone treated with hydrogen peroxide, which reduced the biofilm and increased direct rock surface exposure) and colony fragments may have snagged on these rocks. Both single cells and colony fragments occur in the drift during blooms (cells: Kilroy et al. 2005; fragments: Cathy Kilroy, personal communication 2008); therefore, both can settle on streambed substrates.

Rock size can affect algal biomass, but was not a factor in this experiment. The sandstone and shale rocks had a similar range of rock size and no correlation was found between rock size and *Didymosphenia* density. Similarly Kilroy et al. (2005) found rock size unrelated to *Didymosphenia* density during blooms in New Zealand.

Didymosphenia colonization was greater on intact diatom-dominated biofilms than on disrupted biofilms, which is consistent with the general colonization pattern of biofilms on submerged surfaces. Diatoms are generally not the first colonizers of bare surfaces in streams; rather a microbial and organic layer first forms (Barranguet et al. 2005), followed by colonization of adnate and short-stature diatoms (Hudon and Bourget 1981). Thus, Peterson and Stevenson (1989) and Sekar

et al. (2004) found that diatoms colonized non-algal biofilms faster than cleaned substrates. Following this initial diatom assemblage, stalked and branched colonies become more abundant (Hudon and Bourget 1981; Hoagland et al. 1982; Ács et al. 2000), which was observed in this study by the greater colonization of *Didymosphenia* on intact biofilms. Korte and Blinn (1983) found that upright diatoms, including those with stalks, become abundant on introduced substrates after 2 weeks in riffles—a longer colonization period than used in our study.

Rock roughness may interact with biofilm development and indirectly affect *Didymosphenia* colonization. During colonization, rougher substrates accrue greater biofilm biomass than smoother substrates (Blinn et al. 1980; Clifford et al. 1992). By promoting biofilm development, the rougher sandstone may be more conducive to *Didymosphenia* colonization than the smoother shale.

Sites with invasive or nuisance *Didymosphenia* blooms are typically rivers that are regulated, occur below lakes, or have low variation in flow (e.g., Kawecka and Sanecki 2003; Kirkwood et al. 2007; Beltrami et al. 2008), where the biofilm is little disturbed by rain-associated spates. This study indicates that habitat conditions in these rivers may promote *Didymosphenia* establishment by maintaining a biofilm suitable for *Didymosphenia* colonization.

The apparent selection of surface traits by settling *Didymosphenia* (e.g., sandstone > shale and intact biofilm > reduced biofilm) implies the possibility that diatoms can select surfaces. Once individual diatoms reach a surface, adhesion, movement, and release are active processes (Cooksey and Wigglesworth-Cooksey 1995; Wetherbee et al. 1998) that could allow substrate selection. Studies using marine diatoms have demonstrated responses to physical and chemical cues (Wigglesworth-Cooksey and Cooksey 1992; Falciatore et al. 2000; Thompson et al. 2008) and such cues may be involved in diatom colonization. Texture characteristics are a factor—more diatoms settle on rougher artificial substrates than on smoother substrates (Sekar et al. 2004; Patil and Anil 2005), and this study demonstrated greater colonization of the rougher sandstone than the smoother shale.

Because *Didymosphenia geminata* colonized rougher rocks with intact biofilms, the use of introduced artificial substrates (especially smooth substrates like glazed tiles) in survey or surveillance programs is not appropriate. Instead, surveillance programs should target rougher in situ rocks.

Conclusions

1. *Didymosphenia geminata* colonized the rough rocks (sandstone) faster than the smooth rocks (shale). This experimental result was consistent with field observations and indicates that surveillance programs for this species might target the rougher rock types. There is a paucity of information on rock type and roughness in studies of *Didymosphenia* and this information might prove useful in better understanding the species' distribution.
2. Rock size, within the range of large gravel to cobble, did not affect colonization by *Didymosphenia*.
3. *Didymosphenia* colonized rocks with intact biofilms faster than rocks with disrupted biofilms. This pattern is consistent with *Didymosphenia*'s ability to colonize and persist in regulated rivers, where biofilm-disrupting spates are suppressed.
4. Greater understanding of the colonization dynamics of this species may help explain the geographical and habitat expansion of *Didymosphenia* and help model its potential range.

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