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Diatom viability in insect fecal material: comparison between two species, *Achnanthidium lanceolatum* and *Synedra ulna*

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Abstract

We examined differences in digestibility and viability following gut passage through water penny larvae (*Psephenus herricki*) of *Synedra ulna* and *Achnanthidium lanceoloatum*, two common diatom taxa that differ in growth habit and autecological characteristics. Prior to the experiment, diatoms were cultured in Chu-10 media in petri plates to establish a monospecific biofilm to offer grazers. After collection, insects were left to clear their guts over night, allowed to graze for 3 hours on diatom biofilms, and then placed in vials over 1-mm mesh to defecate. Samples from source material and from insect feces were mounted in syrup media and the ratio of chloroplast-containing to empty diatom frustules was microscopically assessed. In addition, subsamples from source material and feces were sprayed onto agar plates prepared with Chu-10 and individual cells were mapped and tracked for 5 days to quantify reproduction. Cells of both *S. ulna* and *A. lanceolatum* taken from source material formed colonies on agar. *Achnanthidium lanceolatum* cells from insect feces also formed colonies, but with lower densities than those from source material. In contrast, none of the *S. ulna* cells tracked from fecal cultures formed colonies, and the percentage of *S. ulna* cells that were dead was significantly greater in feces relative to source material. Dead cell percentages of *A. lanceolatum* were also higher in feces relative to source material, but to a lesser degree than observed for *S. ulna*. These findings have potential implications for linking patterns of energy transfer in stream ecosystems and the structure and dynamics of benthic microalgal communities.

Introduction

In lotic systems, benthic microalgae are a key source of autocthonously fixed carbon to primary consumers. By far, algae provide the largest fixed-carbon source in streams that are continually well lit (Minshall, 1978), and are seasonally important in temperate systems shaded by deciduous riparian canopies (Berg & Hellenthal, 1992; Mulholland et al., 2000). Even in heavily shaded streams benthic algae can be important in some invertebrate diets (Rounick et al., 1982; Mayer & Likens, 1987). Diatoms are ubiquitous, occurring in all freshwater habitats, and often are the dominant component of microbial assemblages (Sheath & Wehr, 2003). Of the algal divisions common in stream benthos, diatoms are seemingly the most important dietary component for many algivores. Many cyanobacterial and chlorophyte species are enveloped in thick mucilage, reducing their susceptibility to digestion by herbivores (Porter, 1976; Gladyshev et al., 2000). Cyanobacteria also often contain secondary chemicals that limit their palatability (Porter, 1977). When fed diatoms, however, mayflies grow faster (Fuller & Desmond, 1997) and tadpoles metamorphose earlier (Kupferberg et al., 1994) compared to those fed non-diatom algal diets. When given access to both diatom- and non-diatom dominated algal mats, algivorous fish preferentially and voraciously consume mats domin-

ated by diatoms (Power et al., 1988; Napolitano et al., 1996).

Benthic diatom species differ autecologically, varying in the abilities to proliferate in newly cleared space (Peterson & Stevenson, 1989; Peterson, 1996), exploit localized and/or transient inorganic nutrient supplies (Pringle, 1985; Cox, 1988), and resist displacement by current shear or scour (Cazaubon, 1988; Biggs & Thomsen, 1995). As such, the distribution and abundance of different species in streams are heterogeneous in space (Stevenson & Hashim, 1989; Passy, 2001) and time (Rout & Gaur, 1994; Peterson et al., 2001a). Variation in the taxonomic content of diatom assemblages would be of little consequence to consumers if all species were equally consumable and/or equally digestible. Inequity among species exists on both of these fronts. Diatom susceptibility to ingestion by grazers can vary with cell size and growth habit (Barnese et al., 1990; Alverson & Courtney, 2002). Diatom species differ in their ability to grow in culture from grazer feces (Underwood & Thomas, 1990; Brendleberger, 1997), and in the degree to which chloroplast condition within cells is degraded by passage through grazer guts (Peterson et al., 1998; Peterson & Boulton, 1999), suggesting interspecific variation in digestibility, as well.

Here we present results of a laboratory study that assessed differences in digestion and cell viability following gut passage through water-penny larvae (*Psephenus herricki* Dekay, Coleptera: Psephenidae) of two common diatom species, *Synedra ulna* (Nitz.) Grun. and *Achnanthidium lanceolatum* (Bréb.) Kütz.. These species differ in size, growth habit, and the environmental conditions in which they thrive. *Synedra ulna* is a large (80–200 μm long), araphid diatom that forms apically attached colonial rosettes or unattached cell aggregates (Biggs et al., 1998). This species is often abundant in early successional algal assemblages (Stevenson et al., 1991; Peterson et al., 1993), and can be susceptible to ingestion by grazers (Steinman et al., 1987; Hill & Knight, 1988). *Achnanthidium lanceolatum* is a smaller (10–30 μm long), monoraphid diatom with an adnate growth habit. This species can attach strongly to solid surfaces (Rosowski et al., 1986), is tolerant of low light (McIntire, 1968; Steinman & McIntire, 1986), and is relatively resistant to ingestion (Hill & Knight, 1988; Peterson et al., 2001b).

Studies have shown that *Synedra ulna* exhibits little or no viability in fecal material of snails (Underwood & Thomas, 1990) or tadpoles (Peterson & Boulton, 1999) that have grazed on taxonomically diverse periphyton assemblages. Peterson et al. (1998) found that 60–75% of *Achnanthidium lanceolatum* cells in periphyton exposed to heavy grazing pressure by larval insects in a small montane stream were dead, suggesting low resistance to digestion. However, 10–12% of *A. lanceolatum* cells in feces produced by caddis larvae and mayfly nymphs that grazed on this assemblage contained full, healthy-looking chloroplasts (Peterson, unpubl. data), indicating some cells of this taxon remained viable. In diatom-rich assemblages containing both *S. ulna* and *A. lanceolatum*, an invasive bacterial pathogen decimated *S. ulna* populations but had little apparent effect on *A. lanceolatum* (Peterson et al., 1993).

Our primary goals in the research reported here are two-fold. First, by offering grazers monospecific cultures of *A. lanceolatum* and *S. ulna*, we wished to directly compare the digestibility of these two taxa by a single algivore in the absence of any potentially confounding environmental factors (i.e., variation in biofilm thickness, taxonomic composition, detrital/inorganic sediment content). Second, by using spray-plate culture techniques and microscopically tracking clonal colony development from individual algal cells, we hoped to confirm that cells containing healthy-looking chloroplasts were, in fact, viable.

We used two criteria to select an algivore for use in this experiment: (1) confirmation that our selection consumed microalgae as a primary food source and, (2) local availability in sufficient numbers to allow us to conduct the experiment. Because our goal was to evaluate digestibility of our two diatom species under the same ecological conditions, specific grazer identity was not important as long as it met our selection criteria. Larvae of *Psephenus herricki* met both. This taxon is common, geographically widespread, and consumes microalgae as the primary component of its diet (Murvosh, 1971; Courtney et al., 1996).

Materials and methods

Mono-cultures of *S. ulna* and *A. lanceolatum* were maintained in 12, 250-ml Erlenmeyer flasks (6 for each species) containing Chu-10 growth medium (Nichols 1973) in a Percival incubator at 11 °C and 12:12 L:D cycle. Two weeks before starting the experiment, a portion of each culture was transferred to shallow (1.3-cm deep) Petri plates (5.4-cm diam.) to develop monospecific diatom biofilms for ingestion by grazing insects. On 15 February, 2000, water-

penny larvae were collected from Hickory Creek, Will County, IL and brought to the laboratory where they were placed in a basin atop 1-mm mesh and allowed to clear their guts, at ambient lab temperature (21 °C) over night. The next morning, samples were taken from each culture plate to characterize live/dead cell ratios in the diatom food source available to grazers, and to determine cell viability. To start the experiment, 3 larvae were added to each of the 12 Petri plates (6 for each diatom species) and allowed to graze for 3 h, after which larval groups were placed in 25-ml vials atop 1-mm mesh to defecate for 3 h. Larval acclimation and grazing trials were done at room temperature because water-penny larvae apparently do not feed at temperature below ca. 13 °C (Murvosh, 1971). Fecal samples were concentrated via slow centrifugation for 10 min. From each 3-larvae group, sub-samples of both fecal and source material were preserved in Lugol's solution and mounted in syrup medium (Stevenson, 1984). The ratio of chloroplast-containing cells (live cells) to dead cells (the sum of single diatom valves bearing a central area and those in intact but empty frustules, divided by 2) was then microscopically quantified from counts of at least 100 cells at 1250× magnification. A second sub-sample was sprayed, using an atomizer, onto Chu-10-infused agar, producing a total of 24 spray-plate cultures (2 diatom species × 2 sample types [source & feces] × 6 replicates). Plates were secured to a microscope stage and 5–10 individual cells per plate were located using a long-working distance 40× objective (500× magnification), and coordinates of their positions recorded. No clumping of cells was observed for either diatom species. Cell coordinates were relocated after 2 and 5 days to quantify change in the size of clonal colonies. In some instances, colonies initiated from different cells that were in close proximity at the initial mapping coalesced after 5 days making it impossible to partition clones among initial parent cells. In such cases, data from colonies involved were pooled, yielding initial cell numbers of 2–6 rather then one.

We used *t*-tests to detect changes in live-cell percentages between source and fecal material and differences in the magnitude of change in live-cell percentages between the two species. Growth rates (r) of the two species on agar cultures initiated from either source or fecal material were calculated as the slope of a regression line reflecting change in natural-log transformed cell densities over time. Separate repeated-measures ANOVA were used (1) to assess differences, for each species, in cell accrual in source versus fecal cultures, and (2) to assess between-species differences in accrual in both source and fecal cultures.

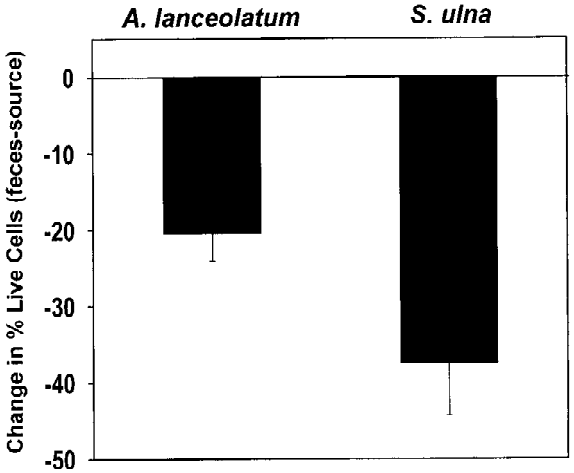

Figure 1. Change in live-cell percentages (− 1 s.e.) for each diatom species induced by passage through the guts of *P. herricki* larvae.

Results

In source material fed to water-penny larvae, 90.5% (± 2.3 s.e.) of *Synedra ulna* cells contained chloroplasts (i.e., were presumed live). This was a significantly ($p < 0.05$) higher percentage than the 67.2% (± 2.1 s.e.) noted in *Achnanthidium lanceolatum* cultures. Gut passage through *P. herricki* larvae reduced live-cell percentages in both diatom species (Fig. 1), but changes were significantly greater (*t*-test; $p = 0.039$) for *S. ulna* (57.1% live, ± 6.8 s.e. in feces) than for *A. lanceolatum* (47.0% live, ± 2.6 s.e. in feces).

Both diatom species produced discrete clonal colonies that spread, in a mono-layer, laterally over the agar surface. *Synedra ulna* did not form apically attached rosettes on agar. Although colonies of *A. lanceolatum* were more diffuse at the peripheries as the motile cells dispersed outward from colony margins, this did not compromise our ability to identify each colony as a distinct entity and to quantify cell densities therein. Both species grew equally well in cultures initiated from source material (Fig. 2, Table 1). Gut passage reduced growth in *A. lanceolatum* by 22.7%, but cells of this species remained viable in fecal material and produced colonies that increased in cell density over the 5-d incubation period (Table 1). In contrast, no *S. ulna* cells mapped on spray culture plates from insect feces produced colonies,

Table 1. Growth rates ($r \pm$ s.e.) for two diatom species in agar cultures initiated from source material or from feces of *P. herricki*. Probabilities reflect significance, determined with Repeated-Measures ANOVA, of between-species differences for each inoculum type, or within-species differences between inoculum types

	Achnanthidium Lanceolatum	*Synedra ulna*	$\text{Prob.}_{ac=sy}$
Source	0.511 (0.038)	0.608 (0.026)	0.145
Feces	0.395 (0.036)	−0.001 (0.017)	<0.001
$\text{Prob.}_{source=feces}$	0.025	<0.001	

despite the fact that these cells contained chloroplasts, albeit reduced in size, when mapped on day 0 (Fig. 2).

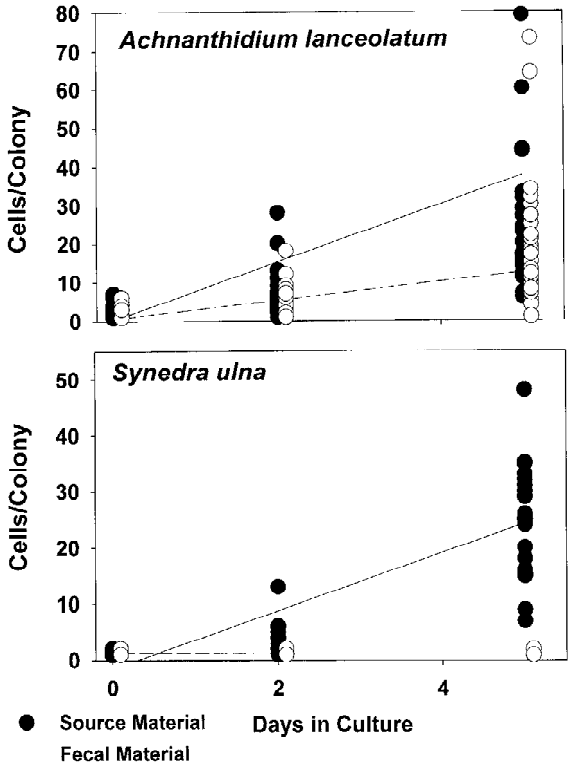

Figure 2. Change in colony size and associated regression lines for each diatom species on agar plates inoculated with source material (closed symbols, solid lines: $R^2_{synedra} = 0.802$, $R^2_{Achnanthidium} = 0.546$) or *P. herricki* feces (open symbols, dashed lines: $R^2_{Achnanthidium} = 0.396$). The number of cells/colonies tracks is reflected by the number of data points on day 5. On days 0 and 2, many symbols are obscured due to overlap. For *S. ulna* fecal cultures, symbol overlap is extensive on all days.

Discussion

Our results are consistent with previous observations of autecological characteristics of these two diatom species. Among 18 taxa cultured by Underwood & Thomas (1990) from taxonomically diverse source material and from feces of two species of freshwater snail, *Synedra ulna* was most digestible, exhibiting only minimal growth in cultures inoculated with snail feces. Similarly, changes in live/dead cell ratios induced by gut passage of 12 diatom taxa through algivorous tadpoles revealed *S. ulna* as the only species for which conversion of chloroplast-containing cells to empty frustules was complete (Peterson & Boulton, 1999). In contrast, many *A. lanceolatum* cells ingested by Limnephilid caddisfly larvae and Ameletid mayfly nymphs retained full or reduced chloroplasts in insect feces (Peterson et al., 1998). *Synedra ulna* and *Achnanthidium lanceolatum* differed markedly in their resistance to infection by a pathogenic bacterium during two post-spate diatom blooms in a Sonoran Desert stream (Peterson et al., 1993). Populations of *S. ulna*, and various *Nitzschia* spp. of similar size and shape and likely location in the upper strata of the community, were decimated by both of these events. *Achnanthidium lanceolatum* was unaffected by the first of these episodes, protected, we surmised, within an algal mat containing algal mucilage, detritus, and mineral particles that may have impeded transmission of the pathogen from cell to cell. During a second outbreak, when organic matter within rapidly developing algal mats was comprised almost exclusively of diatoms, *A. lanceolatum* experienced a pathogen-induced reduction in live-cell percentages, but to a much lesser degree than *Synedra*, during a second outbreak. The similar pattern we observed in our gut passage study, in the absence of potentially confounding factors associated with multi-species biofilms in nature, suggests that these species represent different points on the lower portion of a digestion-resistance spectrum, with *S. ulna* lying at the non-resistant extreme.

Algal taxa differ considerably in resistance to digestion. Some species, typically chlorophytes or cyanobacteria that produce thick, mucilaginous envelopes around individual cells or colonies are actually stimulated by gut passage, presumably benefiting from increased availability of nutrients (Porter, 1976; Soto & Hurlbert, 1991; Gladyshev et al., 2000). Porter

(1977) argued that temporal change in phytoplankton assemblage structure in lentic systems stem, at least in part, from differential loss and reproductive stimulation of algal taxa associated with grazing activity. Little work has been conducted to assess the implications of differential digestibility of algae in benthic systems.

Diatoms, while they do produce mucilage for many different purposes (Hoagland et al., 1993), are rarely surrounded by thick mucilage envelopes. Exceptions include those taxa that produce mucilaginous tubes (Carr & Hergenrader, 1987). Differences in cell size, shape, and thickness of cell-wall silicification among diatom species likely generates differences in gut-passage induced mortality. Digestive enzymes should access a larger percentage of cellular contents in long, thin diatoms (like *S. ulna*) than in cells that are either much smaller (i.e. with less actual surface area for enzyme transfer) or large but wider (i.e. much of cellular contents displaced from outer membrane surfaces). Our results show that *A. lanceolatum* was susceptible to digestion, but to a much lesser degree than *S. ulna*. However, data presented by Peterson et al. (1998) indicate that when grazing pressure is intense and individual cells likely pass through insect guts multiple times, populations of *A. lanceolatum* do not fare well (60–75% dead). In contrast, negative effects of gut passage on small, heavily silicified *Fragilaria* (=*Staurosirella*) species appear to be minimal (Peterson et al., 1998). Observational evidence, from both laboratory and field studies, suggests that these taxa may benefit from gut passage, despite lacking copious mucilage. In Rio Calaveras, a heavily grazed 1st-order stream in New Mexico, small *Fragilaria* (primarily *F. pinnata*, *F. pinnata* var. *lancetula*, and *F. leptostauron*) form dense, healthy looking aggregates in flocculent material of presumed fecal origin on stream bottoms (Peterson, pers. obs.). In the culture study that generated results reported here, we also attempted to assess response of *Fragilaria pinnata* to gut passage through *Psephenus*. The small cell size and rapid expansion of colonies of this species in spray plates initiated from both fecal and source-material inoculum made quantification of results untenable, providing qualitative evidence of digestion resistance and, perhaps, reproductive stimulation by gut passage.

While impacts of differential susceptibility of algal taxa to ingestion by algivorous macroinvertebrates on benthic algal community structure and function have been well studied (reviewed by Steinman, 1996), ecological ramifications of interspecific differences in digestibility among diatom taxa have received less attention. Digestion efficiencies differ among grazer species (Brendelberger, 1997; Peterson et al., 1998) and between developmental stages within species (Skoog, 1978; Peterson & Boulton, 1999). Thus, temporal and spatial variation in densities of more digestible diatom species could provide transient periods of high resource availability that would be particularly important to consumers with low digestion efficiencies. Such interactions could significantly affect the pattern and extent energy transfer and nutrient flux between trophic levels, and the content and taxonomic structure of assemblages of primary consumers.

The biomass and taxonomic structure of benthic algal communities varies considerably at several spatial and temporal scales. The presence and degree of dominance of highly digestible species like *Synedra ulna* differ among streams based on system-specific differences in frequency of high discharge events or stream-water nutrient content (Biggs & Gerbeaux, 1993; Peterson & Boulton, 1999). Within streams, transient, localized proliferations of *S. ulna*, accounting for 25–40% or more of benthic algal biovolume (Marker & Casey, 1982; Rout & Gaur, 1994; Peterson et al., 2001a), can arise in association with stochastic physical and chemical variation in environmental conditions. Spatially localized aggregates of *Synedra ulna* have been noted in areas of relatively high current (Ghosh & Gaur, 1991; Passy, 2001), and at zones of upwelling where nitrogen-rich groundwater enters the surface stream (Peterson et al., 2001a). As a common early successional species, transient proliferations of *S. ulna* can also occur following spates (Stewart, 1988; Peterson et al., 1993) or in other circumstances in which clean, non-colonized substrata becomes available for algal colonization (Oemke & Burton, 1986; Stevenson et al., 1991).

The resource base available to primary consumers in lotic systems may vary considerably with changes in benthic algal species composition, even when coarser measures of algal standing crop, such as chlorophyll or ash-free dry mass, remain constant (cf. Peterson & Grimm, 1992). Equivalent biovolumes of algal taxa that differ in digestibility would clearly not be of the same ecological value to primary consumers, even if the energy and nutrient content of the two cell aggregates were the same. Within and among years, the habitat template that dictates resource availability to benthic algae shifts in response to larger scale drivers related to precipitation patterns that influence the extent of groundwater recharge (Peterson et al.,

2001a) and the frequency and magnitude of spates (Grimm & Fisher, 1991). The ecological implications of changes in the species composition of periphyton in response to such environmental stochasticity merit closer attention.

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