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Mineral grains in caddisfly pupal cases and streambed sediments: Resource use and its limitation through conflicting resource requirements

Bernhard Statzner[1] *and Sylvie Mérigoux*
CNRS-Ecologie des Hydrosystèmes Fluviaux, Université de Lyon I, 69622 Villeurbanne Cedex, France

Maria Leichtfried
Biologische Station Lunz, A-3293 Lunz am See, Austria

Abstract

Sand and fine gravel in streambed sediments are considered to be an overabundant resource for caddisflies that build cases from it. However, larvae of *Rhyacophila* and *Hydropsyche* build pupal cases with material collected near the pupation site and typically attach these cases to cobbles in riffles, where the rapid flow facilitates respiration but decreases the availability of case-building material through erosion. Analyzing mineral grain sizes of the pupal cases and the streambed in samples taken from cobbles in a stream riffle, we found that the overall mass use in pupal cases of *Rhyacophila* and particularly *Hydropsyche* significantly increased with local mass availability of building material, indicating that mineral grains can be a limited resource for these taxa. In addition, the most abundant species (*Hydropsyche siltalai* Döhler) significantly changed the case architecture if the preferred grain fraction (2.5–3.15 mm) was a limited resource. Under resource limitation of the preferred fraction, *H. siltalai* used the 1.6–2-mm fraction instead, which could reduce the resistance of the cases to damage resulting from floods that move coarser bottom material. Our findings suggest that, in streams or near shores of lakes and oceans, water currents that modify grain availability can create conflicts in resource requirements for invertebrates, particularly if they need locally available fine grains for the building, coarse grains for attachment, and high oxygen renewal rates for metabolic needs.

There can be little doubt that resource limitations play a key role in the understanding of the biology and ecology of living systems. Therefore, resource limitations figure prominently in studies that address the potential role of intra- or interspecific competition for resources, which in turn determine niche dimensions, community composition, and diversity (e.g., Begon et al. 1986). Corresponding to the importance of the topic, recent research in limnology and oceanography focused on the question of how seemingly overabundant resources can be limited because subtle constraints interfere with their use. For example, dissolved inorganic carbon (C) in seawater is so abundant, but the discovery of carboxylating enzyme activities suggests that inorganic C may be limiting for marine algae (Buitenhuis et al. 2003; Rost et al. 2003). Likewise, there is growing evidence that the usability of overabundant organic C for aquatic bacteria, zooplankton, and fish depends on size, morphology, mineral composition, toxicity, and/or other biochemical features of the C source (Becker and Boersma 2003; Castillo et al. 2003; Holzman and Genin 2003; Strom et al. 2003). This paper contributes to the research on the subtleties in the use of overabundant resources in aquatic systems, as it examines the use of seemingly large quantities of sand and fine gravel in streambed sediments by caddisfly larvae that build pupal cases from it.

Building tubes or cases from mineral or organic particles is a technique that is used by a taxonomically diverse group of aquatic animals (Dudgeon 1990). For example, protozoans, rotifers, molluscs, annelids (particularly polychaetes), and arthropods (crustaceans and insects) all have species that build such structures, and the degree of selectivity in the use of particles for building in relation to particle availability has been a major thread in studies of their biology and ecology (Dudgeon 1990). Among the insects, caddisfly larvae build cases from mineral or organic particles that are cemented with silk threads, and this ability is viewed to be a key for the evolutionary success of this order (Mackay and Wiggins 1979).

For a given development stage, the architecture of caddisfly cases is naturally often so typical that it can be used for the identification of families, genera, or even species (e.g., Waringer and Graf 1997). In experiments, however, larvae typically use case-building material that is as close as possible to the preferred one, if the preferred material is not available (Gorter 1931). For example, species building mineral cases will switch to grain sizes near the upper or lower range limit of the unavailable, normally preferred one (Hanna 1961; Tolkamp 1980). If the available grain sizes are too different from the preferred ones or mineral grains are not available in such experiments, the larvae build irregular silk constructions, or, if provided, some (but not all) species use plant material of appropriate size (Haller 1948; Hanna 1961; Tolkamp 1980). In addition, larvae that prefer a particular mineral material (e.g., travertine) may switch to a different

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[1] Corresponding author (statzner@biomserv.univ-lyon1.fr).

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mineral (e.g., quartzite) if the preferred one is unavailable (Gaino et al. 2002).

Given that lotic caddisflies in a certain development stage often build cases with a typical architecture, the availability of the preferred mineral case material has been viewed as a limiting factor at the scale of stream or habitat types (e.g., Hanna 1961; Tolkamp 1980). However, lotic larvae that carry their cases with them can migrate ~1–4 m d^{-1} (Elliott 1971; Jackson et al. 1999) to locations containing the preferred grain size when building cases (Mackay 1977; Podgornyi and Nepomnyashchikh 1999) and use few grains from those that are available in their natural habitats (Tolkamp 1980). Therefore, there is no solid evidence that suitable mineral case-building material is limiting to caddisflies in natural streams (Dudgeon 1990). In contrast with these mobile itinerant species, which acquire the building material for their cases where it can be found, other caddisfly species are more resident in that they depend on local grain availability. Among the latter, hydropsychid and rhyacophilid larvae construct an entirely new pupal case immediately before pupation, using sand and fine gravel in the neighborhood (i.e., near the building place; see Materials and methods), where they fix these grains to the surface of coarser particles (Haller 1948; Waringer and Graf 1997). Typically, species of *Hydropsyche* and *Rhyacophila* fix their pupal cases to cobbles in fast-flowing stream riffles (Sattler 1958; Lepneva 1970; Waringer and Graf 1997). Cobble habitats in stream riffles facilitate the provision of abundant oxygen (the fast flow facilitates oxygen uptake) and provide solid surfaces for case attachment, whereas the erosive flow forces prevailing near the bottom of cobbles reduce the amount of sand and fine gravel (Hynes 1970; Newbury and Gaboury 1993). Thus, *Hydropsyche* and *Rhyacophila* potentially have conflicting resource requirements, suggesting that finer mineral grains needed for the construction of the pupal cases could be a limited resource for them. To assess this potential resource limitation, we examined a riffle of a French stream that had abundant populations of *Hydropsyche* and *Rhyacophila* and analyzed mineral grain size in their pupal cases and the streambed (focusing on the most abundant species *H. siltalai* Döhler).

Materials and methods

Study site—We studied a riffle in the Furan River because it had an abundant, monospecific hydropsychid population (*H. siltalai*) that co-occurred with *Rhyacophila.* The studied riffle is described in detail by Statzner and Bretschko (1998) and Statzner et al. (1999). It is situated ~22 km downstream of the principal source of the Furan River. Many groundwater sources feed the stream along its main channel, so the water temperature at the study site is stable and rather low during summer (mean ~12°C). At baseflow, the riffle is ~12 m wide and ~0.3 m deep and has a relatively uniform near-bottom flow (~90% of the near-bottom flow in the shear-stress range 0.8–2.3 N m^{-2}, assessed with FST-hemispheres; Statzner et al. 1991). Previous to and during the period of this study (June to mid-August 1998), the discharge was high in April (maximum: 9 $m^3\ s^{-1}$) and returned to baseflow in early May. It remained at baseflow until mid-August, so that the daily mean (±1 standard error; SE) from the beginning of June until mid-August was 0.97 ± 0.04 $m^3\ s^{-1}$.

The caddisflies: Identification and essentials of their biology—We identified immature pupae using the characteristics of the last larval instar (key: Waringer and Graf 1997) and mature ones using the structure of the genitalia (key: Malicky 1983). *H. siltalai* was the only hydropsychid at our site, whereas identifications of rhyacophilid pupae were difficult (see below).

Larvae of *Hydropsyche* migrate before they start to build their pupal cases, using the surface of larger particles (e.g., cobble) as foundations (i.e., bedplate). Observations by Haller (1948) and Sattler (1958) suggest that the larvae never leave their building site and collect the building material for the entire case from a surface of ~15 cm^2. However, Mogel et al. (1985) observed that larvae make short excursions into the neighborhood of their building site to collect sand and fine gravel. The larvae need ~1 d to construct their pupal cases (Sattler 1958; Mogel et al. 1985). The final products are domed cases on larger bedplates, with the domes made of mineral grains that are cemented together with silk threads. Depending on the temperature, pupae emerge after ~2–4 weeks (Schuhmacher 1970).

We used data from our study site that were collected previously (Statzner and Bretschko 1998; Statzner et al. 1999) to obtain site-specific information on pupae of *H. siltalai.* In samples covering a gradient of near-bottom baseflow conditions, the pupal case density of *H. siltalai* increased with shear stress so that the highest pupal densities occurred across a shear-stress range of ~1–2.5 N m^{-2}. This shear-stress range is critical for sand and fine gravel, as it erodes mineral particles with an approximate diameter of <1 to <2.5 mm (Newbury and Gaboury 1993). Inspecting pupal cases collected in these previous studies, we noticed variation of individual pupal case masses in individual samples, which was not caused by the sex of the pupae (*U*-test on 33 females vs. 33 males: $p = 0.63$). Assessing this variation using Monte Carlo simulations showed that it would require ≥ 10 cases to estimate mean case mass with an error $<5\%$. Knowing that *H. siltalai* was always abundant at the study site (during our study, 250.0 ± 18.9 (mean ± 1 SE) pupal cases m^{-2}), ≥ 10 cases could be obtained by sampling a relatively small bottom area.

The two rhyacophilid species occurring in the Furan River are from the subgenus *Rhyacophila* sensu stricto, which build pupal cases resembling that of *Hydropsyche* (Waringer and Graf 1997). Confirmed species identifications of larvae are currently impossible in this subgenus (Waringer and Graf 1997), so it was impossible to assign prepupae or immature pupae (from the larval exuviae) to species using larval characteristics, and we refer to all pupae (including mature ones) as *Rhyacophila* spp. Among the mature pupae, ~70% were *Rhyacophila dorsalis* Curtis and the others were *Rhyacophila fasciata* Hagen. Prepupae and pupae of *R. dorsalis* and other *Rhyacophila* species can be found over many months of the year (Cereghino et al. 1997; Sangpradub et al. 1999). In our study, these two rhyacophilids had a mean density of 99.3 ± 7.2 pupal cases m^{-2}.

Beyond these hydropsychids and rhyacophilids, mineral pupal cases of other caddisflies species were found (mean density: 136.7 ± 12.6 pupal cases m^{-2}). These species were *Odontocerum albicorne* (Scopoli) (Odontoceridae), *Potamophylax cingulatus* (Stephens) (Limnephilidae), *Potamophylax luctuosus* (Piller and Mitterpacher), and *Silo nigricornis* (Pictet) (Goeridae). All these species pupate in their larval cases, which are built with material distant from the pupation site throughout later stages of their larval life (Lepneva 1971; Waringer and Graf 1997).

Sampling and analyses—To have a sufficient number of samples with large numbers of *H. siltalai* pupal cases, the riffle in the Furan River was stratified and cobble substrates with a shear stress >0.8 N m^{-2} (assessed with FST-hemispheres; Statzner et al. 1991) were randomly sampled. It is important to note that cobble substrate alone does not imply complete absence of finer sediments, as smaller patches of sand and finer gravel occurred locally among the cobbles. Fifty samples were taken on each of two occasions during the period of highest pupal density (16 June and 10 July 1998). Furthermore, 30 samples were collected during each of the three subsequent months, but only the samples collected on 7 August 1998 contained sufficient identifiable pupal cases to be included in our analyses.

The area of each sample was marked with a metal frame (0.3 m × 0.2 m) and a net with a fine mesh size (0.1 mm^2) was positioned downstream of the frame. The surface layer and approximately the first subsurface layer of cobbles were carefully removed from the frame (note that deeper layers had cobble interstices filled with finer sediments and lacked caddisfly pupal cases), and all finer sediments from these layers were retained with the net. Obviously, not all of these finer sediments were really accessible for caddisflies, which makes our tests of resource limitation more conservative. The silk of the pupal cases attaching them to the cobble surfaces was cut using sharp knives, so that the entire cases could be conserved in ethanol (70%).

In the laboratory, the pupal cases of each sample were sorted and identified. Fragments of empty pupal cases were also sorted and other case-bearing caddisflies were removed from the sample. The silk of the cases was dissolved using a NaOH solution and the case material was dried for 48 h at 105°C. Likewise, the remaining sediment of the sample was dried. The dried case material (pooling all cases per taxon and sample) and the dried remaining sediments were separated with a set of 13 sieves having a mesh size range of 5–0.125 mm, and each fraction was weighted. We followed the suggestion of Leichtfried (1986) and transformed the mass values to the number of grains per sieved fraction assuming a grain density of 2.7 g cm^{-3}, a spherical grain shape, and a mean grain mass per fraction (as mean of the upper and lower size limit of the fraction). Both *H. siltalai* and *Rhyacophila* spp. sometimes built their cases among larger gravel (using these as foundation for the case). Assuming that the larvae were unable to collect this larger gravel, we ignored the material >5 mm in our analyses.

We used all the remaining sediments of a sample, plus the fragments of empty pupal cases, plus half of the material used in the pupal cases of *H. siltalai* and *Rhyacophila* spp. to determine a building-material availability measure. The rationale for this calculation related to the observation that the pupal cases of both taxa in a sample could contain all development stages (larva to mature pupa). Thus, some of the cases were built shortly before sampling, whereas others were built weeks before sampling, i.e., the latter should have had material available for building that was unavailable for the former. Therefore, adding half of the material found in the pupal cases of *H. siltalai* and *Rhyacophila* spp. to the available sediment quantities was an arbitrary correction for the temporal differences in building activities.

Overall, we could analyze sediment use and availability for 129 samples (we lost one sample through a mishap). However, when analyzing case architecture, we avoided additional scatter created by interindividual case variability (see above, Monte Carlo simulations) by reducing the data to samples having ≥ 10 pupal cases of *H. siltalai* (77 samples) or ≥ 8 pupal cases of *Rhyacophila* spp. (many samples had 8 cases, so that we could analyze 39 samples including them). We used Systat-10® for (1) Kruskal-Wallis tests in comparisons among sampling dates, (2) regression analyses of relations between material availability and use and of relations between the use of particular grain fractions, and (3) multiple stepwise forward regression to check if grain use by *H. siltalai* interfered with that by *Rhyacophila* spp. or vice versa (independent variables: availability of each grain fraction and its use by the other taxon). Normalized principal component analysis (PCA) (Thioulouse et al. 1997; ADE-4 2001 Release) served for the assessment of correlations among grain fractions in terms of availability or use.

Results

The overall quantities of mineral grains in the size range used in the pupal cases (Table 1) did not differ significantly among the three sampling dates in terms of grain availability (Kruskal–Wallis test, assuming a Chi-square distribution with 2 degrees of freedom here and below, and indicating the Kruskal–Wallis test statistic associated with the *p*-value; grain mass: $p = 0.31$, 2.36; number of grains: $p = 0.85$, 0.33), pooled grain use by *H. siltalai* and *Rhyacophila* spp. ($p = 0.55$, 1.18; $p = 0.45$, 1.58), and grain use by *H. siltalai* ($p = 0.78$, 0.51; $p = 0.21$, 3.12). Whereas one would expect temporal variation in sediment availability and use for three sampling dates covering a period of ~2 months, the Furan River remained at baseflow throughout our study period (i.e., we had no sediment changes or pupal mortality through flood disturbances) and the relatively low summer temperature of the Furan River slowed the pupal development. Regardless, temporal differences of grain use by *Rhyacophila* spp. were significant for grain mass ($p = 0.02$, 7.51) but insignificant for the number of grains used ($p = 0.38$, 1.94). Given these findings, we pooled data from all three sampling dates for subsequent analyses.

Overall pupal case architecture and grain availability—On average, *Rhyacophila* spp. had heavier pupal cases than *H. siltalai* (Fig. 1a). Grains >2 mm contributed most to the mean case mass of both taxa. *Rhyacophila* spp. also used more grains in the pupal cases than *H. siltalai* (Fig. 1b), with

Table 1. Overall mass and number of mineral grains (mean±1 SE) in the size range (0.125–5 mm) used in the pupal cases by *Hydropsyche siltalai* and *Rhyacophila* spp. on three sampling occasions in 1998.

	16 Jun (n=50)	10 Jul (n=49)	7 Aug (n=30)
Grain mass (kg m^{-2})			
Availability	1.708±0.127	1.656±0.127	1.932±0.153
Use by *H. siltalai*	0.121±0.014	0.124±0.014	0.112±0.020
Use by *Rhyacophila* spp.	0.081±0.009	0.049±0.005	0.062±0.007
Number of grains (×10^{7}m^{-2})			
Availability	1.6578±0.1990	1.6925±0.1943	1.8329±0.2694
Use by *H. siltalai*	0.0056±0.0008	0.0084±0.0010	0.0073±0.0016
Use by *Rhyacophyila* spp.	0.0039±0.0009	0.0023±0.0002	0.0047±0.0010

grains <1 mm contributing most to the overall number of grains in the cases of both taxa.

On average, across the grain-size range used in the pupal cases by *H. siltalai* and *Rhyacophila* spp., ~1.7 kg or ~1.7 × 10^7 grains m^{-2} were available for them (Fig. 2). In terms of grain mass, ~50% each were available as grains <2 or >2 mm. In terms of number of grains, grains >1 mm were rare compared with smaller grains (Fig. 2).

Grain availability and use: H. siltalai *versus* Rhyacophila *spp.*—The mass of grains used by *H. siltalai* increased significantly with the available mass of grains in the size range used in the pupal cases (Fig. 3a). Although mass use by *Rhyacophila* spp. also increased with mass availability, this relation was less meaningful (Fig. 3b). As illustrated by Fig. 1, the number of grains in finer fractions was high and rather variable in pupal cases of *H. siltalai* and *Rhyacophila* spp., whereas the mass of these finer fractions was unimportant in their cases. Plotting use versus availability of the number of grains for all 129 samples (not shown here), the data scattered more in comparison with the mass plot (see Fig. 3), and the regression between use and availability of the number of grains had a relatively low significance level (*H. siltalai*: $F_{1,127} = 4.9$, $p = 0.03$, $r^2 = 0.04$) or was insignificant (*Rhyacophila* spp.: $F_{1,127} = 2.2$, $p = 0.14$, $r^2 = 0.02$). Thus, we focus the analyses in subsequent sections on grain mass and assess the number of grains only in visualizations of case architectures.

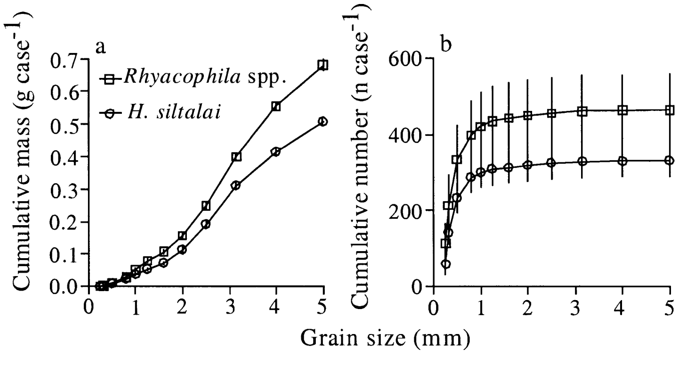

Fig. 1. Overall architecture of pupal cases of *H. siltalai* and *Rhyacophila* spp., showing mean ± 1 SE of the cumulative (a) mass and (b) number of grains for all samples each taxon was found (samples per taxon: *H. siltalai*: 110; *Rhyacophila* spp.: 121). Here and in subsequent figures, grain size indicates the upper size limit of a given fraction.

The similarity of the cumulative distributions of mass use over grain size in *H. siltalai* and *Rhyacophila* spp. (Fig. 1) suggests that both taxa potentially competed for similar building material. On average, *H. siltalai* and *Rhyacophila* spp. used ~15–20% of the available mass of the 2.5–4-mm grain fraction in the pupal cases at elevated case densities (Fig. 4a). However, a certain (but unknown) proportion of the individuals of *Rhyacophila* spp. could have built the pupal cases before *H. siltalai* started to build theirs (see Materials and methods), i.e., we assume at least some temporal segregation of building activities between these two taxa.

Mass use of the various grain-size fractions by one of these two taxa was primarily related to grain-size availability and not ($p > 0.05$; multiple stepwise forward regressions with varying degrees of freedom) to grain-size use by the other taxon. Overall, however, we never observed an elevated grain mass use of both taxa at the same location (Fig. 4b), i.e., the grain mass in pupal cases of *Rhyacophila* spp. was high when that in the cases of *H. siltalai* was relatively low and vice versa.

Grain availability and case architecture—The mean total mass of *H. siltalai* pupal cases decreased with decreasing available mass case^{-1} in the 77 samples having ≥10 cases. However, this decrease was not significant ($F_{1,75} = 1.5$, $p = 0.23$, $r^2 = 0.02$).

Analyzing grain-size fractions using the samples with ≥10 cases, normalized PCA illustrates that the mass per

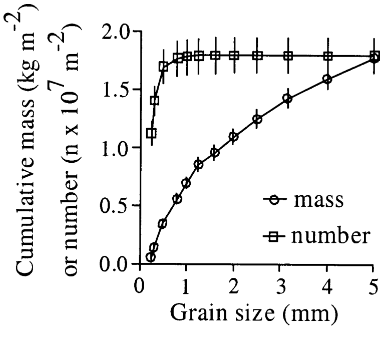

Fig. 2. Overall grain availability across the grain-size range used in the pupal cases (see Fig. 1), showing mean ± 1 SE of the cumulative mass and number of grains for all 129 samples.

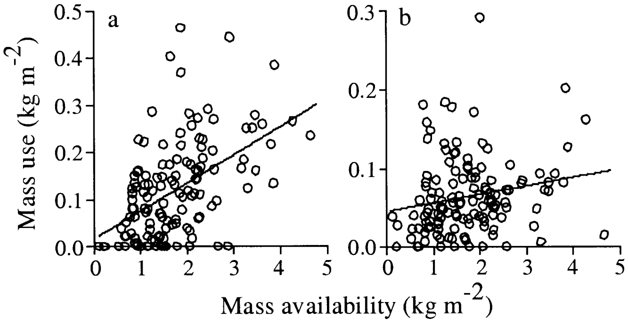

Fig. 3. Mass availability and use by (a) *H. siltalai* and (b) *Rhyacophila* spp. across the entire grain-size range (0.125–5 mm) used in the pupal cases (including all 129 samples; note the differently scaled *y*-axes). Statistics (±1 SE) are: (a) $y = 16.8 \pm 17.3 + (0.059 \pm 0.009)x$, $F_{1,127} = 44.7$, $p < 10^{-9}$, $r^2 = 0.26$; and (b) $y = 45.6 \pm 9.4 + (0.011 \pm 0.005)x$, $F_{1,127} = 5.0$, $p = 0.03$, $r^2 = 0.04$.

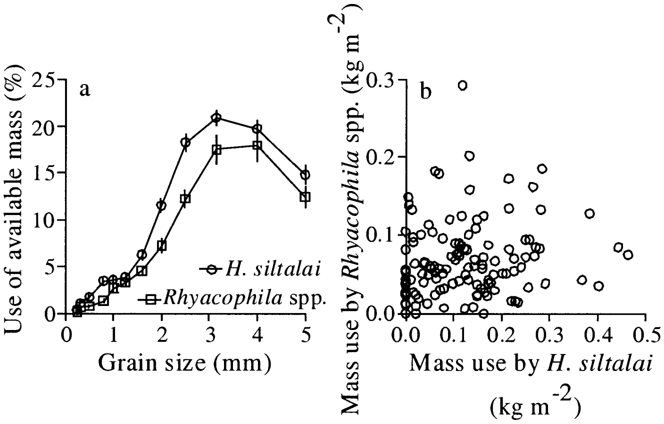

Fig. 4. (a) Mean (±1 SE) percentage of available mass of grain fractions used in the pupal cases by *H. siltalai* and *Rhyacophila* spp. if these were abundant (*H. siltalai* ≥10 cases per sample, i.e., $n = 77$; *Rhyacophila* spp. ≥8 cases per sample, i.e., $n = 39$). (b) Mass use by *H. siltalai* and *Rhyacophila* spp. across the entire grain-size range (0.125–5 mm) used in the pupal cases (including all 129 samples; note the differently scaled axes).

grain fraction available for *H. siltalai* correlated more among fractions of similar grain size than among fractions of different grain size (Fig. 5a). Therefore, PCA ordinated the availability of fractions gradually in the order of grain size (Fig. 5a). In contrast, correlations among the mass of the grain fractions used by *H. siltalai* formed three distinct groupings (Fig. 5a), indicating a correlated use of grain fractions within each of them. The directions of the arrows in the three groupings illustrate that samples with an elevated mean mass in the grain size 2.5–5 mm had less mass in the size 1.25–2 mm and vice versa, whereas use in the size 0.25–1.25 mm was intermediately correlated with the two other groupings (Fig. 5a).

The correlated use of grain fractions within each of the three groupings illustrated by Fig. 5a suggests analyzing grain mass use versus availability for these three groupings. Alternatively, Fig. 5a indicates that one could focus on a representative fraction of each grouping, e.g., on the three fractions that were the most distant among each other in the PCA on grain use (2.5–3.15 mm; 1.6–2 mm; 0.315–0.5 mm). Here, we communicate the analyses on these three fractions, as they provided the most significant results and clearest patterns.

The relation between the mean used and available mass case^{-1} for the 2.5–3.15-mm fraction was by far the most significant ($F_{1,75} = 22.9$, $p = 0.000008$, $r^2 = 0.23$) among all 12 individual fractions assessed by us. In this fraction, use increased logarithmically with availability (Fig. 5b). Likewise, use increased logarithmically with availability in the 0.315–0.5-mm fraction, but use of the 1.6–2-mm fraction was not related to availability (Fig. 5b), rather it decreased significantly in a linear relation with use of the 2.5–3.15-mm fraction (Fig. 5c). Furthermore, use of the 0.315–0.5-mm fraction increased linearly with use of the 1.6–2-mm fraction (Fig. 5c).

To translate these assessments of grain-fraction masses into a statistically based visualization of changes in case architecture, we used the relations shown in Fig. 5c, plus other relations between the use of other fractions and the use of the three fractions included in Fig. 5c. We solved this set of regression equations starting with the relation between use and availability of the 2.5–3.15-mm fraction to obtain the mass of this fraction that was used at the lowest and highest availability shown in Fig. 5b. Based on these two extreme uses of the 2.5–3.15-mm fraction, we subsequently calculated the grain mass used in all other fractions. We transformed grain mass to number of grains, rounding decimals to the nearest integer. Using the median size of each fraction as grain diameter, we thus could visualize case architecture for the two situations by arranging the grains in a shape corresponding to that of a *Hydropsyche* pupal case (Fig. 6).

At both lowest and highest availability of the 2.5–3.15-mm fraction, *H. siltalai* used 265 grains of identical size from different grain fractions (Fig. 6). To these, it added two grains of the 2.5–3.15-mm fraction and one grain of the 3.15–4-mm fraction if the availability of the 2.5–3.15-mm fraction was the highest. At the lowest availability of that fraction, it added 29 smaller grains instead. As a result, if all grains would be in contact with other grains and finer grains would be arranged among coarser grains, the pupal case would be larger and would have a better mixture of coarser and finer grains at the highest if compared with the lowest availability of the 2.5–3.15-mm fraction (Fig. 6). Alternatively, for a similar case size, the gaps among the grains would be larger at the lowest if compared with the highest availability of the 2.5–3.15-mm fraction.

Given that *Rhyacophila* spp. included two species and that individuals of these species may have built their cases months before they were sampled (see above), it was foreseeable that finer analyses of the case architecture of this taxon would provide less evidence than for *H. siltalai.* Indeed, using the 39 samples with ≥8 pupal cases, normalized PCA on grain fraction availability and use by *Rhyacophila* spp. revealed very similar patterns (for both availability and use the pattern resembled the availability pattern shown in Fig. 5a for *H. siltalai*). Therefore, we do not communicate further analyses on grain availability and its use by *Rhyacophila* spp. here.

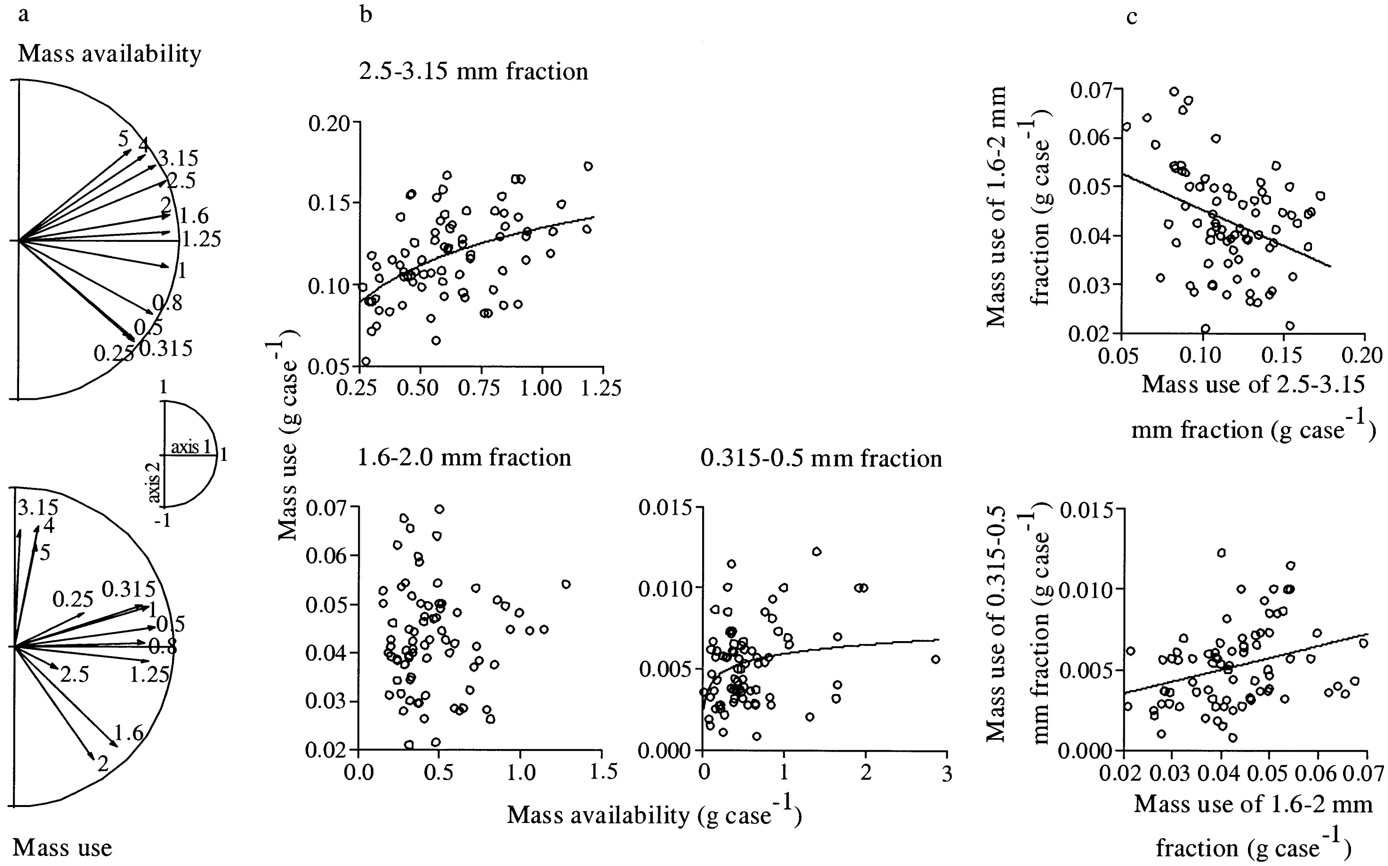

Fig. 5. Grain-fraction mass availability and use in the pupal cases by *H. siltalai* in the 77 samples having ≥10 cases per sample. (a) Results of normalized principal component analyses (PCA) of 12 grain fractions for the available and used mass (g case^{-1}), showing only half of the correlation circles (i.e., where the fractions were ordinated; see smaller half of the correlation circle for scales and axes). Long vectors in the same direction indicate positive correlations between fractions, whereas long vectors in the opposite direction indicate negative ones. Inertia related to the first/second axes were 69.4/19.8% for grain availability and 37.1/21.9% for grain use. (b) Relation between grain use and availability for the three size fractions that were the most distant among each other and thus the most representative for each of the three groupings of mass use in Fig. 5a. Statistics (±1 SE) are: (2.5–3.15 mm) $y = 0.139 \pm 0.005 + (0.033 \pm 0.007) \ln x$, $F_{1,75} = 22.9$, $p = 0.000008$, $r^2 = 0.23$; (1.6–2.0 mm) $F_{1,75} < 0.1$, $p = 0.93$, $r^2 < 0.01$; and (0.315–0.5 mm) $y = 0.0059 \pm 0.0004 + (0.0008 \pm 0.0003) \ln x$, $F_{1,75} = 6.7$, $p = 0.01$, $r^2 = 0.08$. (c) Relations between the use of the three grain-size fractions included in Fig. 5b. Statistics are: (1.6–2.0 vs. 2.5–3.15 mm) $y = 0.060 \pm 0.005 - (0.147 \pm 0.044)x$, $F_{1,75} = 11.4$, $p = 0.001$, $r^2 = 0.13$; and (0.315–0.5 vs. 1.6–2.0 mm) $y = 0.002 \pm 0.001 + (0.074 \pm 0.025)x$, $F_{1,75} = 8.4$, $p = 0.005$, $r^2 = 0.10$.

Discussion

Stratifying our sample universe to cobbles and shear-stress conditions that were critical for sand and fine gravel (Newbury and Gaboury 1993), we assessed locations that were preferred by *H. siltalai* and *Rhyacophila* spp.. These locations had less building material for their pupal cases in the bed sediments when compared with locations up- and downstream of our studied riffle or near its banks. Despite this shortage of finer sediments, the average availability in the grain-size range used in their pupal cases was ~1.7 × 10^7 grains with a mass of ~1.7 kg m^{-2}. Whereas, this appears indeed to be an overabundant resource for the pupal cases of both taxa, at elevated pupal case densities, both taxa used ~15–20% of some grain fractions (Fig. 4a) and, in extreme cases, *H. siltalai* used even >50% of a fraction (e.g., 2.5–3.15-mm fraction in Fig. 5b). Given that our sampling technique overestimated grain availability and *Hydropsyche* larvae collect grains from a very small surface in their neighborhood, these values suggest that *H. siltalai* may use certain grain fractions near the limit of their availability. Correspondingly, the increase of the overall mass of the pupal cases with the available mass of building material was more significant in *H. siltalai* than in *Rhyacophila* spp. (Fig. 3).

Thus, why did these larvae not build their pupal cases in slower flowing parts of the Furan River, where building material was very abundant? First, the foundation of the case of *H. siltalai* and *Rhyacophila* spp. is a larger, solid surface, which is typically found on cobbles in fast-flowing riffles. Second, the immobile prepupa of *H. siltalai* is unable to ventilate its case (Sattler 1958) and is a phase with high mortality rates (Schuhmacher 1970), suggesting that passive water renewal in the case is essential for the prepupa; this would also require fast flows near the case front. Because of these conflicting resource requirements, larvae of both

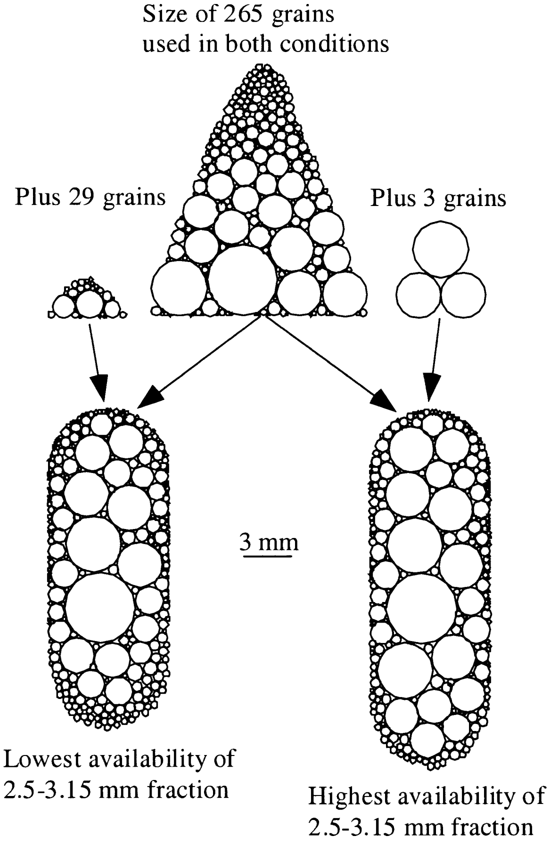

Fig. 6. Visualization of case-building material and case architecture of *H. siltalai* for the lowest and highest availability of the 2.5–3.15-mm grain fraction shown in Fig. 5b (see text for details on the visualization procedure).

taxa had to build pupal cases at locations where building material was rare because of the erosive flow forces, so that the overall case mass depended on the local availability of building material. Similarly, the abundance of lotic midge larvae using fine detritus to build tubes attached to the surface of stones depends significantly on the overall quantities of local building material (Brennan et al. 1978; Brennan and McLachlan 1979). In addition, the upper surfaces of stones and rocks provide other resources (e.g., algae for grazers) that are only temporarily exploited by stream invertebrates because they also have to use the interstitial space of the streambed to satisfy other resource requirements (reviewed in Statzner et al. 1988).

Hydropsyche and *Rhyacophila* larvae could avoid a shortage of building material by building their pupal cases prior to other conspecific larvae or to other species within these taxa, as is done by *R. dorsalis* and other species in this genus (see Cereghino et al. 1997; Sangpradub et al. 1999). Another possibility to avoid a shortage of building material would be to build the pupal case far from locations where other larvae are building pupal cases, which would explain the aggressive interactions between *Hydropsyche* larvae working too close to each other on their pupal cases (Mogel et al. 1985).

For elevated case densities, data on the overall mean case mass of *H. siltalai* do not indicate resource limitations, as mean total case mass did not significantly change with the locally available mass of building material. In contrast, patterns of grain-size use by *H. siltalai* indicate resource limitations, as the mass of individual grain fractions in the pupal cases changed significantly with local grain availability. In comparison, the finer analysis of the case architecture of *Rhyacophila* spp., i.e., the taxon including two species, provided no evidence for resource limitations, suggesting that such subtle details may be missed if working with groups of species.

However, the changes in grain-size use by *H. siltalai* were not as simple as those reported from laboratory experiments, in which caddisfly larvae switch to grain sizes near the upper or lower range limit of the unavailable, normally preferred range (Hanna 1961; Tolkamp 1980). Clearly, *H. siltalai* used the mass of coarser (>2.5-mm) or intermediate (1.25–2-mm) grains as alternatives in the pupal cases (Fig. 5), but each of these alternatives required the use of finer grains (<1.25 mm, see the intermediate position of finer grains in Fig. 5a). Our data provide evidence that the 2.5–3.15-mm grain fraction was preferred over all other fractions by *H. siltalai*: this fraction (1) produced the steepest slope in the cumulative mass use distribution over grain size (Fig. 1); (2) was, in relation to mass availability, the most used among all fractions (Fig. 4a); and (3) had the most significant relation between mass use and availability case^{-1} (Fig. 5b). Obviously, use of neighboring fractions (2–2.5 mm; 3.15–4 mm) had no strong negative correlation with the use of the preferred fraction (Fig. 5a). Thus, why did *H. siltalai* not switch to material in the neighboring fractions if the preferred fraction was rare at a location?

Our data provide three complementary answers to this question. First, at our field conditions, the availability of the preferred fraction was more correlated with the availability of its upper and lower neighboring fraction than with any other fraction (Fig. 5a), i.e., locations with a shortage in the preferred fraction also had less building material in the neighboring fractions. Second, the relation between the available number of grains in the upper neighboring fraction to the preferred fraction was ~1 : 2 (for samples with ≥10 cases), i.e., switching to the upper neighboring fraction would increase the effort to find and to transport these coarser grains (this argument does not hold for the grains in the lower neighboring fraction, which had about twice as many grains as the preferred fraction). Third, as mentioned before, the use of either coarser or intermediate grains required the use of finer grains.

Consequently, the more grain availability and use in the preferred 2.5–3.15-mm fraction indicated resource limitation (Fig. 5b), the more the larvae used the 1.6–2-mm fraction instead (Fig. 5c), which was an unlimited resource (Fig. 5b). It seemed that using more of the 1.6–2-mm fraction required more of the 0.315–0.5-mm fraction (Fig. 5c), which seemed to be another unlimited resource (available mass used only ~2%; Fig. 4a). Given that ~98% of the 0.315–0.5-mm fraction was not used, we consider the relation between use and availability of this fraction (Fig. 5b) as a spurious relation. If so, resource limitation of the preferred grain size 2.5–3.15 mm produced chained effects across other grain fractions, as the alternative use of the 1.6–2-mm fraction induced an increased use of the 0.315–0.5-mm fraction.

The visualization of the case architectures in Fig. 6 enables speculation about potential causes of these chained effects as well as about their potential biological significance. To achieve a grain arrangement that reduces gaps among the grains to the minimum, the use of a certain coarser grain size would require the use of a certain finer grain size (Fig. 6). This could have been a cause for these chained effects.

Concerning the biological significance of these effects, the amount of silk required to cement particles together increases with decreasing particle size (Smart 1976; Becker 2001), which increases the energetic costs for case construction (Dudgeon 1990). However, we cannot imagine that adding 29 finer grains (i.e., using more silk) instead of three coarser ones to 265 other grains would make a significant difference in the costs of the silk-thread production for a *Hydropsyche* specimen. First, larvae that have finished building their pupal case have their silk glands tightly filled with secretion (Haller 1948). Second, the production of silk threads by caddisfly larvae can correspond energetically to ~20% of their body tissue production (Huryn and Wallace 1988). Thus, through its entire life, a *Hydropsyche* larva uses plenty of silk for safety threads when moving or for frequently renewed larval retreats and filter nets (Sattler 1958; Schuhmacher 1970; Statzner et al. 1999). Third, forcing caddisflies in experiments to use plenty of silk prior to pupation causes only minor (though significant) decreases in the mass of the emerging adults in comparison with control groups (Stevens et al. 1999, 2000). Therefore, the additional silk required to add 29 finer instead of three coarser grains to the pupal case should be a marginal cost in the silk budget of a *H. siltalai* specimen. In contrast, we can imagine that the differences in case architecture at the lowest and highest availability of the preferred grain fraction would have consequences for the case stability, e.g., the resistance of the case to damage resulting from floods that move coarser bottom material. Obviously, these speculations need confirmation by solid data.

This is among the first examples of limitations in a seemingly overabundant resource used by lotic caddisflies to build mineral cases (Dudgeon 1990). Obviously, the constraints interfering in this resource limitation were quite subtle so that its discovery required a careful study design (guided by analyses of preliminary data) as well as some luck (patterns were not obscured by flood disturbances).

Generalizing our findings beyond the system examined by us, we focus on aquatic systems, mineral particles, and benthic invertebrate builders. First, the risk that mineral grains become a limited resource for aquatic invertebrates that use them to build cases or tubes should be related to the space that can be exploited during grain acquisition, i.e., this risk should be greater for resident builders (depending on local grain availability) than for mobile itinerant builders (acquiring grains where they can be found). Typical representatives of the former are polychaetes or amphipods in marine and insects (caddisflies, midges, moths) in freshwater systems (Dudgeon 1990). Second, particularly in streams but also near shores of lakes and oceans, water currents can create conflicts in the resource requirements for invertebrates building with mineral grains. Obviously, increasing flow facilitates respiration but also erodes mineral grains of increasing size, until the grains become so large that invertebrates cannot use them, except for the attachment of their cases or tubes. This conflict of resource requirements is extreme for invertebrates that need locally available fine mineral grains for their cases or tubes, coarse grains to attach their cases or tubes, and high oxygen renewal rates (i.e., caddisflies, midges, and moths that attach their case or tube to stones in rapidly flowing stream riffles; Dudgeon 1990). For marine invertebrates that construct within finer sediments, this conflict should be less extreme, as they do not need large grains for firm attachment (Dudgeon 1990). Third, water currents in natural environments simultaneously create a shortage in mineral grain sizes that are in or near the grain-size range preferably used by an invertebrate builder. Thus, in contrast with experimental conditions, switching to grain sizes neighboring the preferred grain-size range is difficult in natural habitats.

References

Becker, G. 2001. Larval size, case construction and crawling velocity at different substratum roughness in three scraping caddis larvae. Arch. Hydrobiol. **151:** 317–334.

———, and M. Boersma. 2003. Resource quality effects on life histories of *Daphnia.* Limnol. Oceanogr. **48:** 700–706.

Begon, M., J. L. Harper, and C. R. Townsend. 1986. Ecology. Blackwell.

Brennan, A., and A. J. McLachlan. 1979. Tubes and tube-building in a lotic chironomid (Diptera) community. Hydrobiologia **67:** 173–178.

———, ———, and R. S. Wotton. 1978. Particulate material and midge larvae (Chironomidae: Diptera) in an upland river. Hydrobiologia **59:** 67–73.

Buitenhuis, E. T., K. R. Timmermans, and H. J. W. De Baar. 2003. Zinc-bicarbonate colimitation of *Emiliania huxleyi.* Limnol. Oceanogr. **48:** 1575–1582.

Castillo, M. M., G. W. Kling, and J. D. Allan. 2003. Bottom-up controls on bacterial production in tropical lowland rivers. Limnol. Oceanogr. **48:** 1466–1475.

Cereghino, R., T. Boutet, and P. Lavandier. 1997. Abundance, biomass, life history and growth of six Trichoptera species under natural and hydropeaking conditions with hypolimnetic release in a Pyrenean stream. Arch. Hydrobiol. **138:** 307–328.

Dudgeon, D. 1990. Functional significance of selection of particles and their use by aquatic animals in the construction of external structures, p. 263–288. *In* R. S. Wotton [ed.], The biology of particles in aquatic systems. CRC.

Elliott, J. M. 1971. Upstream movements of benthic invertebrates in a lake district stream. J. Anim. Ecol. **40:** 235–252.

Gaino, E., F. Cianficconi, M. Rebora, and B. Todini. 2002. Case-building of some Trichoptera larvae in experimental conditions: Selectivity for calcareous and siliceous grains. Ital. J. Zool. **69:** 141–145.

Gorter, F. J. 1931. Köcherbauversuche an Trichopterenlarven. Z. Morph. Ökol. Tiere **20:** 443–532.

Haller, P. H. 1948. Morphologische, biologische und histologische Beiträge zur Kenntnis der Metamorphose der Trichopteren (*Hydropsyche*). Mitt. Schweiz. Entomol. Ges. **21:** 301–359.

Hanna, H. H. 1961. Selection of materials for case-building by larvae of caddis flies (Trichoptera). Proc. R. Entomol. Soc. Lond. (A) **36:** 37–47.

Holzman, R., and A. Genin. 2003. Zooplanktivory by a nocturnal coral-reef fish: Effects of light, flow, and prey density. Limnol. Oceanogr. **48:** 1367–1375.

Huryn, A. D., and J. B. Wallace. 1988. Community structure of

Trichoptera in a mountain stream: Spatial patterns of production and functional organization. Freshw. Biol. **20:** 141–155.

HYNES, H. B. N. 1970. The ecology of running waters. Liverpool Univ. Press.

JACKSON, J. K., E. P. MCELRAVY, AND V. H. RESH. 1999. Long-term movements of self-marked caddisfly larvae (Trichoptera: Sericostomatidae) in a California coastal mountain stream. Freshw. Biol. **42:** 525–536.

LEICHTFRIED, M. 1986. Räumliche und zeitliche Verteilung der partikulären organischen Substanz (POM—Particulate Organic Matter) in einem Gebirgsbach als Energiebasis der Biozönose. Ph.D. thesis, Univ. of Vienna.

LEPNEVA, S. G. 1970. Larvae and pupae of Annulipalpia. Israel Program Sci. Translations.

———. 1971. Larvae and pupae of Integripalpia. Israel Program Sci. Translations.

MACKAY, R. J. 1977. Behavior of *Pycnopsyche* (Trichoptera: Limnephilidae) on mineral substrates in laboratory streams. Ecology **58:** 191–195.

———, and G. B. Wiggins. 1979. Ecological diversity in Trichoptera. Annu. Rev. Entomol. **24:** 185–208.

MALICKY, H. 1983. Atlas of European Trichoptera. Junk.

MOGEL, R., N. RIEDER, AND B. STATZNER. 1985. Ein Gerät zur Freilandbeobachtung des nächtlichen Verhaltens von benthischen Bachtieren, mit Befunden aus der Gattung *Hydropsyche* (Trichoptera, Insecta). Carolinea **42:** 121–128.

NEWBURY, R. W., AND M. N. GABOURY. 1993. Stream analysis and fish habitat design. Newbury Hydraulics.

PODGORNYI, K. A., AND V. A. NEPOMNYASHCHIKH. 1999. Effects of behavioral variability on optimization of caddis fly (*Chaetopteryx villosa*) larvae behavior. Adv. Curr. Biol. **119:** 218–222 (in Russian, with English abstract).

ROST, B., U. RIEBESELL, S. BURKHARDT, AND D. SÜLTEMEYER. 2003. Carbon acquisition of bloom-forming marine phytoplankton. Limnol. Oceanogr. **48:** 55–67.

SANGPRADUB, N., P. S. GILLER, AND J. P. O'CONNOR. 1999. Life history patterns of stream-dwelling caddis. Arch. Hydrobiol. **146:** 471–493.

SATTLER, W. 1958. Beiträge zur Kenntnis von Lebensweise und Körperbau der Larve und Puppe von *Hydropsyche* Pict. (Trichoptera) mit besonderer Berücksichtigung des Netzbaues. Z. Morph. Ökol. Tiere **47:** 115–192.

SCHUHMACHER, H. 1970. Untersuchungen zur Taxonomie, Biologie und Ökologie einiger Köcherfliegenarten der Gattung *Hydropsyche* Pict. (Insecta, Trichoptera). Int. Rev. Gesamten Hydrobiol. **55:** 511–557.

SMART, K. 1976. A progress report on the building motivation in the caddis larva, *Lepidostoma hirtum*. Proc. 1st Int. Symp. Trichoptera. **1974:** 185–186.

STATZNER, B., M.-F. ARENS, J.-Y. CHAMPAGNE, R. MOREL, AND E. HEROUIN. 1999. Silk-producing stream insects and gravel erosion: Significant biological effects on critical shear stress. Water Resour. Res. **35:** 3495–3506.

———, AND G. BRETSCHKO. 1998. Net-building of caddis fly (*Hydropsyche siltalai*) in a French stream: Relations with larval density and physical conditions. Arch. Hydrobiol. **144:** 87–102.

———, J. A. GORE, AND V. H. RESH. 1988. Hydraulic stream ecology: Observed patterns and potential applications. J. N. Am. Benthol. Soc. **7:** 307–360.

———, F. KOHMANN, AND A. G. HILDREW. 1991. Calibration of FST-hemispheres against bottom shear stress in a laboratory flume. Freshw. Biol. **26:** 227–231.

STEVENS, D. J., M. H. HANSELL, J. A. FREEL, AND P. MONAGHAN. 1999. Developmental trade-offs in caddis flies: Increased investment in larval defence alters adult resource allocation. Proc. R. Soc. Lond. B **266:** 1049–1054.

———, ———, AND P. MONAGHAN. 2000. Developmental trade-offs and life histories: Strategic allocation of resources in caddis flies. Proc. R. Soc. Lond. B **267:** 1511–1515.

STROM, S., G. WOLFE, A. SLAJER, S. LAMBERT, AND J. CLOUGH. 2003. Chemical defense in the microplankton II: Inhibition of protist feeding by β-dimethylsulfoniopropionate (DMSP). Limnol. Oceanogr. **48:** 230–237.

THIOULOUSE, J., D. CHESSEL, S. DOLÉDEC, AND J.-M. OLIVIER. 1997. ADE-4: A multivariate analysis and graphical display software. Stat. Comput. **7:** 75–83.

TOLKAMP, H. H. 1980. Organism-substrate relationships in lowland streams. Agric. Res. Rep. Wageningen **907:** 1–211.

WARINGER, J., AND W. GRAF. 1997. Atlas der österreichischen Köcherfliegenlarven unter Einschluß der angrenzenden Gebiete. Facultas.

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