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RESEARCH PAPER

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Changes in periphyton abundance and community structure with the dispersal of a caddisfly grazer, *Micrasema quadriloba*

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Abstract We examined the larval population densities and biomass of a caddisfly grazer, *Micrasema quadriloba*, and the abundance and community structures of periphyton at a segment scale (7.4 km with four study sites), along a second- to fourth-order Japanese mountain stream throughout the grazer's life cycle. In the uppermost riffle of the study segment (site 1), periphyton abundance was kept at low levels when the larvae occurred. The larval distribution spread downstream as larvae developed from first instars in May to fifth instars in January. We performed multiple regression analyses to test the effects of environmental variables and larval biomass on periphyton abundance in both the riffle of site 1 and the study segment; the results revealed that the larval biomass was significantly negatively correlated with periphyton abundance similarly in both the riffle and the study segment. In addition, both the correlation and community analyses showed that the larval biomass was significantly negatively correlated to the relative abundance of large and/or filamentous microalgae, which appeared in the uppermost layer of the periphyton mat, and that larval biomass was significantly positively correlated to the relative abundance of small diatoms, which strongly adhered to the substrate. Thus, the present study implied that the grazing of *M. quadriloba* larvae would regulate the abundance of periphyton in a riffle and also regulate the abundance and community structure of periphyton at the segment scale with the expansion of their longitudinal distribution.

Key words Segment · Riffle · Distribution · Seasonal change · Life cycle

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Introduction

In stream ecosystems, the strong trophic interactions between stream grazers and periphyton have been widely recognized. In the regulation of periphyton biomass and/or productivity, grazing may have an impact that is equal to, or greater than, that of abiotic factors such as light penetration, nutrient supply, current velocity, and water temperature (Rosemond 1993; Feminella and Hawkins 1995). Many studies have reported that grazers selectively immigrate to patches with abundant periphyton, and that periphyton biomass is decreased for several days afterward as a result of this grazing pressure (Lamberti and Resh 1983; Vaughn 1986; Richards and Minshall 1988; Katano et al. 2005b, 2007; Doi et al. 2006). These interactions, however, have not been extensively studied at both large spatial and long temporal scales. To our knowledge, only a few studies have investigated periphyton–grazer relationships at a reach spatial scale (i.e., several riffle–pool units; Taylor et al. 2002; Katano et al. 2005c), and no studies have been performed at the segment spatial scale (i.e., of the order of 10^3 to 10^4 m). Nearly all studies have been performed at the microhabitat scale – for instance, on a cobble or an experimental clay tile – within specific seasons (e.g., Lamberti and Moore 1984; Hill and Knight 1987, 1988; Steinman et al. 1987; Wellnitz and Ward 2000). Therefore, the large-scale dynamics of the relationship between stream grazers and periphyton remains unclear.

In general, benthic invertebrates (including grazers) repeatedly drift to the downstream reaches of streams by means of the continuous and unidirectional flow of water from upstream to downstream (Allan 1995). As a result of

this process, the segment-scale distribution of invertebrates was predicted to expand downstream over time. However, the recording of changes in segment-scale invertebrate distribution has seldom been reported (Fujitani 2002), although the distances traveled by drifting invertebrates have been reported (Larkin and McKone 1985; Allan and Feifarek 1989; Elliott 2002). As a result of grazer dispersal and drifting, the intensity of regulation of periphyton (i.e., the grazing pressure) would shift downstream as well. However, the relationships between changes in segment-scale grazer distribution and periphyton biomass and community structures are not well known. To fully understand the dynamics of periphyton–grazer interactions, researchers need to investigate grazer distribution and the relationships between grazers and periphyton abundance across the entire life cycle of grazers.

Larvae of the case-bearing caddisfly *Micrasema quadriloba* Martynov (Brachycentridae) are small and slow-moving grazers that are widely distributed in the mountain streams of central Honshu Island, Japan (Katano et al. 2002; Tanida et al. 2005). This species is univoltine, with juveniles molting in March, adults emerging in May, and eggs hatching in June (Isobe et al. 1994). *Micrasema quadriloba* larvae often dominate in communities of benthic invertebrates (Katano et al. 2005a,c). In addition, these larvae markedly expand their distribution to downstream habitats as they develop (Isobe 2000). Therefore, *M. quadriloba* is a suitable study species for examining the relationships between the changes in grazer distribution and periphyton biomass and community structure.

In the present study, we examined temporal changes in the distribution of *M. quadriloba* larvae and the periphyton biomass and community structures at the segment scale. In addition, we also examined temporal changes in density and biomass of the larvae and the periphyton biomass at a riffle of the study segment to interpret the segment-scale results in detail. To investigate the dynamics of the relationships between grazers and periphyton, we conducted field investigations during four seasons throughout the life cycle of *M. quadriloba*. In addition, we estimate whether biotic and abiotic factors affected on periphyton abundance at riffle and segment scales.

Methods

Study area

We chose four sampling sites in the Shigo-gawa and Takami-gawa Streams in Nara Prefecture, Japan (Fig. 1, Table 1). Site 1 and sites 2–3 are second- and third-order mountain streams, respectively. Because the Shigo-gawa Stream flows into the Takami-gawa Stream at 50 m upstream of site 4, site 4 represents a fourth-order mountain stream. The distance between sites 1 and 4 is 7.4 km; stream width and water temperature at each site naturally tended to increase downstream. The changes in environmental variables among the sites are shown in Table 1. Site 1 is located where early-instar *M. quadriloba* larvae have been recorded at a high population density (18320 larvae m^{-2} in July; Isobe 2000) and account for the majority (80%) of the benthic invertebrates (in December; Katano et al. 2005a). The riparian forest surrounding all the study sites is a commercial cedar (*Cryptomeria japonica*) forest, but the stream-

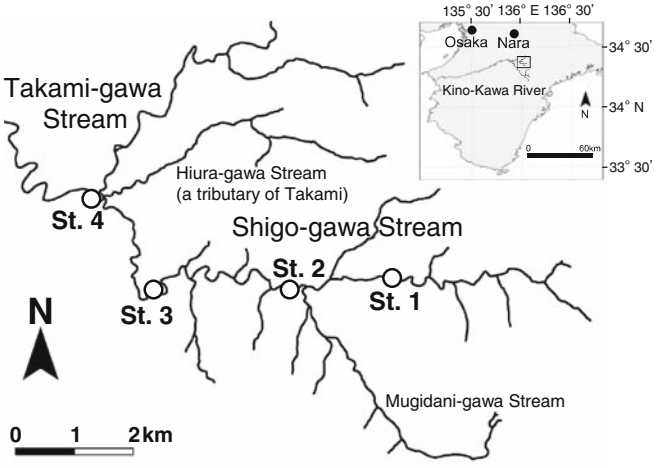

Fig. 1. Map of the study area with sampling sites 1–4 along the Shigo-gawa Stream and the Takami-gawa Stream, Nara Prefecture, Japan. *Open circles* show sampling sites (*St.*). Field surveys were performed within second- to fourth-order streams

Table 1. Environmental variables measured at sites 1–4, in where segment-scale investigations were performed

		Site 1	Site 2	Site 3	Site 4
Location		34°22′64″ N 136°02′38″ E	34°22′69″ N 136°01′00″ E	34°22′60″ N 135°59′97″ E	34°23′34″ N 135°59′28″ E
Elevation (m a.s.l.)		425	365	313	259
Stream width (m)		6.3 ± 0.5 (4)	13.0 ± 0.8 (4)	14.5 ± 0.7 (4)	18.1 ± 0.6 (4)
Water depth (cm)		14.8 ± 3.3 (4)	11.5 ± 1.3 (4)	15.3 ± 5.3 (4)	14.0 ± 2.6 (4)
Current velocity ($cm\,s^{-1}$)		22.4 ± 2.2 (4)	25.9 ± 3.0 (4)	34.5 ± 10.8 (4)	32.9 ± 6.1 (4)
pH		7.4 ± 0.1 (4)	7.4 ± 0.1 (4)	7.4 ± 0.1 (4)	7.4 ± 0.1 (4)
Electric conductivity		9.9 ± 0.9 (4)	9.1 ± 1.2 (4)	9.3 ± 0.8 (4)	9.3 ± 0.9 (4)
Water temperature (°C)	July 1998	19.1	20.5	22.0	24.2
	Oct 1998	14.0	15.0	15.5	16.2
	Jan 1999	4.6	4.9	5.0	5.2
	May 1999	15.2	18.2	18.7	20.8

Riffle-scale investigations were performed at site 1
Values for water temperature are shown for each sampling date; the other variables represent mean ± 1 SD (sample size)

beds of all sites, which are composed mainly of cobbles and pebbles, are not shaded by the canopy. Predatory fish such as minnow (*Phoxinus oxycephalus*) and dace (*Tribolodon hakonensis*) occur throughout the year (Mizuno and Gose 1993).

Investigation at a riffle

We performed field surveys on July 10, 1998, October 17, 1998, February 26, 1999, and May 23, 1999 at a riffle of site 1 (see Fig. 1). A 5-m stretch of the stream was selected as the study riffle. Equally spaced transects (1-m intervals) with evenly spaced collection points (0.75-m intervals) were established in the study riffle, and a total of 15–26 points (n) were established per sampling date. A 20 × 20-cm quadrat was set up at each collecting point on the streambed. At the center of the quadrat, we measured water depth using a ruler and surface current velocity using a portable current meter (model CW-7WP; Cosmo Riken, Tokyo, Japan). In the quadrat, all cobbles and pebbles were lifted and sampled for macroinvertebrates. During the sampling, a hand net with 0.5-mm mesh was placed immediately downstream of the quadrat to catch dislodged macroinvertebrates. Macroinvertebrates were brushed from the substrate into a pan of water, and the contents of the pan were sieved with the hand net. Macroinvertebrates were preserved in 4% formaldehyde in the field, sorted in the laboratory, and identified to species level if possible, and the number of individuals in each taxon was counted. We also determined the instar stages of *M. quadriloba* larvae on the basis of head width (Isobe et al. 1994). Then, the biomass of *M. quadriloba* larvae at each sampling point was calculated according to the relationships between the head widths and body dry weight of each instar of the larvae (Miyasaka et al., submitted).

The periphyton biomass was estimated by measuring the chlorophyll *a* content. We wiped the periphyton in a 3-cm-diameter circle from the upper surface of the largest cobble in each quadrat (maximum length, 10.5–17.0 cm) using acrylic fiber cloths, in accordance with the sampling method of Tanida et al. (1999). Each cloth was placed into a vial containing 10 ml 99.5% ethanol. After placing the vials in a dark refrigerator at 4°C for 24 h, we measured the extracted pigments using a spectrophotometer (model MPS-2000; Shimadzu, Kyoto, Japan). We determined the chlorophyll *a* content from these data according to the method of UNESCO (1966) and expressed the abundance of the periphyton as micrograms chlorophyll *a* per square centimeter.

Investigations at the segment scale

We performed field surveys at the segment scale on July 9, 1998, October 6, 1998, January 16, 1999, and May 31, 1999 at sites 1–4 (see Fig. 1, Table 1). On each survey date, we measured the stream width as well as water temperature using a thermometer placed at the approximate center of the riffle. The pH and electric conductivity of the surface stream water were measured with a pH Color Comparison Kit (Advantec; Toyo, Tokyo, Japan) and a portable conductivity meter (model CM-14P; Toa Electric, Tokyo, Japan), respectively.

At each station, we set up a 20 × 20 cm quadrat on the streambed approximately at the center of the riffle. At the center of the quadrat, water depth and surface current velocity were measured using a ruler and the portable current meter, respectively. In the quadrat, macroinvertebrates and periphyton biomass (chlorophyll *a*) were sampled using the same methods as described above for the measurements at a riffle.

To examine the periphyton community structure, periphyton within a 5 × 5-cm quadrat near the circles that had been wiped to collect periphyton for chlorophyll *a* measurements was brushed off and placed in a container with 250 ml water. The periphyton sample was then preserved in 4% formaldehyde in the field. To obtain the relative proportions of all algal taxa, including unicellular diatoms and multicellular filamentous algae, in the laboratory we measured the occupied area by each algal taxon (μm^2). To estimate the relative abundances of the algae, the area occupied by each diatom taxon was calculated using the following equation:

$$A_i = (RA_i \times CA_i)/TDA$$

where A_i represents the area occupied by diatom taxon i (μm^2), RA_i is the relative abundance of diatom taxon i among the diatom assemblages (%), CA_i is the mean cell area of diatom taxon i (μm^2), and TDA is the total area occupied by diatoms (μm^2).

To measure TDA, we took five photographs of each well-homogenized periphyton sample under a light microscope (400× magnification) and analyzed the photographs using NIH-Image (National Institutes of Health, Bethesda, MD, USA). For the relative abundance (RA) and the mean cell area of each diatom taxon (CA), a part of the homogenized periphyton sample was cleaned with concentrated sulfuric acid and potassium permanganate, and the cleaned samples were mounted on glass slides with Mountmedia (Wako Chemical, Osaka, Japan) (Nagumo and Osada 1999). The glass slides were examined under a light microscope (1000× magnification), and the diatom taxa were identified following Krammer and Lange-Bertalot (1986–1991). On the basis of counts of at least 400 cells, the relative abundance of each diatom taxon among the diatom assemblages (RA, %) was obtained. In addition, we used photographs of the slides (1000× magnification) to measure the mean cell area of each diatom taxon (CA, μm^2) with NIH-Image. In the case of filamentous algae (i.e., blue-green and green algae), we directly measured the areas occupied by each taxon in the first set of five photographs (400× magnification). Thus, the areas of each algal taxon (diatom, blue-green, and green) were converted into relative areas (μm^2).

Periphyton mats are frequently formed by several layers tangled together (Steinman 1996; Johnson et al. 1997; Tsuji 2000), and we classified the algal taxa into three groups on the basis of their life forms and vertical locations in the periphyton mat (Katano et al. 2002; cf. Steinman 1996).

Group 1 included small diatoms, which were located in the lowest layer of the mat and adhered strongly to the substrate. Group 2 included diatoms of various life forms, such as rosette, motile, stalk-forming, and tube-forming, which were located in the middle layer of the mat. Group 3 consisted of large and/or filamentous taxa, including diatoms, green algae, and blue-green algae, which appeared in the uppermost layer, called the canopy.

Statistical analysis

To analyze seasonal changes in the density of *M. quadriloba* larvae and periphyton biomass at the riffle of site 1, these parameters were separately tested by one-way analysis of variance (ANOVA) and Scheffe's multiple comparison ($\alpha = 0.05$) among the sampling dates using the software package Statcel 2 with Microsoft Excel 2003 (OMS, Tokorozawa, Japan).

To examine the effects of *M. quadriloba* biomass and environmental factors on the periphyton abundance at the both riffle and segment scales, we performed multiple regression analysis using the following equation:

$$Y = C_0 + C_bX_1 + C_vX_2 + C_dX_3 + C_tX_4 + C_lX_5$$

where Y is chlorophyll *a* content, C_0 is a regression constant, X_1, X_2, X_3, X_4, and X_5 represent *M. quadriloba* biomass, current velocity, water depth, water temperature, and mean light intensity, respectively, and C_b, C_v, C_d, C_t, and C_l are the regression coefficients. The mean light intensity (mol photons m^{-2} day^{-1}) for the sampling month and the preceding month was obtained by calculations using irradiance data of the Nara observation station (34°41′60″ N, 135°49′60″ E), which is the station nearest the study sites, from Japan Metrological Agency (http://www.jma.go.jp). Because there was no canopy in our study sites (I. Katano, personal observations), we treated data of irradiance for all the sites. For the analyses, we used the whole data set (riffle: $n = 74$, July, October, February, and May; segment scale: $n = 16$, July, October, January, and May) after $\log_{(x+1)}$ transformation.

At the segment scale, the correlation between chlorophyll *a* content and the total area of periphyton (μm^2) was also tested by a correlation analysis using the whole data set ($n = 16$; July, October, January, and May). Then, the relationships between the larval biomass and the relative areas occupied by group 1, group 2, and group 3 (μm^2) were also tested by a correlation analysis after $\log_{(x+1)}$ transformation.

We used multivariate statistics to compare the community structure of the periphyton within sites and seasons. Bray–Curtis coefficients were used to calculate a similarity matrix between samples on the basis of the areas of different taxa in the periphyton assemblages (Clarke and Warwick 1994). We used analysis of similarities (ANOSIM) to test for differences between periphyton assemblages on the basis of the rank similarities from Bray–Curtis similarity matrices, with 999 iterations (Clarke and Warwick 1994). Using seasons (July, October, January, and May) and sites (sites 1, 2, 3, and 4) as factors, we performed a one-way ANOSIM on all samples for each factor separately, because the sample numbers were too small to analyze by a two-way crossed ANOSIM (season × site, $N = 16$). When analyses revealed significant differences, pairwise comparisons were also performed on the factors. We performed these multivariate analyses using PRIMER v.5.0 software (Clarke and Warwick 1994).

In addition, we used the species contributions to the similarity (SIMPER) function in PRIMER to identify which taxa were responsible for the observed differences in assemblages. SIMPER uses the Bray–Curtis similarity matrix to calculate how much each taxon contributes to the observed dissimilarities between groups. Finally, we used nonmetric multidimensional scaling (nMDS) to visualize how periphyton assemblages differed across seasons and sites. This technique is the preferred method to portray community patterns graphically in two dimensions (Clarke 1993).

Results

At the riffle of site 1, *M. quadriloba* larvae accounted for up to 90% of the macroinvertebrate density, except in May (Fig. 2A). The density of *M. quadriloba* larvae at the site decreased from July to May, over the course of their development (one-way ANOVA: $F_{3,70} = 11.80$, $P < 0.0001$). *M. quadriloba* larvae were mainly second, fourth, and fifth instars in July, October, and February, respectively. On May 23, we collected no first instars at site 1, although many *M. quadriloba* egg masses were observed. Chlorophyll *a*

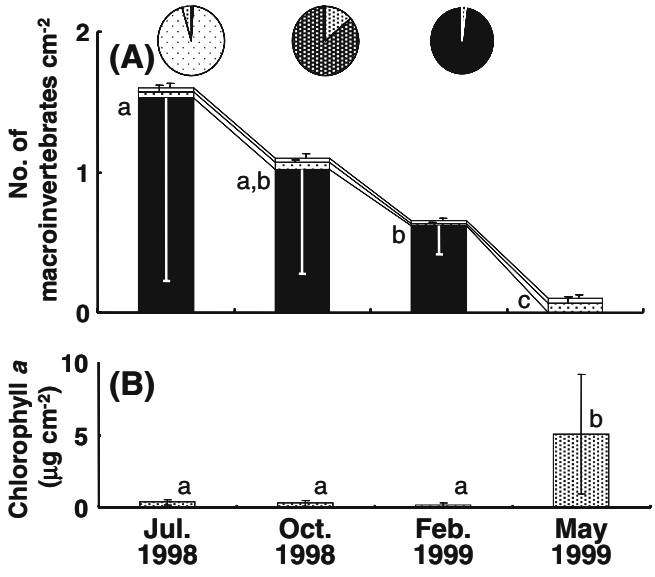

Fig. 2. Seasonal changes of (**A**) macroinvertebrates and (**B**) periphyton abundance at site 1. In **A**, the density of the *Micrasema quadriloba* larvae, that of the other grazer taxa, and that of the other taxa are shown in *closed*, *dotted*, and *open bars*, respectively. *Circle graphs* show the relative proportions of each *M. quadriloba* instar stage: first, *open*; second, *light shading*; third, *medium shading*; fourth, *dark shading*; fifth, *black*. Error bar = S.D. *Same lowercase letters* indicate no significant differences at $P = 0.05$ (Scheffe's test)

content in July, October, and February was significantly lower than that in May (Fig. 2B; one-way ANOVA: $F_{3,70}$ = 26.51, $P < 0.0001$). Multiple regression analysis ($n = 74$, $R^2 = 0.62$, $P < 0.001$ for full model) showed that periphyton abundance was significantly related to the larval biomass and current velocity (regression coefficient: –0.27 and –0.22, $P_r < 0.001$ and 0.01, respectively), but was not significantly related to water depth, water temperature, and light intensity (–0.05, –0.20, and 0.14, $P_r > 0.05$, respectively).

At the segment scale, the densities of *M. quadriloba* larvae tended to decrease from site 1 to site 4 throughout the seasons (Fig. 3). During the study seasons, the relative proportions of each *M. quadriloba* instar stage were nearly equal to those in the riffle of site 1 (see Fig. 2), except on May 31, when the first instars were hatching. Because first instars of the next generation were observed only at site 1 on May 31 (Fig. 3D), the distribution of the larvae might gradually expand downstream over the course of their development (Fig. 3A–C). In contrast to larval distribution, periphyton chlorophyll *a* content tended to increase from site 1 to site 4 (Fig. 3). Multiple regression analysis ($n = 16$, $R^2 = 0.73$, $P < 0.05$ for full model) showed that periphyton abundance was significantly related to larval biomass and water depth (regression coefficient: –0.30 and 1.76, $P_r < 0.01$ and 0.05, respectively), but was not significantly related to current velocity, water temperature, and light intensity (–0.80, 0.20, and –0.49, $P_r > 0.05$, respectively).

As the community components of periphyton, we obtained 29 identified algal taxa (Table 2). A significant relationship between the total area of the taxa (μm^2) and chlorophyll *a* concentration was observed ($R = 0.94$, $P < 0.0001$); thus, the area of periphyton can be considered as the relative abundance of periphyton. The abundance of each algal group by total chlorophyll *a* content showed different tendencies among sites and sampling seasons (see Fig. 3). The relative proportions of group 2 (e.g., dominant taxa: *Synedra inaequalis* and *Cymbella minuta* v. *silesiaca*) and group 3 (e.g., *Phormidium* sp. and *Homeothrix varians*) increased downstream, whereas group 1 (e.g., *Cocconeis placentula* and *Achnanthes lanceolata*) decreased. No members of group 3 were collected at site 1, except on May 31. The biomass of *M. quadriloba* larvae was significantly positively correlated with the area of group 1 but was negatively correlated with the area of group 3 ($R = 0.60$ and 0.74, $P < 0.05$ and 0.001, respectively; Fig. 4). On the other hand, no significant relationship was found between the biomass and the area of group 2.

We used nMDS ordinations and ANOSIMs to examine community-level differences among seasons and sites (Fig. 5). The periphyton assemblages among sites were not

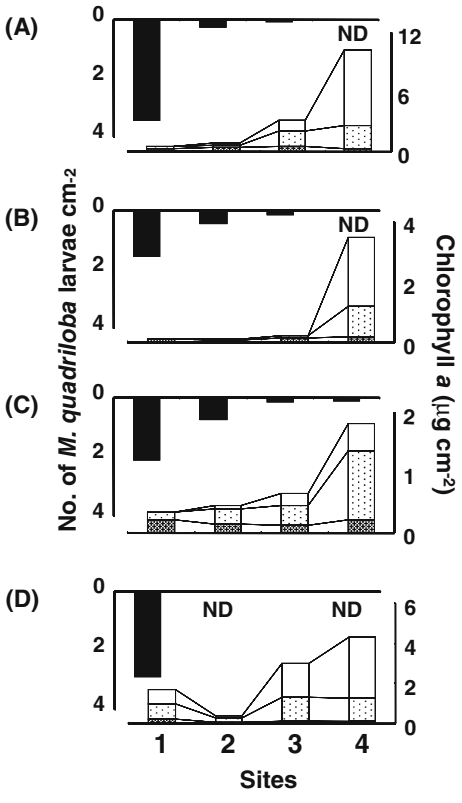

Fig. 3. Seasonal changes in longitudinal distribution of *Micrasema quadriloba* larvae and periphyton abundance in July 1998 (**A**), October 1998 (**B**), January 1999 (**C**), and May 1999 (**D**). *Closed bars* (*top*) show larval density. *Dark shaded*, *light shaded*, and *open parts of bars* show the proportions of area occupied by algal groups 1, 2, and 3, respectively, within the total periphyton abundance (estimated using the micrographs). The total height of the lower bars shows the chlorophyll *a* content of the periphyton. *ND*, *M. quadriloba* larvae were not observed

Table 2. Grouping of each algal taxon observed in the present study, according to their life forms and vertical locations in the periphyton mat

Class	Group	Species
Diatom	1	*Achnanthes lanceolata, Amphora inariensis, Cocconeis placentula*
	2	*Achnanthes minutissima, Ach. japonica, Ach. convergens, Synedra inaequalis, Fragilaria vaucheriae, Cymbella minuta v. silesiaca, Cym. sinuata, Cym. turgidula, Gomphonema clevei, Gom. vibrio* v. *pumilum, Gom. quadripunctatum, Navicula capitatoradiata, Nav. cryptotenella, Nav. oppugnata, Nav. viridula, Nav. decussis, Navicula* spp., *Nitzchia frustulum, Nit. linearis, Nit. palea, Nit. dissipata*
	3	*Synedra ulna*
Blue-green, Green	3	*Phormidium* sp., *Homoeothrix varians*, *Stigeoclonium* sp.

Group 1 were small diatoms located in the lowest layer of the mat and were adhered strongly to the substrate; group 2 were diatoms of various life form types, such as rosette, motile, stalk-forming, and tube-forming, which were located in the middle layer of the mat; group 3 were large and/or filamentous taxa, including diatoms, green algae, and blue-green algae, which appeared in the uppermost layer of the mat
Source: Katano et al. 2002; cf. Steinman 1996

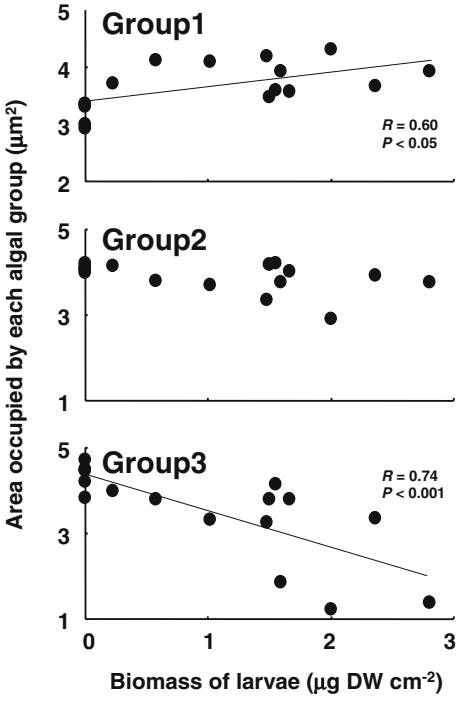

Fig. 4. Results of correlation analyses between the biomass of *M. quadriloba* larvae and each periphyton group at the segment scale. All data were normalized by $\log_{(x+1)}$ transformations. If the regression is significant, a regression line was drawn in the figure

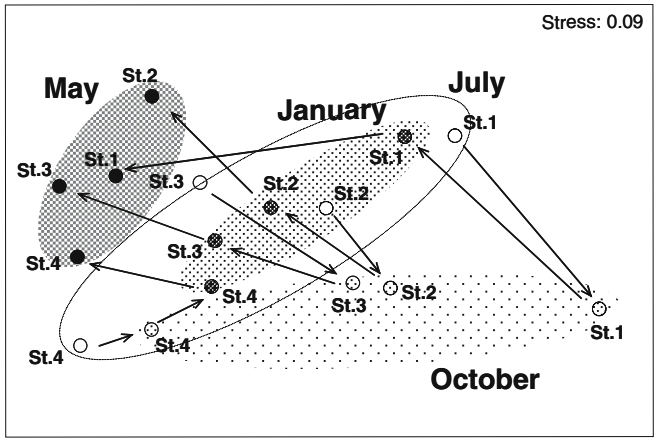

Fig. 5. Nonmetric multidimensional scaling (nMDS) ordination among periphyton assemblages at the segment scale. Site circles and shaded areas: *clear*, July 1998; *light shading*, October 1998; *medium shading*, January 1999; *dark shading*, May 1999. *Arrows* indicate seasonal change within the same site

significantly different (ANOSIM, global $R = 0.171$, $P = 0.09$), whereas those among seasons were significantly different (ANOSIM, global $R = 0.411$, $P = 0.003$). According to pairwise comparison tests, the assemblages were significantly different between the October and May samples ($R = 0.688$, $P = 0.029$) and between the January and May samples ($R = 0.833$, $P = 0.029$). SIMPER showed that increases in *C. minuta* v. *silesiaca* (group 2) and *H. varians* (group 3) abundance and a decrease in *A. lanceolata* (group 1; these taxa are the three largest contributors to dissimilarity) abundance accounted for 24.2% of the observed dissimilarity between October and May. SIMPER also showed that increases in *Synedra ulna* and *H. varians* (group 3) abundance and a decrease in *A. lanceolata* (group 1) abundance accounted for 21.5% of the observed dissimilarity between January and May (Fig. 5).

Discussion

At the riffle of site 1, periphyton abundance was kept at significantly low levels when *M. quadriloba* larvae occurred, regardless of instar stage and larval density. Similarly, the longitudinal trends in periphyton abundance and the density of the larvae were opposite at the segment scale (7.4 km in this study). That is, periphyton abundance tended to be higher at downstream sites than at upstream sites; larval density tended to be higher at upstream sites than at downstream sites throughout the study seasons, although the distribution of the larvae extended from upstream to downstream during their life cycle (first to fifth instars). Also, significant negative relationships between larval biomass and periphyton abundance were observed at both riffle and segment scales. Thus, the present study would indicate that the grazing of *M. quadriloba* larvae regulated periphyton abundance in the field, not only at the riffle but also at the segment scale, which is larger spatial scale than previous studies (e.g., in a reach; Taylor et al. 2002). In other environmental factors, current velocity and water depth were each also significantly correlated to periphyton abundance at the riffle and segment scale, respectively, probably because high or low water turbulence and the resultant disturbance affected periphyton abundance (Allan 1995). Also, light intensity and water temperature were not significant factors in determining periphyton abundance at either riffle or segment scale. This observation indicates seasonal changes such as light and temperature would not strongly affect periphyton abundance. Although the nutrients in the stream water were also important factors in periphyton abundance (Pringle 1987), an experiment by Tank and Dodds (2003) showed that periphyton abundance was not limited by nutrients in streams with moderate nutrient concentrations [nitrate, 2.3–59; soluble reactive phosphorus (SRP), 2.9–13 $\mu mol\,l^{-1}$]. At these study sites, nitrate and SRP were 28.8–51.9 and 1.4–3.4 $\mu mol\,l^{-1}$, respectively (in August; I. Katano, unpublished data), and thus nutrients may be not limiting to periphyton abundance in the study sites according to Tank and Dodds (2003).

Grazing pressure of *M. quadriloba* larvae significantly decreased the relative abundance of group 3 taxa (large and filamentous algae) and significantly increased that of group 1 taxa (small diatoms adhered strongly to substrate) in the periphyton mat. The results of SIMPER analyses also followed the results of the aforementioned correlation analyses. In brief, the periphyton assemblage was significantly different between the periods when the larvae were in late instar (October and January) and when the larvae were not

present (May); and the community structure changed with the decrease of group 1 and increases of groups 2 and 3 from October and January to May. These findings imply that the periphyton community structure was regulated by late instars more strongly than by early instars in July. Previous microhabitat-scale studies have shown that various caddisfly grazers facilitate growth of the group 1 algal taxa and that they remove the group 3 algal taxa from the periphyton mat (Hart 1985a,b; Jacoby 1987; Katano et al. 2002). Thus, the present study showed that not only at the microhabitat scale, but also at the segment scale, the tiny grazing larvae were able to alter the vertical structure of the periphyton mat as their distribution expanded downstream over time and as their development proceeded.

On the basis of nMDS ordination, the seasonal trends of periphyton assemblages were different between sites 1–3 and site 4 throughout the study period. In brief, the relative patterns of arrows in Fig. 5 illustrate the seasonal changes of the periphyton assemblage within each site; the pattern in sites 1, 2, and 3 goes from the lower right (July to October), to the upper left (October to January), and to the left (January to May), whereas this pattern was not observed at site 4, where *M. quadriloba* larvae did not occur in July, October, and May. This result might indicate that these seasonal trends were explained not only by the seasonal changes of environmental factors, although the *P* value of the ANOSIM among the study sites was only marginally significant. Thus, the nMDS results also suggested that the periphyton community structure is altered not only by the seasonal physio-ecological changes in algal species but also by the grazing of *M. quadriloba* larvae.

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