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Differential vulnerability of benthic algae to grazing by the loach, *Niwaella delicata* (Niwa)

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Abstract

Algal vulnerability to the grazing loach, *Niwaella delicata*, was investigated in the Ogawa stream, a tributary of the Kiso River flowing through Nagano Prefecture, Japan. To evaluate the vulnerability, Chesson's electivity index was calculated using the relative abundance of ten dominant algal taxa in the gut contents of the loach and benthic algal assemblage. The index values differed significantly among the algal taxa and the index values of diatoms increased with their size. The results showed that vulnerability to the loach differed among algal taxa. Prostrate filamentous cyanobacteria and the large diatom, *Ulnaria ulna*, were susceptible to loach grazing, while upright filamentous cyanobacteria and small diatoms such as *Achnanthidium convergens*, *Fragilaria perminuta*, *Gomphonema parvulum*, *Cymbella turgidula* var. *nipponica* and *Encyonema* sp. were less susceptible to loach grazing.

Key index words : diatoms, filamentous cyanobacteria, gut contents, loach, *Niwaella delicata*, vulnerability

Introduction

Benthic algae in streams and rivers provide a large quantity of organic matter for many aquatic consumers as they cover almost all submerged surfaces and multiply at high turnover rates. However the whole benthic algal mass is not always available as food because it consists of algal taxa with different vulnerability to grazing ; some algae are susceptible to grazing, but others are resistant to grazing (Hill & Knight 1988, Power *et al.* 1988, Abe *et al.* 2006). Furthermore, the ability to dislodge benthic algae from the substrate differs among the consumers with different feeding apparatus and behavior (Lamberti *et al.* 1987, Hill & Knight 1988, Karouna & Fuller 1992). Therefore, the overall availability of the benthic algal assemblage as food will differ among the consumers. To estimate the actual food availability, understanding the vulnerability of benthic algae to the consumers is a matter of great importance.

The grazing loach *Niwaella delicata* (Niwa) is a freshwater fish endemic to Japanese mountain streams and in danger of extinction due to habitat deterioration (Goto & Goto 1971, Kobayashi *et al.* 2004). The loach has no teeth in its mouth, but has sucker-like semicircular lips to feed on benthic algae and sticking to substrates in the rapids. Benthic algae are a significant food resource for the loach, although the algal vulnerability to the loach has not been investigated. This study investigated algal vulnerability

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to *N. delicata* by comparing the algal composition between the gut contents of loach samples and the benthic algal assemblage in their habitat. Benthic algae were classified into susceptible or less susceptible taxa when the loach ingested disproportionately larger or lower quantities, respectively, than that expected from their abundance.

Materials and Methods

Study site

This study was carried out in August 2006 in the Ogawa stream which is a third-order tributary of the Kiso River, Nagano, Japan. All samples were collected in a riffle (35°46′N; 137°39′E) located 800 m up from the confluence with the Kiso River. The riffle has an area of 396 m^2 and consists mainly of boulder and cobble substrata. Benthic algal biomass on the substrates was 17.0±6.9 chlorophyll *a* mg m^{-2} (mean±standard deviation, $n=10$). Average stream width and depth are 14 m and 43 cm, respectively. Discharge was 0.27 m^3 s^{-1} and water temperature reached 22℃ in the daytime. Stream water was clear and total dissolved nitrogen and phosphorus concentrations were 0.205 mg L^{-1} and 0.004 mg L^{-1}, respectively. The canopy of riparian vegetation did not overhang the stream and therefore full sunlight reached to the streambed. At the upper end of the study site a weir was constructed and prevented the upstream movement of fish. The density of the grazing loaches was estimated at 0.31±0.01 individuals m^{-2} (mean±standard error) from three fish capturing surveys conducted in August 2006 (80, 18 and 18 individuals at the first, second and third sampling, respectively) using the population estimation program with a variable probability removal estimator (Pollock & Otto 1983, http://www.mbr-pwrc.usgs.gov/software/capture.html). Besides the loach, *Tribolodon hakonensis* (Günther), *Oncorhynchus masou ishikawae* Jordan & McGregor, *Salvelinus leucomaenis japonicus* (Oshima), *Rhinogobius flumineus* (Mizuno) and *Liobagrus reini* (Hilgendorf) were observed in the riffle.

Sampling procedure

The loaches were collected using a back-pack electrofishing unit (Model 12B, Smith-Root Inc., Vancouver, WA, USA) adjusted at DC 200-400V. Twenty five loaches were collected and preserved with 10% formalin following anesthetization using 2-phenoxyethanol to ensure ethical use of fish (Huntingford *et al.* 2006). Standard length and body weight of the loach samples were 82±21 mm (mean±standard deviation) and 3.5±2.0 g, respectively. Samples of the benthic algal assemblage were collected from 10 boulders. Benthic algae was removed from a quadrat (5×5 cm) placed on a boulder using a nylon brush and preserved immediately with 5% formalin for the analysis of algal composition. In the laboratory the entire gut was removed from each loach. Then the gut contents were extracted from the front part of the gut (one-fourth of a gut) to minimize any bias due to differential digestibility of some algal taxa and preserved in 5% formalin to examine the algal composition in the diet.

Analysis of benthic algal composition

The densities of algal taxa in the samples of the gut contents and assemblages were calculated by counting using a Palmer cell at ×400 under a light microscope. The trichomes of filamentous cyanobacteria and cells of green algae, diatoms and unicellular cyanobacteria were counted as individual units. Cyanobacteria and green algae were identified at this step. In the gut content samples many empty mucilaginous sheaths of filamentous cyanobacteria were observed due to digestion. Therefore, the empty mucilaginous sheaths were also counted and classified on the basis of their thickness. Diatoms were identified at ×1000 with a light microscope (LM) until counting up to 400 frustules after cleaning in a domestic drainpipe cleaner (Pipe-Unish, Uni-Charm Inc.) according to the method of Nagumo (1995). Biomass of each algal taxon was expressed as the biovolume calculated by multiplying the algal density of each taxon by the estimated volume per cell or trichome. The volume was estimated using average dimensions (length, breadth, diameter and thickness) of cells or trichomes by applying an algal cell to appropriate geometric form such as a sphere and pillar with the base of a drop-form, semicircle, oval, and so on. Thickness was measured in the center of cells. At least 10 individuals of each taxon were measured to the nearest

0.1 μm under LM to determine the average dimensions of cells or trichomes. Relative abundance of each taxon was expressed as the percentage of its biovolume to the total algal biovolume.

Data analysis

Vulnerability of algal taxa to loach grazing was estimated using Chesson's α which is an electivity index measuring the utilization of food items in relation to their abundance in the environment. Chesson's α of each algal taxon was calculated in each individual of loach using the following equation (Chesson 1978).

$$\alpha_i = \frac{r_i / p_i}{\sum_i r_i / p_i}$$

Where r_i is the relative abundance of algal taxa i in the gut content of a loach individual and p_i is the mean relative abundance of algal taxa i in the ten samples of the benthic algal assemblage. Values of the index range from 0 to 1. The expected value for random feeding is the reciprocal of the number of algal taxa (a). The index values above and below $1/a$ indicate the ingestion of disproportionately larger and lower

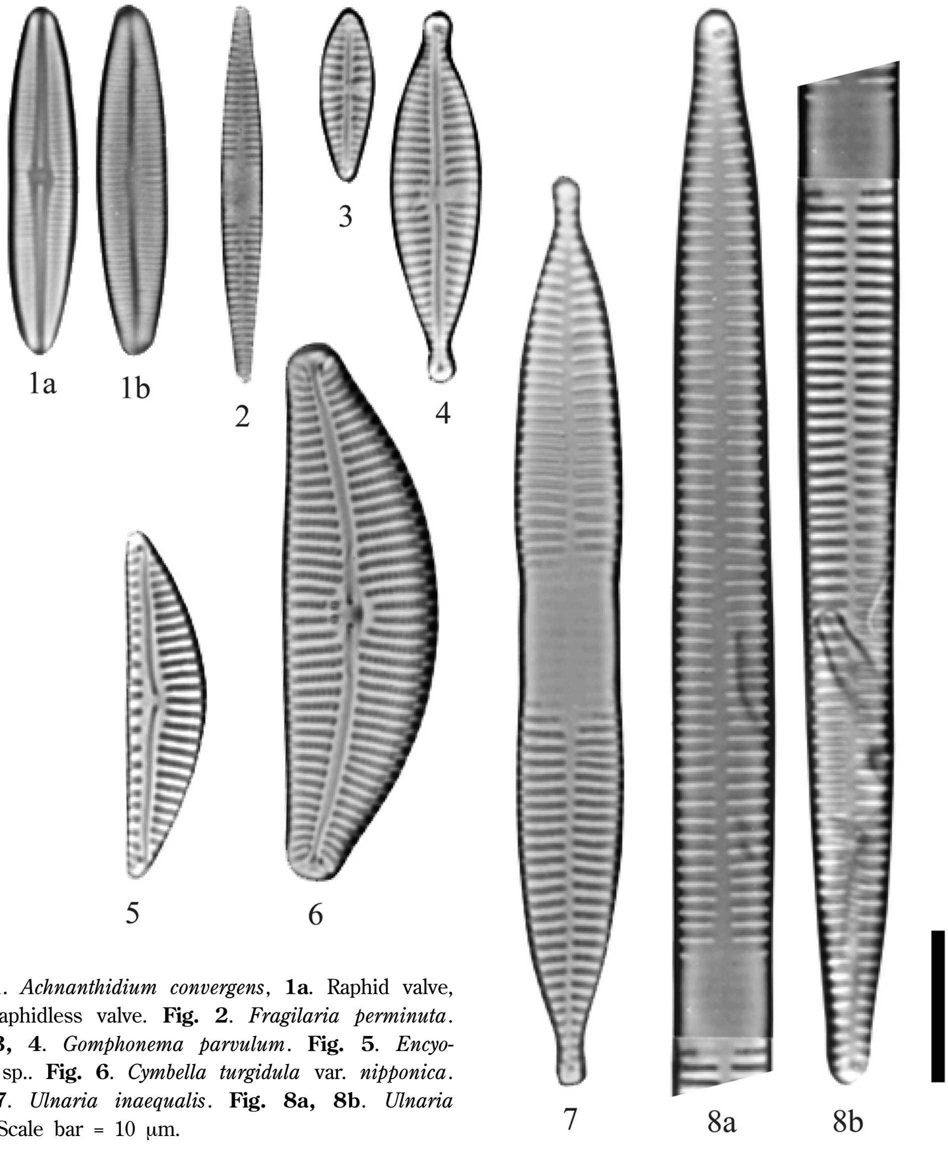

Fig. 1. *Achnanthidium convergens*, **1a**. Raphid valve, **1b**. Raphidless valve. **Fig. 2**. *Fragilaria perminuta*. **Fig. 3, 4**. *Gomphonema parvulum*. **Fig. 5**. *Encyonema* sp.. **Fig. 6**. *Cymbella turgidula* var. *nipponica*. **Fig. 7**. *Ulnaria inaequalis*. **Fig. 8a, 8b**. *Ulnaria ulna*. Scale bar = 10 μm.

quantity, respectively, than that expected from their abundance in the assemblages.

Difference in Chesson's α among algal taxa was examined by a repeated measures analysis of variance with setting up the algal taxa as a repeated measures factor ($n = 25$). Mauchly's test was conducted to examine the sphericity which relates to the equality of the variances of the differences between levels of the repeated measures factor. When the significance level of the Mauchly's sphericity test is ≤0.05, Greenhouse-Geisser adjustment was applied. Vulnerability of each algal taxon was estimated using a 95% confidence interval of the index on the base of $1/a$. Effect of biovolume on the index values of dominant diatom taxa was examined using Page's trend test.

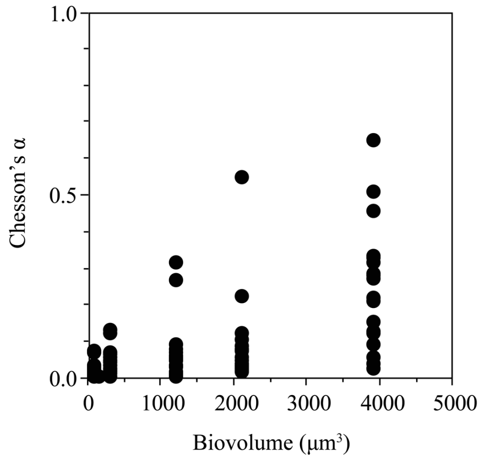

Fig. 10. Relationship between Chesson's α and biovolume of seven dominant diatom taxa.

Results

The benthic algal assemblage was mainly composed of diatoms (15 – 65% in relative abundance) and the upright filamentous cyanobacterium, *Homoeothrix janthina* (Bornet et Flahault) Starmach (23 – 81%), but rarely included the prostrate filamentous cyanobacterium, *Phormidium* sp. (0 – 7%). On the other hand, the loach guts contained mainly diatoms (9 – 93%) and the prostrate filamentous cyanobacterium (7 – 77%) but rarely the upright filamentous cyanobacterium (0 – 12%). Seven diatoms, *Achnanthidium convergens* (H.Kobayasi) H.Kobayasi (Fig. 1a, b), *Fragilaria perminuta* (Grunow) Lange-Bert. (Fig. 2), *Gomphonema parvulum* (Kütz.) Kütz. (Fig. 3, 4), *Cymbella turgidula* var. *nipponica* Skvortsov (Fig. 5), *Encyonema* sp. (Fig. 6), *Ulnaria inaequalis* (H.Kobayasi) M.Idei (Fig. 7) and *Ulnaria ulna* (Nitzsch) Compère (Fig. 8a, b), were predominated (relative abundance >1%) in the gut contents and assemblage. Insect larva and ciliate were observed in one individual loach.

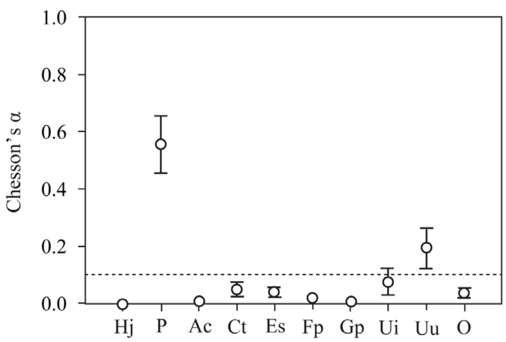

Fig. 9. Chesson's α of *Homoeothrix janthina* (Hj), *Phormidium* sp. (P), *Achnanthidium convergens* (Ac), *Cymbella turgidula* var. *nipponica* (Ct), *Encyonema* sp. (Es), *Fragilaria perminuta* (Fp), *Gomphonema parvulum* (Gp), *Ulnaria inaequalis* (Ui), *U. ulna* (Uu) and others (O). Circles indicate the mean values of the index. The upper and lower ends of vertical bars indicate the upper and lower values of 95% confidence intervals, respectively. A dotted line is drawn at the expected value (0.1) for random feeding.

Chesson's α was calculated for the ten dominant algal taxa (the upright and prostrate filamentous cyanobacterium, seven dominant diatoms and others, Fig. 9). Results of a repeated measures analysis of variance indicated the significant difference in the index values among the algal taxa ($F_{1.658,\ 39.782} = 54.353$, $p < 0.001$). Of these algal taxa, the prostrate filamentous cyanobacterium and *U. ulna* were more ingested than expected from their abundance in the assemblage (95% confidence interval of the index was above 0.1), while the upright filamentous cyanobacterium, *A. convergens*, *F. perminuta*, *G. parvulum*, *C. turgidula* var. *nipponica* and *E.* sp. were less ingested (95% confidence interval was below 0.1). *U. inaequalis* was selected almost at random (the 95% confidence interval included 0.1). Furthermore, the index values of the seven dominant diatoms increased with their biovolume ($z = 2.414$, $p < 0.01$, Fig. 10).

Discussion

The results showed that prostrate filamentous cyanobacterium and *U. ulna* were positively selected, whereas upright filamentous cyanobacterium, *A. convergens*, *F. perminuta*, *G. parvulum*, *C. turgidula* var. *nipponica* and *E.* sp. were negatively selected. When resources are used disproportionately to their abundance, the usage is considered as selective (Johnson 1980). In this case, however, active selection is not plausible as it is unlikely that large-sized consumers like the loach can actively select a particular microscopic alga from the mixed assemblage (Lowe & Hunter 1988). It is more reasonable that differential consumption would result from the difference in vulnerability to grazing by the loach among algal taxa. Hence, prostrate filamentous cyanobacteria and large diatoms are susceptible to loach grazing, while upright filamentous cyanobacteria and small diatoms are more difficult to remove from the substrate by the loach.

Differential vulnerability will result from differences in size and attachment manner among algal taxa. In general, large, loose or overstory component of benthic algal assemblages are more susceptible to grazing (Hill & Knight 1987, Wellnitz & Ward 2000), while small and tightly attached algae are more resistant to grazing (Abe *et al.* 2001). In the present study prostrate filamentous cyanobacteria were more susceptible, but upright filamentous cyanobacteria were less susceptible to loach grazing. This difference would reflect in the difference in attachment manner. The susceptible prostrate filamentous cyanobacteria form cohesive laminar mat in the overstory of benthic algal assemblage (Stuck & Ward 1991), but resistant upright filamentous cyanobacteria are firmly attached to the substrate with their basal cells (Abe *et al.* 2001). Furthermore, among the diatoms, large diatoms were more susceptible to loach grazing compared with small diatoms. Large diatoms may reach into overstory of the assemblage and then be more easily dislodged from the substrate and/or more efficiently taken by consumers.

Intensive grazing on susceptible algae by the loach probably enhanced the dominance of upright filamentous cyanobacteria, which were difficult to ingest. Difficulty to ingest upright filamentous cyanobacteria is a general outcome of grazing fishes despite the different mouthpart morphologies and feeding behaviors (Power *et al.* 1988, Abe *et al.* 2006). Consequently, the upright filamentous cyanobacteria often predominated under fish grazing conditions (Power *et al.* 1988, Gelwick *et al.* 1997, Pringle & Hamazaki 1997, Abe *et al.* 2006).

Availability of prey items to consumers is often estimated from the prey abundance (e.g. Angermeier & Karr 1983, Feminella *et al.* 1989, Abe *et al.* 2003). However this simple assumption is not accurate because the availability is a composite variable determined by the vulnerability of prey items as well as the prey abundance (Gawlik 2002). The present study indicated that the benthic algal assemblage was composed of algal taxa with different vulnerabilities to loach grazing. Resistant algae such as the upright filamentous cyanobacterium accounted for the majority of the benthic algal assemblage in the Ogawa stream. Hence, this study suggested that estimating food availability from the algal abundance will result in a severe overestimation of the actual availability to grazing loaches.

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References

Abe, S., Iguchi, K., Ito, S., Uchida, Y., Ohnishi, H. & Ohmori, K. 2003. Habitat use of the grazing goby (*Sicyopterus japonicus*) in response to spatial heterogeneity in riparian shade. Journal of Freshwater Ecology **18**: 161-167.

Abe, S., Kiso, K., Katano, O., Yamamoto, S., Nagumo, T. & Tanaka, J. 2006. Impacts of differential consumption by the grazing fish, *Plecoglossus altivelis*, on the benthic algal composition in the Chikuma River, Japan. Phycological Research **54**: 94-98.

Abe, S., Uchida, K., Nagumo, T., Ioriya, T. & Tanaka, J. 2001. Effects of a grazing fish, *Plecoglossus altivelis* (Osmeridae), on the taxonomic composition of freshwater benthic algal assemblages. Archiv für Hydrobiologie **150**: 581-595.

Angermeier, P.L. & Karr, J.R. 1983. Fish communities along environmental gradients in a system of tropical streams. Environmental Biology of Fishes **9**: 117-135.

Chesson, J. 1978. Measureing preference in selective predation. Ecology **59**: 211-215.

Feminella, J.W., Power, M.E. & Resh, V.H. 1989. Periphyton responses to invertebrate grazing and riparian canopy in three northern California coastal streams. Freshwater Biology **22** : 445-457.

Gawlik, D.E. 2002. The effects of prey availability on the numerical response of wading birds. Ecological Monographs **72** : 329-346.

Gelwick, F.P., Stock, M.S. & Matthews, W.J. 1997. Effects of fish, water depth, and predation risk on patch dynamics in a north-temperate river ecosystem. Oikos **80** : 382-398.

Goto, M. & Goto, T. 1971. Fish fauna in River Nagaragawa and its change for the last several years in relation to river pollution. Japanese Journal of Ecology **21** : 254-264. (in Japanese with English summary)

Hill, W.R. & Knight, A.W. 1987. Experimental analysis of the grazing interaction between a mayfly and stream algae. Ecology **68** : 1955-1965.

Hill, W.R. & Knight, A.W. 1988. Concurrent grazing effects of two stream insects on periphyton. Limnology and Oceanography **33** : 15-26.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøre, P & Turnbull, J.F. 2006. Current issues in fish welfare. Journal of Fish Biology **68** : 332-372.

Johnson, D.H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology **61** : 65-71.

Karouna, N.K. & Fuller, R.L. 1992. Influence of four grazers on periphyton communities associated with clay tiles and leaves. Hydrobiologia **245** : 53-64.

Kobayashi, S., Kitano, S., Yamagata, T. & Uehara, T. 2004. Distribution of *Niwaella delicata* in the upper Kiso River drainage, Nagano Prefecture. Bulletin of Nagano Nature Conservation Research Institute **7** : 23-28. (in Japanese with English summary)

Lamberti, G.A., Ashkenas, L.R., Gregory, S.V. & Steinman A.D. 1987. Effects of three herbivores on periphyton communities in laboratory streams. Journal of the North American Benthological Society **6** : 92-104.

Lowe, R.L. & Hunter, R.D. 1988. Effects of grazing by *Physa integra* on periphyton community structure. Journal of the North American Benthological society **7** : 29-36.

Nagumo, T. 1995. Simple and safe cleaning methods for diatom samples. Diatom **10** : 88. (in Japanese)

Pollock, K.H. & Otto, M.C. 1983. Robust estimation of population size in closed animal populations from capture-recapture experiments. Biometrics **39** : 1035-1049.

Power, M.E., Stewart, A.J. & Matthews, W.J. 1988. Grazer control of algae in an Ozark mountain stream : effects of short-term exclusion. Ecology **69** : 1894-1898.

Pringle, C.M. & Hamazaki, T. 1997. Effects of fishes on algal response to storms in a tropical stream. Ecology **78** : 2432-2442.

Stuck, M.S. & Ward, A.K. 1991. Blue-green algal mats in a small stream. Journal of Phycology **27** : 692-698.

Wellnitz, T.A. & Ward, J.V. 2000. Herbivory and irradiance shape periphytic architecture in a Swiss alpine stream. Limnology and Oceanography **45** : 64-75.