

Usefulness of Chironomid Larvae as Physicochemical and Biological Indicators

~A case study of the mountainous streams in the Gono River System~

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Abstract: Relationships among environmental factors, ecological parameters of aquatic insect communities, and occurrences of chironomid species were examined in mountainous streams in the Chugoku Region. A significant negative relationship was observed between water quality and larval density of *Polypedilum tamasemusi*. A significant positive relationship was observed between taxonomic diversity and feeding style diversity of aquatic insect communities. Larval densities of *Polypedilum tamasemusi* and *Tvetenia tamaflava* also showed significant positive and negative relationships, respectively, with taxonomic diversity. Similarly, larval density of *Polypedilum takaoense* showed a significant negative relationship with lifestyle diversity. Furthermore, significant positive relationships were observed between the larval densities of *Neobrillia longistyla* and *Polypedilum tamasemusi*, and *Tvetenia tamaflava* and *Virgatanytarsus arduennensis*, suggesting that the same factor determines the larval occurrence.

These results suggest the possibility of some chironomid species as promising indicators of biological and physicochemical conditions in mountain streams.

Keywords: chironomid larvae, environment, indicator, river

I. Introduction

Freshwater benthos, including aquatic insects, have been widely used to assess water quality (Morishita, 1978). However, identification of all the organisms to species level is difficult. Chironomids are commonly found in aquatic insect communities, and there are as many as 2,000 species in Japan (Nihon yusurika kenkyukai, 2010). Chironomids can live in different types of waters, and factors that limit their occurrence differ depending on the species (Inoue et al., 2005). In our previous studies, some species that belong to 4 genera *Cricotopus*, *Polypedilum*, *Rheotanytarsus* and *Tanytarsus*, were proven to be reliable indicators of water quality when the occurrence of each species was examined in 3 electric conductivity ranges (Kawai et al., 1998). Further, a recent study showed some chironomid community parameters or occurrences of some taxa in relation to various environmental factors such as dissolved oxygen concentration, turbidity and electric conductivity (Odume and Muller, 2011). However, this study was not performed at the species level but at the genus level. Furthermore, the relationships between the occurrence of chironomid

species and various ecological parameters of aquatic insect communities are still unclear.

Community parameters such as species diversity and some biotic indices have been widely used as a useful biological indicator (Morishita, 1978). However, measurements of these parameters require a lot of labor and are expensive; thus simpler parameters are required.

In this study, we examined the relationships among environmental factors, ecological parameters, and occurrences of some chironomid species in aquatic insect communities in mountainous streams in the Chugoku Region, and we discussed the usefulness of chironomids as physicochemical and biological indicators.

II. Materials and Methods

1. Study sites

The study sites were limited to mountain streams in order to exclude some effects of the scale or topographic type of rivers on the aquatic insect communities. Thirteen sampling stations were selected at the sites of Aa to Aa-Bb type (mountain streams), according to the classification by Kani (1944), and they covered almost the entire basin

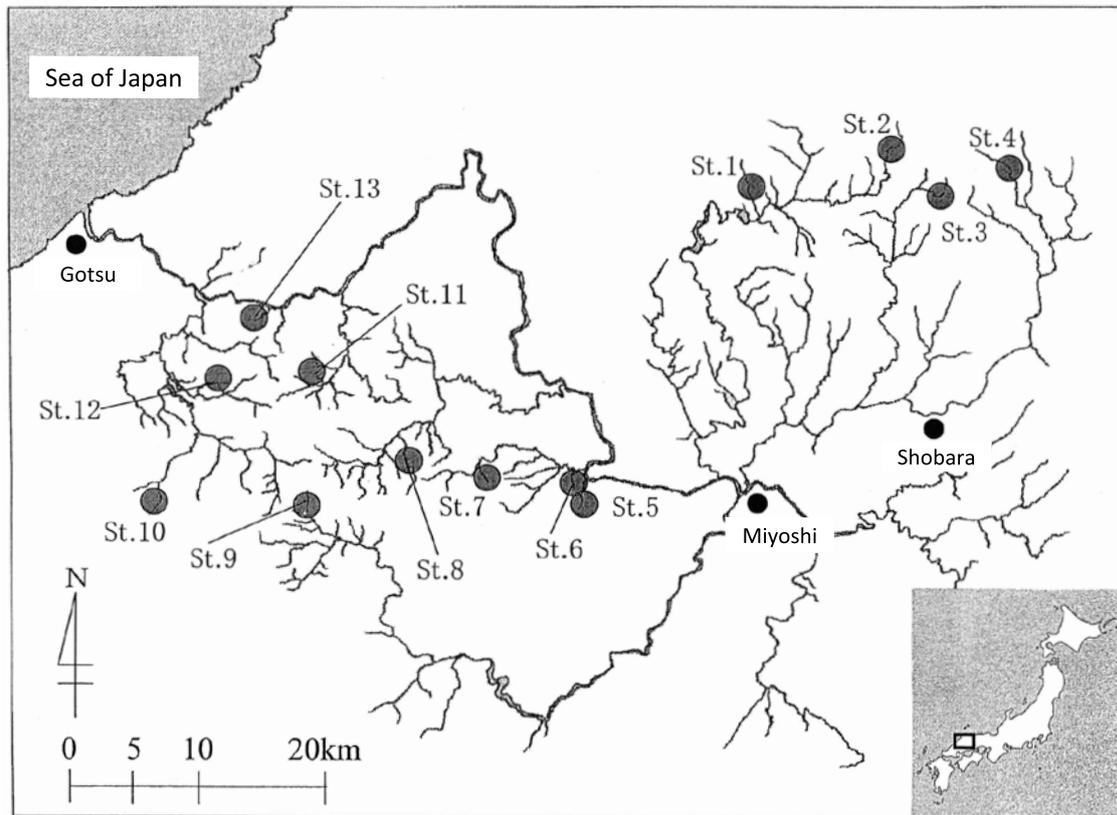


Fig. 1 Map of the Gono River Basin that shows 13 sampling stations.

of the Gono River System (Fig. 1). Gono River is the largest river in the Chugoku Region; it originates in Mt. Asa (1,218m) in the western Chugoku Mountain Chains and flows into the Sea of Japan. The catchment area is 3,870km³, and the main stream is 194km in length.

2. Sampling methods

Sampling was performed in May, July, September and November 2003, after considering the seasonal emergence of each species. Sampling was not performed in winter because of snow. Samples of the aquatic insect communities were collected using a quadrat of 30cm × 30cm in the middle of a rapid. Organisms attached to rocks or gravel, those dwelling in the sand, and those trapped using a frame net (0.25mm in diameter) set downstream to the quadrat were collected into a plastic bag and fixed with 5% formalin. Samples of the larval chironomid communities were similarly collected 4 times at rapids and put into a plastic bag. Because of the difficulty in identifying chironomid larvae accurately to the species level, sediment samples containing chironomid larvae, were put into a plastic container (φ15cm × H9cm) covered with a nylon net on the top and aerated using an air stone (φ1.5cm). The

emerging adult male chironomids were collected every other day for at least a month.

3. Identification

The aquatic insects were identified according to the classification of Kawai (1992). They were categorized on the basis of family as collector-filterers, collector-gatherers, scrapers, shredders and predators (feeding styles) and stickers, creepers, burrowers, swimmers and case-bearers (life styles), according to the classification of Tsuda (1962), Kawai (1992), Niina (1996), Merrit and Cummins (1996) and Fukawa and Inoue (1999). Adult male chironomids were identified mainly on the basis of the classification of Wiederholm (1989) and Sasa and Kikuchi (1995). Because it is difficult to identify chironomid larvae to the species level, larval density in a quadrat sample was represented as the number of adult males emerging from the sample. Taxonomic, feeding style and life style diversities of the aquatic insect communities were represented using the Shannon-Weaver index (H').

4. Measurement of environmental factors

Electric conductivity was measured as an indicator

of water quality. Electric conductivity as well as water temperature were measured using YSI Model 30/50 FT (YSI Co. Inc., Ohio, USA). Water velocity and depth was measured at the sampling sites. Velocity was measured with an electromagnetic velocity meter (VE10/VET-200-10; Kenek Co. Inc., Tokyo, Japan).

5. Statistical analysis

Significance of the correlation coefficients among various parameters was analysed using Student's *t*-test.

III. Results

1. Environmental data

Average electric conductivity, average water temperature, water temperature range, average depth, and average water velocity for each station are shown in Table 1. Average electric conductivity was in the range of 29.8-70.9 μ S/cm, and it was the lowest at Sta. 4 and the highest at Sta. 13. Water temperature range was 3.3-9.3°C, and the range was the narrowest at Sta. 3 and the widest at Sta. 6. Average depth was in the range of 14.5-

25.3cm, and average water velocity was in the range of 0.28-0.54m/s.

2. Data on the aquatic insect communities and adult male chironomids

A total of 23,916 aquatic insects belonging to 45 families and 7 orders were collected. A total of 681 adult male chironomids belonging to 63 species, 28 genera, and 3 subfamilies were collected from the sediment samples.

H' of average taxonomic, feeding style, and life style diversities in aquatic insect communities are shown in Table 2. Because of usually scarce numbers of emerging males from each seasonal sample, the total numbers of adult male chironomids collected from 4 seasonal sediment samples are shown. Average taxonomic diversity was in a rather narrow range of 2.67-3.37. Average life style diversity was also in a rather narrow range of 1.75-2.25. Average feeding style diversity was in the range of 1.56-2.27, and it was the highest at Sta. 1 and the lowest at Sta. 10. *Polypedilum tamahosohige* was the most

Table 1 Environmental data and some diversity data of aquatic insect communities.

Sta.	Average electric conductivity (μ S/cm)	Average water temperature (°C)	Water temperature range (°C)	Average depth (cm)	Average water velocity (m/s)	Average Taxonomic diversity	Average Lifestyle diversity	Average Feeding style diversity
Sta.1	32.5	14.0	4.6	23.9	0.54	3.33	2.13	2.27
Sta.2	43.6	13.9	5.3	25.3	0.45	3.14	1.78	1.97
Sta.3	38.0	14.3	3.3	21.9	0.42	2.81	1.93	1.92
Sta.4	29.8	12.5	4.7	20.8	0.35	3.19	1.86	2.17
Sta.5	53.6	17.7	9.2	18.2	0.33	2.77	2.09	1.92
Sta.6	50.7	15.2	9.3	21.9	0.30	2.88	1.87	1.98
Sta.7	48.5	14.8	7.7	18.1	0.31	2.96	1.91	1.90
Sta.8	54.3	13.5	6.7	19.4	0.30	3.26	2.25	1.99
Sta.9	50.2	13.7	7.5	20.9	0.37	2.90	2.08	2.11
Sta.10	57.2	13.7	7.6	20.5	0.36	2.67	1.75	1.56
Sta.11	70.9	14.8	7.8	14.5	0.39	2.80	2.20	2.11
Sta.12	54.7	13.7	7.4	17.9	0.28	3.37	2.18	2.00
Sta.13	70.8	15.7	7.7	17.9	0.28	3.08	1.90	2.06

Table 2 Occurrence data of chironomid species at 13 stations.

Sta.	No. adult males emerging from the sample										
	<i>Brillia japonica</i>	<i>Neobrylia longistyla</i>	<i>Tvetenia tamaflava</i>	<i>Polypedilum takaoense</i>	<i>P. tamahosohige</i>	<i>P. tamanigrum</i>	<i>P. tamasemusi</i>	<i>P. tsukubaense</i>	<i>P. unifascium</i>	<i>Virgatanytarsus arduennensis</i>	
Sta.1	9	13	0	1	18	2	13	1	3	0	
Sta.2	0	1	0	1	2	6	5	0	1	0	
Sta.3	7	1	4	3	6	10	2	4	0	1	
Sta.4	1	3	0	5	2	1	7	2	0	0	
Sta.5	0	0	4	0	1	2	0	0	12	0	
Sta.6	0	0	0	2	1	6	0	0	0	0	
Sta.7	6	2	2	4	5	7	3	13	7	0	
Sta.8	5	1	0	0	3	1	2	1	1	3	
Sta.9	0	1	2	0	2	2	1	2	0	9	
Sta.10	1	2	2	3	10	1	1	0	2	0	
Sta.11	3	1	7	0	0	2	0	10	6	10	
Sta.12	0	1	0	0	5	2	1	2	1	0	
Sta.13	4	3	0	6	23	0	1	1	0	0	

abundant, and *P. tamanigrum* and *P. tamahosohige* were found at almost all stations. In contrast, *Tvetenia tamaflava* was the rarest species, and *T. tamaflava* and *Virgatanytarsus arduennensis* occurred only at limited sites.

3. Relationships between environmental factors and ecological parameters or occurrence of chironomid species

There was a significant negative relationship between only average electric conductivity and larval density of *Polypedilum tamasemusi* in terms of 4 seasonal samples (Fig. 2). However, there were no significant relationships between other physicochemical factors and ecological parameters or larval density.

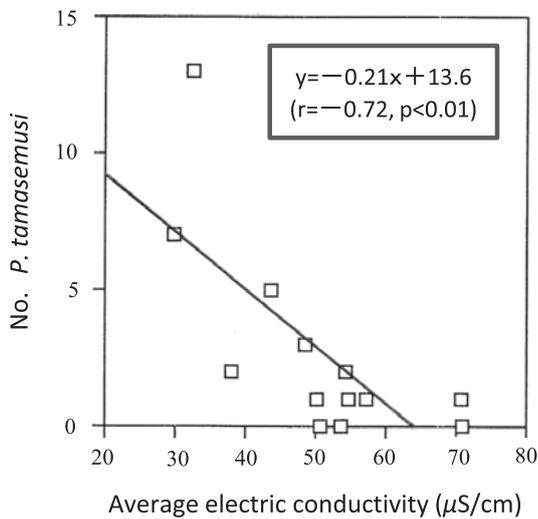


Fig. 2 Relationship between average electric conductivity and larval density of *P. tamasemusi*.

4. Relationships among ecological parameters

There was a significant positive relationship between taxonomic diversity and feeding style diversity of aquatic insects (Fig. 3). However, there were no significant relationships between taxonomic or feeding style diversity and lifestyle diversity.

5. Relationships between ecological parameters and species occurrences

Larval densities of *Tvetenia tamaflava* and *Polypedilum tamasemusi* showed significant negative and positive relationships with taxonomic diversity, respectively (Figs 4 & 5). Similarly, larval density of

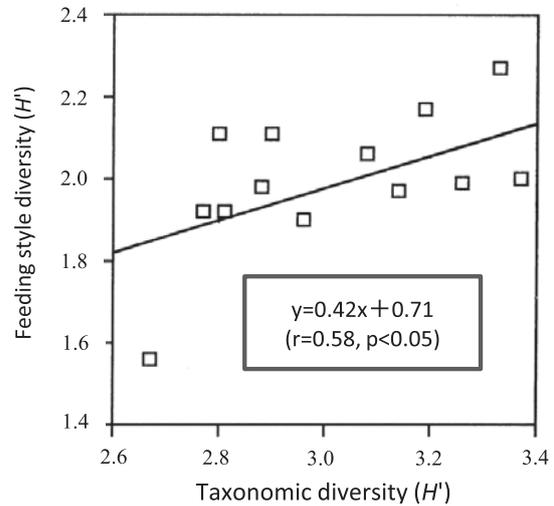


Fig. 3 Relationship between average taxonomic diversity (H') of the aquatic insect community and average feeding style diversity (H').

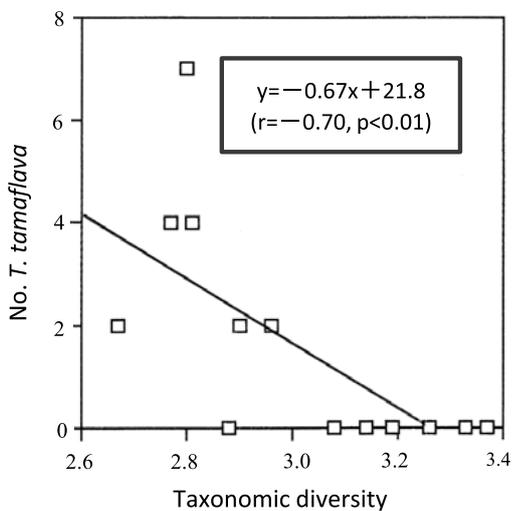


Fig. 4 Relationship between average taxonomic diversity and larval density of *T. tamaflava*.

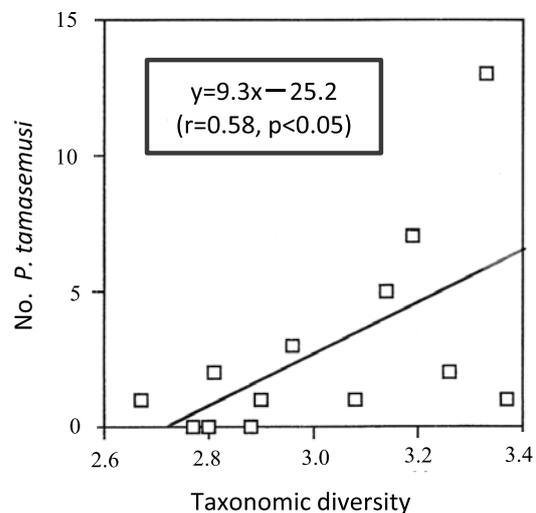


Fig. 5 Relationship between average taxonomic diversity and larval density of *P. tamasemusi*.

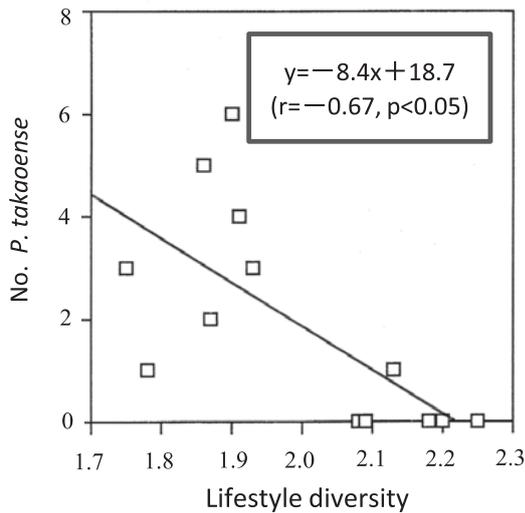


Fig. 6 Relationship between average lifestyle diversity and larval density of *P. takaense*.

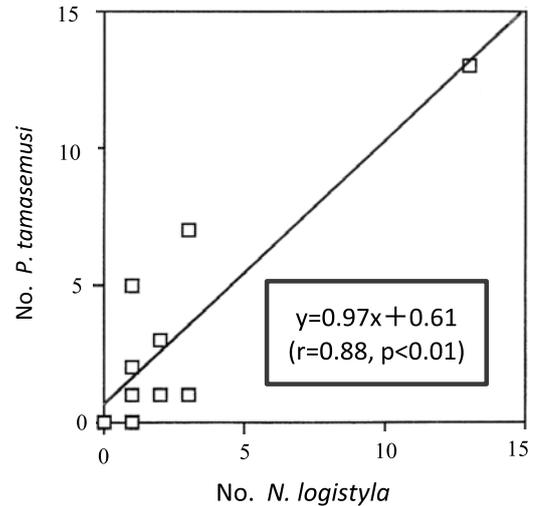


Fig. 7 Relationship between larval densities of *N. longistyla* and *P. tamasemusi*.

Polypedilum takaense showed a significant negative relationship with lifestyle diversity (Fig. 6).

6. Relationships between species occurrences

There was a significant positive relationship between the larval densities of *Neobrillia longistyla* and *Polypedilum tamasemusi* (Fig. 7) and *Tvetenia tamaflava* and *Virgatanytarsus arduennensis* (Fig. 8).

IV. Discussion

Because of their ubiquitous occurrence in different types of waters, including fresh, brackish, and marine waters, chironomids are considered to be the most promising indicators for various environmental conditions (Nihon yusurika kenkyuu-kai, 2010). However, difficulty in identifying larvae to the species level has hindered their practical utilization. In this study, however, some chironomid species were proven to be reliable indicators of physicochemical or biological factors of mountainous streams by easy identification of emerging male adults from sediment samples to the species level.

Differences in some environmental requirements among species of the genus *Polypedilum* have already been demonstrated in our previous study (Kawai et al., 1998; Kawai et al., 1999), suggesting the possibility of practically using the species composition in this genus as biological indicators. Among physicochemical factors, in this study, electric conductivity showed a significant relationship with the larval density of *Polypedilum*

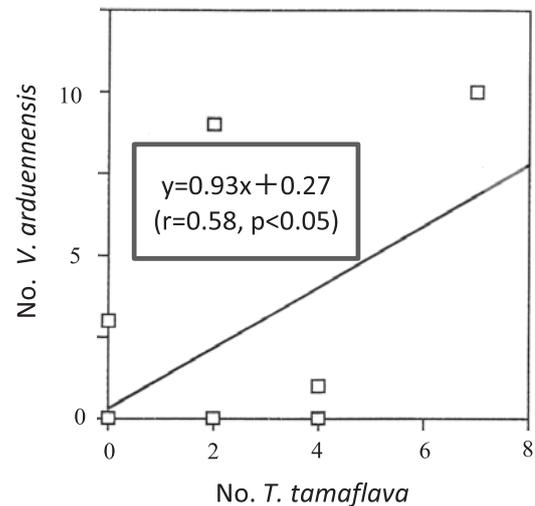


Fig. 8 Relationship between larval densities of *T. tamaflava* and *V. arduennensis*.

tamasemusi. This suggests the possibility of using the densities of some species as sensitive indicators.

In this study, however, only electric conductivity was used as a convenient indicator of water quality because other physicochemical indicators such as concentrations of $\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ have low sensitivity and are often “under the detectable limit” in mountainous rivers. However, electric conductivity can not necessarily represent the eutrophication level correctly in case of rivers that flow through granite and originally contain a high amount of minerals. Therefore, other more sensitive and reliable physicochemical parameters are required.

The relationship between taxonomic diversity and feeding style diversity was significantly positive. There were no significant relationships between taxonomic or

feeding style diversity and lifestyle diversity. This means that taxonomically related species are likely to have a similar food habit and that there is some convergence in lifestyle among the aquatic insect taxa.

What is the meaning of a significant positive relationship between the taxonomic diversity of the aquatic insect community and larval density of *Polypedilum tamasemusi*? A high taxonomic or lifestyle diversity could show the stability or resilience of the community or ecosystem to climatic or human disturbances through a fine mesh-like food web structure and an efficient material circulation (Wilson and Bossert, 1971). Therefore, this species can be an indicator of a stable community that is sound, not disturbed and has a high potential biological productivity.

On the other hand, what is the meaning of a significant negative relationship between the taxonomic diversity of aquatic insects and larval density of *Tvetenia tamaflava*, and lifestyle diversity and *Polypedilum takaoense* density? Because a community at the early stages of succession usually shows low taxonomic and lifestyle diversities (Begon *et al.*, 1999), these species could be dominant or transiently dominant only in an immature or disturbed community or a community that is being restored.

There was a highly significant positive relationship between the larval densities of *Neobrillia longistyla* and *Polypedilum tamasemusi*; this shows that both species could be sharing a microhabitat. Indeed, *Polypedilum tamasemusi* is a species of the subgenus *Uresipedilum* and the larvae of this subgenus are considered to usually dwell on the surface of rocks that are affected by direct strong water currents (Nihon yusurika kenkyu-kai, 2010). Similarly, there was a significant positive relationship between the larval densities of *Tvetenia tamaflava* and *Virgatanytarsus arduennensis*. Since the larvae of *V. arduennensis* are considered to use the spaces under rocks, the top parts of which are exposed to strong currents (Inoue *et al.*, 2005), the occurrence of these species could suggest natural riverbed structures composed of multiple layers of rocks at rapids.

In this study, larval densities of some chironomid species in the river bottom itself could be used as convenient, sensitive, and reliable indicators of the riverine environment. However, we could only show significant relationships between some environmental or ecological

parameters and occurrences of some chironomid species with a relatively high number of individuals emerging from the samples. This is partly attributable to larval death during transportation or the rearing process in the laboratory. Therefore, improvement in rearing conditions with respect to water quality, temperature, illumination and larval foods, especially diatoms, may lead to the use of additional species as reliable indicators of various conditions in mountainous streams.

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