UTILITY OF LARVAL REARING FOR ASSESSMENT OF LOTIC CHIRONOMID ASSEMBLAGES: A CASE STUDY IN A RIFFLE/POOL SECTION OF THE MIDDLE REACHES OF THE SHINANO RIVER, JAPAN

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With 3 figures and 2 tables

ABSTRACT: A laboratory rearing technique was used to estimate the species distribution of chironomid larvae on a riffle/pool section of a Japanese fifth-order river. Imagines of 31 species were obtained by rearing of quantitative benthic samples of immatures (larvae and pupae). Significant differences in species composition were associated with the riffle/pool gradient. However, emergence ratio *Em*/*Bn*, where *Em* is the number of imagines obtained by rearing and *Bn* is the number of immatures obtained by direct counts of benthic samples replicated at the same site, for the representative sites ranged from 0.3-1.9 % at the riffle and from 1.3-3.9 % at the pool, indicating that the in situ distribution and density of species would be underestimated.

RESUMO: Foi investigada a composição específica de larvas de quironemídeos num rio de ordem cinco. Os imagos emergiram por cultivo larvar tendo sido identificadas 31 espécies, a sua composição está significativamente associada ao gradiente riffle/pool. No entanto, o rácio de emergência variou entre 0,3-1,9% no riffle e 1,3-3,9% no poll, indicando que a distribuição das espécies in situ foi subestimada.

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INTRODUCTION

Chironomid communities play a major role in the processing of material in stream ecosystems, and chironomid species are a large component of the richness and biodiversity in streams and rivers (ARMITAGE *et al*., 1995). Therefore it is important to clarify community composition and species distribution pattern of chironomids to draft plans for conservation and restoration of river environment. In Japan, most studies of chironomid community composition have relied on rearing of larvae (e.g. INOUE *et al*., 2005), because samples often include many indistinguishable low-instar larvae, and especially because many Japanese species still remain to be described. However, significant larval mortality during rearing would be a severe problem in using this technique to estimate larval density and species distributions in situ.

Studies conducted in low-order streams have established that species distribution and assemblage composition of chironomids and other benthic macroinvertebrates, reflect a riffle/pool habitat gradient (e.g. LENCIONI & ROSSARO, 2005) (e.g. DAVY-BOWKER *et al*., 2006). In contrast, studies of middle and high order rivers which are deep and/or fast-flowing, are limited to measuring species distributions at the reach or larger spatial scale (e.g. INOUE *et al*., 2005).

The purposes of the present study were to examine the utility of the larval rearing technique for assessing the density and distribution of chironomids in a riffle/pool section of a fifth-order river. The emergence ratio during the rearing was estimated by direct counts of benthic larvae and pupae from several samples to evaluate any potential biases in the rearing technique for estimating larval densities in nature.

MATERIAL AND METHODS

The study was conducted on the middle reaches of the Shinano River (Fig. 1; 36º25’40" N, 138º11’19" E) which is the longest river in Japan that has 367 km of stretched main flow length. Larval sampling and measurement of environmental variables was conducted on 30-31 Jan. 2006, on a riffle/pool section of the river in Sakaki Town, Nagano Prefecture, Central Japan.

The maximum depth and current at the study reach exceeded 1.4 m and 1.2 ms^{-1}, respectively, so that usual sampling devices and methods were unavailable. Therefore, in order to take replicate quantitative samples of larvae by Surber sampler (30 cm × 30 cm, 450 μm mesh), we excavated upstream riverbed using a backhoe and directed the main flow away from the study sites, then stopped the remaining flow temporarily to lower the water level of a 300 m long section (Fig. 1). We also took drift samples at the section using the same sampler as above during and one day before the flow manipulation. As a result, we confirmed that drifting macroinvertebrates were rare and were not significantly different between before and during the manipulation, which indicated that macroinvertebrate migration during the flow manipulation work did not affected the larval density in the study reach.

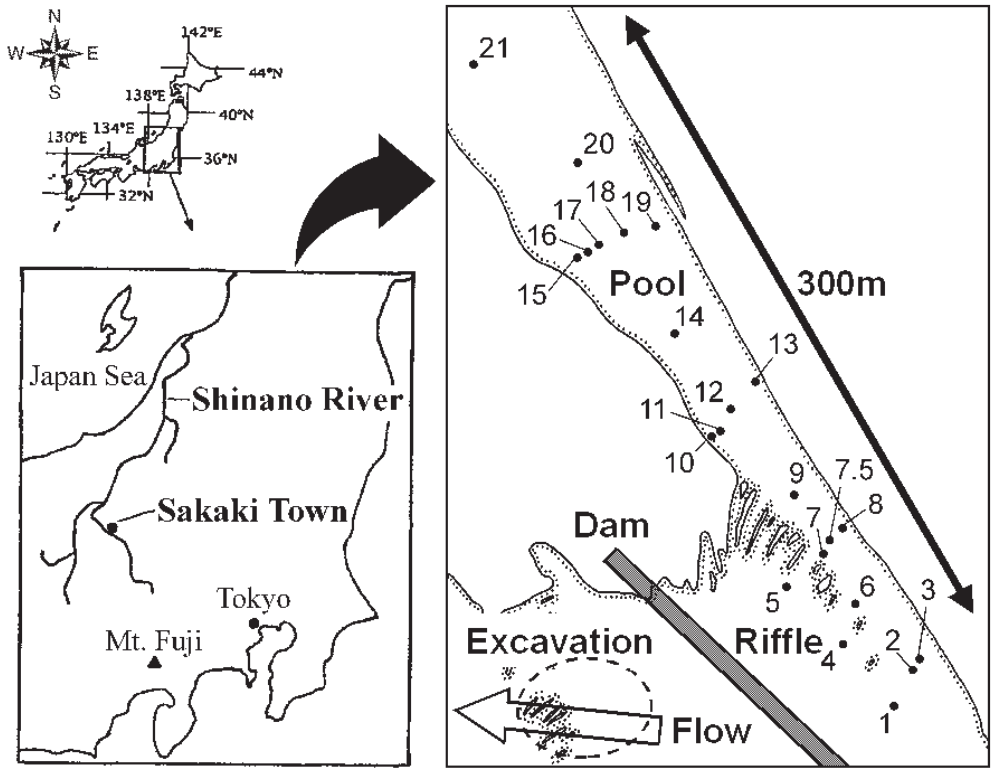

Fig. 1. Location of the study reach and arrangement of the 22 sampling sites.

Twenty two sampling sites were selected in the reach, arrayed along a riffle/pool gradient (Fig. 1). The following variables were measured to represent the gradient between riffle and pool: current velocity and water depth (before it was lowered), plus ash-free dry mass (AFDM), chlorophyll *a* and phaeophytin content of epilithon. We collected four benthic samples at each site, three of which were fixed in 10 % formalin for direct counts of larvae, while one was kept alive for rearing of chironomid larvae. The unpreserved samples were reared with aeration at room temperature (10-20 ºC) and imagines were collected every day for 70 days, until emergence ceased. Imagines were preserved as dried specimens and only males were used for identification because of the difficulty in accurate identification of females and immature stages. Males were mounted on slides with gum chloral under a binocular dissecting microscope (max. ×40), examined under a high-powered biological microscope (max. ×600) and identified to species using taxonomical keys by PINDER (1978), SASA & KIKUCHI (1995) and SÆTHER *et al*. (2000).

Relationships between the chironomid assemblage composition and the five environmental variables were quantified with Canonical Correspondence Analysis (CCA; TER BRAAK, 1986) using CANOCO 4.5 software for Windows (TER BRAAK & ŠMILAUER, 2002). In the CCA, in order to interpret especially species distribution patterns on the environmental variables measured, biplot scaling with a focus on inter-species distances and no data transformations were used besides downweighting of rare species. Sites 4, 15 and 16 were not included in the analysis due to lack of either male

emergence or missing environmental data. Significance of the first and sum of all canonical axes were evaluated with Monte Carlo permutation tests (1,000 runs). The test for the second canonical axis alone was not provided in CANOCO, however, the test for the first canonical axis has maximum power that explains significance of the relationship between the species and environment (TER BRAAK & ŠMILAUER, 2002).

Emergence ratio in the laboratory rearing was estimated for five sites (1, 3, 11, 14 and 20) that represent the riffle/pool gradient as *Em*/*Bn*, where *Em* is the number of imagines obtained by rearing and *Bn* is the number of larvae plus pupae obtained by direct counts of benthic samples replicated at the same site. As for the rest of the study sites, formalin-preserved samples are under sorting and currently unavailable, although the data will be used in our future study on chironomid distribution pattern.

RESULTS AND DISCUSSION

A total of 397 males belonging to 31 species and 458 females were obtained by larval rearing (Table 1). The most abundant species was *Microtendipes shoukomaki* (14.9 % of total abundance), followed by *Rheopelopia maculipennis* (12.6 %), *Tanytarsus tamaundecimus* (11.3 %), *Polypedilum asakawaense* (10.1 %). Orthocladiinae were relatively rare, comprising 5.8 % of total abundance. Both species richness and abundance of imagines from riffle sites tended to be lower than at pool sites (Table 1).

Table 1. Abundance of chironomid species obtained by larval rearing.

Species and abbreviation		Total abundance
Subfamily Tanypodinae		
Ablabesmyia monilis (Linnaeus, 1793)	Abmon	2
Hayesomyia tripunctata (Goetghebuer, 1922)	Hatri	18
Macropelopia paranebulosa Fittkau, 1962	Mapne	13
Rheopelopia maculipennis (Zetterstedt, 1838)	Rpmac	50
Rheopelopia ornata (Meigen, 1839)	Rporn	1
Subfamily Orthocladiinae		
Cricotopus (*Cricotopus*) *triannulatus* (Macquart, 1826)	Crtri	2
Diplocladius cultriger Kieffer, 1908	Dipcu	9
Orthocladius (*Orthocladius*) *glabripennis* (Goetghebuer, 1921)	Orgla	12
Subfamily Chironominae		
Tribe Chironomini		
Cryptochironomus albofasciatus (Staeger, 1840)	Ccalb	13
Cryptotendipes oyabeprimus Sasa, Kawai & Ueno, 1988	Ctoya	1
Demicryptochironomus chuzequartus Sasa, 1984	Dechu	2

Table 1. (Cont.)

Species and abbreviation		Total abundance
Microtendipes shoukomaki Sasa, 1989	Mtsho	59
Microtendipes truncatus Kawai & Sasa, 1985	Mttru	1
Polypedilum (*Polypedilum*) *asakawaense* Sasa, 1980	Poasa	40
Polypedilum (*Polypedilum*) *nubeculosum* (Meigen, 1804)	Ponub	8
Polypedilum (*Polypedilum*) *parviacumen* Kawai & Sasa, 1985	Popva	5
Polypedilum (*Tripodura*) *japonicum* (Tokunaga, 1938)	Pojpn	9
Polypedilum (*Tripodura*) *tamahinoense* Sasa & Ichimori, 1983	Potam	5
Polypedilum (*Uresipedilum*) *convictum* (Walker, 1856)	Pocon	1
Polypedilum (*Uresipedilum*) *cultellatum* Goetghebuer, 1931	Pocul	1
Polypedilum (*Uresipedilum*) *paraviceps* Niitsuma, 1992	Popvc	16
Stictochironomus akizukii (Tokunaga, 1940)	Staki	19
Tribe Tanytarsini		
Cladotanytarsus vanderwulpi (Edwards, 1929)	Clvan	8
Cladotanytarsus sp.	Clasp	2
Micropsectra tamaprima Sasa, 1980	Mptam	4
Rheotanytarsus kyotoensis (Tokunaga, 1938)	Rtkyo	2
Rheotanytarsus tamatertius Sasa, 1980	Rtter	5
Tanytarsus takahashii Kawai & Sasa, 1985	Tatak	6
Tanytarsus tamagotoi Sasa, 1983	Tagot	27
Tanytarsus tamakutibasi Sasa, 1983	Takut	7
Tanytarsus tamaundecimus Sasa, 1980	Taund	45
Not identified (key parts for identification damaged or lost		4
Total male abundance		397
Total female abundance		458
Total abundance		855
Total No. of male species		31

The first canonical axis of the CCA ordination was significant ($p = 0.007$, Monte Carlo permutation test 1,000 runs) and sum of all the axes ($p = 0.048$). The first two axes were used to explain the species distribution pattern, and they explained 22.3 % of the variance in the species data and 65.1 % of the species-environment relationship. The biplot of sites and environmental variables showed that current, AFDM and chlorophyll *a* were positively associated with the axis I, whereas phaeophytin (a decomposition product of chlorophyll) and depth showed a negative association (Fig. 2). In addition, the riffle sites had high scores on axis 1, whereas pool sites had lower scores on axis 1. Therefore, we interpreted axis I to represent the riffle/pool gradient. In relation to species distribution, species with high axis 1 scores, e.g. *Polypedilum paraviceps* and *R. maculipennis*, were abundant in the riffle (Fig. 3). On the other hand, species with lower axis 1 scores, e.g. *P. asakawaense*, *Stictochironomus akizukii* and *Tanytarsus* species, were abundant in the pool. Thus, the imaginal assemblage composition obtained by larval rearing reflected the riffle/pool gradient.

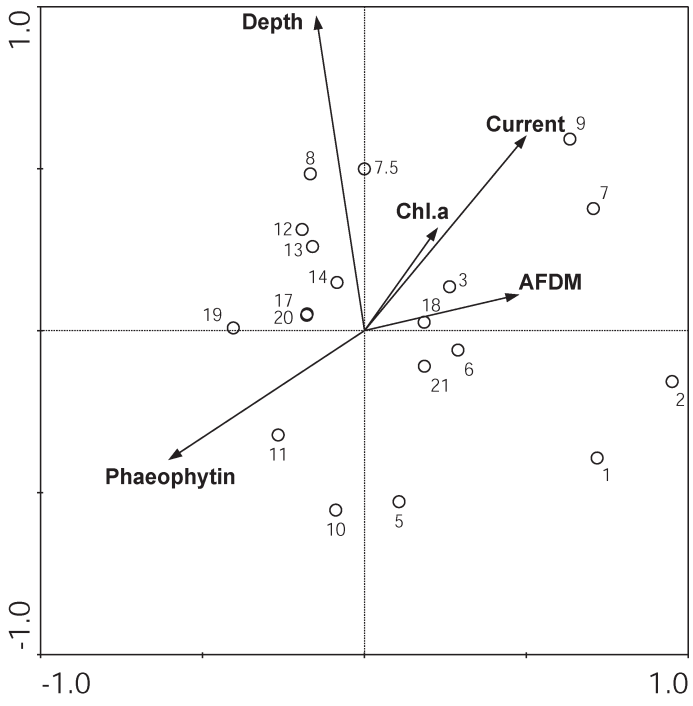

Figure 2. Biplot diagram of CCA results, indicating ordination site scores (circles) and environmental variables (arrows) on the axes I and II. Weighted average scores are plotted for sites.

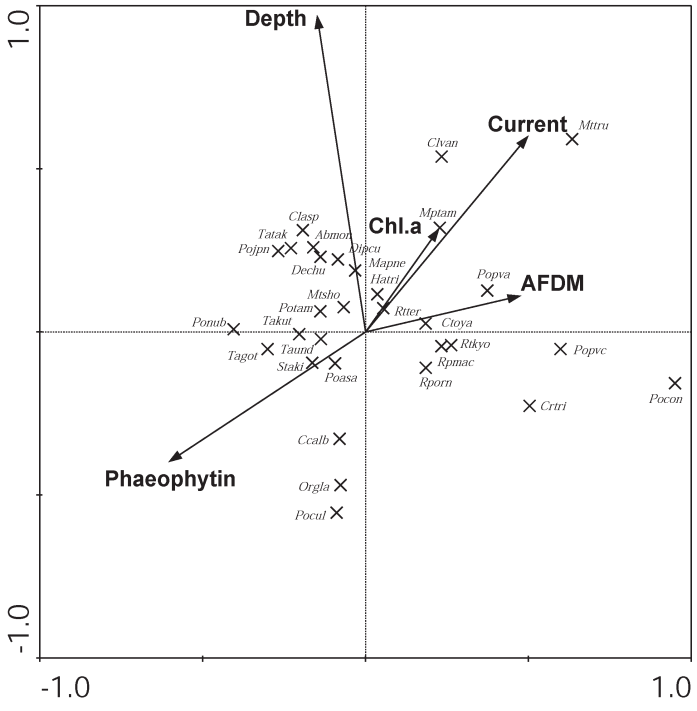

Figure 3. Biplot diagram of CCA results, indicating ordination species scores (crosses) and environmental variables (arrows) and on the axes I and II. Weighted average scores are plotted for species. See Table 1 for species abbreviations.

TABLE 2. Ratio of successful emergence (%) at the five sampling sites estimated from chironomid densities (indiv.m^{-2}) of in situ larvae and pupae, and imagines emerged from benthic samples.

Site	Riffles		Pools		
	1	3	11	14	20
Imagines emerged (*Em*)	167	78	78	400	156
Larvae	8.73	26.57	1.99	24.12	12.29
Pupae	133	56	22	156	56
Immatures (*Bn*)	8.86	26.62	2.01	24.28	12.35
Successful emergence (*Em*/*Bn*)	1.89	0,29	3.88	1.65	1.26

However, Orthocladiinae species were unusually rare in our study. In Japanese temperate rivers, they usually compose about 20 % or more of total abundance (INOUE *et al*., 2005). In addition, species richness and abundance of Orthocladiinae were lower in the riffle than the pool. Rearing stress would bias the imaginal assemblage composition if there was significant mortality of rheobiontic larvae during rearing. Moreover, fairly high abundance of Tanypodinae (21.2 % of total abundance), especially *R. maculipennis*, would also bias the composition through their predation for other chironomid larvae in the rearing (ARMITAGE *et al*., 1995). Many Orthocladiinae larvae, normally abundant at riffles, appeared not to survive to emergence. At five representatives of sampling sites, the estimated emergence ratio for the riffle sites ranged 0.29-1.88 % (n = 2), much less than those for the pool sites (1.26-3.87 %; n = 3) (Table 2). In contrast, the ratio estimated with lentic chironomid assemblages, from IWAKUMA *et al*. (1993) was 35.5 % on average, much higher than our study. In addition, Tanypodinae collected by IWAKUMA *et el*. (1993) comprising only 2.2 % of total abundance, much lower than our study. These results suggest that it was more difficult for lotic larvae to develop to imagines than for lentic larvae, under the rearing conditions of this study, and that impact of predation in vitro by Tanypodinae larvae on emergence of other chironomid larvae could not be neglected if they were abundant in rearing samples. Application of larval rearing data to field samples would underestimate the habitat distribution of some species, as well as their density in situ.

Currently, the larval rearing technique is the only method for species identification of chironomids of which immatures are unknown. Despite the density and distribution data should not be used quantitatively, our results indicate that larval rearing is a useful technique to examine correlation between chironomid assemblage and environmental conditions. It would be also valuable for qualitative work, e.g. making a list of major species, clarifying rough habitat of larvae and exploring unknown species. Future studies may improve rearing conditions, e.g. replicating physicochemical factors in situ more accurately and inhibiting predation in vitro, to raise emergence ratio enough to provide reliable quantitative data.

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