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Effect of copper and zinc on the growth and emergence of *Epeorus latifolium* (Ephemeroptera) in an indoor model stream

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Abstract

Effects of Cu and Zn through food as well as through the water on the growth and emergence of the young larvae of *Epeorus latifolium* (Ephemeroptera), were investigated using an indoor model stream. The critical lowest concentrations of Cu which have significant effects on the growth of the larvae were between 10 and 15 $\mu g\,l^{-1}$ Cu. Growth and emergence of the larvae fed on the algae (diatoms) which accumulated more than 1000 μg Cu g^{-1} (dry wt.) were significantly impaired. Growth of the mayfly larvae exposed to 100 or 300 $\mu g\,l^{-1}$ Zn ceased after the second week, and all died before emergence. At 30 $\mu g\,l^{-1}$ Zn, the growth rate decreased gradually and many larvae died before emergence. The molt interval also nearly doubled that of the control at these concentrations. Growth and emergence of the mayfly fed the algae which accumulated more than 2000 $\mu g\,g^{-1}$ of Zn were significantly affected.

Introduction

There are many reports on the effects of heavy metals on the aquatic insect community in the rivers or mountain streams which receive effluent from mines or abandoned mines (Gose, 1960; Machida & Ishizaki, 1975; Watanabe & Harada, 1976; Brown, 1977; Ishizaki & Machida, 1980; Moore, 1980; Winner, 1980; Peckarsky & Cook, 1981). The effects of heavy metals on the aquatic insect community have been assessed mainly based on the density and diversity of aquatic organisms by results of field investigation.

Rivers receiving the effluents from these abandoned mines are polluted usually with many kinds of heavy metals rather than any one special kind of metal. Therefore, to assess the effects of any special heavy metal on aquatic insects, some model experiments are needed other than field investigations. Moreover, heavy metal concentrations in algae and/or substrates, the main food of many aquatic insects, were very high in rivers polluted with heavy metals (Watanabe & Sumita, 1976; Eyrest & Pugh-Thomas, 1978; Foster, 1982; Hatakeyama *et al.*, 1985).

The aquatic insect larvae used in the present study was a species of the mayfly, *Epeorus latifolium* (Ephemeroptera, Ecdyonuridae). This species is one of the most common mayflies in Japan, and distributes widely from the upper reaches of rivers to downstream. Heavy metal tolerance of this species has been reported to be comparatively high by field investigation (Machida & Ishizaki, 1975; Watanabe & Harada, 1976). They usually dwell on the surface of stones in the river and use attached diatoms as their main food.

Therefore, indoor model streams were prepared to assess the effects of heavy metals (Cu and Zn) on the growth and emergence of young larval *E. latifolium* through food (diatoms) as well as through the water containing metals.

Materials and methods

Two stainless steel streams are arranged in a room which was controlled at 15 ± 1 °C and 12 L (2500 Lux, fluorescence light) and 12 D cycles, with a gradient of 2 degrees. Figure 1 is an outline of a model stream and exposure-cage. The length, width and height of the channel measured 600, 40 and 30 cm, respectively. Underground water, temperature controlled at 12 ± 1 °C, was used, and the flow rate of the water was 7 l min^{-1} (per channel) through the experiment. The pH, total hardness and electric conductivity of the water was 7.9 to 8.0, 83 ± 1 $\mu g\ l^{-1}$ as $CaCO_3$ and 190 $\mu s\ cm^{-1}$ at 25 °C, respectively.

The channel was partitioned with plastic plates (7 cm high) like three dams (Fig. 1) to provide 3 different metal concentration zones per channel.

Preceding the introduction of the mayfly larvae, spherical ceramic stones (diameter; 4.5 cm) were put in the upper part (Fig. 1, u) of the channel for 40 to 50 days, to let algae grow on the surface of the stones. After 40 to 50 days, diatoms grew sufficiently as food of single mayfly larva, usually 2 to 3 mg chl.a per ceramic stone.

E. latifolium larvae were collected from a sampling station of the Kinu River about 65 km to the north of our Institute, about 55 km north-east from Tokyo, Japan. There was no any heavy metal pollution to refer at the sampling station. The larvae were put into a polyethylene bag with sufficient oxygen and carried to our laboratory cooled in an ice box. Before the metal-exposure, body length of the larvae were measured and put individually into an exposure-cage (7x7x8 cm, Fig. 1, c), which was made stainless netting of

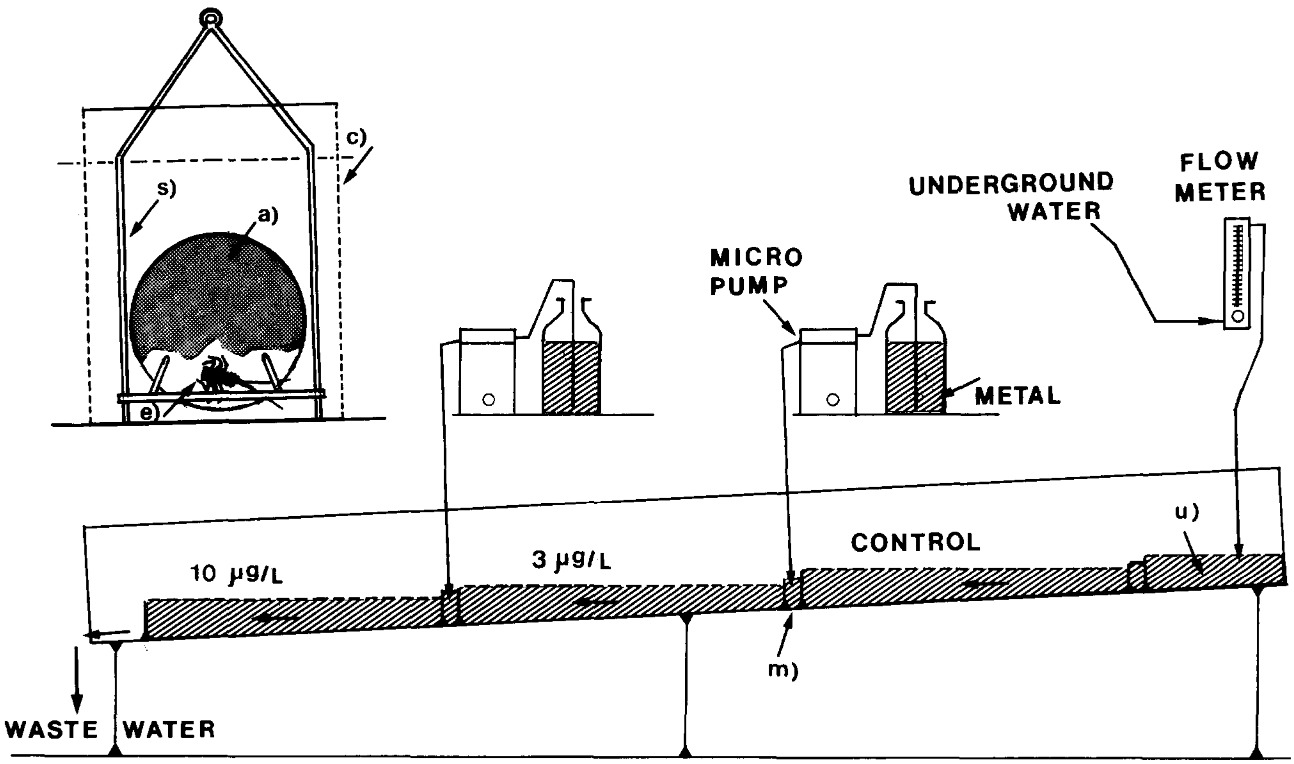


Fig. 1. Outline of model stream and exposure-cage. Underground water (12 °C, 7 l/min^{-1}) was mixed with stock solution of metal in mixing part (m) to prepare the nominal concentration. Mayfly larva was individually exposed to metals in a stainless cage (c). As food for mayflies (e), diatoms (a) attached to the ceramic stone, which was put on the stone-supporter (s), were used.

1 mm mesh size. Then, twelve cages per concentration were arranged in the channel with 4 rows and 3 ranks along the water flow to acclimatize the larvae for 5 days to 1 week. Seventy two larvae were used in each experiment, because six concentrations were used in one experiment. As food for mayfly larvae, algae (diatoms) attached on the ceramic stone were put into a stainless cage with a stone-supporter (Fig. 1, s). Body length of mayflies used for the experiment was around 4 mm, as described later.

The mayfly larvae were exposed to metals as follows. A stock solution of Cu (as $CuSO_4$) or Zn ($ZnSO_4$), both from Wako Pure Chemical Ind. Ltd., was continuously introduced into the mixing part (Fig. 1, m) of each concentration zone (Fig. 1) by a micro peristaltic-pump (220 ml d^{-1}) and mixed there with the underground water (7 l min^{-1}) homogeneously by vigorous aeration so as to become the nominal concentration.

Effects of copper

Twelve mayflies were used per concentration. In the first experiment, mayflies were individually exposed to 3, 10, 30, 100 and 300 $\mu g\ l^{-1}$ Cu in the cage with attached algae as food for 8 weeks.

Usually, mayfly larva was found on the bottom of the stone and removed from the exposure-cage with the stone-supporter. The stone was shaken vigorously together with the stone-supporter in the underground water in a plastic vat to detach the larvae from the stone. Then, the larva was put on a watch-glass using the pipette with flat tip, and body length was measured every one week under binoculars in a room controlled at 15 °C, and immediately returned to the stainless cage in the channel. From 3 weeks after Cu-exposure, emergence was also checked every 1 to 2 days to 8 weeks after the experiment.

On the other hand, algae attached on the surface of the ceramic stones were also exposed to the same Cu concentrations for 2 weeks. Thereafter, the Cu-accumulated algae were transferred to clean water zones of the channel. The Cu-concentrations of the algae decreased very slowly, although the Cu-concentration in water dropped quickly. Effects of Cu on the growth and emergence of mayfly larvae through the algae were examined in the same way mentioned above using the Cu-accumulated algae as food.

In the third experiment, mayflies were exposed to 5, 10, 15, 20 and 25 μg Cu l^{-1} for 8 weeks and effects on the mayfly larvae were examined by the same methods mentioned above to assess the effect of Cu in the water at lower concentration levels.

Effects of zinc

The nominal Zinc-concentrations used for the experiment were 3, 10, 30, 100 and 300 μg Zn l^{-1}, respectively. Effects of Zn were observed for 6 weeks by the same procedures mentioned above, except that water temperature was 15.5 °C instead of 12.5 °C. Molt interval of the larvae was also examined during the initial 2 weeks by checking the exuviae floating on the water surface in the cage (Fig. 1, c), because delay in the molt-intervals were noticed from the end stage of the third Cu-experiment (5 to 25 μg Cu l^{-1}).

At 4 weeks after the start of Zn-exposure, the ceramic stones covered upside with Zn-accumulated algae for 4 weeks were transferred to clean water zones of the channel, and effects of Zn on the growth and emergence of mayfly larvae through the algae were examined per above.

Analysis of heavy metals

Algae on the surface of the ceramic stone were collected by nylon brush into glass vials and washed 3 times in 0.1 mM EDTA by centrifugation (5000 r.p.m.) for 15 minutes. Finally, clots of algae (mainly diatoms and partially green algae) were put in acid washed glass vials and dried at 100 °C for overnight. Then, dried samples were digested with HNO_3 and $HClO_3$ (Wako Pure Chemical Ind., Ltd.) mixture. The digested solution was diluted with Milli-Q (Millipore Corp.) water, and Cu or Zn concentration was measured by an atomic absorption instrument equipped D^2 lamp (Shimadzu 640-A).

Results

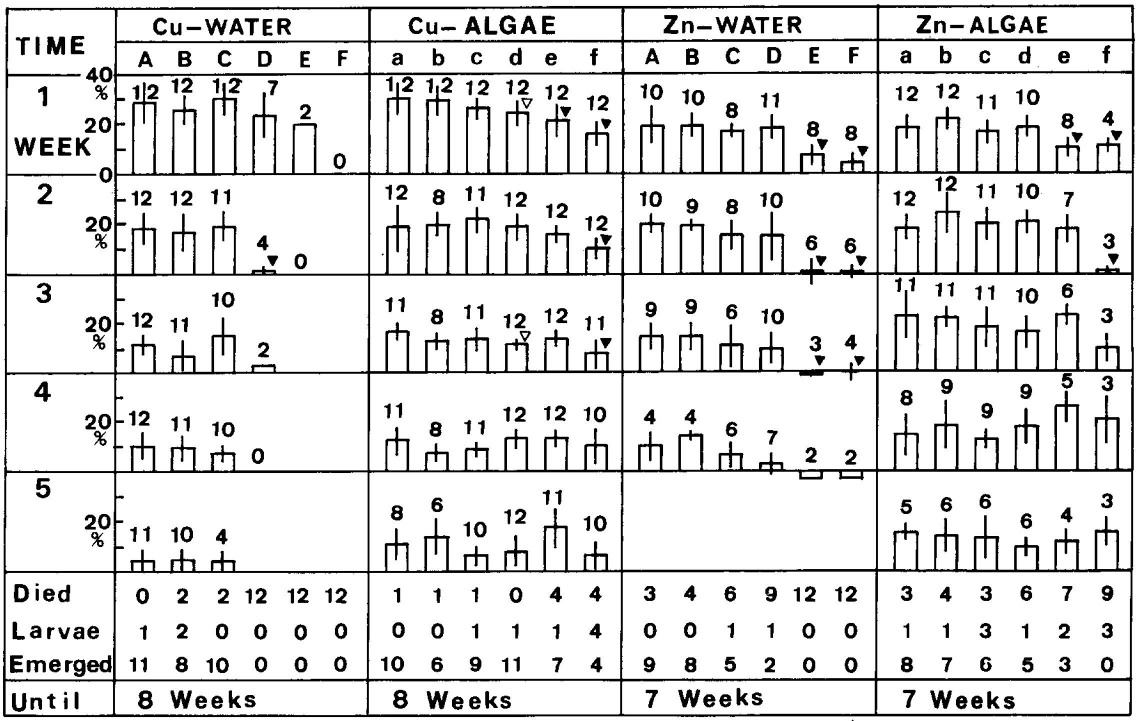
Effects of Cu on growth and emergence of E. latifolium larvae through water and algae

Effects of Cu (3 to 300 μg l⁻¹) through water

Mayfly larvae were collected in February, and the water temperature of the river water was 11.5 °C. The mean body length at the start of acclimation was 4.52 ± 0.31 mm (n = 72). After one week, at the start of Cu-exposure, body length increased to 5.48 ± 0.38 mm, that was a 21.2% increment per week. There were no differences in growth rates in 6 partitions of 2 channels during acclimation, although the water temperature in the upper sections (control and 30 $\mu g\, l^{-1}$) was about 0.5 °C lower than in the lower sections (10 and 300 $\mu g\, l^{-1}$). These values were 21.6 ± 4.8 (mean ± S.D., n = 24), 21.3 ± 3.9, and 21.6 ± 5.1% in the upper, middle, and lower sections of the 2 channels, respectively.

At 300 $\mu g\, l^{-1}$, all mayflies died within a week after the start of Cu-exposure. At 100 $\mu g\, l^{-1}$, only 2 larvae survived over one week, and they died within two weeks. However, at 30 $\mu g\, l^{-1}$, 7, 4 and 2 mayflies survived after 1, 2 and 3 weeks, respectively, but all died within 4 weeks. There was no definite increase in mortality at 3 and 10 $\mu g\, l^{-1}$.

Figure 2 (1st column) show the mean values of body length increment (%, per week), based on the mean body length of the surviving larvae, from the start of Cu-exposure to 5 weeks. The growth



Cu (or Zn)-Water: A; control, B; 3, C; 10, D; 30, E; 100, F; 300 $\mu g\, l^{-1}$.

Cu-Algae: a; 3 ± 2, b; 63 ± 11, c; 110 ± 30, d; 240 ± 68, e; 590 ± 175, f; 1140 ± 250 (μg Cu g^{-1} dry weight), as mean values of Fig. 5.

Zn-Algae: a; 510 ± 30, b; 600 ± 20, c; 780 ± 42, d; 940 ± 80, e; 1380 ± 170, g; 2170 ± 180 (μg Zn/g^{-1} dry weight), as mean values of Fig. 6.

▽; $p < 0.05$, ▼ < 0.01 (ANOVA)

Fig. 2. Body length increment (%, per week, mean ± S.D.) of mayfly larvae (12 individuals at start) individually exposed to Cu or Zn in model stream through water or algae. Total number of died, stayed in the larval stage and emerged during the experimental period are shown in under part of each column.

was not impaired at 10 $\mu g\,l^{-1}$. However, at 30 $\mu g\,l^{-1}$, growth decreased significantly from one to two weeks after the Cu-exposure as did the survival rates.

In the first week after Cu-exposure, body length increased about 30% in the control and the larvae exposed to 3 or 10 $\mu g\,l^{-1}$. However, as the mayfly larvae developed to mature, the growth rate decreased gradually. From 2 to 3 weeks, the wing pad started to develop and became dark. Then, from 3 weeks, the first emergence occurred at 10 $\mu g\,l^{-1}$. Finally, after 8 weeks of Cu-exposure, 11, 8 and 10 out of 12 larvae emerged in the control, and at 3 and 10 $\mu g\,l^{-1}$ Cu, respectively (Fig. 2).

Figure 3 shows composition (%) of the attached algae at 2 weeks after Cu-exposure and 3 weeks after the end of the Cu-exposure. Algal flora was composed of 4 species of diatoms. In the control, *Navicula minima* and *Gomphonema parvulum* were dominant at 2 weeks after the Cu-exposure. However, in the algae exposed to 30 to 300 $\mu g\,l^{-1}$ Cu, *N. minima* was exclusively dominant, although being accompanied by *Nitzschia frustulum* and *Synedra rumpens* lower than 10%. Moreover, algal composition was still different even at 3 weeks after Cu-exposure ended between the control algae and those exposed to high Cu-concentrations.

Effect of Cu (5 to 25 $\mu g\,l^{-1}$) through water

Mayfly larvae in this experiment were collected in August and water temperature in the River was 23.0 °C. Body length of the larvae at the start of acclimation was 3.60 ± 0.62 mm (n = 72). After 5 days, at the start of Cu-exposure, mean body length increased to 3.75 ± 0.64 mm (n = 63). During the acclimation of 5 days, 9 larvae out of 72 died and those were substituted for other acclimated larvae just before the Cu-exposure.

Table 1 and Fig. 4 show the mean values of body length increment (%, per week) and growth curve of the larvae after the Cu-exposure, respectively. Growth rate was low and mortality (survivor number in parentheses in Table 1) was high even in the control, compared to the case in which winter or spring materials were used. This may be attributed to unsuccessful acclimation of mayflies, because the water temperature gap was too large between the river (23.0 °C) and model stream (12.5 °C).

There was no significant effect at 5 and 10 $\mu g\,l^{-1}$ in growth rate. At 15 $\mu g\,l^{-1}$ Cu, the growth rate was also low during the first 3 weeks, although it restored gradually from 4 weeks. However, growth rates of the larvae exposed to 20 or 25 $\mu g\,l^{-1}$ Cu stayed at lower than 7% through the experiment. Finally, until 10 weeks, from 7 (control) to all larvae died, and only 4 (control), 3, 3, 2, 1 and 0 mayflies emerged in order of Cu-concentration. Finally, one mayfly stayed in the larval stage at all concentrations except 25 $\mu g\,l^{-1}$ at 10 weeks.

Effect of Cu through algae

After 2 weeks of the first experiment (3 to 300 μg Cu l^{-1}), the ceramic stones upside covered with Cu-accumulated algae were put in the stainless cage (Fig. 1), and set in the clean water zones of the channel. From the next day, 12 mayflies (per concentration) were introduced individually into the cages. The Cu-concentrations of the attached algae decreased slowly even in the clean water (Fig. 5). Mean Cu-concentrations of the algae in the clean water during 3 weeks were 3 (control), 60, 110, 240, 590 and 1140 $\mu g\,g^{-1}$ (dry weight), respectively. Hereafter, these mean values will be used for abbreviation in describing the results.

The larvae were collected in March and water temperature of the river was 13 °C. They were used for the experiment after 1 week's acclimation at 12.5 °C. Mean body length of the larvae at the start of Cu-exposure was 5.84 ± 0.71 mm. Figure 2 (2nd column) shows the mean values of body length increment (%, per week) after the Cu-exposure through algae. At 1 week, the values decreased to 80% ($p < 0.05$, ANOVA), 70% ($p < 0.01$) and 52% ($p < 0.01$) of the control in the larvae fed the algae accumulated 240, 590 and 1140 μg Cu g^{-1} (dry wt.), respectively. Growth rate of mayfly larvae fed the algae which accumulated 240 or 590 μg Cu g^{-1} approximated gradually to the control from 1 week, although the rate

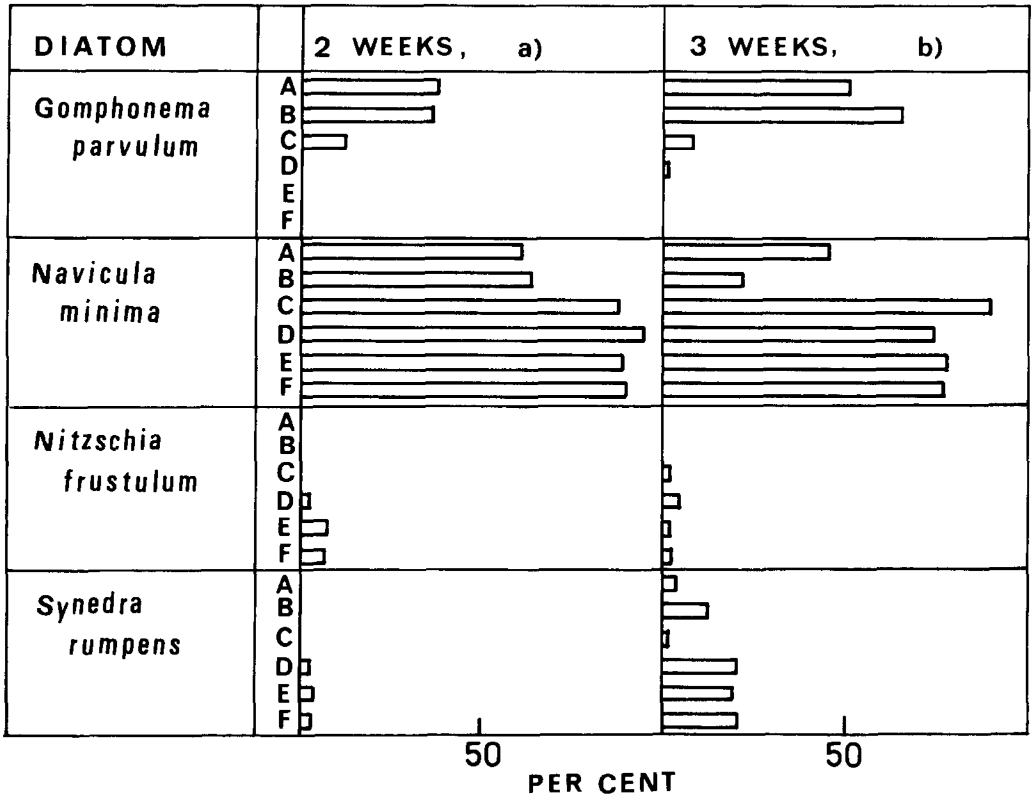


Fig. 3. Algal composition (%) of diatoms on the surface of ceramic stones. a), 2 weeks after the start of Cu-exposure; b), 3 weeks after the end of Cu-exposure.

A; Control, B; 3, C; 10, D; 30, E; 100, F; 300 μg Cu l^{-1}

Table 1. Mean body length increment (%, per week) of mayfly larvae exposed to Cu in an indoor model stream for 7 weeks. Attached algae were used as food.

Nominal conc. of Cu ($\mu g\, l^{-1}$)	Weeks after start of Cu-exposure						
	1 W	2 W	3 W	4 W	5 W	6 W	7 W
Control	9.2 ± 7.4	11.6 ± 7.4	12.2 ± 6.4	10.1 ± 7.7	12.8 ± 3.9	18.2 ± 1.9	14.6 ± 7.8
	(11)	(8)	(8)	(8)	(6)	(6)	(6)
5	5.7 ± 5.2	14.0 ± 8.7	12.2 ± 7.6	15.1 ± 3.9	16.4 ± 2.0	19.4 ± 5.2	20.8 ± 7.7
	(10)	(9)	(8)	(8)	(7)	(5)	(5)
10	9.6 ± 7.7	8.2 ± 6.4	10.7 ± 5.1	12.0 ± 6.6	14.2 ± 8.6	10.7 ± 9.6	12.1 ± 9.7
	(12)	(12)	(8)	(8)	(8)	(8)	(4)
15	6.8 ± 4.0	4.5 ± 5.1*	4.8 ± 5.4*	7.2 ± 5.3	12.2 ± 8.4	6.8 ± 5.5**	10.2 ± 7.9
	(11)	(10)	(7)	(7)	(6)	(5)	(4)
20	7.8 ± 7.4	3.5 ± 2.9**	5.6 ± 5.4*	2.1 ± 2.6*	5.5 ± 7.4	6.3 ± 4.4**	6.8 ± 4.6**
	(11)	(9)	(8)	(7)	(6)	(6)	(5)
25	8.4 ± 5.7	4.0 ± 4.4*	4.6 ± 6.1*	4.1 ± 9.2	6.9 ± 7.7	1.1	3.9
	(8)	(7)	(5)	(4)	(3)	(2)	(2)

Body length at the start of Cu-exposure was 3.75 ± 0.64 mm (Mean ± S.D., n = 63). Mean ± S.D. (n), Numerals in parentheses are number of survivors among 12 mayflies. *; $p < 0.05$, **; $p < 0.01$ (ANOVA).

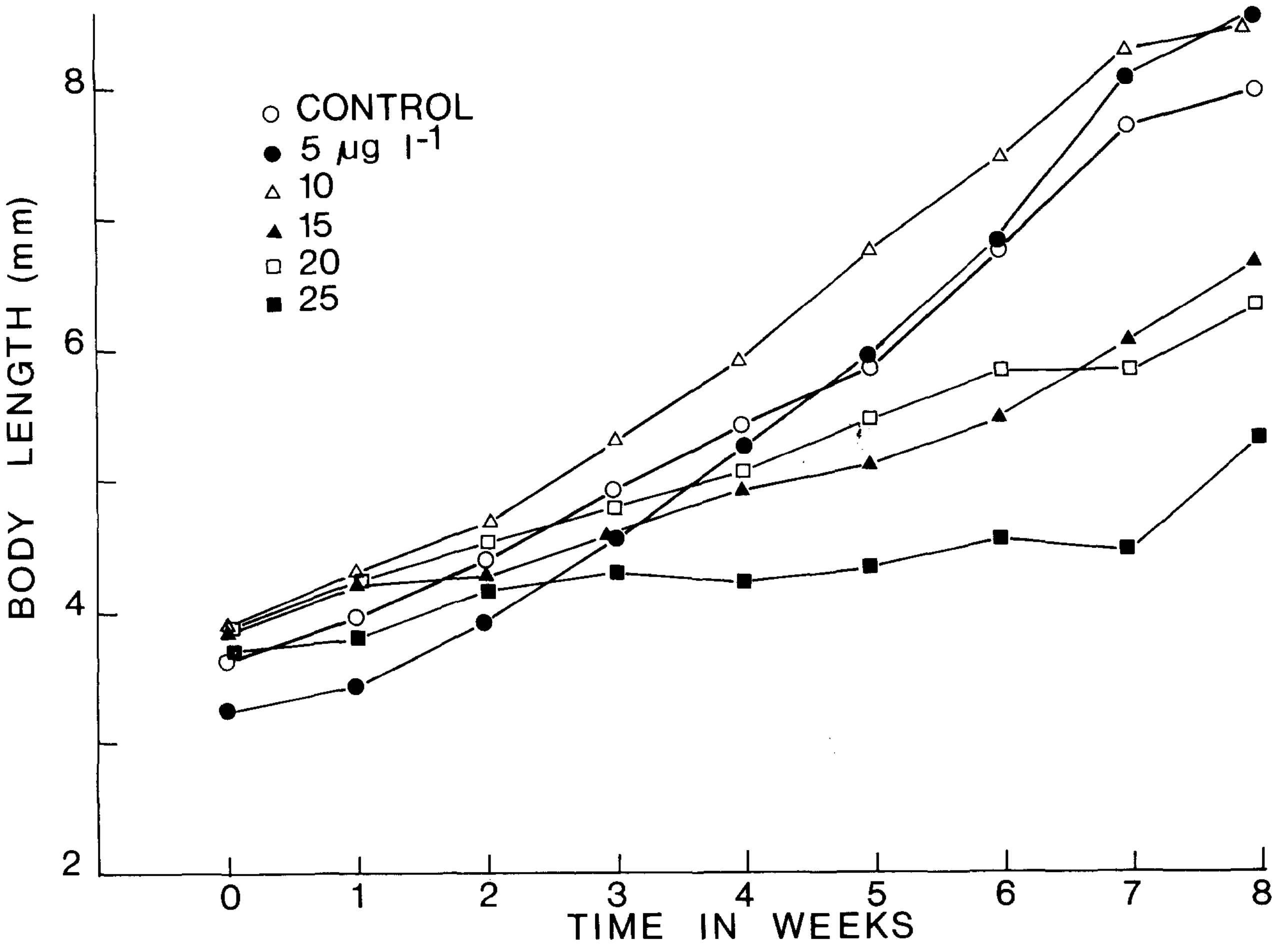


Fig. 4. Growth curves of mayflies exposed to Cu, 5 to 25 $\mu g\ l^{-1}$, in a model stream based on mean body length of surviving larvae (number shown in Table 1).

still remained at low a level ($p < 0.05$) during 2 weeks compared to the control. Growth rate of mayfly larvae fed the algae which accumulated 1140 μg Cu g^{-1} stayed at about 50% of the control level for 3 weeks, although mortality did not increase as shown by survival numbers in Fig. 2. After 8 weeks, 10 (control), 6, 9, 11, 7 and 4 out of 12 larvae emerged in the order of Cu-concentration in the algae.

Four individuals died among the larvae fed on the algae which accumulated 590 and 1140 μg Cu g^{-1}, respectively, and 4 stayed in the larval stage in the latter at 10 weeks. However, the Cu-effect on the emergence was not observed in the larvae fed the algae which accumulated 240 μg Cu g^{-1} or lower levels.

Effects of Zn on growth and emergence of E. latifolium through water and algae

Effect of Zn through water

The larvae were collected in June and water temperature in the river was 22 °C. The larvae were acclimated for 5 days at 15.5 °C before the Zn-exposure. Mean body length of larvae at the start of Zn-exposure was 5.75 ± 0.45 mm. Figure 2 (3rd column) shows the mean values of body length increment (%, per week) after the Zn-exposure. The Zn-concentration of the underground water was $8.8 \pm 2.3\ \mu g\ l^{-1}$ (n = 7, number of days analyzed) during the experiment. Thus, actual concentration should be added with $8.8\ \mu g\ l^{-1}$ to nominal concentration. But, the

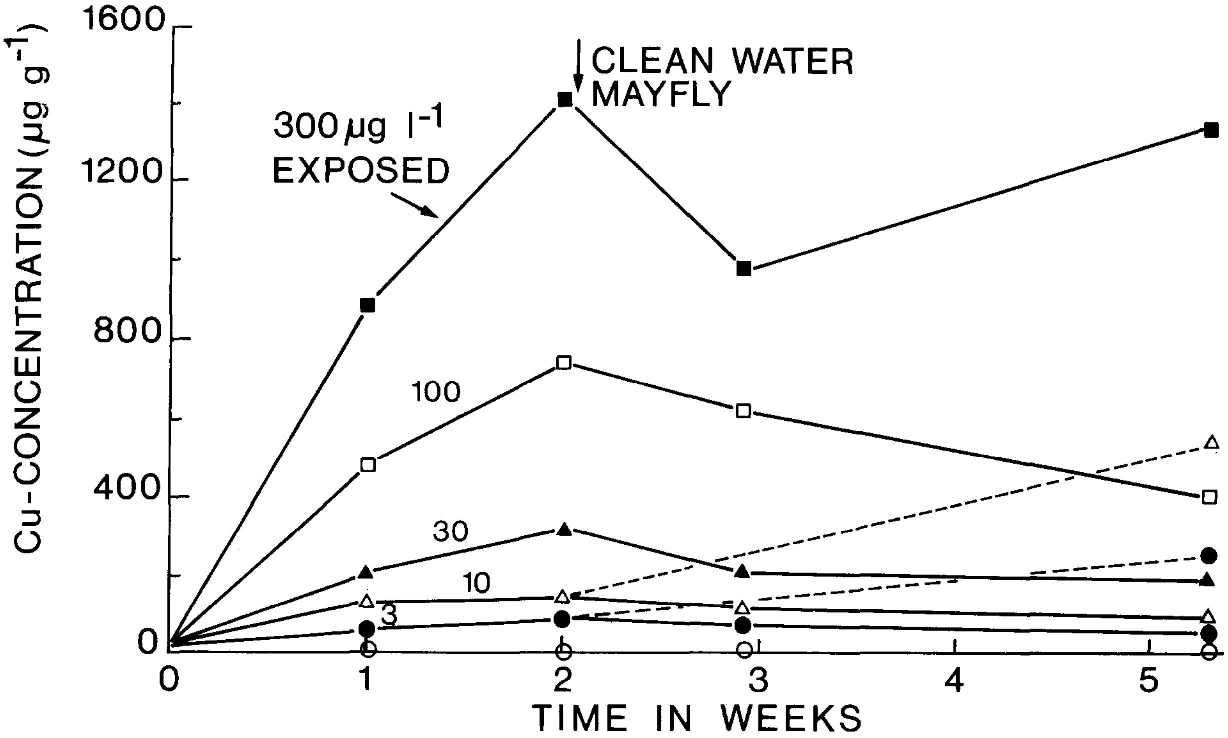


Fig. 5. The Cu-concentration of attached diatoms exposed to 3, 10, 30, 100 and 300 $\mu g\, l^{-1}$ Cu for 2 weeks in a model stream. At the arrow-indicated time, these algae were transferred to clean water zones of the channel with ceramic stones. Individual mayfly larvae were continually put in a stainless cage with this algae as food. Broken lines show Cu-concentrations of the algae exposed to Cu for 5 weeks at 3 and 10 $\mu g\, l^{-1}$ Cu, respectively.

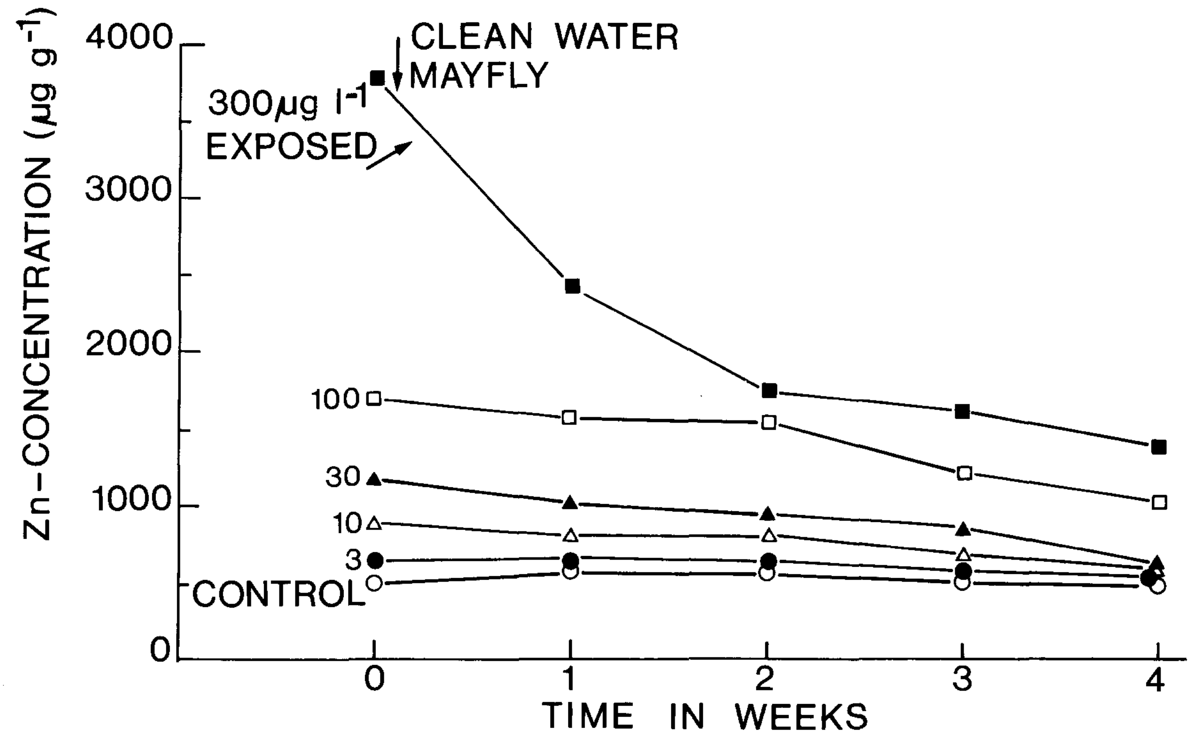


Fig. 6. The Zn-concentrations of attached algae put in clean water zones of the channel after 4 weeks exposure to 3, 10, 30, 100 and 300 $\mu g\, l^{-1}$ Zn in the channel. From the arrow-indicated time, mayfly larvae were individually introduced into a stainless cage with this algae as food.

Table 2. Molt intervals of mayfly larvae exposed to Zn in an indoor model stream.

Nominal Zn concs. ($\mu g\,l^{-1}$)	Molt interval (time in days)
Control	6.9 ± 0.4 (n = 10)
3	7.2 ± 0.7 (n = 9)
10	8.2 ± 2.5 (n = 8)
30	8.7 ± 2.2 (n = 10)
100	11.4 ± 5.4 (n = 11)
300	15.1 ± 9.0 (n = 10)

Mean ± S.D. (n).

author will describe the result based on the nominal concentration, just for a convenience to illustrate.

With the control and at 3 $\mu g\,l^{-1}$, 9 and 8 out of 12 larvae emerged, although 3 and 4 died during the experiment. At 10 and 30 $\mu g\,l^{-1}$, growth rates were not significantly different from the control within 1 week, however, from 2 weeks they decreased gradually to less than 10% (per week) at 4 weeks. Finally, 6 and 9 larvae died, although 5 or 2 mayflies emerged at 10 and 30 $\mu g\,l^{-1}$, respectively.

At 100 and 300 $\mu g\,l^{-1}$, the growth rate decreased to 37 and 24% of the control at 1 week after the Zn-exposures (Fig. 2). The rate become nearly zero after 2 weeks and finally, all died before emergence. At 100 and 300 $\mu g\,l^{-1}$, molt interval also significantly prolonged to about twice that of the control (Table 2).

Effect of Zn through algae

The larvae were collected in August and water temperature in the river was 26 °C. They were acclimated for 4 days at 15.5 °C before the Zn-exposure. Mean body length of the larvae at the start of Zn-exposure was 4.53 ± 0.69 mm (n = 72).

The Zn-concentrations of the attached algae decreased very slowly in the algae accumulated low levels of Zn, although the decrease was relatively fast in the algae accumulated the highest concentration of Zn (Fig. 6). Mean values of Zn-concentrations of the algae were 510 ± 30, 600 ± 20, 780 ± 42, 940 ± 80, 1380 ± 170 and 2170 ± 180 (μg Zn g^{-1} dry wt.), with the control, and at 3, 10, 30, 100 and 300 $\mu g\,l^{-1}$ Zn, respectively. Hereafter, these mean values will be used to describe the results. Figure 2 (4th column) shows the effect of Zn on the growth and emergence of the mayfly larvae exposed to Zn through the Zn-accumulated algae. Until 7 weeks after the start of Zn-exposure through algae, 8, 7 and 6 out of 12 larvae emerged in the control and those fed the algae accumulated 600 and 780 μg Zn g^{-1} (dry wt.), respectively. Growth rate of the larvae fed the algae accumulated 940 μg Zn g^{-1} did not decrease and 5 larvae emerged, although 6 larvae died. The growth rate of larvae fed the algae accumulated 1380 μg Zn g^{-1} decreased to 55% of the control within 1 week, although from 2 weeks, it restored. Finally, 7 died, 2 stayed in larval stage, and 3 larvae emerged. At 2170 μg Zn g^{-1}, the growth rate stayed at significantly low levels for 2 weeks ($p < 0.01$), although the rate of the surviving 3 larvae recovered as Zn-concentration of the algae decreased gradually to 1500 $\mu g\,g^{-1}$ level until 4 weeks. Finally, until 7 weeks, 9 larvae died and 3 still remained as larvae (Fig. 3).

Discussion

The purpose of the present investigation was to assess the effect of Cu and Zn on the growth and emergence of the mayfly larvae through food (algae) in contrast with the effect through the water. A test to assess the effects of metals through the water of the aquatic organisms is inevitably accompanied by metal-contamination of their food, since metals are easily adsorbed and/or accumulated to their food. However, as shown in Fig. 5, the Cu-concentration of algae exposed to 100 $\mu g\,l^{-1}$ Cu for 1 week was about 450 μg Cu g^{-1}. Therefore, the main cause of high mortality of the mayfly larvae exposed to 100 $\mu g\,l^{-1}$ Cu could be attributed to Cu in water, because at this algal Cu level, mayfly larvae did not die at all (Fig. 2, 2nd column).

On the other hand, it is relatively easy to expose organisms to metals exclusively through metalcontaminated food, although the metal concentration of algae gradually decreased in the present experiment. Therefore, recovery in growth rate observed after 2 weeks in the larvae which were exposed to Cu through the accumulated algae (Fig. 2, 2nd column) might be attributed to a decrease in the algal metal concentrations (Fig. 6). To assess a more chronic effect of metals through algae, the metal concentration of algae would have to be maintained, for example, by replacing the attached algae accumulating constant metal levels.

In the present investigation, growth rate of *E. latifolium* larvae exposed to 25 $\mu g\, l^{-1}$ Cu stayed at almost several% (per week) for 7 weeks (Table 1). While, from the results of field investigations undertaken at several heavy metal polluted rivers in Japan, it was shown that density of *E. latifolium* was zero or very low where the Cu-concentration in water exceeded ca. 25$\mu g\, l^{-1}$, although concentrations of other metals, such as Zn or Cd were high (our unpublished data, Hatakeyama *et al.*, 1986). Therefore, it was considered that Cu in water at 25 $\mu g\, l^{-1}$ level is so toxic as low density of *E. latifolium* in these rivers could be attributed to solely Cu in the water. Moreover, the Cu-concentration of the attached substances, mainly composed of diatoms, usually exceeded 1000 μg Cu g^{-1} (dry weight), where Cu-concentrations in water were higher than around 20 $\mu g\, l^{-1}$. Therefore, it was considered, based on the results of the present study, that the mayfly might be also significantly affected in either existence or density by the Cu-accumulated algae as well as Cu dissolved in the river water.

The growth rate was low and mortality was high in the third experiment (Cu, 5 to 25 $\mu g\, g^{-1}$) even in the control, in which mayfly larvae were collected in summer. As one of the possible causes, it was considered that the larvae could not adapt successfully to the difference of water temperature between the river (23 °C) and the model stream (12 °C). After that, in the Zn-experiment, water temperature in the channel was elevated to 15 °C from 12 °C, and growth rates in the larvae collected in summer were improved. Further, body length of the larvae used in the third Cu-experiment was shorter than in the other experiments, although it is not clear whether this is related to the results mentioned above. *E. latifolium* stripped the attached algae as food and disclosed the surface of ceramic stone as areas or patches of white in contrast to the dark brown color of diatoms (Fig. 1). Consequently, activity of food consumption by the larvae could be known indirectly. In the algae accumulating a high concentration of Cu, the area or patches were very scarce. Therefore, decrease in food consumption might also affect the growth rate of the larvae fed the Cu-accumulated algae at high levels.

Zinc was also very toxic to *E. latifolium*, although less so than Cu (Fig. 1st and 3rd column). However, usually, Zn-concentrations were much higher than that of Cu, in the river water receiving the effluents from the abandoned mines (McLean *et al.*, 1975; Brown, 1977; Foster, 1982; Yasuno *et al.*, 1985; Fukushima *et al.*, 1986). Therefore, it is difficult to assess which is more hazardous to aquatic insects in the rivers where Zn-concentrations are several times higher than Cu, although effects of Cu on growth and emergence of mayfly larva was higher than Zn in the present experiment.

Heavy metal-tolerance of *E. latifolium* was relatively high according to the results of the field investigations conducted in Japan (Machida & Ishizaki, 1975; Watanabe & Harada, 1976). They adapted well to the environment of the present model stream, in which water current velocity was very low, notwithstanding the fact their habitat is in running water. In another report, the correlation between the actual density of *E. latifolium* and metal (Cu and Zn) concentrations in water and attached substances, mainly composed of attached algae, will be examined based on the data gained in the present investigation and collected in the several metal polluted rivers.

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