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Chronic Effects of an Insecticide, Fenobucarb, on the Larvae of Two Mayflies, *Epeorus latifolium* and *Baetis thermicus*, in Model Streams

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Abstract. Effects of the insecticide fenobucarb on 2 mayfly species, *Epeorus latifolium* and *Baetis thermicus*, were examined in indoor model streams. Aqueous concentrations of fenobucarb residues in the model streams were 69–71% of its nominal level until 24 h, except at low concentrations (less than 2 μg l^{-1} in the water) in the water. Chronic effects of fenobucarb on the growth and emergence of Ephemeropteran larvae were examined over 2 months. For concentrations of 1 and 2 μg l^{-1}, the numbers of individuals of the third larval growth stage (average head width ≥ 3.0 mm) gradually decreased up to 20 days after the applications; the number of emergent individuals also remained 20 to 25 by the end of the experiment. The number of individuals to emerge at 1 to 2 μg l^{-1} fenobucarb was restricted in the long-term experiment. These results suggest that fenobucarb affects the emergence of this species through disruption of the endocrine system. On the other hand, the number of emergent individuals of *B. thermicus* at low concentrations of fenobucarb was not significantly less than that in the control.

Keywords: chronic; fenobucarb; *Epeorus latifolium*; *Baetis thermicus*; emergence

Introduction

Rice paddies line both sides of most rivers in Japan and are sprayed with pesticides during the planting and growing season. Most rivers tend to suffer pesticide contamination, although the concentrations are generally low relative to rice paddies and transient (Maru, 1985; Iwakuma et al., 1988a; Hatakeyama and Shiraishi, 1994; Hatakeyama et al., 1994; Hatakeyama, 1995). The pesticides can reach peak concentrations (1 to 10 μg l^{-1}) within several hours to several days and can also reach low concentrations (< 1 μg l^{-1}) within a similarly short period Hatakeyama et al., 1990; Takamura et al., 1991a; Iwakuma et al., 1993; Tada and Shiraishi, 1994; Hatakeyama and Yokoyama, 1997). Concentrations can reach acute sublethal levels (Takamura et al., 1991a) and this sustained contamination for more than a month can impact macroinvertebrates (Iwakuma et al., 1988a, 1988b).

Fenobucarb (a carbamate insecticide) is the main pesticide used for protection of rice plants in Japan. Changes in benthic macroinvertebrates and concentrations of pesticide residues were surveyed in the Kajinashi River, where fenobucarb reached its peak concentration (3.9 to 22.4 μg l^{-1}) soon or one month after rice transplantation (Tada and Shiraishi, 1994). Other pesticides, such as fenitrothion, fenthion, and chlornitrofen, were detected at peak concentrations below 1 μg l^{-1}. During a study of the River Kawamata, Ibaraki

Prefecture where bentiocarb, oxadiazon, fenobucarb, and fenthion reached concentrations of 3 to 4 $\mu g\ l^{-1}$, the density of *Baetis sahoensis* decreased to one-tenth of that during the period of low pesticide contamination (Iwakuma et al., 1988b). These results were possibly due to latent and subtle overall pesticide effects during the spraying period.

The acute effects of fenobucarb have been examined using indoor model streams (Tada, 1998). However, the sublethal and chronic effects of pesticides (EC_{50}) in relation to ecology or behavior and the effects of acutely lethal levels on aquatic organisms have only rarely been reported (Hatakeyama et al., 1997; Hatakeyama et al., 1990; Takamura et al., 1991a, 1991b). The present study was carried out using the model streams under more simplified and controlled conditions to investigate the chronic effects of fenobucarb on the growth and emergence of two species of mayflies (*Epeorus latifolium* and *Baetis thermicus*) (Ephemeroptera). These species are two of the most common mayflies in Japan.

Methods and materials

Collection of Ephemeropteran larvae

Larvae of *E. latifolium* were collected in March 1998 from the upper reaches of the Kinu River 65 km north of National Institute for Environmental Studies (NIES); the institute is 55 km north-east of Tokyo. This site was considered to be unpolluted with pesticides (Tada, 1998).

Larvae of *B. thermicus* were collected in March 1998 from the middle reaches of the Kozakura River at the northern foot of Mt. Tsukuba 8 km north of the institute. This site was considered to be minimally polluted with pesticides because of its fauna (Tada and Hatakeyama, unpublished).

The larvae were collected using a D-frame net sampler (width 25 cm, mesh size 1 mm, Rigosha Ltd, Japan). The surfaces of pebbles were rubbed with fingers to dislodge the benthos into the net along the current. Each six samples for Ephemeroptera (*E. latifolium* and *B. thermicus*) were collected using the net sampler from each river for six indoor model streams described below. Each sample was discharged into each polyethylene bag containing 500 ml of river water. The bags were filled with compressed O_2 during transport to the laboratory in the institute (NIES). In these studies mortality of the larvae during transport (1.5 h) was negligible. The Ephemeropteran larvae were hand-sorted upon at the laboratory and identified to species (*E. latifolium* and *B. thermicus*) using a binocular microscope ($10\times$ to $64\times$ magnification) and keys recommended by Gose (1985).

Indoor model streams

Six polyvinylchloride model streams were arranged in a room (Tada, 1998) that was controlled at 15 ± 1°C, 12 l (2500 Lux, fluorescence light), and 12 D cycles. Each channel measured 130 cm long, 13 cm wide, and 10 cm high with no gradient. Stock solutions of fenobucarb (methylcarbamate insecticide, 99%, Wako Pure Chemical Industries Ltd, LTV) were prepared using distilled water (20 mg l^{-1}). This stock solution was mixed with the tap water (34 l) of a tank (tank-a: 40 cm, 50 cm, 30 cm height, polyvinylchloride) in the following nominal concentrations: control (0), 1, 2, 4, 8, and 16 $\mu g\ l^{-1}$. The solutions were introduced into the head of the channel using a peristaltic pump (8 l min^{-1}) and circulated between the tank-a (the water level: 17 cm) and the channel (the water level: 5 cm). The water in each tank-a was renewed every 2 or 3 days by the addition of 20 l of tap water from a stock (200 l) tank (tank-B) and the stock solution mixed with the water of each tank-a. The pH of the water was 7.6 ± 0.1, the temperature 15 ± 1°C, and conductivity was 130.1 ± 0.5 $\mu S\ cm^{-1}$ at 25°C; the flow rate was 2.1 cm s^{-1} (per channel). These parameters were measured every Monday throughout the experiment.

Fenobucarb analysis in the channel water sample

Water sample from each channel was collected from the tank-a in duplicate 500-ml glass beakers 24 h and 48 h after the fenobucarb application. Each sample (500 ml) in the tank was filtered through a Whatman GF/C glass fibre filter (pore size < 1.2 μm) that had been heated at 470°C for 2 h. The filtrate was passed through a C18-bond Elut column (3 ml, Analytichem International, Varian) at a flow rate of 5 ml min^{-1}. The column

was then centrifuged at 3000 r.p.m. for 12 min to remove excess water. The pesticides in the column were eluted with 0.5 ml of analytical grade acetone (> 99.8%, Wako Pure Chemical Industries, Ltd) and centrifuged at 1000 r.p.m. for 12 min in a glass conical centrifuge tube (10 ml). This procedure was repeated once and followed by a final centrifugation with 0.5 ml acetone at 3000 r.p.m. for 15 min (a total of 1.5 ml acetone was used). The combined samples were analyzed with a gas chromatograph (Hewlett-Packard HP-5890 A) fitted with a capillary column (SPB-5; 0.25 μm length, 30 m inside diameter, and 0.32 mm fused silica) and equipped with an NPD-detector, together with an inner standard (50 μl of 1 mg l^{-1} azobenzene) for the pesticide analysis. The column temperature of the carrier gas (He) was 50 to 300°C and the flow rate was 54 ml·min^{-1}, respectively (Shiraishi et al., 1988).

Chronic effects on mayfly larvae

We examined the chronic (30–60 days) effects of fenobucarb on the growth and emergence of *E. latifolium* and *B. thermicus* at concentration of 1–16 μg l^{-1} levels. The six indoor model streams described above were used to examine the effects of fenobucarb on these two species. To begin the study, the head widths of the larvae were measured using a binocular microscope, then several larvae (36 to 41 per D-frame net) of each Ephemeropteran species were introduced into stainless steel net cages (18 × 8 × 8 cm, 0.8 mm mesh/net size).

The head widths of the larvae were measured using an ocular micrometer of a binocular microscope and separated into three groups based on head size (first growth stage: head width of ≤ 1.9 mm; second growth stage: head width of 2.0 to 2.9 mm; third growth stage: head width of ≥ 3.0 mm). These three groups of the larvae were introduced into the same cages described above settled in each channel (control, 1, 2, 4, 8, and 16 μg l^{-1} concentration) after one hour application of fenobucarb. An unglazed white tile (12 × 6 cm, 6 mm thick) on which periphyton had been grown for 2 weeks as food for the test organisms was submerged in each cage.

The larvae in each cage were transferred to plastic trays filled with tank-a water two or three times a week (*E. latifolium*) or every day (*B. thermicus*). The head widths of the larvae were then measured using the ocular micrometer of the binocular microscope after transferring each larva from the tray to a watch glass (diameter 4 cm) using a flattened pipette for *E. latifolium* and common pipette for *B. thermicus*. The larvae were then returned to their cages together with new tiles on which periphyton had been grown. The cage nets were brushed daily with a toothbrush to remove periphyton, which tended to clog the mesh.

The experiments continued until all individuals had either died or emerged (29 days for *E. latifolium*, 69 days for *B. thermicus*) in the controls. The existence of emerged adults, which were often found inside the cages screened by acrylic resin board (20 × 10 cm, 3 mm thick) as subimago and/or their exuviae, was checked daily. Dead individuals were removed during daily cage maintenance.

Results

Fenobucarb concentration residues in the channel water

Table 1 shows concentrations of fenobucarb residues in the water of model streams 0, 24, and 48 h after each one application (0, 1, 2, 4, 8, 16 μg l^{-1} concentrations). Measured concentrations of fenobucarb in the model streams were between 74% and 83% for the nominal concentrations immediately after application and decreased to 52% and 64% of their nominal concentrations of 1 and 2 μg l^{-1} after 24h application. However,

Table 1. Concentration (μg l^{-1}) of fenobucarb residues in the waters of the model streams

Nominal concentration (μg l^{-1})	Concentrations (μg l^{-1}) of fenobucarb residues (%)		
	0 h	24 h	48 h
0	0	0	0
1	0.7 (74)	0.5 (52)	0.4 (43)
2	1.6 (80)	1.2 (64)	0.9 (45)
4	3.3 (83)	2.8 (70)	2.2 (55)
8	6.1 (76)	5.5 (69)	5.0 (63)
16	13.3 (83)	11.4 (71)	10.1 (63)

the nominal concentrations at greater than 4 μg l^{-1} in the model streams remained high (61–71%) after 24 h application. The fenobucarb residues decreased to 55–63% of the nominal concentrations after 48 h application (Table 1).

Chronic effects of fenobucarb on the growth of E. latifolium

The chronic effects of fenobucarb on the growth of the larval populations of *E. latifolium* in model streams are shown in Figure 1. In the control, the individuals of the first growth stage (head width $\leq$ 1.9 mm) were observed after 24 days, but their numbers decreased after the applications of fenobucarb in the treated streams.

Individuals of the second growth stage (head width 2.0 to 2.9 mm) gradually decreased in number by growing to the third growth stage and disappeared by 67 days. Although the individuals of the second growth stage also gradually decreased in number up to 58 days after the applications (1 and 2 μg l^{-1}), 5 to 10 individuals were still present by the end of the experiment.

The individuals of the third growth stage (head width ($\geq$ 3.0 mm) gradually decreased in number up to 20 days after the applications and the number of emergent individuals also remained at 20 to 25 by the end of the experiment. On the other hand, the individuals of the third growth stage in the control also gradually decreased by emerging and disappeared within 69 days after the beginning of the experiment.

The number of larvae for the nominal concentrations of 1 and 2 μg l^{-1} showed a gradually decrease and the number of emergent individuals increased to 21 and 18 individuals, respectively 21 days after the application (Fig. 1). The number of larvae for the nominal concentrations of 4 and 8 μg l^{-1} showed a marked decrease ten days after the application. For 4 μg l^{-1}, the number of emergent individuals increased to 8 individuals sixty days after the application. For 8 μg l^{-1}, the number of emergent individuals increased to 3 individuals eleven days after the application. For 16 μg l^{-1}, all of the larvae were dead within 24 h after the application except for one emergent individual.

Chronic effects of fenobucarb on the growth of B. thermicus

The chronic effects of fenobucarb on the growth of the larval populations of *B. thermicus* in model streams are shown in Figure 2. Larval individuals of the first growth stage (head width $\leq$ 0.7 mm) disappeared for the first three days for each concentration and the control. For the control, the larvae of the second growth stage (head width 0.8 to 0.9 mm) and the third growth stage (head width $\geq$ 1.0 mm) decreased in number by growth and emergence until 19 and 29 days, respectively. The number of larvae at 1, 2, and 4 μg l^{-1} decreased in 10 to 15 days after the applications because of the increase in larval mortality. The number of emergents appeared at 28 (1 μg l^{-1}), 23 (2 μg l^{-1}), and 9 (4 μg l^{-1}) individuals. All of the larvae were dead within 3 days after applications of 8 and 16 μg l^{-1} and only one emergent appeared at 8 μg l^{-1}.

Chronic effects of fenobucarb on the emergence of E. latifolium

The changes in the emergence and larval mortality of *E. latifolium* are shown in Figure 3. For the control experiment, the number of emergents increased gradually during the 30 days after the application and almost all of them had emerged completely by 69 days (larval mortality 5%, rate of emergence 95%).

For 1 and 2 μg l^{-1}, the number of emergents increased over the first 20 days after application. The emergent rates were 63.4% at 1 μg l^{-1} and 57.5% at 2 μg l^{-1}, respectively by the end of the experiment. Larval mortalities were 10% to 20% and 20% to 30% (Fig. 3). For 4 μg l^{-1}, the rate of emergence increased to 10% and larval mortality increased to 60% 11 days after the application. The rates of emergence and the larval mortality reached 22.5 and 77.5%, respectively after 59 days. For 8 μg l^{-1}, the rate of emergence was 7.7% and larval mortality was 64.1% 11 days after the application. The rate of emergence remained at 7.7% and the larval mortality reached 92.3% 31 days after the application. For 16 μg l^{-1}, the rate of emergence reached 2.5% (one emergent

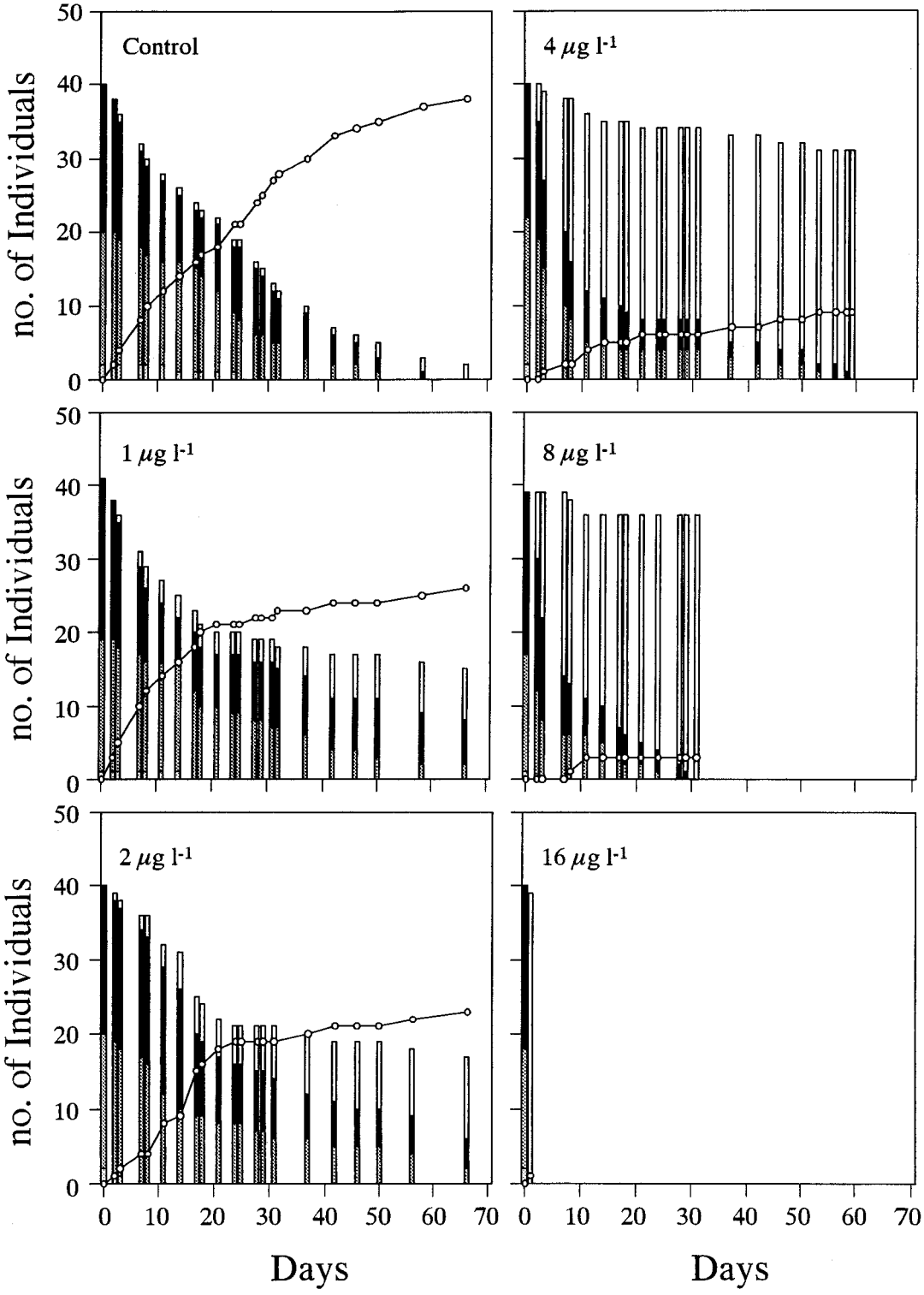

Figure 1. Changes in three larval growth stages of individuals and emergence of individuals of *E. latifolium* introduced into the model streams after applications of fenobucarb. ▫ First larval growth stage (head capsule width of ≤ 1.9 mm); ▨ Second larval growth stage (2.0–2.9 mm); ■ Third larval growth stage (≥ 3.0 mm); □ Dead individuals; –○– Emergence of individuals.

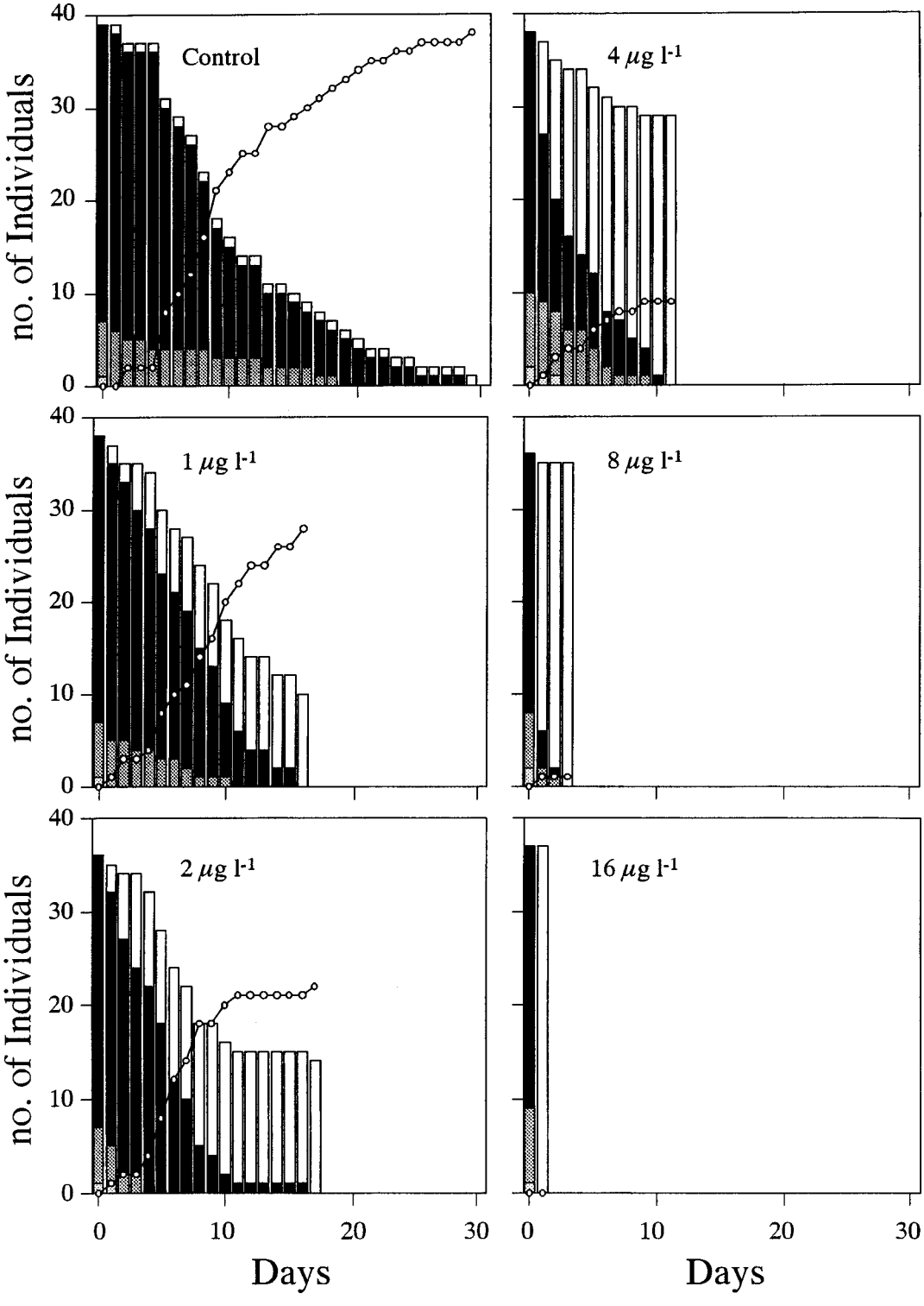

Figure 2. Changes in three larval growth stages of individuals and emergence of individuals of *B. thermicus* introduced into the model streams after applications of fenobucarb. □ First larval growth stage (head capsule width of ≤ 0.7 mm); ▨ Second larval growth stage (0.8–0.9 mm); ■ Third larval growth stage (≥ 1.0 mm); □ Dead individuals; –○– Emergence of individuals.

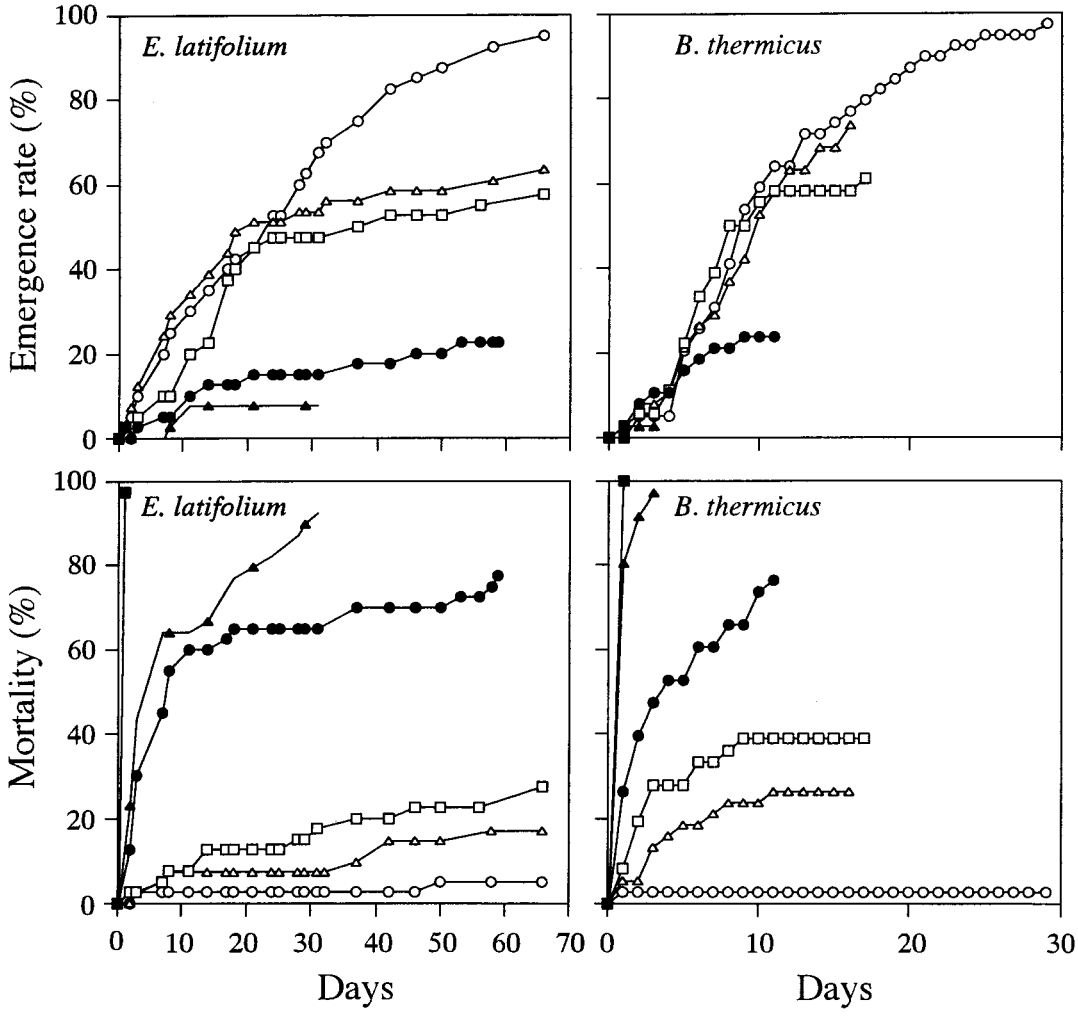

Figure 3. Changes in the rate of emergences and larval mortality of *E. latifolium* and *B. thermicus* after applications of fenobucarb. –○– 0 μg l^{-1}; –●– 1 μg l^{-1}; –□– 2 μg l^{-1}; –■– 4 μg l^{-1}; –△– 8 μg l^{-1}; –▲– 16 μg l^{-1}.

individual) and the larval mortality reached 97.5%, one day after the application.

Chronic effects of fenobucarb on the emergence of B. thermicus

The changes in the emergence and larval mortality of *B. thermicus* are shown in Figure 3. For the control experiment, the rate of emergence greatly increased for the first ten days and reached 97.4% in 29 days after the beginning of the experiment; the final larval mortality was 2.6%.

The rate of emergences increased for the first ten days and reached 52.6% at 1 μg l^{-1} and 55.6% at 2 μg l^{-1} (59% for the control). Larval mortality gradually increased for the first ten days and reached 26.3% at 1 μg l^{-1} and 38.9% at 2 μg l^{-1}. Mortalities at 1 and 2 μg l^{-1} increased five days after the applications and leveled out at the end of the experiment. For 4 μg l^{-1}, the rate of emergence increased after five days and reached 15.8% (20.5% for the control), after which the rate of emergence slowly increased to 23.7% at the ninth day (53.8% for the control). The larval mortality was rapidly increased and reached 76.3% 11 days after the application. For 8 and 16 μg l^{-1}, the rates of emergence were 2.8 and 0%, respectively. Larval mortality reached 97.2% after

three days and 100% the day after the application. The emergent individuals increased constantly with each concentration.

Discussion

The effects of pesticides on benthic communities in rivers have been investigated previously using model streams (Hatakeyama et al., 1994; Yasuno et al., 1985; Hatakeyama et al., 1997). Hatakeyama et al. (1997) reported that the mortality of the larvae that had been introduced into an outdoor channel carrying water from the Kokai River in early June increased to 100% within 2 weeks when the concentrations of fenobucarb, malathion, and pyridaphenthion increased to a peak of 5.9 $\mu g\ l^{-1}$.

We studied the chronic effects of an insecticide, fenobucarb, on benthic macroinvertebrates, namely Ephemeropteran larvae, dwelling in a site considered to be unpolluted with pesticides. The concentrations of fenobucarb residues in the water of the model streams have remained at relatively high levels, that is, 69% to 71% of the nominal concentrations 24 h after the applications, except for the low concentrations (1 and 2 $\mu g\ l^{-1}$) (Table 1). On the other hand, for 10 to 160 $\mu g\ l^{-1}$ the rate of residues in the model streams remained high: 78% to 85% of nominal concentrations 24 h after the applications (Tada, 1998). These results indicate that fenobucarb is relatively water-soluble (660 $mg\ l^{-1}$ water at 20°C) and can not be decomposed by microorganisms in tap water (Kanazawa, 1992), although it is reported to be decomposed by microorganisms in stream water (Kanazawa, 1992).

The three main species of Ephemeropteran larvae (*I. sonychia japonica*, *E. latifolium*, and *Ecdyonurus yoshidae*) were collected at the Kinu River sampling site. The *E. latifolium* larvae, which were dwelling in riffles, were relatively sensitive (24 h-LC_{50}: 13 $\mu g\ l^{-1}$; 48 h-LC_{50}: 11 $\mu g\ l^{-1}$) to fenobucarb (Tada, 1998).

In this study, the chronic effects of fenobucarb on the growth and the emergence of the larval populations of *E. latifolium* in model streams were examined (Figs. 1 and 3). The number of larvae for the nominal concentrations of 4 and 8 $\mu g\ l^{-1}$ showed a marked decrease ten days after the application (Fig. 1). For 4 $\mu g\ l^{-1}$, the rates of emergence and the larval mortality reached 22.5 and 77.5%, respectively after 59 days (Fig. 3). For 8 $\mu g\ l^{-1}$, the rates of emergence and the larval mortality reached 7.7 and 92.3%, respectively after 31 days.

For 1 and 2 $\mu g\ l^{-1}$, the emergent rates were 63.4% at 1 $\mu g\ l^{-1}$ and 57.5% at 2 $\mu g\ l^{-1}$, respectively by the end of the experiment. Larval mortalities were 10% to 20% and 20% to 30%, respectively (Fig. 3). So the numbers of 20% to 30% of larvae remained mature and did not emerge until 69 days after the beginning of the experiment. These results suggest that some of the larvae of *E. latifolium* could not emerge normally by chronic effects of fenobucarb at 1 and 2 $\mu g\ l^{-1}$.

On the other hand, Iwakuma et al. (1988a, b) reported for the River Kawamata, Ibaraki Prefecture that when bentiocarb, oxadiazon, fenobucarb, and fenthion reached between 3 and 4 $\mu g\ l^{-1}$, the density of *B. sahoensis* decreased to one-tenth that during the period of low pesticide contamination. These results were possibly due to latent and subtle overall pesticide effects during the pesticide spraying period. In the latest study (Tada, 1998), the larvae of *B. thermicus* collected in the Kozakura River were so highly sensitive to fenobucarb (24 h-LC_{50}: 4 $\mu g\ l^{-1}$; 48 h-LC_{50}: 2 $\mu g\ l^{-1}$) that they were considered to be directly affected by pesticides in rivers.

In this study, the chronic effects of fenobucarb on the growth and the emergence of the larval populations of *B. thermicus* in model streams (Figs. 2 and 3). The number of larvae at 1, 2, and 4 $\mu g\ l^{-1}$ decreased in 10 to 15 days after the applications because of the gradual increase in larval mortality for the first ten days and reached 26.3% at 1 $\mu g\ l^{-1}$ and 38.9% at 2 $\mu g\ l^{-1}$. The rate of emergences reached 52.6% at 1 $\mu g\ l^{-1}$ and 55.6% at 2 $\mu g\ l^{-1}$ (59% for the control). For 4 $\mu g\ l^{-1}$, the larval mortality was rapidly increased and reached 76.3% 11 days after the application. The rate of emergence reached 15.8% (20.5% for the control). These results suggest that the emergence of the larvae was so highly sensitive to fenobucarb by using model streams in the present study.

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