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Overall pesticide effects on growth and emergence of two species of Ephemeroptera in a model stream carrying pesticide-polluted river water

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The overall pesticide effects on mortality, growth and emergence of two dominant species of Ephemeroptera in Japanese rivers, *Epeorus latifolium* and *Ecdyonurus yoshidae*, were assessed using an outdoor channel carrying water from the Kokai River. Young larvae collected from another river were introduced into cages in the channel after their body lengths were measured. The concentrations of 17 pesticides were measured three times a week from April to August 1993. A shrimp mortality test on water samples was conducted concurrently. A relationship between the high mortality of the shrimp in the water samples and of *E. latifolium* in the channel was recognized. The mortality of *E. yoshidae* increased only when the shrimp mortality increased drastically in early June, reflecting the difference in insecticide susceptibility between the two mayfly species. Almost all the larvae which had been introduced into the channel in winter and/or early spring, when pesticides had cleared from the river, emerged in spring, although their growth rate during the winter was low. The shrimp mortality in the river water samples was caused by the overall pesticide toxicity. The increase in the mortality of the mayfly larvae in the channel might be due to the overall pesticide toxicity, although their concentrations were low and varied independently.

Keywords: mayfly larvae; pesticides; assessment; shrimp mortality; biomonitoring.

Introduction

Mayfly larvae are the main, primary consumer, grazing periphyton attached on pebbles and/or stones in rivers (Cummins, 1979). Investigation of the pesticide effects on these primary consumers is essential in assessing the overall pesticide effects on river ecosystems. However, it is difficult to simulate pesticide-polluted conditions in rivers, because the concentrations of many pesticides vary unpredictably at low levels from spring to summer (Maru, 1991; Iwakuma *et al.*, 1993; Hatakeyama *et al.*, 1994). Two species of mayflies were collected from a river and exposed directly to pesticide-polluted river water in a channel deployed beside the Kokai River. *Epeorus latifolium* is one of the dominant species among aquatic insects in Japanese rivers. *Ecdyonurus yoshidae* is also a dominant species and preferably inhabits the margins of rivers where the flow rate is relatively low. Both species are easily distinguished from other species, even in their fairly young stages, based on characteristic spots covering the gill lobes of the former and the body surface of the latter (Gose, 1985). The overall pesticide effects on these mayfly larvae were assessed in comparison with shrimp mortality in water samples and frequent pesticides analyses which were all conducted concurrently.

It is well known that the insecticide susceptibility of the shrimp is generally high. A freshwater shrimp, *Paratya compressa improvisa*, has been used as a biomonitoring test organism to assess the overall pesticide effects on aquatic organisms in rivers (Hatakeyama and Sugaya, 1989; Hatakeyama *et al.*, 1991). The correlation between the shrimp mortality in the river water samples and the overall pesticide effects on mayfly larvae (present investigation) and/or benthic communities (Hatakeyama, 1995) was investigated to extrapolate the shrimp biomonitoring results to assess the pesticides effects at the ecosystem level. The effects of insecticides on benthic communities in rivers have been investigated largely in mountain or forest streams (Symons, 1977; Eidit, 1981; Yasuno *et al.*, 1982; Hatakeyama *et al.*, 1990). Control areas were selected from the upper reaches and/or rivers adjacent to those being investigated, because generally mountain streams were not polluted with pesticides. However, assessment of the pesticide effects on benthic communities in rivers flowing past farm land is difficult, because many environmental factors affect benthic communities and, therefore, it is difficult to find suitable control rivers.

Experiments conducted during the winter season were regarded as controls for pesticide-polluted rivers in the present investigation, because pesticides had almost cleared from the Kokai River in winter or early spring just before the pesticide application season.

Materials and methods

The Kokai River and the water channel

The Kokai River flows through a rural district mainly composed of rice paddy fields south from its headwaters, ~160 km northeast of metropolitan Tokyo and joins the Kinu River, 110 km downstream. The annual mean flow rate was ~30 $m^3 s^{-1}$ at the station selected for the present investigation, 18 km upstream from its confluence with the Kinu River. The river width (on the water surface) was usually ~50 m and the water depth ranged from 1 to 3 m around the station, following water supply to the paddy fields in the rice planting season and/or rainfalls. The river water was continuously pumped up into a channel made of polyvinyl chloride boards (length 4 m, width 40 cm and height 40 cm). The current speed on the water surface in the channel was 12–16 $cm\,s^{-1}$ and the depth was 14–16 cm.

Pesticide analysis in the river water samples

River water samples, for pesticide analysis and shrimp mortality tests, were collected from the channel in duplicate 500 ml glass flasks three times a week (on Monday, Wednesday and Friday) from April to August 1993. The temperature, pH and conductivity of the water in the channel were measured by portable meters (for temperature and pH, model HM-10 and for EC, model CM-K1, TOA Electronics Ltd).

Each river water sample (500 ml) was filtered through a Whatman GF/C glass fibre filter which had been heated at 470 8C 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 the excess water. The pesticides in the column were eluted with 0.5 ml of analytical grade acetone (>99.8%, Wako Pure Chemical Industries, Ltd) with centrifugation at 1000 r.p.m. for 12 min in a glass conical centrifuge tube (10 ml). This procedure was repeated once, followed by a final centrifugation with 0.5 ml acetone at 3000 r.p.m. for 15 min (total 1.5 ml acetone used). The combined samples were analysed 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 a NPD-detector, together with an inner standard (50 μl of 1 $\mathrm{mg\,l^{-1}}$ azobenzene) for the pesticides analysis. The column temperature and flow rate of the carrier gas (He) were 50–300 °C and 54 $\mathrm{ml\,min^{-1}}$, respectively.

Shrimp mortality tests of the water samples

The water sample was transported to our laboratory within 30 min and poured into three 100 ml glass beakers. After adjustment of the water temperature to 22 ± 1 °C, seven 4 week old shrimp individuals were introduced into each beaker (21 individuals were used for one sample). Dechlorinated tap water (pH 7.8 and hardness ~80 $\mathrm{mg\,l^{-1}}$ as $CaCO_3$) was used as control water as well as rearing water. The rearing methods for the freshwater shrimp, *P. compressa improvisa* and the test methods have been published previously (Hatakeyama and Sugaya, 1989). The shrimp mortality was checked on days 1, 2, 3, 4, 7 and 14. One grain of dried fish food (~40 mg, Nihon Haigou Shiryou) was added to each beaker on days 4 and 7 to avoid starvation of the test organisms. Dead individuals were removed at observation times. Moribund individuals which were very close to death, which fell down to the bottom of the beaker and could not locomote at all, were counted as dead because most of them died within the same day. The control mortality was negligible throughout the investigation period. Therefore, the shrimp mortality in the water sample was calculated by dividing number of dead individuals by 21 for every water sample.

Collection of mayfly larvae

Mayfly larvae were collected from a sampling station in the upper reaches of the Kinu River, because this site was considered to be unpolluted with pesticides. The dominant benthic species at this station were several Ephemeropteran species such as *E. latifolium*, *E. yoshidae*, *Isonychia japonica*, *Baetis* spp. and *Stenopsyche marmorata* (Trichoptera), which often comprise the maximum benthic biomass in Japanese rivers. The periphyton which developed on the stone surfaces was composed of diatoms.

Young larvae of *E. latifolium* and *E. yoshidae* (both Heptageniidae) were collected using a Surber net (33 × 33 cm and 0.45 mm mesh size). The surfaces of pebbles were rubbed with fingers to dislodge the benthos into the Surber net along the current. Collected samples were discharged into white plastic trays filled with river water and larvae of the desired size were sucked into a flattened pipette and discharged into polyethylene bags containing ~500 ml river water. The bags were bubbled with compressed air for transportation to our institute or directly to the channel. The mortality of the larvae during transport (~1.5 h) was negligible.

Assessment of pesticide effects on mayfly larvae

Experiments were conducted with *E. latifolium* seven times from April to August 1993 during the pesticide spraying season and twice during the winter and early spring 1994, a season in which pesticides had cleared from the river. Experiments were done at the same time with *E. yoshidae*, although two experiments in the pesticide spraying season were omitted due to shortages in the numbers of available individuals.

The body lengths of larvae were measured through a binocular microscope situated beside the channel. For the experiment, the larvae of both species with body lengths ranging from 5 to 7 mm were selected. After measurement of the body length, five (in the case of *E. latifolium*) or six (in the case of *E. yoshidae*) larvae were introduced into stainless steel wire net (1.2 mm mesh size) cages (18 cm × 8 cm × 8 cm high). Five and three replicates were used, usually, for the former (30 individuals) and the latter (18 individuals). These cages were settled onto a cage supporter made of stainless steel wire to adjust the water depth in the cage to ~3 cm.

An unglazed white tile (12 × 6 cm and 6 mm thick), on which periphyton had been grown for 2 weeks as food for the test organisms, were submerged in each cage. Four small tiles (2 cm diameter and 4 mm thick) were inserted between the four corners of the tile and the cage bottom to make space for the larvae, because they prefer to hide under or between pebbles in rivers to escape from feeding pressure by fish during the day.

For weekly measurements of body length, the larvae in the cage were transferred to plastic trays filled with river water. The body lengths of the larvae were measured through a binocular microscope after sucking up each respective larvae from the tray and transferring it to a watch glass (diameter 4 cm) using a flattened pipette. After measurement of the body length, the larvae were returned to their cages with new tiles on which periphyton had been grown as described above. During the several weeks of each experiment, 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. The existence of emerged adults, which were often found around the cages as subimago and/or their exuviae, was checked daily. Dead individuals were removed when found during daily cage maintenance.

Mayfly larvae acute toxicity test

Small indoor flow-through channels (length 40 cm and width 6 cm) made of plastic boards were used to test the acute toxicity levels of fenitrothion (organophosphorus insecticide) to the two species of Ephemeroptera. The flow rate and current speed of the water (groundwater, 22 6 1 8C) were 800 ml min^{-1} and ~12 cm s^{-1}, respectively. Stock solutions of fenitrothion (5–6 concentrations) were prepared using ethanol (99%, Wako Pure Chemical Industries, Ltd). These stock solutions were introduced into the heads of channels using peristaltic pumps (200 ml per day) and mixed with the ground water there by bubbling with air. Seven larvae of *E. latifolium* or *E. yoshidae* were introduced into two replicate stainless steel net cages (12 × 5.5 × 4 (height) cm and 0.8 mm net size) The mortality was checked after 24 h. At the end of the acute toxicity test, the body lengths of the tested organisms were measured. The LC_{50} values were calculated using probit (Finney, 1978) regression equations.

Periphyton growth rate on the tiles

Two tiles (length 12 cm and width 6 cm) were submerged into a channel for 3 days twice weekly (on Monday and Friday) from April to August 1993 to estimate the periphyton growth rates by measuring chlorophyll *a*. A portion of the periphyton which were collected from the two tiles was trapped on a glass fibre filter (Whatman GF/C) and extracted in 90% methanol (10 ml, conical centrifuge test tube) in a refrigerator for ~7 days. The optical density of the cleared samples, centrifuged at 2000 r.p.m. for 10 min, was measured at wavelengths of 664 and 750 nm to calculate the amounts of chlorophyll *a* in the extracted samples (Standard Methods, 1992). The upper part of the channel was continuous fluorescent light (~6000 lux) and was provided on the water surface in the upper part of the channel to estimate the algal growth rate under a constant light regime (Hatakeyama *et al.*, 1994).

Algal species in formalin-fixed periphyton samples were identified without acid treatment and the numbers of cells per unit area (mm^{-2}, after each 50 ìl sample had been spread out under a 24 × 32 mm^2 coverglass) were counted through a microscope (× 600 magnification).

Results

Environmental factors of the Kokai River

The water temperature ranged from 14 to 26 8C from spring to summer 1993 (Fig. 1) and from 4 to 17 8C from winter to early spring 1994 (Fig. 6). The mean water temperature in summer 1993 (from July to August) was 20.4 6 3.2 8C ($n = 57$). The pH between April and August ranged from 7.0 to 8.3 with a mean value of 7.5 6 0.3 ($n = 57$). The electroconductivity varied between 100 and 280 ìS cm^{-1}, which is inversely proportional to the water level of the Kokai River which usually increased following rainfalls. The river bed around the sampling station was covered by silty sand and vegetation along the bank was mainly weeds with a few trees.

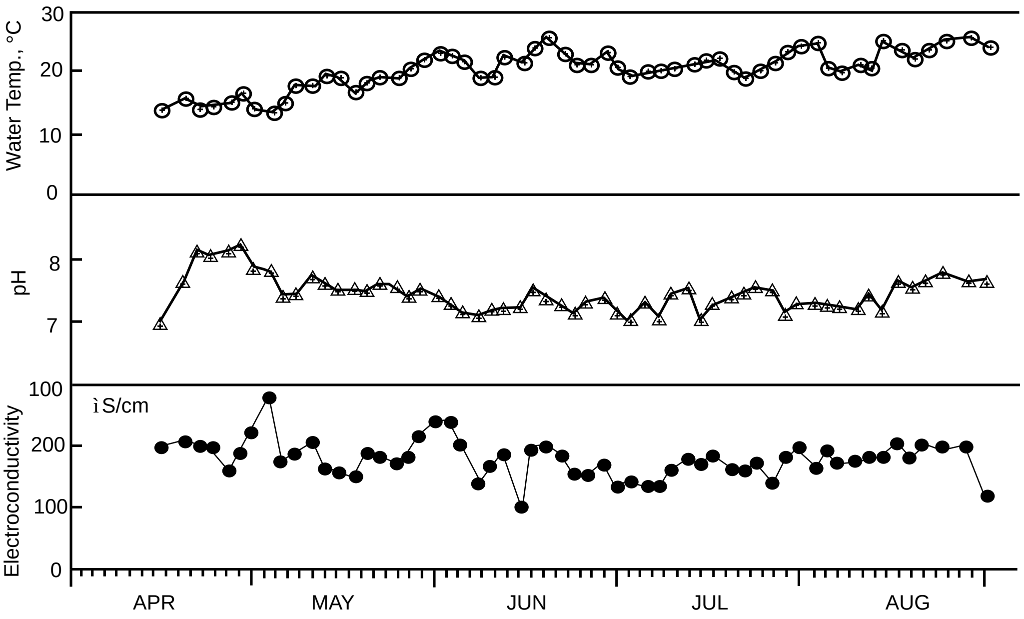

Fig. 1. Water temperature, pH and electroconductivity of the water in the channel carrying the Kokai River water from April to August 1993.

Pesticide concentrations in the river water

Young rice seedlings are transplanted, from late April to mid-May, into the paddy fields spreading out on both sides of the Kokai River. These paddies are cultivated with inputs of river waters, nutrients and pesticides. Several herbicides are sprayed on the paddy fields before and after the rice planting season. The Kokai River was contaminated with many kinds of pesticides from April to August 1993, although their concentrations were generally lower than 10 ìg l^{-1} except for molinate (a herbicide), which increased to 17.2 ìg l^{-1} at its peak (Fig. 2, herbicides, white bars). Four herbicides, simazine, butachlor, oxadiazon and mefenacet, appeared mainly in May. In contrast, the peak concentrations of molinate, simetryn, thiobencarb and chlornitrofen were detected in early June (Fig. 2). The concentrations of these herbicides changed with similar patterns and they continued to be detected for 1–2 months.

Six insecticides were detected occasionally in the period from May to August (Fig. 2, black bars). Of these, five were organophosphorus insecticides and another was the carbamate insecticide, fenobucarb. The concentrations of fenobucarb, malathion and pyridaphenthion increased to peaks of 5.9, 2.4 and 6.9 ìg l^{-1}, respectively. The concentrations of the other insecticides remained lower than 1 ìg l^{-1} throughout the investigation period. Two fungicides, iprobenfos and edifenphos (Fig. 2, grey bars), were detected from July to August at peak concentrations of 5.0 and 1.2 ìg l^{-1}, respectively.

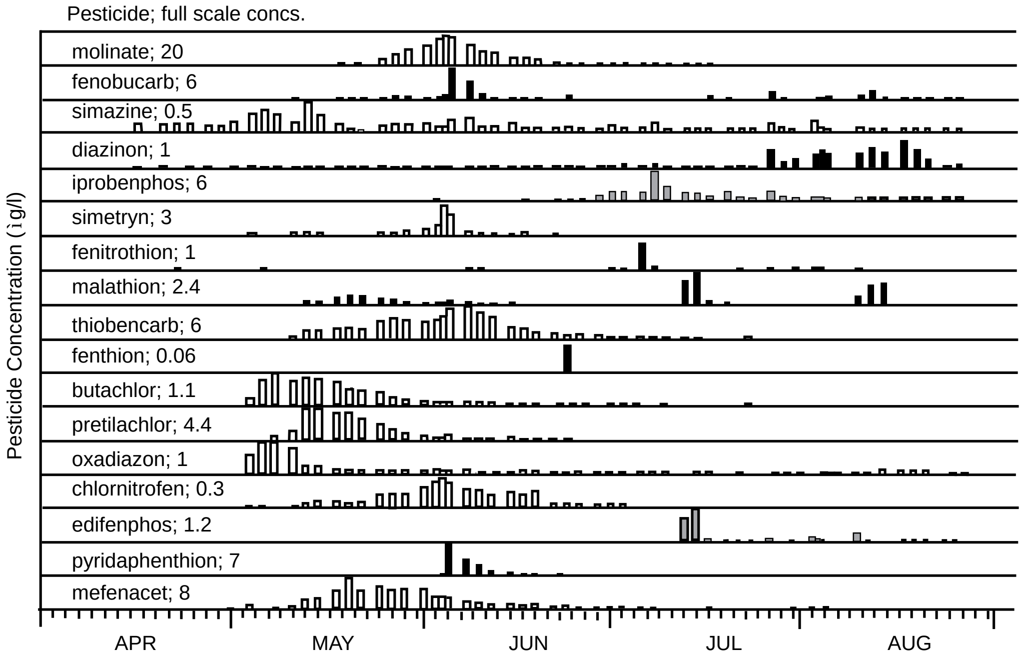

Fig. 2. Temporal changes in the concentrations of 17 pesticides in river water samples of the Kokai River. The numbers adjacent to the pesticide names are full scale pesticide concentrations (ìg l^{-1}). White bars, herbicides; black bars, insecticides; grey bars, fungicides.

Shrimp mortality in the river water

The temporal changes in the shrimp mortality in the river water samples in 1993 are illustrated in Figs 3 (4 days) and 4 (4 days, 1 week and 2 weeks). The shrimp mortality increased markedly in early June. We compared the observed 4 day mortality and the expected mortalities, which were calculated by summing the respective mortalities (Fig. 3, white bars) at the respective insecticide concentrations in the water samples. These respective mortalities were calculated by probit dose–response regression equations (Hatakeyama, 1995). The maximum expected mortality due to the joint effect of fenobucarb and pyridaphenthion reached ~160% on 4 June. The mortalities observed in mid-July and mid-August were much lower than the expected mortalities, which were calculated based on mortalities due to several insecticides, such as fenobucarb, diazinon and malathion (Fig. 3). On the contrary, the 4 day mortality observed on 5 July was lower than the expected mortality owing to fenitrothion.

The remarkable shrimp mortality in early June decreased towards mid-June (Fig. 4). However, 100% mortality (1 week) occurred again in mid-July and mid-August, possibly induced primarily by the several insecticides described above. The shrimp mortality at 2 weeks, ranging from 10 to 50%, was often observed from May to August.

Insecticide susceptibility of two species of mayfly

The fenitrothion susceptibility (24 h LC_{50}) of *E. latifolium* larvae was approximately three times that of *E. yoshidae* larvae of similar body length, 6.7 mm for the former and

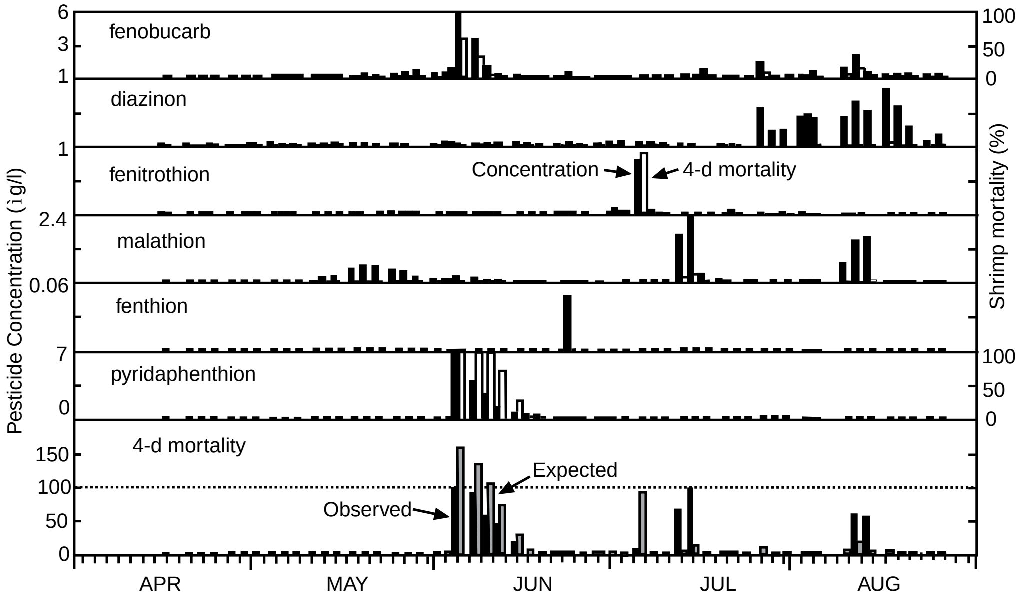

Fig. 3. Upper: insecticide concentrations in the river water samples (left, black bars) and 4 day shrimp mortality (right, white bars) in this water. The mortalities were calculated using probit regression equations. Lower: observed 4 day shrimp mortality in the river water samples (left, black bars) and the expected mortality (right) calculated by summing up the respective mortalities.

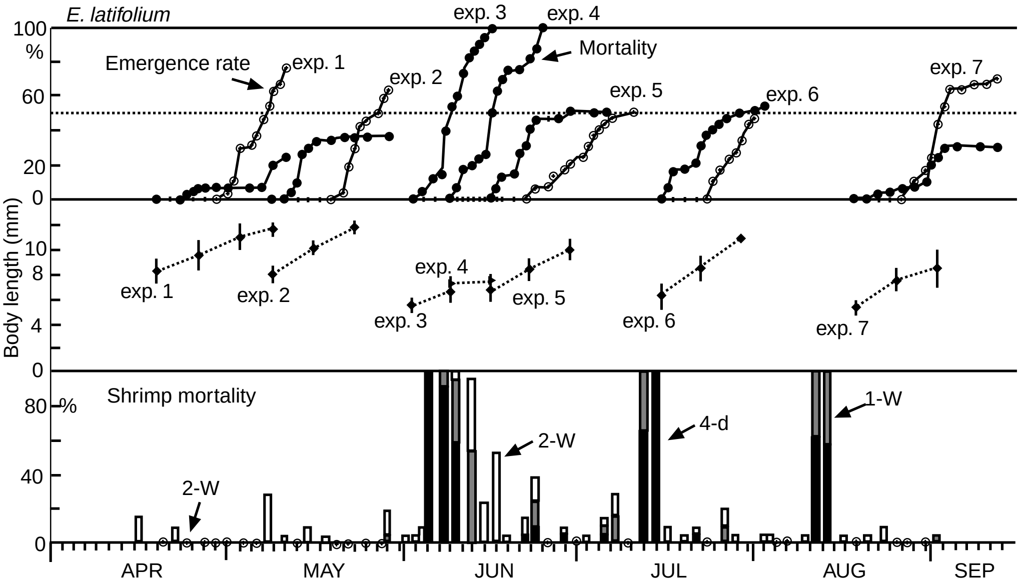

Fig. 4. Growth, mortality and emergence rates of *E. latifolium* larvae in a channel carrying water from the Kokai River from spring through to summer 1993. The 4 day, 1 week and 2 week shrimp mortalities in the river water samples are also illustrated to show the overall pesticide toxicity to aquatic organisms.

5.5 mm for the latter (Table 1). The fenitrothion susceptibility of *E. latifolium* was approximately two times greater in young stage (body length ~6.7 mm) compared to the full grown larvae (body length ~10.7 mm) (Table 1).

Overall pesticide effects on E. latifolium

In the two experiments begun in April and May, the larvae grew ~250 and 270 ìm per day and finally 77 and 63% of initial larvae emerged within 4 weeks, respectively, the other larvae having died (Fig. 4, experiments 1 and 2). In experiment 1, the mortality increased to ~25% in the later stage in early May. In experiment 2, the mortality of the larvae increased to ~30% during the initial stage of the experiment from early to mid-May and it stayed at 40% thereafter. In both experiments, the mortality increased in the period from early to mid-May, when several pesticides started to enter the river (Fig. 2).

In experiments 3 and 4, the mortality of the larvae which had been introduced into

Table 1. The 24 h LC_{50} values of fenitrothion to two species of Ephemeroptera in different growth stages

Species	Body length (mm)	24 h LC_{50} (ìg l^{-1})
E. latifolium	10.73 6 1.22	1.83 (1.27–2.47)
	8.60 6 1.04	1.41 (1.01–1.78)
	6.70 6 1.37	1.09 (2.19–5.01)
E. yoshidae	5.52 6 0.81	3.24 (2.19–5.01)

Body length, mean 6 SD ($n = 14$), LC_{50} values (95% fiducial limit).

the channel in early June increased to 100% within 2 weeks and the growth of the surviving individuals during the initial week was low, ~150 ìm per day (Fig. 4). The shrimp mortality in the river water samples also increased markedly during the same period (Fig. 4). Approximately 50% of the larvae introduced into the channel in mid-June (experiment 5) and mid-July (experiment 6) died. Those surviving emerged within 1 month and 3 weeks, reflecting the differences in growth rate, 240 and 335 per day, in experiments 5 and 6, respectively. In experiment 5, the mortality increased rapidly to ~50% in the initial stage in mid-June, during which period the shrimp mortality was relatively high and the number of emerging individuals increased slowly from mid-June compared to other experiments (Fig. 4). The emergence rate of the larvae which were introduced into the channel in mid-August (experiment 7) reached 70%, the other 30% of the larvae having died before emergence.

On the other hand, the larvae which had been introduced into the channel on 28 January 1994 started to emerge from 1 April and finally 94% of them had emerged by mid-April, although their growth rate during winter was low. The growth rates in February, March and early April were estimated to be ~24, 94 and 110 ìm per day, respectively (Fig. 6, experiment 8). The growth rate of the larvae which were introduced into the channel on 30 March increased with increasing water temperature to ~260 ìm per day (Fig. 6, experiment 9) and 97% of these larvae (28 out of 29) emerged between 24 April and the beginning of May.

Overall pesticide effects on E. yoshidae

Of the larvae introduced into the channel in early May, 75% emerged and 25% (of 24 total individuals) died during the initial stage of the experiment (Fig. 5, experiment 1).

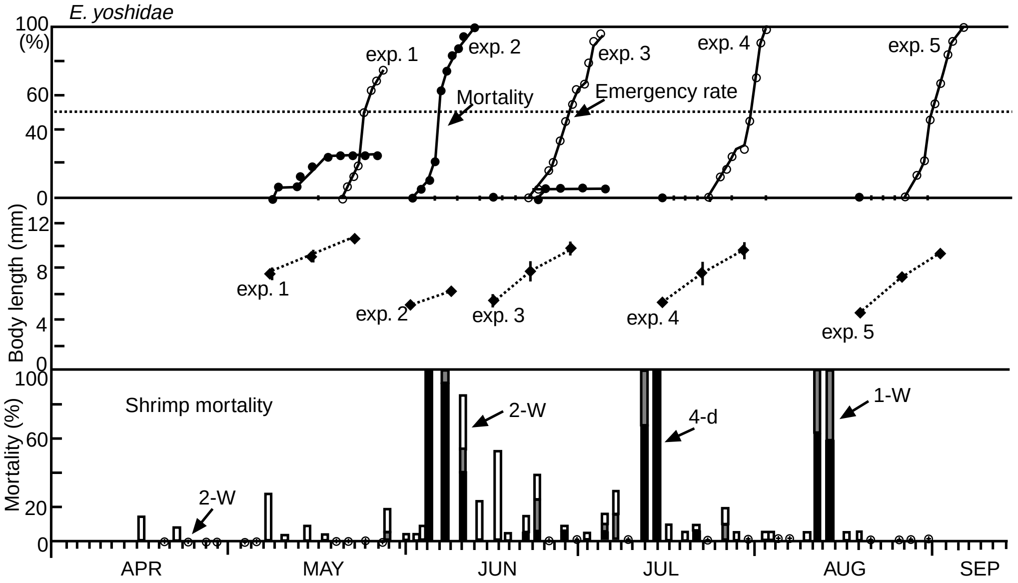

Fig. 5. Growth, mortality (d) and emergence rates (s) of *E. yoshidae* larvae, which were introduced into the channel carrying water from the Kokai River from April to August 1993. The shrimp mortality in the river water samples is also illustrated to show the overall pesticide toxicity.

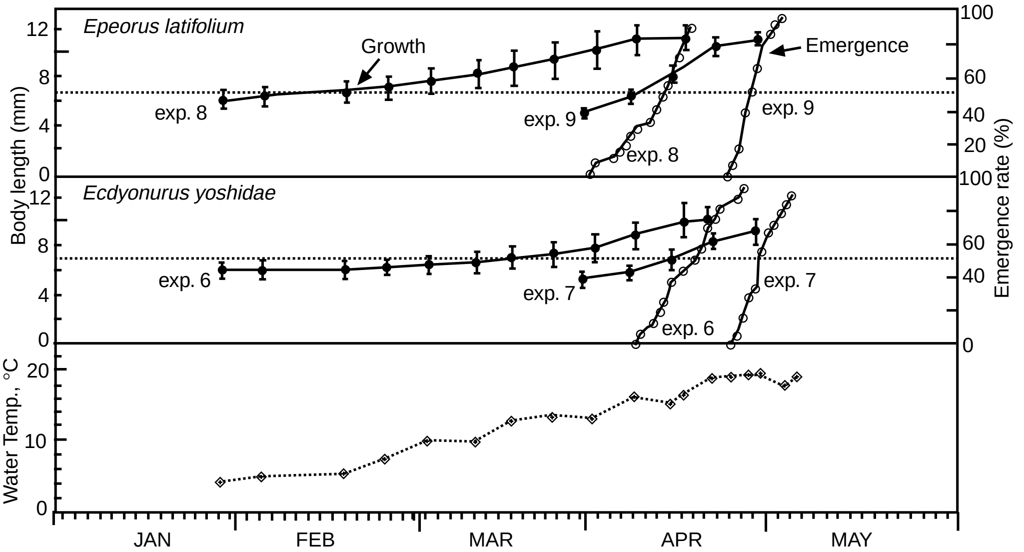

Fig. 6. Growth (d) and emergence rates (s) of two species of mayfly larvae, *E. latifolium* and *E. yoshidae*, introduced into the channel carrying Kokai River water in late January and late March 1994. The river water temperatures in the channel were recorded between 10:00 and 13:00.

The mortality of the larvae introduced into the channel on 1 June increased rapidly to 100% (experiment 2). However, the larvae introduced in mid-June emerged almost 100% (23 out of 24) by early July (experiment 3). Of the larvae introduced in mid-July (experiment 4) and mid-August (experiment 5), 100% emerged, in contrast to the 50% emergence rate for *E. latifolium*, possibly reflecting differences in insecticide susceptibility between the two species (Table 1).

The growth rate of *E. yoshidae* was lower than that of *E. latifolium* in the winter (Fig. 6). The growth rate of the larvae which were introduced into the channel on 28 January was almost negligible from late January to late February (~10 ìm per day), although it increased to 50 ìm per day in March with increasing water temperature. The larvae introduced into the channel in late January started to emerge on 9 April and almost all (26 out of 28) had emerged within 30 days. The larvae introduced into the channel on 30 March started to emerge on 9 April and ~90% (16 out of 18) had emerged by 2 May 1994. The experiment was interrupted by water supply trouble on 4 May, although two larvae still remained (Fig. 6).

Periphyton growth rate and algal species composition

The periphytons which grew on the tiles were mainly composed of several dominant species such as the diatoms *Melosira varians*, *Cyclotella* spp., *Nitzschia frustulum*, *Nitzschia palea* and *Synedra ulna* although around 12 taxa were identified (mostly to species level) through the investigation period (Fig. 7). *Clamydomonas* spp. and *Scenedesmus* spp. were dominant among several green algae present. The amount of periphyton provided as food for the mayfly larvae appeared sufficient, although the periphyton accumulation rate on tiles decreased in mid-May due to the joint effects of

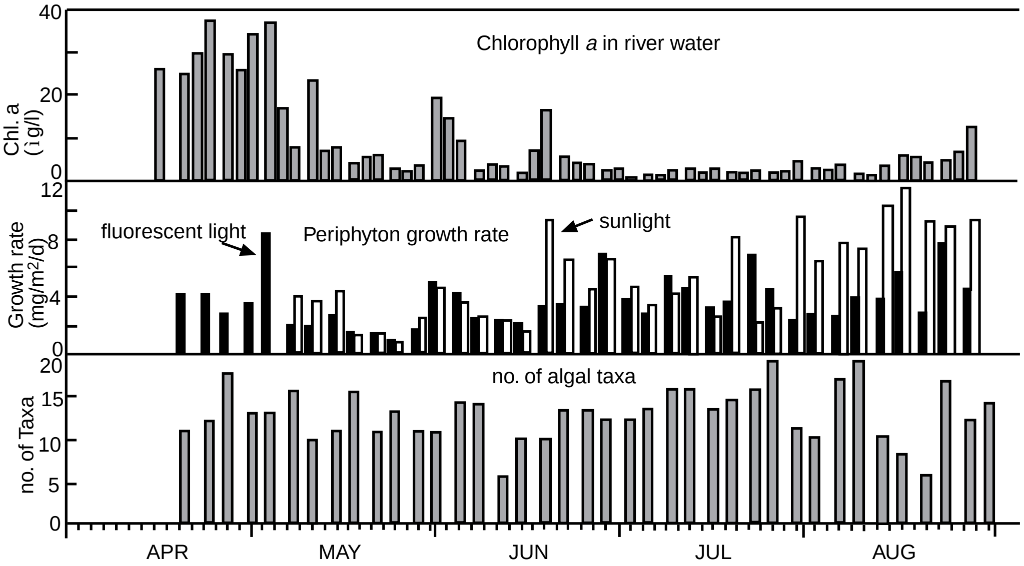

Fig. 7. River water chlorophyll *a* concentrations, rates of periphyton growth rate on the tiles submerged in the channel for 3 days and number of algal taxa in the periphyton samples. Periphyton growth rates (chlorophyll *a*, mg m^{-2} per day) were estimated for samples grown under both continuous fluorescent light (left) and sunlight (right, white bars).

several herbicides (Hatakeyama *et al.*, 1994) under sunshine as well as continuous fluorescent light (Fig. 7).

Discussion

The high mortalities of the shrimp in early June were attributed to the joint effects of pyridaphenthioin and fenobucarb, although the agreement between the observed and expected mortalities was not always satisfactory in the July and August (Fig. 3). However, we believe that the mortality of the shrimp in the river water samples was mainly caused by single, joint and/or synergistic effects of pesticides for the following reasons: (1) the shrimps in the river water samples stopped dying at the same time that pesticides were cleared from the rivers and (2) the shrimp mortality became zero even in the river water samples, in which 100% (1 day) mortality had been observed, after the pesticides were removed by passing through a ODS-column which binds hydrophobic chemicals (S. Hatakeyama, unpublished data).

The main purpose of the present investigation was to assess the relationship between the shrimp mortality in river water samples and the mortality of two species of mayfly larvae in the channel carrying the same river water. The exposure conditions for mayfly larvae in the channel were regarded as similar to those in rivers, although tiles were used in the channel for convenience despite the fact that mayfly larvae inhabit and scrape periphyton from pebbles.

The mortality of *E. latifolium* and *E. yoshidae* rapidly increased to 100% in the channel within several days when the shrimp mortality had increased to 100% in the river water samples in early June.

Generally, it is recognized that the insecticide susceptibility of various shrimps is particularly high among benthic organisms. The effects of pesticides assessed by shrimp mortality therefore have tended to be considered less serious on other aquatic organisms. However, the fenitrothion susceptibility of *E. latifolium* (Table 1) is similar to that of *P. improvisa compressa* (Hatakeyama and Sugaya, 1989).

In the two experiments using *E. latifolium*, the mortality started to increase in early May simultaneously with the increase of several herbicide concentrations such as simazine, butachlor, pretilachlor and oxadiazon. The synergistic effects of these herbicides should also be tested to assess the latent pesticide impacts on susceptible organisms, although the toxicity of each herbicide to the shrimp was low (Hatakeyama and Sugaya, 1989).

The mortality of *E. latifolium* reached 50 (experiment 6) or 30% (experiment 7) although the larvae had been introduced into the channel just after the shrimp mortality in the river water samples had ceased (Fig. 3, mid-July and mid-August). This suggests that some subtle pesticide toxicity remained in the river water and/or periphyton in particular. (Fig. 4). Dietary pesticide effects were first considered, although only pyridaphenthion was detected in the periphyton as an insecticide (unpublished data). The synergistic effects of herbicides accumulated in periphyton should also be evaluated to assess the overall pesticide effects on the grazer type mayfly larvae.

All of the *E. yoshidae* larvae introduced into the channel from mid-June to mid-August emerged although the emergence rate of *E. latifolium* decreased to ~50% during the same period. This difference might be attributable to a several-fold difference in pesticide susceptibility, such as indicated by the ~3-fold difference in the fenitrothion LC_{50} values for the two species (Table 1). Several-fold differences in insecticide susceptibility therefore seem critical for aquatic organisms inhabiting rivers contaminated with various pesticides at low concentrations.

Among medium-sized Japanese rivers, the Kokai River may be one of the most highly polluted with respect to various pesticides, because it flows through paddy field districts for ~110 km from its upper to lower reaches. In addition, its headwaters originate from agricultural fields, in contrast to similar-sized rivers which largely issue from mountainous areas gathering rainwater along the way.

The macrobenthic species which entered the channel together with the river water and inhabited the channel were very limited. They were composed of chironomids, *Cheumatopsyche brevilineata* (Trichoptera) and *Baetis sahoensis* (Ephemeroptera). Several species of chironomids (Sugaya, 1995) and *C. brevilineata* (Konno *et al.*, 1994) collected from the Kokai River and/or other pesticide-polluted rivers showed tremendous insecticide tolerance. The 24 h LC_{50} values of *C. brevilineata* to several insecticides were higher than $1\ \mathrm{mg\,l^{-1}}$ (Hatakeyama, 1995) and the 48 h LC_{50} values were close to $1\ \mathrm{mg\,l^{-1}}$ (Tada and Shiraishi, 1994). The insecticide tolerance mechanism of *C. brevilineata* was shown to be rapid binding of fenitroxon to a specific protein (Konno *et al.*, 1994). *Baetis sahoensis* has a high recovery potential, depending on many cohorts composed of different age groups, a high growth rate and a preference for night drift (Yasuno *et al.*, 1982; Hatakeyama *et al.*, 1990). In addition, the benthic species which was regarded as dominant in the vicinity of our Kokai River sampling station, *Cipangopludian chinensis* (Gastropoda), is also tolerant to insecticides.

One to three orders of magnitude differences in fenitrothion susceptibility have been recognized among ~30 taxa of aquatic insects so far tested (Hatakeyama, 1995). The fenitrothion susceptibility of the two species of mayfly used in the present study was rather higher among ~30 species of aquatic insects. The mortality of *E. latifolium* and *E. yoshidae* increased in the channel, possibly due to latent and subtle overall pesticide effects during the pesticide spraying period. However, most mayfly larvae, which had been introduced into the channel during the winter or early spring, emerged in the early spring suggesting that the effects of other chemicals of domestic or industry origin might be low in the Kokai River.

The freshwater shrimp was netted abundantly until several decades ago in the rivers investigated. The disappearance of this species from the rivers may be due to long-term pesticide impacts as is illustrated by the present investigation. We suggest that even insecticide-susceptible aquatic organisms might inhabit the Kokai River and recovery of the benthic fauna might be greatly accelerated, if the latent pesticide impacts during the spring to summer period were to be prevented hereafter.

Acknowledgements

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