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Correlation between Overall Pesticide Effects Monitored by Shrimp Mortality Test and Change in Macrobenthic Fauna in a River

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The relationship between change in benthic fauna in the Suna River and shrimp mortality in the river water samples (test was conducted two times per week from May to September) was investigated to assess the insecticide impact on the benthic community. The river was selected because pesticide application had been restricted for a long time in the upper reaches, while aerial insecticide spraying had been done four times on paddy fields in the lower reaches in summer. Species richness and density of benthos were high at the stations in the upper reaches. High shrimp mortality was measured in the water samples collected from a downstream station during the pesticide spraying period, from late July to late August. Species richness and density of benthos markedly decreased at the stations in the lower reaches in that period. However, several species of chironomids, *Cheumatopsyche* (Trichoptera), which demonstrated tremendous insecticide resistance, and *Baetis sahoensis* (Ephemeroptera), which has a high recovery potential, were not affected, even in the insecticide spraying period. The benthic fauna in the lower reaches tended to recover from autumn to spring, although not sufficiently. It is considered that the temporal recovery of the benthic community was brought about through recruitment from the benthic community in the 5-km upper reaches. A close correlation was found between the high mortality of shrimp in the water samples and the deterioration in the benthic fauna in this river which was contaminated with insecticides during the summer.

INTRODUCTION

Assessment of short-term and/or repeated impacts of overall pesticide effects on macrobenthic communities is important. Most rivers in Japan have paddies on both sides which are sprayed with many kinds of pesticides during the rice planting and growing season. Young rice seedlings are transplanted from the rice nursery to paddy fields, which have been cultivated after introduction of river water. Therefore, most rivers tend to suffer pesticide contamination although the concentrations are generally low and transient (Hatakeyama *et al.,* 1994; Hatakeyama, 1995).

The insecticides reach peak concentrations within a short period from several hours to several days and also decrease within a short period (Hatakeyama *et al.,* 1990, 1991a; Iwakuma *et al.,* 1993). The short or transient impact of pesticides on aquatic organisms was well documented by a monitoring program using *Selenastrum* (a green alga) for herbicide (Hatakeyama *et al.,* 1992, 1994) and a freshwater shrimp (*Paratya compressa improvisa*) for insecticide (Hatakeyama, *et al.,* 1991a,b).

Substances inhibiting growth of *Selenastrum* and affecting shrimp mortality were documented based on additive, synergistic, and/or antagonistic effects of pesticides detected in the river water samples collected from the two rivers investigated (Hatakeyama *et al.,* 1992, 1994; Hatakeyama, 1995). Biomonitoring using a shrimp mortality test with river water samples is efficient, because the method is simple, the mortality of the control is negligible, and the shrimp-rearing method is easy and stable (Hatakeyama and Sugaya, 1989).

Another important issue was how the shrimp mortality in the water samples indicates or reflects the overall pesticide effects on the macrobenthic community. To investigate a possible correlation, two study areas were needed; a control area and a river area polluted with insecticides at well-known times. From several rivers, the Suna River was selected. It is ca. 360 km NE from this institute, and the paddy fields around its lower reaches were sprayed with insecticide four times from late July at 10-day intervals. On the other hand, the pesticide spraying had been strictly restricted by volunteers living in the upstream reaches over the past 20 years so as to protect human health and the environment.

Effects of insecticide on macrobenthic communities in rivers have been largely investigated in mountain or forest streams to assess the side effects of aerial spraying of insecticides which were conducted to control forest pests and/or human disease vectors (Eidit, 1975; Symons, 1977; Yasuno *et al.,* 1982a,b; Satake and Yasuno, 1987; Hatakeyama *et al.,* 1990; Yameogo *et al.,* 1993). In these studies, the upper reaches of the investigated rivers and/or adjacent rivers are available as control rivers, because benthic communities in mountain streams are usually not exposed to pesticide pollution. However, it was difficult to find control rivers in the agricultural field area in Japan, because almost all rivers are largely lined on either side by paddy fields from their upper to lower reaches. Therefore,

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the Suna River was of particular value for the purpose of the present investigation. Takamura *et al.* (1991) reported that the diversity and density of Odonata larvae are low in the lower reaches compared to those in the upper reaches throughout the year. The present report investigates a possible correlation between shrimp mortality in the water samples and benthic faunal change in the upper to the lower reaches of the river to assess the repeated insecticide impact on river benthic communities. For supplementary data, dynamic faunal changes before, during, and after the insecticide spraying were investigated using a drift net (Hatakeyama *et al.*, 1990).

INVESTIGATION AREA AND METHODS

Characteristics of Pesticide Use

The pesticide application had been voluntarily restricted upstream of St. 6 in Fig. 1 since ca. 20 years ago. Many inhabitants in the area have noticed that too much pesticide spraying was not beneficial to human health and the natural environment. Insecticides and fungicides were aerially sprayed on downstream paddy fields four times by helicopters from late July to late August at about 10-day intervals. Dates of the aerial sprayings and the kinds of pesticides used during the investigations are provided in Table 1. Days of aerial sprayings were almost the same in every year, although several kinds of pesticides were replaced by another insecticide in some cases, and the spraying day was rarely changed according to weather conditions. People in the pesticide spraying area were informed in advance as to the areas and time of aerial spraying, which was usually conducted in the early morning.

Herbicides and insecticides were also sprayed on paddy fields by hand or using various types of spraying equipment from spring to early summer. However, information on the kinds of pesticides and days of spraying by individual farmers was not available.

Suna River

Suna River flows through the rural district of Yamagata Prefecture in northeastern Japan about 500 km north of metropolitan Tokyo. The upper reaches are lined by paddy fields and orchards, and both sides of the lower reaches from St. 5 and/or St. 6 are almost entirely rice paddies. Farmhouses and small orchards are scattered among the rice paddies from the upper to the lower reaches. There appeared to be no particular chemical or organic pollution sources besides the pesticide pollution, at least in the areas of the selected stations. Mean river width (on water surface) and water depth (at the benthos sampling position) were measured at the monthly sampling of benthos (described later) as illustrated in Fig. 2. The riverbeds around St. 3, 4, and 5 are covered mostly with pebbles ranging from 10 to 20 cm in diameter. The riverbed at the lower reach stations are mostly composed of smaller pebbles ranging from ca. 5 to 15 cm in diameter. The usual water current velocity and depth, where benthic samples were collected, were ca. 30 cm sec^{-1} and 30 cm at St. 5 and ca. 25 cm and 30 cm sec^{-1} at St. 9, respectively.

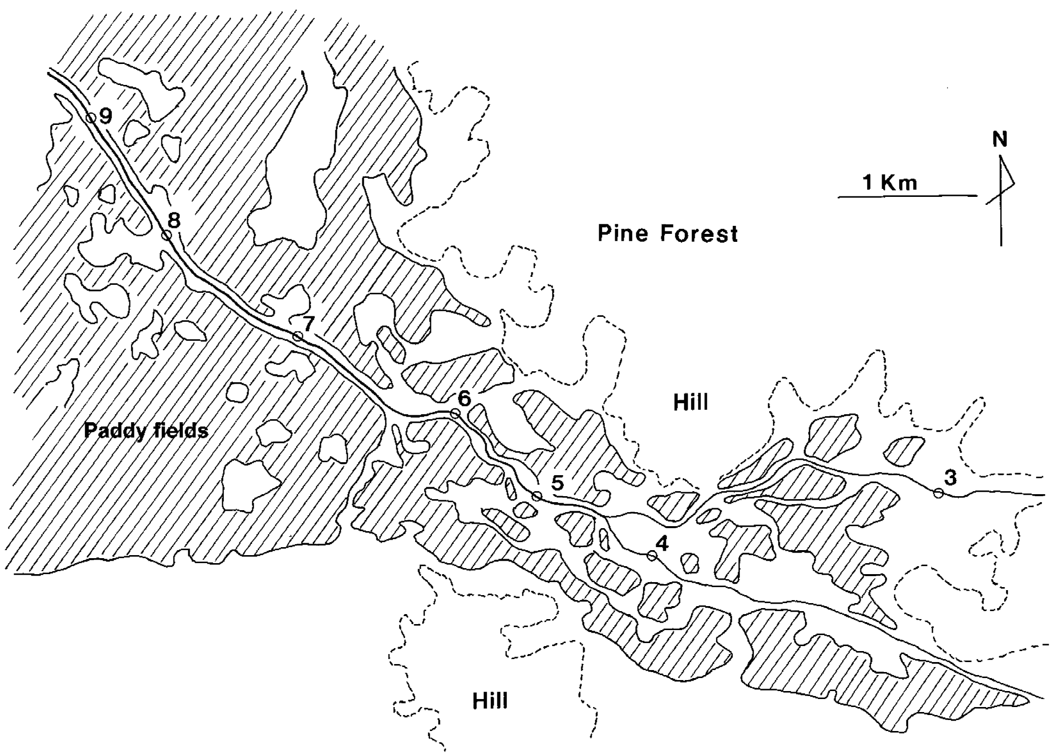

FIG. 1. Seven stations along the Suna River from the upper to lower reaches for monthly benthos sampling. Aerial spraying of insecticides and/or fungicides onto paddy fields was conducted downstream of St. 5 and/or St. 6 four times from late July to late August with ca. 10-day intervals.

TABLE 1

Time Schedule for Aerial Spraying of Pesticides on the Paddy Fields in the Lower Reaches of Suna River from 1989 to 1991

	1988		1989		1990	
1st	20 July	Mepronil	20 July	Edifenphos	20 July	Pyridaphenthion (i1)
		Tricyclazole		Ft halide		Edifenphos (f2)
		Posdiphen (f1)		Mepronil		Mepronil (f3)
2nd	30 July	Fenthion	1 August	Fenthion	29 July	Fenthion (i2)
		Fenobucarb		Edifenphos		Fenobucarb (i4)
		Tricyclazole		—		Edifenphos
3rd	10 August	Fenitrothion	10 August	Fenitrothion	9 August	Fenitrothion
		Fenobucarb		Fenobucarb		Fenobucarb
		Tricyclazole		Tricyclazole		Tricyclazole (f4)
4th	21 August	Fenthion	21 August	Fenthion	21 August	Fenthion
		—		—		Iprobenphos (f5)

Note. The spraying was conducted in the early morning. i1, i2, i3: organophosphorous insecticide; i4: carbamate insecticide; f1–f5: fungicide.

Shrimp Mortality Test with Water Samples

Two water samples, for pesticide analysis and shrimp mortality tests, were collected two times a week (usually on Tuesday and Friday) into 400-ml glass vials from St. 5 and St. 9 from April to September 1990. A farmer living near the river was entrusted with this task, and samples were transported to this institute within 24 hr by an express delivery service under a cooled condition using an ice cooler. Eight individual 4-week old shrimps were introduced into a glass beaker filled with 100-ml water samples of 22 ± 1°C. The rearing methods of freshwater shrimp *P. compressa improvisa* and test methods were described elsewhere (Hatakeyama and Sugaya, 1989; Hatakeyama *et al.*, 1991a,b). The shrimp test was usually conducted on Monday with three replicates per water sample on the four water samples (St. 5 and 9, Tuesday and Friday). One grain of fish food (ca. 40 mg; Nihon Haigou Shiryou) was given on Days 4 and 7 to avoid starvation of test organisms. The mortality of shrimps was checked on Days 1, 2, 3, 4, 7, and 14. Dead individuals were removed at every observation time. Moribund individual shrimps, which had completely lost mobility, were counted as dead because most of them died within the same day.

At the 2nd and 4th insecticide spraying days, water samples for the shrimp mortality test and insecticide analysis, each 500 ml in a glass bottle, were taken with intervals from 2 to 16 hr (for these intervals, see Fig. 8 and 9). These samples, 18 for the 2nd and 22 for the 4th insecticide spraying time, were brought to this laboratory cooling in an icebox. The shrimp mortality tests were conducted as described above, and insecticide analyses were conducted as described below for these samples. Six-hour mortality was recorded for water samples collected at the 2nd insecticide spraying.

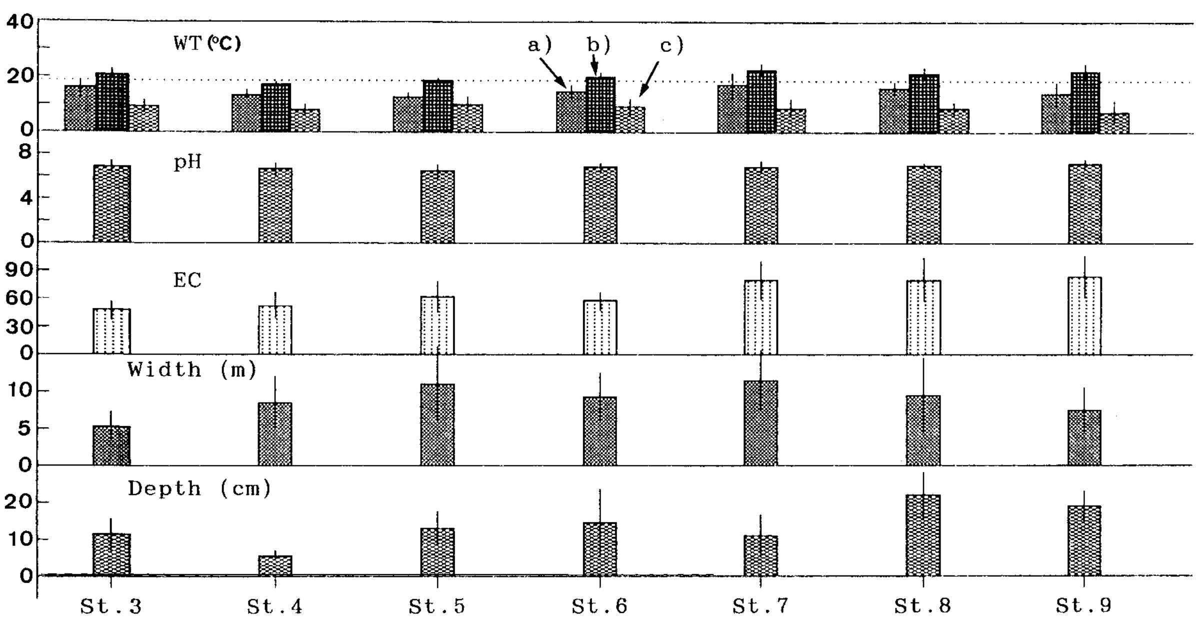

FIG. 2. Mean water temperatures, pH, electroconductivity (EC), width, and depth of the Suna River at the stations (Fig. 1) where benthos samples were collected (n = 9). Mean water temperatures: (a) April, May, June; (b) July, August, September; (c) October, December, March.

Effects of Insecticides on Benthic Communities: Surber Net Sampling

Three Surber net (33 × 33 cm, 0.1 m^{-2} area, 0.45-mm mesh size) samples were collected from the seven stations (Fig. 1) of the Suna River monthly from April to October and in December in 1989 and in March in 1990. These sampling stations were selected based on the preliminary investigation conducted in 1988. Surber net samples were collected from a riffle riverbed, which is composed of pebbles. Pebbles in the unit area surrounded by a frame (0.1 m^{-2}) were rubbed and disturbed with fingers to dislodge the benthos into the Surber net along the current. After that, the riverbed was kicked to dislodge benthos in the bottom sediment into the net. Collected samples were discharged into polyethylene bags (30 × 42 cm) with a little river water and fixed with formalin for species identification and estimation of respective species densities. Individual organisms in the samples were picked up using forceps under binoculars (×20) and were identified to species level as far as possible, except for chironomid larvae, because it is time-consuming to identify larval chironomids.

Cheumatopsyche brevilineata (Trichoptera) and *Baetis sahoensis* (Ephemeroptera) were the exclusively dominant species at the insecticide-polluted stations. Subsamples of these species were collected optionally besides the monthly Surber net samples. Head width of the former and body length of the latter were measured under binoculars to investigate the population dynamics of these species. This is to consider how they can dominantly inhabit the insecticide-polluted river.

Effects of Insecticides on Drift of Macrobenthos

Real time effects of insecticides on benthic communities were assessed using a drift net. Two drift nets were settled in the center of the riverbed at St. 5 and St. 9 the day before the 2nd insecticide spraying (Table 1) in 1990. The depth and current speed of the river water were ca. 30 cm and 30 cm sec^{-1} at St. 5 and ca. 25 cm and 22 cm sec^{-1} at St. 9, respectively. Dimensions of the drift net used were mesh size, 0.45 mm; width and height of the mouse frame of the drift nets, 50 and 30 cm, respectively; and length (lateral view, triangular), 85 cm. A plastic cylinder cup (diameter, 8 cm; length, 12 cm with 0.48-mm netting at the bottom) was attached to the end of the drift net. Benthos collected in the cup were discharged into a plastic tray, and the identified species were counted under live conditions and/or after fixation with formalin. Insecticide spraying was conducted in the early morning on 29 July from a helicopter. The Surber net samples were collected at 2-hr intervals from the previous day to 2 days after the insecticide spraying. Water temperature, pH, and electroconductivity were measured at the same intervals. Water temperatures, measured at the monthly sampling of benthos, were averaged based on the data covering (a) April, May, and June; (b) July, August, and September; and (c) October, January, and March (Fig. 2), because diurnal change in the water temperature was large and sampling time was usually at random between the stations. Water samples for the nutrient analysis were collected at the monthly benthos sampling time.

Pesticide Analysis

The 400-ml river water samples were filtered through a Whatman GF/C glass filter treated at 470°C for 2 hr. The water sample was passed through a C18-bond Elut column (3 ml; Analytichem International, Varian) at a flow rate of ca. 5 ml min^{-1}. The column was centrifuged at 3000 rpm for 12 min to remove the unnecessary water in it. The pesticides in the column were eluted with 0.5 ml of pesticide analytical grade acetone (99.8%; Wako Pure Chem. Ind., Ltd.) by centrifugation at 1000 rpm for 12 min into a conical centrifuge glass tube (10 ml). This procedure was repeated once, followed by the final centrifugation with 0.5 ml acetone at 3000 rpm for 15 min (total 1.5 ml acetone used). The samples were analyzed with a gas chromatograph (Hewlett–Packard, HP-5890 A) equipped with a NPD detector and fitted with a capillary column (SPB-5; 0.25 μm, 30 m, 0.32 mm i.d., fused silica), together with a standard (50 μl of 1 mg $liter^{-1}$ azobenzene) of the pesticides. The column temperature and flow rate of the carrier gas (He) were 50–300°C and 54 ml min^{-1}, respectively.

RESULTS

Environment of Suna River

In the summer season, the water temperature at the downstream stations revealed higher values than those at St. 5 from several to 10°C (Fig. 2). In the cold season, however, not much difference in water temperatures between stations was recognized (Fig. 2). Diurnal changes in the water temperature and pH at St. 5 and St. 9 in late July are presented in Fig. 3 as examples of extreme cases. Water temperature at St. 5 changed from 16 to 21°C during the 3 days in late July. However, the water temperature at St. 9 rose from 21°C in the morning to a maximum of 33°C at midday. The river water temperature rose gradually ca. 10°C while flowing from St. 5 to St. 9 (Fig. 1). On clear summer days, the air temperature ranged from 20°C in the early morning to 33°C at noon.

The pH values increased to nearly 8 at midday at both stations, reflecting the active photosynthesis (Fig. 3). Electroconductivity increased from ca. 50 μS cm^{-1} in the upper reaches to ca. 80 μS cm^{-1} in the lower reaches. Figure 4 presents the seasonal changes in nutrient concentrations at St. 5, St. 9, and mean values of those based on data from St. 6, St. 7, and St. 8 ($n = 3$). The NH_4^+ and NO_3^- concentrations were rather low in summer in the lower reaches compared to those in other seasons (Fig. 4).

Change in Benthic Fauna

Species richness (number of taxa), diversity index (Shannon and Weaver, 1963), and density of aquatic insects at all stations are provided in Fig. 5. The numbers of taxa and diversity indices in the upper reaches (St. 3 to St. 5) revealed fairly steady values except for St. 3 in July, where water weeds grew

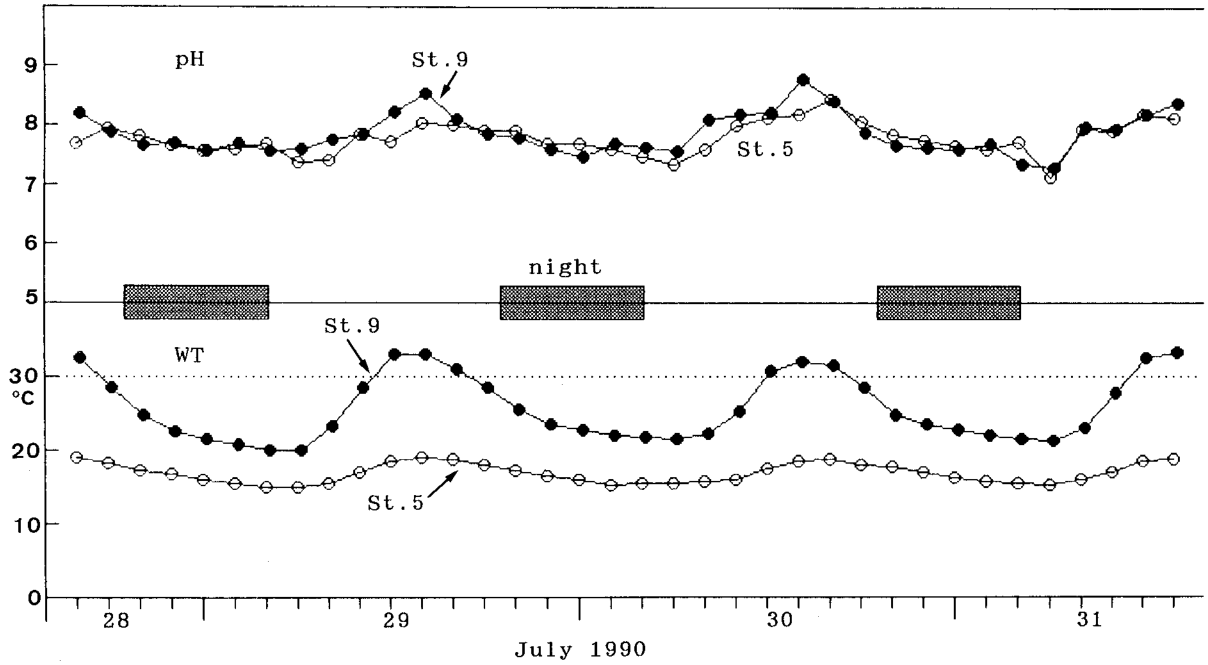

FIG. 3. Change in water temperature and pH at St. 5 and St. 9 on the Suna River in late July 1990.

thick, and the riffle zone was limited and shadowed at that time. The mean number of taxa was 40.4 ± 6.3 ($n = 9$) at St. 5, although only ca. 1 km away from the aerially sprayed area.

On the contrary, the values related to benthic fauna tended to decrease at stations downstream of St. 6 in summer. In particular, the number of taxa decreased to only 3 and 4 at St. 8 and St. 9 (Table 3), respectively, in August. After that, the deterioration in benthic fauna in the lower reaches tended to recover during autumn (Fig. 5), when it was assessed based on the number of taxa. However, the mean diversity indices from autumn to winter in the lower reaches (St. 7 ~ 9) were 2.04 ± 0.72 ($n = 9$), although those in the upper reaches (St. 3, 4, and 5) were 3.17 ± 0.5 ($n = 9$) (Fig. 5), Densities of the recovered species in the autumn at St. 9 were low, suggesting insufficient recovery even in winter (Table 3).

Detailed changes in benthic fauna were compared between

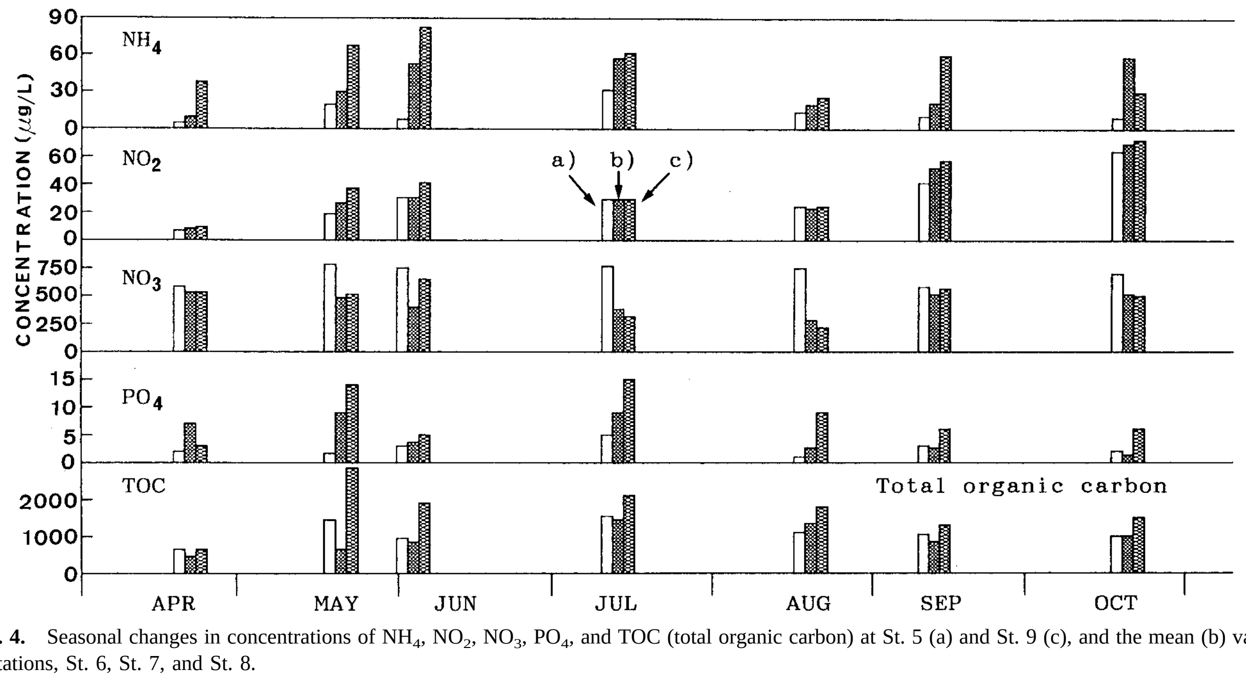

FIG. 4. Seasonal changes in concentrations of NH_4, NO_2, NO_3, PO_4, and TOC (total organic carbon) at St. 5 (a) and St. 9 (c), and the mean (b) values of three stations, St. 6, St. 7, and St. 8.

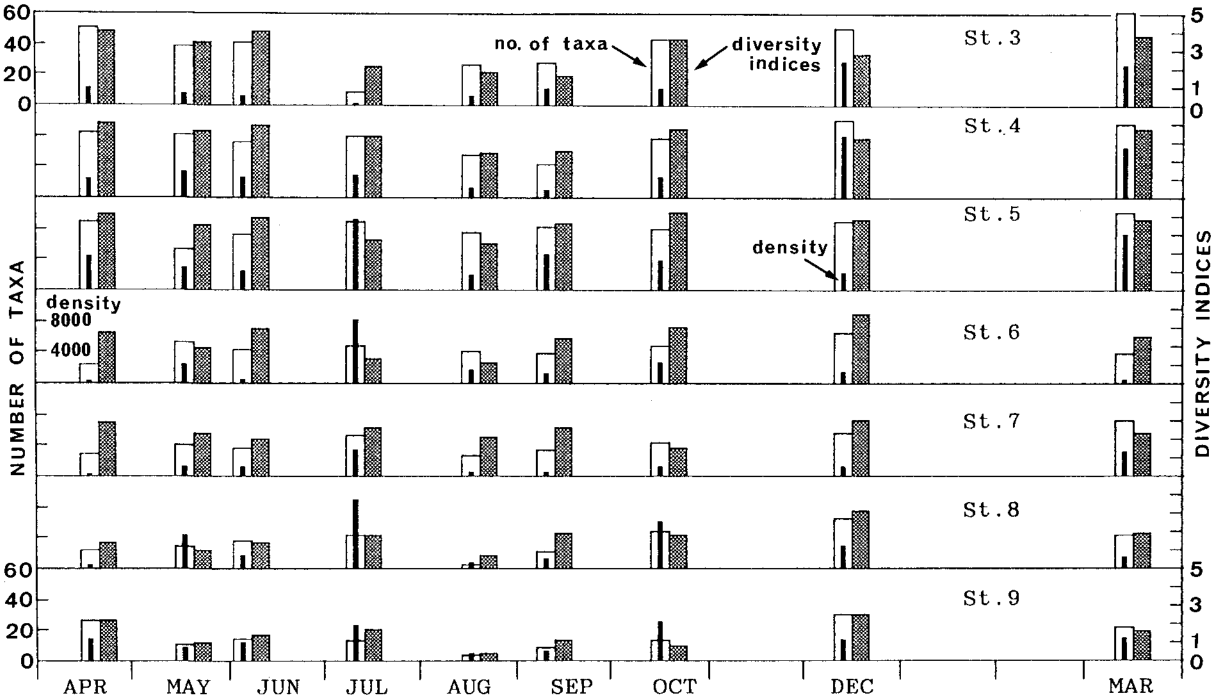

FIG. 5. Number of taxa (mostly as species), density (m^{-2}; full scale, 1200), and diversity indices of benthos collected at the seven stations along the Suna River flowing through the paddy field area. Insecticide spraying was restricted in the upper reaches. Aerial insecticide spraying was conducted four times from late July with ca. 10-day intervals downstream of St. 5 and/or St. 6.

St. 5 and St. 9. Species and density of aquatic insects collected at St. 5 are presented in Table 2. In this table, minor species found only one or two times in the nine monthly samples were neglected for the sake of brevity. The disregard of the minor species had no appreciable effect on the density (Table 2, (a)), although the number of species diminished. Taxa (40.4 ± 6.3; $n = 9$) were found throughout the year at St. 5. Among them, Ephemeropteran and Trichopteran species were abundant and diverse throughout the year compared to the stations in the lower reaches, although several species disappeared from the summer samples in accordance with their inherent life cycles. The total number of species in the respective order of aquatic insects at St. 5 and St. 9 are provided in Fig. 6. The numbers of taxa in each order were relatively steady at St. 5 throughout the year compared to those at St. 9, which decreased significantly in summer. Others in Fig. 5 were mainly composed of Coleoptera as insects. Other than insects, the benthic communities were composed of Oligocheata, exclusively Naididae and Tubificidae, *Erpobdella lineata* (Hirudinea) and *Asellus hirgendorfii* (Crustacea), in almost all stations (Table 4, St. 5 and St. 9). However, *Dugesia japonica* (Turbellaria) were completely absent in the reaches lower than St. 6, although they were abundant in the upper reaches (Table 4).

Species found throughout the year were very few at St. 9 (Table 3). They included several species of chironomids, *C. brevilineata* (Trichoptera), *B. sahoensis* (Ephemeroptera), and *Antocha* spp., although the latter almost disappeared from August to September.

Several species of Ephemeroptera disappeared at St. 9 during the late summer, although they were found with significant density before August (Table 3). However, many species which had disappeared in the summer recolonized in the St. 9 area from late autumn to winter, although the density was not always high (Fig. 5, Table 3). Only *B. sahoensis* was found at St. 9 among the Ephemeroptera in summer (Table 3).

Assessment of Pesticide Effects on Benthos

The temporal changes in the mortality of *P. compressa improvisa* in the river water samples, which were collected from St. 5 and St. 9, are presented in Fig. 7. Shrimp mortality increased rapidly at each pesticide spraying day (Table 1) at St. 9, although it also increased at St. 5 on the second and third spraying days. This might be attributable to aerial drift of insecticides, because St. 5 and the spraying area (below St. 6) were close. At St. 9, the 2-week mortality of the shrimp ranged from 10 to 100% in the insecticide spraying period. However, the shrimp no longer died from the day following the fourth insecticide spraying, because the fenthion concentration decreased quickly from 7.0 μg liter^{-1} to the not-detected level (lower than 0.01 μg liter^{-1}) at the river water sampling time for the toxicity test (Fig. 9; 13:00 on 21 August).

Besides the mortality caused by the aerially sprayed insecticides, the 2-week shrimp mortality ranging from 10 to 70%

TABLE 2

Species Composition of Benthos (Three Surber Net Samples; $3 \times 0.1\ m^{-2}$) Collected from the Riverbed at St. 5

Family	Taxa	April	May	June	July	August	September	October	December	March
Ephemeroptera										
Baetidae	*Baetis chocoratus*	16	19	25	3	1	3	—	4	13
	Baetis sahoensis	—	3	3	27	8	312	19	7	4
	Baetis thermicus	—	—	10	8	—	6	61	4	153
	Baetis yoshinensis	1	—	1	—	—	1	—	—	13
	Baetis sp. E	—	—	1	3	—	1	—	—	—
	Baetis sp. H	—	—	—	3	—	8	24	—	4
Heptageniidae	*Ecdyonurus yoshidae*	49	10	21	21	7	33	93	87	68
	Epeorus latifolium	24	7	21	7	3	26	11	—	8
Leptophlebiidae	*Paraleptophlebia chocolata*	34	9	12	—	—	—	51	211	128
	Paraleptophlebis westoni	—	—	—	1	2	2	—	—	—
Ephemeridae	*Ephemera strigata*	43	3	2	4	102	58	16	39	7
Potamanthidae	*Potamanthodes kamonis*	2	—	—	6	4	—	—	2	1
Ephemerellidae	*Cincticostella okumai*	10	—	—	—	—	17	65	2	9
	Durenlla cryptomeria	166	150	117	76	3	—	6	—	37
	Ephemerella denticula	68	—	—	—	—	—	—	61	78
	Ephemerella imanishi	—	—	4	21	1	—	—	—	—
	Ephemerella spp.	—	—	4	692	9	31	—	—	—
	Serratella rufa	195	114	101	30	13	48	101	69	270
	Torleya japonica	3	1	1	17	2	7	4	6	11
Caenidae	*Caenis* sp.	163	57	84	175	—	—	3	17	20
Odonata										
Gomphidae	*Davidius moniwanus*	—	—	—	1	—	—	—	4	1
Plecoptera										
Nemouridae	*Nemura* sp.	—	—	—	8	14	12	—	3	3
Perlidae	*Kamimuria tibialis*	3	—	—	—	7	35	28	1	9
Megaroptera										
Sialidae	*Sialis* sp.	—	—	—	3	1	—	1	—	—
Corydalidae	*Parachauliodes continentalis*	1	—	1	—	1	—	—	—	—
Trichoptera										
Stenopsychidae	*Stenopsyche marmorata*	12	4	2	17	4	78	70	4	8
	Stenopsyche sauteri	22	22	28	11	5	31	72	6	15
Philopotamidae	*Dolophilodes* sp. DC	—	—	—	—	—	3	3	4	7
Polycentropodidae	*Plectrocnemia* sp.	6	24	18	73	5	2	7	13	1
Hydropsychidae	*Cheumatopsyche brevilineata*	30	9	10	2	3	294	188	19	50
	Hydropsyche orientalis	37	13	16	10	—	33	70	—	18
Rhyacophlidae	*Rhyacophila brebicephalla*	—	—	—	—	—	1	2	—	10
	Rhyacophila kawamurae	2	—	2	—	1	—	1	—	2
	Rhyacophila yamanakaensis	—	—	—	4	—	—	4	—	1
Glossosomatidae	*Glossosoma* spp.	139	191	97	42	11	82	36	1	245
	Oxyethira sp.	—	—	—	11	2	2	1	12	1
Limnephilidae	*Apatania* sp.	91	73	21	97	1	9	—	4	291
	Goera japonica	18	—	—	2	—	4	—	—	2
Sericostomatidae	*Gumaga okinawaensis*	1	—	—	—	—	3	1	5	1
Leptoceridae	*Ceraclea* sp.	4	12	—	—	1	—	13	6	29
	Mystacides sp.	—	1	—	—	2	1	1	4	—
	Oecetis sp.	—	—	2	3	1	3	—	4	—
Coleoptera										
Dytiscidae	Gen. spp. (larva)	—	—	—	11	2	1	—	4	—
Gyrinidae	*Orectochilus* sp.	3	—	1	—	—	—	3	6	1
Psehenidae	*Eubrianax granicollis*	10	—	—	—	1	14	29	3	6
Elimidae	Gen. spp.	19	16	12	15	1	2	29	7	24
Diptera										
Tipulidae	*Antocha* spp.	84	34	36	155	29	39	39	18	78
	Hexatoma sp.	12	20	12	3	5	5	8	1	16
Simullidae	*Simulium* spp.	1	—	—	2	—	—	—	1	3
Ceratopogonidae	*Bezzia* spp.	2	1	1	—	—	—	—	—	—
Chironomidae	Gen. spp.	170	162	149	1462	352	287	173	92	635
Empidae	*Hemerodromia* sp.	—	1	5	—	1	1	—	3	—
	Wiedemannia sp.	2	1	3	—	—	—	—	—	—
Number of taxa:		44	27	36	44	37	41	40	44	51
Number of taxa[a]:		35	26	33	36	33	38	34	36	41
Density:		1466	958	828	3037	608	1501	1267	751	2364
Density[a]:		1442	957	823	3026	605	1495	1232	734	2281

[a] Minor species collected only 1 or 2 times among the 9-month samples have been omitted for the sake of brevity.

TABLE 3
Species Composition of Benthos Collected from the Riverbed at St. 9 (Three Surber Net Samples; 3 × 0.1 m^{-2})

Family	Species	April	May	June	July	August	September	December	January	March
Ephemeroptera										
Siphlonuridae	*Ameletus costalis*	1	—	—	—	—	—	—	1	—
Baetidae	*Baetis sahoensis*	—	15	17	584	4	53	8	5	—
	Baetis thermicus	29	—	2	—	—	—	1	12	79
	Baetis sp. H	26	—	—	55	—	1	11	11	46
	Baetis sp. L	—	—	—	—	—	—	—	138	15
	Pseudocloeon sp. B	—	—	—	—	—	—	1	—	—
Hetageniidae	*Ecdyonurus yoshidae*	1	—	—	—	—	—	—	5	2
	Ecdyonurus kibunensis	—	—	—	—	—	—	—	1	—
	Epeorus aesculus	—	—	—	—	—	—	—	—	1
	Epeorus ikanonis	—	—	—	—	—	—	—	—	3
	Epeorus latifolium	5	—	1	—	—	—	—	—	—
Potamanthidae	*Potamanthodes kamonis*	4	4	20	5	—	—	12	17	4
Ephemerellidae	*Acerella longicaudata*	—	—	—	—	—	—	—	7	—
	Cincticostella okumai	11	—	—	—	—	1	14	19	4
	Durnella basalis	—	—	—	—	—	—	—	1	—
	Durnella cryptomeria	20	8	16	—	—	—	—	—	3
	Ephemerella denticula	6	—	—	—	—	—	—	15	3
	Ephemerella imanishi	—	1	17	4	—	—	—	—	—
	Ephemerella spp.	—	—	31	2	—	—	—	—	—
	Serratella rufa	9	—	1	4	—	—	—	3	1
Caenidae	*Caenis* sp.	3	3	34	—	—	—	—	1	1
Plecoptera										
Capniidae	Gen. spp.	—	—	—	—	—	—	—	10	—
Perlodidae	*Isoperla aizuana*	3	—	—	—	—	—	—	—	—
	Isoperla asakawae	1	—	—	—	—	—	—	—	—
	Stavsolus japonica	—	—	—	—	—	—	1	2	4
Trichoptera										
Stenopsychidae	*Stenopsyche marmorata*	—	—	—	—	—	—	—	1	—
Hydropsychidae	*Cheumatopsyche brevilineata*	91	15	5	127	18	25	173	111	23
	Hydropsyche orientalis	4	—	—	—	—	—	1	2	2
	Hydropsyche setensis	—	—	—	—	—	—	—	1	—
Rhyacophillidae	*Rhyacophila brevicephala*	—	—	1	—	—	—	—	—	—
	Rhyacophila clemans	1	—	—	—	—	—	—	—	—
	Rhyacophila kawamurae	—	—	—	—	—	—	—	—	1
Glossosomatidae	*Glossosoma* spp.	—	2	—	—	—	—	1	1	—
Hydroptilidae	*Hydroptilia* sp.	—	—	—	—	—	—	—	1	—
Limnephilidae	*Apatania* sp.	1	—	—	—	—	—	—	1	—
	Goera japonica	1	—	—	—	—	—	1	—	—
Coleoptera										
Dytiscidae	Gen. sp. (larva)	—	—	—	—	—	—	—	1	—
Psehenidae	*Ectopria* sp.	6	—	—	1	—	1	—	1	—
Elimidae	Gen. spp.	13	2	5	10	—	2	—	4	8
Diptera										
Tipulidae	*Antocha* spp.	158	28	16	18	1	—	1	60	42
Simullidae	*Simulium* spp.	1	—	—	—	—	—	—	4	8
Psychodiae	*Psychoda*	—	—	—	—	—	—	—	—	1
Ceratopogonidae	*Bezzia* spp.	2	2	1	—	—	—	—	—	1
	Gen. spp.									
Chironomidae	*Gen. spp.*	561	490	601	744	312	292	1459	449	746
Tabanidae	*Tabanus* sp.	—	—	—	1	—	—	—	—	—
Empidae	*Clinocra* (Hydrodromia) sp.	—	—	—	—	—	—	—	—	1
	Hemerodromia sp.	1	—	—	—	—	—	—	1	—
	Wiedemannia sp.	1	—	—	—	—	—	—	—	—
Dolichopodidae	Gen. sp.	1	—	—	—	—	3	—	—	—
	Number of Taxa:	28	11	15	13	4	8	13	30	22
	Density[a]	961	570	768	1556	335	378	1684	886	991

[a] Total no. of individuals per 3 Surber net samples (3 × 0.1 m^{-2}).

lasted from mid-June to early July (Fig. 7). The 2-week mortality ranging from 10 to 30% also often observed at St. 5 from early June to mid-July at St. 5 suggested that pesticide spraying took place even in the upper reaches although aerial spraying had been prohibited.

Figure 8 presents the change in fenthion concentrations and shrimp mortality in the water samples collected at St. 9 after the second aerial spraying of an emulsifiable mixture of fenthion and edifenphos in 1990 (Table 1). The fenthion concentration increased quickly to ca. 50 μg liter^{-1} and then decreased to ca. half the concentration after 2 hr. On the 3rd spraying day (Table 1), fenitrothion and fenobucarb were detected at the concentrations of 12.4 and 1.9 μg liter^{-1}, respectively, in the water samples collected at 8:00 AM. These concentrations de-

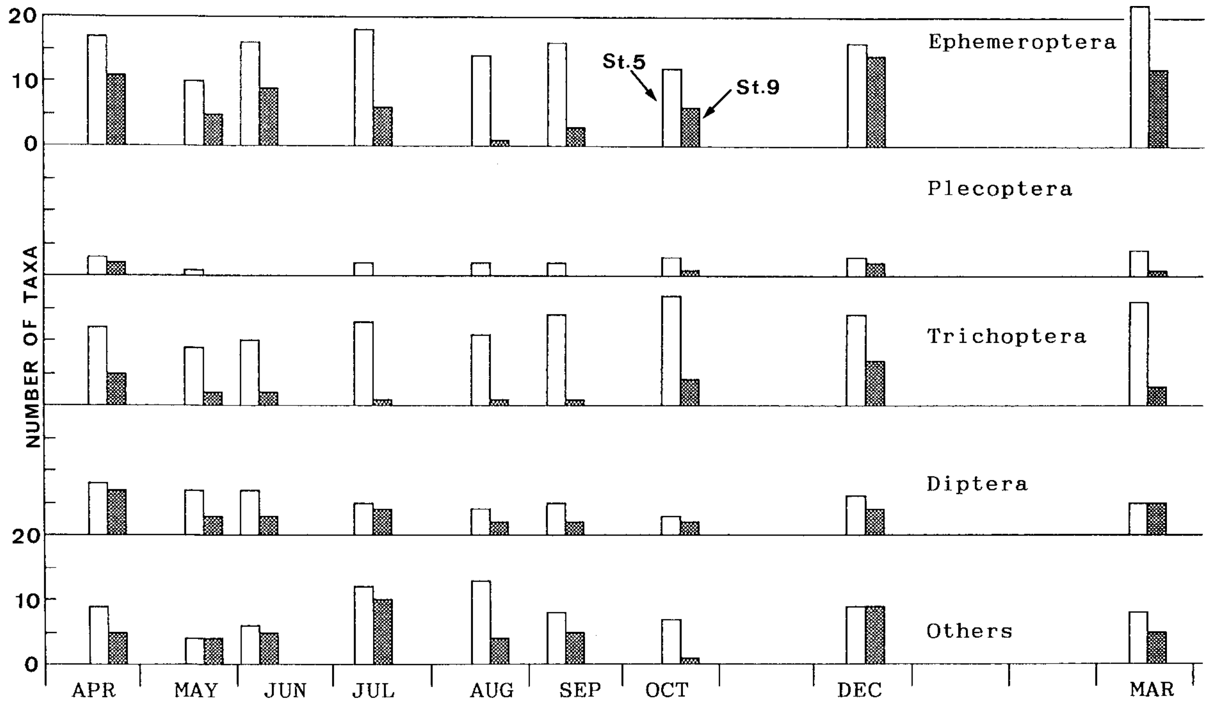

FIG. 6. Change in numbers of taxa (mostly species) of the respective order of aquatic insects in the three Surber net samples (0.1 $m^2 \times 3$) collected monthly or bimonthly from St. 5 and St. 9 on the Suna River from April 1989 to March 1990.

creased to 0.6 and 1.0 μg liter^{-1}, respectively, after 2 days. On the 4th insecticide spraying day, the fenthion concentration increased to 7 μg liter^{-1} at 11:00 AM and thereafter decreased rapidly with a half reduction time of ca. 1.5 hr (Fig. 9).

The change in shrimp mortality in these water samples also indicated fairly good correlation with the insecticide toxicity recorded in the laboratory test (Figs. 8 and 9; Hatakeyama and Sugaya, 1989). The insecticide impact was most severe at the 2nd spraying, as illustrated by the 100% shrimp mortality within 6 hr in the water samples collected within several hours after the aerial spraying (Fig. 8).

Figure 10 presents the number of drifting individuals and species collected at St. 5 and St. 9 before and after the second insecticide spraying. At St. 5, 10.5 ± 3.6 ($n = 24$) taxa drifted in the daytime, whereas 17.3 ± 3.7 ($n = 12$) taxa drifted in the night (20:00 ~ 4:00). The drift pattern did not change significantly after the second insecticide spraying day at St. 5 (Fig. 10). However, several species, such as *B. thermicus*, *Centroptilium* sp. (both Ephemeroptera), and *Nemoura* sp. (Plecoptera), were found in the daytime drift samples. The daytime drifts of these species significantly obscured the typical night–daytime drift pattern, in which most drifts occur at night.

On the other hand, only 2.4 ± 0.9 ($n = 36$) species drifted throughout 3 days at St. 9 (Fig. 10). This means that benthic fauna around St. 9 had already lost its diversity before the second insecticide spraying. The number of species was very few in the drifting samples at St. 9, although the number of individuals before the second insecticide spraying was rather higher than that at St. 5. However, the species were almost exclusively chironomids and *B. sahoensis* (Fig. 9). Other species collected in the drift net were *C. brevilineata, Antocha* sp., Naididae, and Elmidae, although only several or less were obtained per drift net (per 2 hr), and they were detected only several times among 36 drift net samples. The night drift pattern of *B. sahoensis* (Ephemeroptera) before the insecticide spraying almost disappeared in the two nights after the spraying. Contrary to expectations, no day drift of *B. sahoensis* following the insecticide spraying was observed. The drift pattern of Chironomids was not changed significantly, although little change was noted in the second night after spraying (Fig. 8).

DISCUSSION

Changes in Benthic Fauna and Shrimp Mortality

It was considered that the shrimp mortality in the river water samples was entirely caused by the pesticide toxicity mainly attributable to several insecticides sprayed on the paddy fields. The mortality of *P. compressa improvisa* correlated well with the insecticide toxicity in the river water samples collected from St. 9. Interestingly, the shrimp mortality which had lasted during the insecticide spraying period suddenly disappeared after the last insecticide spraying. This strongly suggests that other toxic chemicals did not exist in the Suna River, at least during the present investigation. Reported changes in shrimp mortality reflected well the overall joint and/or synergistic toxicity of several insecticides and/or fungicides (Hatakeyama *et al.*, 1991a,b; Hatakeyama, 1995).

TABLE 4

Species Composition of Benthos (Numbers per Three Surber Net Samples; $3 \times 0.1\ m^{-2}$) Other Than Aquatic Insects Collected from the Riverbed at St. 5 (a) and St. 9 (b)

St. 5 (a) and St. 9 (b)		April	May	June	July	August	Setember	October	December	March
Turbellaria										
Dugesiidae	*Dugesia japonica*	(a) 85	39	69	49	2	24	2	3	115
		(b) —	—	—	—	—	—	—	—	—
Gastropoda										
Lymnaeidae	*Radix auricularia*	(a) —	—	—	—	—	—	—	—	—
		(b) —	—	—	1	—	—	—	—	—
Physidae	*Physa acuta*	(a) —	—	—	—	—	—	—	—	—
		(b) —	—	—	1	—	—	—	—	—
Planorbiidae	*Gyraulus chinensis*	(a) —	—	—	—	1	—	—	—	—
		(b) —	—	—	—	—	—	—	—	—
Ferrissidae	*Pettancylus nipponicca*	(a) —	—	—	—	—	—	—	—	—
		(b) —	—	—	—	—	—	—	1	—
Oligochaeta										
Naididae	Gen. spp.	(a) 32	5	28	62	2	4	—	45	356
		(b) —	6	389	79	46	56	—	2086	999
Tubificidae	Gen. spp.	(a) 35	13	9	5	1	—	—	2	10
		(b) 362	42	3	32	42	103	—	34	17
Hirudinea										
Erpobdellidae	*Erpobdella lineata*	(a) 2	—	—	2	4	9	19	1	—
		(b) 5	—	2	138	1	30	—	4	1
Glossiphoniidae	*Alboglossiphonia lata*	(a) —	—	—	—	—	—	—	—	—
		(b) —	—	—	1	—	—	—	1	—
Arachnida										
Hydrachnellae	Gen. sp.	(a) 3	—	—	2	1	—	1	1	—
		(b) —	—	—	1	—	—	—	—	—
Crustacea										
Asellidae	*Asellus hirgendorfii*	(a) —	—	—	1	1	4	—	—	2
		(b) 6	1	5	18	1	—	1	5	1

The main purpose of the present investigation was to assess the relationships between shrimp mortality in the river water samples and changes in benthic communities. Obviously, the species richness and density of benthos markedly decreased in the period in which the shrimp mortality lasted at high levels. However, there are several environmental and/or biological factors which cause changes in benthic fauna. Some differences were recognized between St. 5 and St. 9 in the nutrient concentrations, riverbed structure (size of pebbles), and vegetation, as described in Figs. 2 and 4. Several kinds of trees are distributed around St. 5, and small apple orchards dotted the downstream area. The concentration of NH_4, which was the most toxic to aquatic organisms among the measured nutrients, was rather low in the summer compared to that in other seasons (Fig. 4). Concentrations of PO_4 at St. 9 (8.3 ± 4.2 mg liter^{-1}, $n = 7$) were always higher than those at St. 9. However, aquatic organisms may not undergo the deleterious effects of these inorganic phosphate levels. Therefore, these factors did not seem to cause the almost complete destruction of the benthic community downstream. Moreover, the benthic community tended to recover after the summer in which the pesticide impact on the benthos was so severe.

Except for the pesticides, the environmental factor which most affected the benthos might be the summer water temperature. Water temperature at St. 9 reached 33°C at midday in summer. However, the present authors are not able to judge whether the warm water temperature induced the elimination of most species at St. 9 except for several dominant species, such as *B. sahoensis*, *C. brevilineata*, and chironomids. To assess this factor, the diurnal change in water temperature at St. 9 (Fig. 3) was reproduced in small indoor streams (40 cm long). Significant mortality was not observed in the three species of aquatic insects, *Epeorus latifolium*, *Ecdyonurus yoshidae*, and *Stenopsyche marmorata*, in the channels in a 7-day experiment (Hatakeyama, unpublished observations). Logging, impoundment, and fish culture ponds around and/or in the rivers are accompanied with water temperature increase, and there are many investigations on the effects of these human activities on benthic communities including water temperature (Ward, 1982; Inverarity *et al.*, 1983; Webb and Walling, 1993).

However, there are few reports on the effects of water temperature which exceeds 30°C on benthic fauna, because most field investigations were conducted in the northern European countries and North America. Among those studies, high water temperature to benthos was assessed in a stream by artificially increasing water temperature using hot-spring heat sources

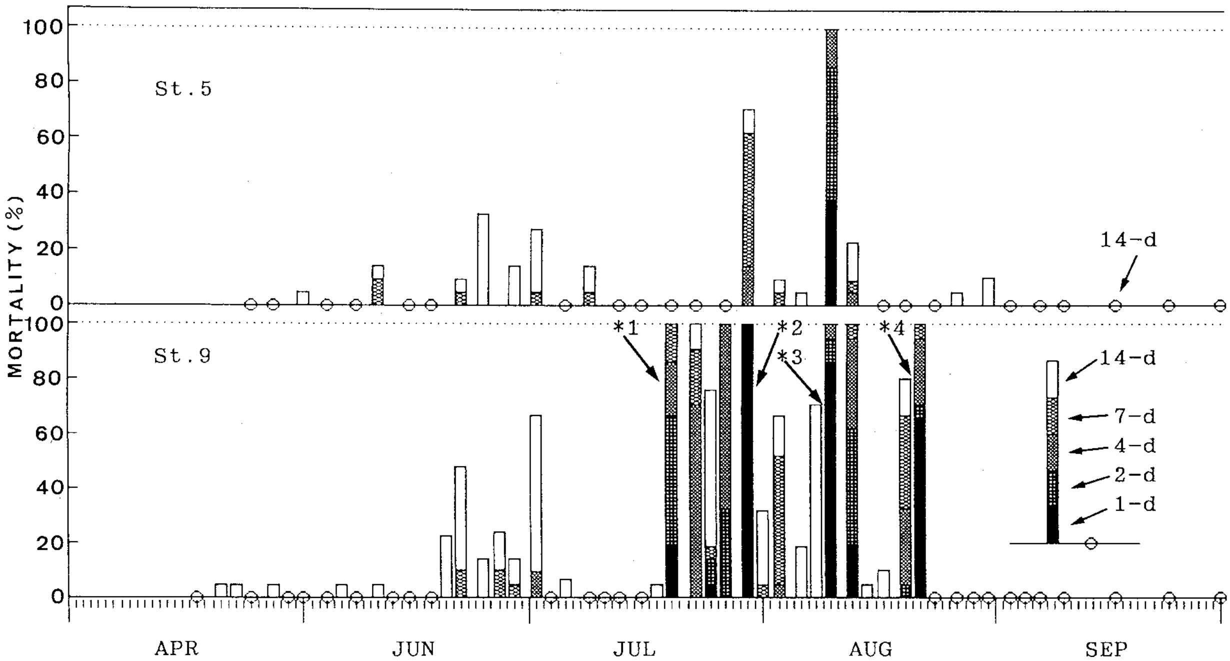

FIG. 7. Mortality of the freshwater shrimp *Paratya compressa improvisa* in the water samples collected from the two stations on the Suna River. The aerial insecticide spraying was conducted in the early morning four times at ca. 10-day intervals from 20 July (Table 1). Pesticides were sprayed on the marked (∗) days.

(Lamberti and Resh, 1983). It is noteworthy for the present investigation that the species richness of the benthos was not affected although the water temperature was increased to produce conditions similar to those of the Suna River (Fig. 3) for 1 month. Effects of over 30°C water temperature should be investigated further, because rivers surrounded by paddy fields receive warmed effluents from paddies, and water temperature tends to increase by direct sunshine, because there are almost no trees around the rivers streaming the paddy field area.

Seasonal Distribution of Benthos According to Life Histories

The change in population density of most aquatic insects depends on their inherent life history and water temperature conditions (Wise, 1980). Most univoltine species emerge

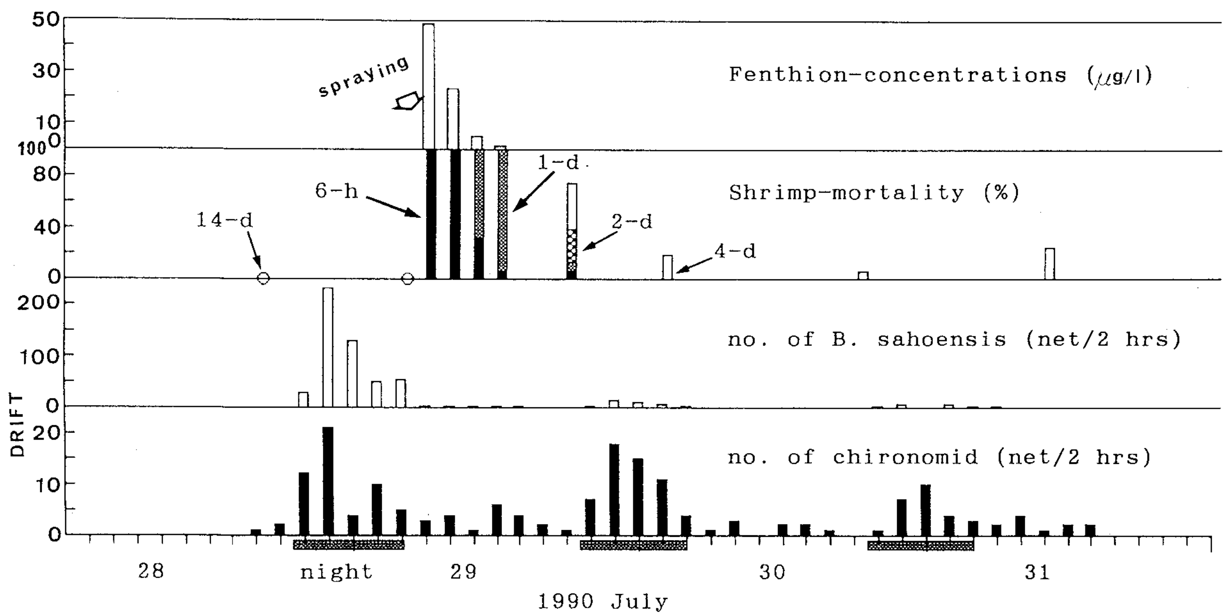

FIG. 8. Changes in fenthion concentrations and the shrimp mortality in the water samples collected from St. 9 on the Suna River at 2- to 16-hr intervals after the 2nd insecticide spraying. Drift patterns of *Baetis sahoensis* (Ephemeroptera) and chironomids are based on samples collected by a drift net per 2 hr at St. 9. Aerial spraying of fenthion was conducted around 6:00 AM over the paddy fields.

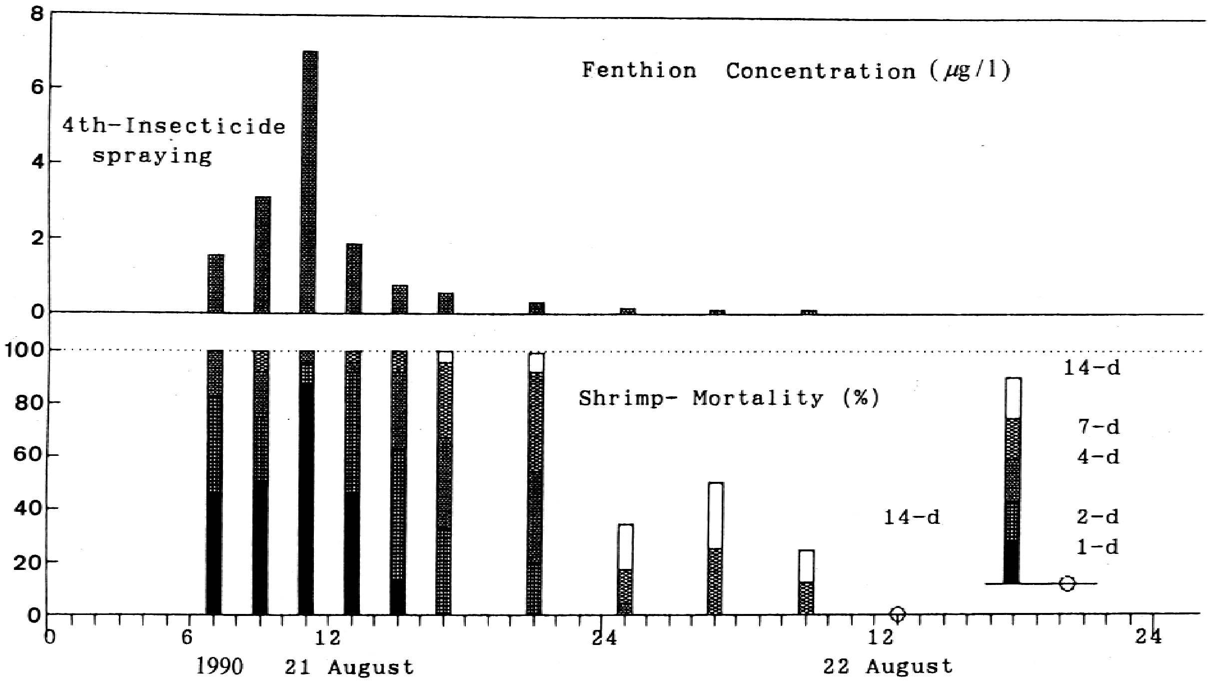

FIG. 9. Fenthion concentrations and shrimp mortality in the river water samples collected at 2- and/or 4-hr intervals after the 4th aerial insecticide spraying.

from spring to early summer. Therefore, many species should disappear temporally from the riverbed to hatch and grow to the size of benthos caught in the Surber net (mesh size, 0.45 mm). Various Ephemeropteran and Trichopteran species were not detected at St. 5 in August, suggesting that they were in the egg stage and/or passed through the Surber net at that time. As there are remarkable differences in benthic fauna between St. 5 and St. 9 in August, this might be explained by insecticide effects, especially because the benthic community tended to recover from the autumn after the pesticide impact completely vanished from the Suna River. The recovery might be mainly attributable to the night drift from upstream (Yasuno *et al.*, 1982a,b; Satake and Yasuno, 1987; Hatakeyama *et al.*, 1990). The possibility of a complete recovery of the benthic

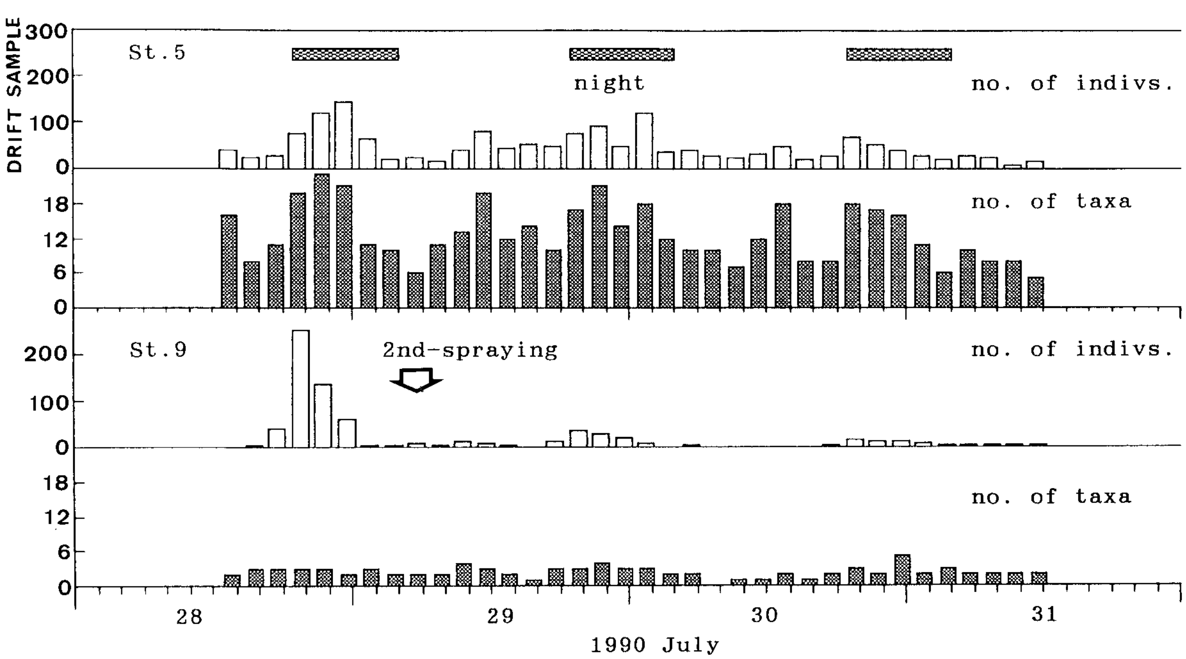

FIG. 10. Number of species and individuals per drift net per 2 hr settled at St. 5 and St. 9 on the Suna River.

community around St. 9 in the Suna River may be low if the insecticide impact in the summer, as clarified by the present investigations, was repeated year by year.

Dominant Species at Insecticide-Polluted Sites

In addition, species differences in the insecticide susceptibility and/or recovery potential from its effect are also important for assessing pesticide effects on benthic fauna. *C. brevilineata* (Trichoptera) was a representative dominant species among several detected at St. 9 (Table 3). After the investigations on the Suna River, it was confirmed that *C. brevilineata* was also often found in pesticide-contaminated environments such as rivers or ditches in paddy field districts as well as several insecticide-tolerant species of chironomids. The 24-hr LC_{50} values of insecticides, fenitrothion and fenthion, to *C. brevilineata* were higher than 1 mg liter^{-1}, and 48-hr LC_{50} values are close to 1 mg liter^{-1} (Tada and Shiraishi, 1994), although the values of most aquatic insects are lower than 0.1 mg liter^{-1}. The mechanism of remarkable insecticide tolerance of *C. brevilineata* was investigated, and it was found that fenitroxon is rapidly bound to specific protein before it binds acetylcholine esterase (Konno *et al.*, 1994). A search was made for insecticide-susceptible strains of *C. brevilineata* in several districts of Japan. However, it has not been found yet, and it cannot be judged whether the extreme high resistance is an intrinsic characteristic or a mutant strain. In addition, the *Antocha* sp. was one of the dominant species at St. 9, and its insecticide susceptibility was close to that of *C. brevilineata* (Tada and Shiraishi, 1994). Several insecticide-resistant strains were also detected in chironomids (Sugaya, 1995), but not searched for in the Suna River's species. However, the possibility of the existence of other insecticide-resistant strains is high in the Suna River, because chironomids were the most consistent species found throughout the year at St. 9, and their drift pattern was not disturbed, even after the second insecticide spraying (Fig. 8).

On the other hand, *B. sahoensis* (Ephemeroptera) was another dominant species at St. 9, despite its high susceptibility to insecticides (Hatakeyama, 1995). However, this species has a great potential for population recovery based on a high growth rate, many cohorts of different ages (Fig. 11), and night drift as illustrated in Fig. 8. The cohorts in various growth stages suggest that there were sequentially egg and adult stages in the insecticide spraying period. Adults may escape insecticide exposure in water, and eggs might be more tolerant to insecticides than those at the larval stage. In addition, the coexistence of many cohorts suggests that emergence was always occurring at St. 9, and the growth rate of each cohort was high in summer. These biological characteristics of the two dominant species at St. 9 suggest that other species which are susceptible to insecticides, and/or uni- or bivoltine organisms, could not as well inhabit the insecticide-polluted site such as St. 9, where the continuous impact of insecticides lasted throughout the summer as indicated by shrimp mortality.

Biomonitoring Using P. compressa improvisa

By prolonging the test period from 4 days to 1 or 2 weeks, the latent toxicity of the insecticide at 4 days was revealed. *P. compressa improvisa* was the test organism preferred for the prolonged mortality test, since they were not carnivorous, in

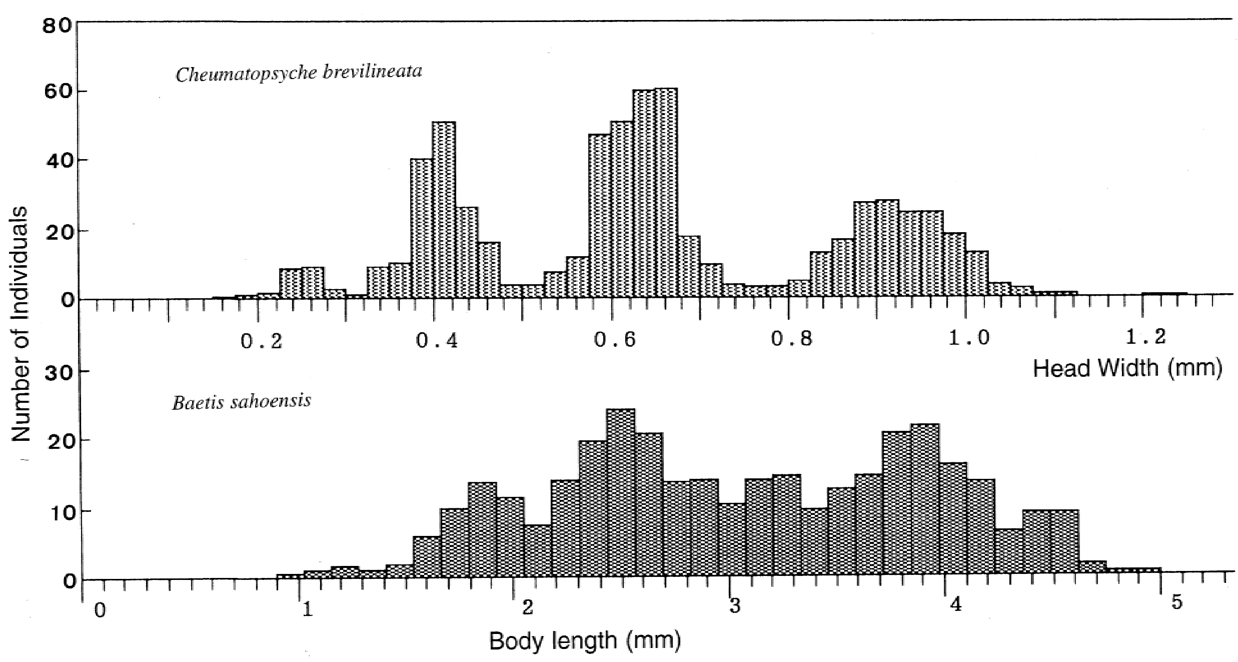

FIG. 11. Histogram of body length of *Baetis sahoensis* (Ephemeroptera) and head width of *Cheumatopsyche brevilineata* (Trichoptera) collected from the riverbed at St. 9 using the 3 Surber net samples collected on 28 July 1990.

contrast to similar freshwater shrimp dominant in the Japanese freshwater environments, as *Palaemon paucidens* DE-HAAN. In another test method, the pesticide concentration in the water sample was increased by freeze evaporation instead of prolonging the test period (Kariya *et al.*, 1988; Suzuki *et al.*, 1991). Other advantageous aspects of *P. compressa improvisa* as a test organism for monitoring are ease of culture, low mortality in the control, and easy discrimination as to whether dead, moribund, or alive (Hatakeyama and Sugaya, 1989). *P. compressa improvisa* is also an adequate test organism for assessing sediment-bound insecticides, because its main food is surface bottom sediment. The effects of the sediment-bound fenthion on mortality and growth of the shrimp were assessed in a flowthrough aquarium (Hatakeyama and Shiraishi, 1994).

Conclusion

The correlation between insecticide effects on benthic communities and shrimp mortality in water samples was assessed. The benthic communities were severely deteriorated at downstream stations of the Suna River in the insecticide spraying period from late July to late August. High shrimp mortality also lasted in the insecticide spraying period, indicating the devastating insecticide impact on aquatic organisms. Significant correlations were found between changes in benthic organism drift and temporal variation in shrimp mortality in the river water samples. However, the increased summer water temperature should also be assessed to ascertain the real insecticide impact on benthic communities, including synergistic effects between both factors.

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