SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 5, pp. 1281–1286, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

ACUTE CONTAMINATION WITH ESFENVALERATE AND FOOD LIMITATION: CHRONIC EFFECTS ON THE MAYFLY, *CLOEON DIPTERUM*

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(*Received* 20 *May* 2004; *Accepted* 29 *November* 2004)

Abstract—Results of environmental risk assessments based merely on toxic effects of contaminants at the individual level, without consideration of population-level effects, may be questionable. The aim of the present study was to investigate how limited food resources, resulting in intraspecific competition, could interact with the chronic effect of short-term contamination with the insecticide esfenvalerate. Larvae of the mayfly, *Cloeon dipterum*, were exposed to esfenvalerate (0.001–100 μg/L) for 1 h and then transferred to indoor microcosms containing insecticide-free water, where they were maintained at various food levels until emergence. The results showed that short-term exposure to 10 or 100 μg/L resulted in acute mortality. Chronic effects on survival occurred at concentrations up to three orders of magnitude lower than that causing the acute effect (0.01 μg/L). Food limitation increased effects on organisms during medium-term observation (8–15 d), but assessment of long-term survival rates suggested that the chronic effects of low insecticide concentrations could be compensated for, at least regarding some endpoints. The authors assume that in limited-food conditions, lethal and sublethal effects reduced competition between individuals, resulting in significantly increased final survival.

Keywords—Esfenvalerate *Cloeon dipterum* Population level Compensation Intraspecific competition

INTRODUCTION

Results of environmental risk assessments based merely on toxic effects of contaminants at the individual level may be questionable if indirect, population-level effects and natural stressors are not considered. Standard toxicity test systems aim to reduce any population-level effects (interaction) and natural stressors (food deficiency) to obtain low control mortality. Apart from the fact that food may have a substantial effect on the growth, body weight, and fecundity of an organism [1–4], nutritional state may affect the sensitivity of an organism to toxicants [5,6]. At the population level, intraspecific interactions often include competition for a certain food as an important limited resource. Interactions both among and within species are a fundamental component of how communities and ecosystems function [7]. Although disagreement still exists regarding the prevalence and importance of competition in natural communities [8], both intra- and interspecific competition is considered to be a relevant factor determining population and community structure [7–10].

Competition usually depends on population density and may modify toxicant effects. Density-dependent responses could be expected when contamination is studied at the population level. These responses could compensate for the influence of contaminants to various degrees. The typical density-dependent compensatory mechanism, as hypothesized by Postma et al. [11], is based on the idea that the mortality produced by a toxicant, though reducing density-dependent competition, results in more favorable conditions for the survivors. Nevertheless, only a few studies have considered such effects of toxicants on the interaction between individuals [12–16]. The common finding of these studies is that the density-dependent compensation is present when the toxicant is strong enough to reduce density without causing mortality in all individuals. However, the question remains regarding the reasons for compensation and the concentrations at which it occurs.

The aim of the present study was to investigate how the intraspecific competition for food resources, supplied in a broad range of different quantities, could interact with the chronic effect of short-term contamination by the insecticide esfenvalerate. The species chosen for the present experiment was the mayfly, *Cloeon dipterum* L., a typical inhabitant of lowland ponds, pools, lake littoral zones, and slow-running waters [1]. This species has a wide geographical distribution throughout the whole Palaearctic [17], and it is characterized by high resistance to environmental changes (e.g., temperature, dissolved oxygen, pH) but low tolerance for insecticides [18,19]. Because this species also inhabits ponds with an agricultural catchment, *C. dipterum* is among the aquatic invertebrates likely to be exposed to pesticides such as the pyrethroid esfenvalerate, which is widely applied all over the world.

MATERIALS AND METHODS

After short-term (1-h) exposure to esfenvalerate, test organisms (larvae of *C. dipterum* L., Baetidae, Ephemeroptera) were transferred to indoor microcosms containing insecticide-free water, where they were maintained until emergence. Larval survival, emergence success, body weights of adult mayflies, female fecundity, and total egg production were evaluated at three different levels of food supply. The experimental design was chosen for the estimation of combined and separate effects of the insecticide and food shortage in conditions relevant to small, lowland ponds contaminated by agricultural pesticides.

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Presented at the 14th Annual Meeting, SETAC Europe, Prague, Czech Republic, April 18–22, 2004.

Table 1. Water-quality parameters (mean ± standard deviation) from microcosm series with high-, medium-, and low-food treatments

Parameter	Food treatment		
	High	Medium	Low
Total ammonia (mg/L)	1.79 ± 0.17	1.70 ± 0.15	1.74 ± 0.16
Nitrite (mg/L)	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001
Nitrate (mg/L)	2.23 ± 0.28	2.20 ± 0.25	2.20 ± 0.29
Orthophosphate (mg/L)	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Hardness (mg $CaCO_3$/L)	85.20 ± 5.25	88.12 ± 6.30	80.86 ± 7.00
Oxygen (mg/L)	3.68 ± 0.92	3.71 ± 0.52	3.65 ± 0.54
BOD5[a] (mg/L)	4.66 ± 1.00	4.44 ± 1.10	4.40 ± 1.22
pH	7.5 ± 0.70	7.6 ± 0.52	7.4 ± 0.64
Temperature (°C)	20.5 ± 2.66	20.6 ± 2.50	20.8 ± 2.42

[a] BOD5 = biological oxygen demand in 5 d.

Experimental conditions and short-term exposure of organisms

Larvae of *C. dipterum* were collected in mid-May from a small, uncontaminated pond near Novosibirsk Zoo in western Siberia, Russia. They were stored in large, indoor aquaria for approximately two weeks. The number of instars does not appear to be constant for the species, so age of the larvae could not be identified exactly. Larvae were at approximately the third or fourth instar [1] and approximately 5.5 mm in length (excluding cerci). Only active and externally undamaged specimens were selected for the test.

Contamination was performed on June 2, 3, and 4, 2003, in glass beakers containing 1 L of filtered (pore diameter, 1–2.5 nm) pond water. Esfenvalerate (mixture of four stereoisomers enriched with the S,S-isomer (S)-α-cyano-3-phenoxybenzyl(S)-2-(-4-chlorophenyl)-3-methylbutyrate) was applied as an emulsifiable concentrate (Sumi-alfa®; Sumitomo, Osaka, Japan; produced and packed by ZAO Avgust, Moscow, Russia) containing 50 g/L of active ingredient. All concentrations given here refer to the active ingredient. The emulsifiable concentrate rather than the pure substances (technical grade) was used to approximate conditions in the field. The 1-h exposure was done with the following concentrations: 0 (control), 0.001, 0.01, 0.1, 1, 10, and 100 μg/L. The concentrations were obtained by serial dilution of a stock solution containing 1 g/L of esfenvalerate. Thirty larvae per beaker and three replicates per concentration were used except for exposure involving 100 μg/L, which was tested only in two replicates. Larvae were rinsed after exposure with pesticide-free water. Then, each group of 30 larvae was divided, and three groups of 10 larvae each were exposed to high-food treatment (HFT), medium-food treatment (MFT), and low-food treatment (LFT). A mixture of dried autumn leaves of pendent white birch, *Betula verrucosa*, and willow, *Salix* sp., was provided for food. Leaves were collected on the shore of the pond where the larvae were obtained. Before use, the leaves were crumbled and kept in pond water for 7 d in the dark. The general scheme of food supply was as follows: HFT, 20 mg/microcosm every day; MFT, 20 mg/microcosm every other day; and LFT, 20 mg/microcosm every fifth day (all three values are dry wt). These amounts were chosen to provide a relative abundance of food in the HFT series and a slight and a strict shortage in the MFT and LFT series, respectively. Preliminary experiments and literature data were used to decide on the amounts of food provided [1,2,20]. Conditions created in the microcosms of the present study could cause not only quantitative but also qualitative differences in food between the treatments. Larvae of *C. dipterum* feed on detritus and, to a lesser degree, on algae; a main component of the nutrients assimilated by an organism originates from bacteria, mycelial fungi, and various microorganisms inhabiting the detritus [20]. Therefore, the time required for food materials to disappear was longer in HFT microcosms than in MFT and, in particular, LFT microcosms. As a result, food quality as well as food quantity might have been increased in the HFT series. In any case, larvae in the HFT were in more favorable conditions compared to the MFT and, of course, the LFT.

Amount of per capita food in the HFT was ad libitum. In the MFT, it was slightly lower than ad libitum. In the LFT, a pronounced food deficiency was present.

Glass, indoor microcosms contained 2 L of pond water and five glass sticks as a substrate. Chemical parameters of microcosm water were measured twice (June 13 and 27, 2003) (Table 1). All the analyses were done by the Western Siberian Center for Environmental Monitoring according to standard methods [21]. Every week, approximately 50% of microcosm water was exchanged for fresh pond water to reduce the likelihood of differences in water chemistry between series with different food treatments. Temperature and pH were measured weekly (Table 1).

Real exposure concentrations (0.1, 1, and 10 μg/L only) were measured at the laboratory for organic chemistry of the Andess Company (Novosibirsk, Russia). Solid-phase extraction of 1-L volumes was carried out with Bakerbond PolarPlus C18 columns (Baker, Philipsburg, NJ, USA). Two liters of each exposure solution were prepared: One liter was used for the exposure, and the other liter was extracted for analyses. This scheme was used to prevent a decrease in measured concentrations resulting from insecticide adsorption on the larval bodies. The measurements were made with a gas chromatograph (model 6890; Hewlett-Packard, Avondale, PA, USA) coupled with a mass spectrometer detector (model 5972; Hewlett-Packard). Nominal and measured concentrations are shown in Table 2.

Observation of effect

After contaminant exposure, larvae were rinsed with pesticide-free water by transferring groups of 30 larvae (from particular replicates) to 1 L of pond water for 15 min and stirring once slowly. As described above, each replicate (n = 3 replicates of 30 larvae) was divided into three groups of 10 larvae each, and these groups were transferred to the microcosms, where they were maintained under different food treatments. Animals were allocated at random to reduce the like-

Table 2. Nominal and measured exposure concentrations of esfenvalerate (μg/L)

Series	Concentration	
	Nominal	Measured
1	10	10.11
2	10	10.15
3	10	10.12
1	1	1.06
2	1	1.08
3	1	1.05
1	0.1	0.10
2	0.1	0.11
3	0.1	0.11

lihood of differences in age and body size. Nine microcosms (each with 10 larvae) were set up for the control series at every level of food treatment. Total number of microcosms was 78 (27 for control and 51 for exposed animals).

Larval survival was monitored every day during the first 4 d of the experiment and then every week thereafter until the first emergence took place. Emergence was monitored every day until the last specimen emerged and molted from subimago to imago. Adult mayflies (imago) were removed daily and preserved in 75% ethanol. After the experiment, adult mayflies were sexed and weighed (precision, ±0.01 mg; weight balance VLA200M; MMZ, Moscow, Russia). Specimens were dried on dry filter paper for approximately 15 min before weighing. Quantity of eggs in the abdomens of female imagoes was calculated using a stereoscopic microscope (MBS10; OAO LZOS, Lytkarino, Russia).

Statistical analyses were performed using Statistica® 6.1 and Statistica® 5.5 A (StatSoft, Tulsa, OK, USA) software. Significance of differences was examined using one-way and two-way analyses of variance with post-hoc Dunnett's, Scheffe's, and least-significant-difference tests. Trimmed Spearman–Karber method [22] was used in the computer program Spearman® (Montana State University, Bozeman, MO, USA) for calculating median lethal concentrations (LC50s) and 95% confidence intervals.

RESULTS

Larval survival

After 1 h of exposure and 96 h of observation, trimmed Spearman—Karber method [22] was used to calculate LC50s and 95% confidence intervals (Table 3). Analyses of confidence interval overlaps showed no statistical difference between LC50s derived for the series with different food supplies.

Table 3. Median lethal concentration (LC50) and 95% confidence interval (95% CI) of esfenvalerate (μg/L) for larvae of *Cloeon dipterum* exposed to the insecticide during 1 h and observed during 96 h in microcosm series with high-, medium-, and low-food treatments

Food treatment	Series	LC50	95% CI
High	1	10.00	4.33–23.08
	2	12.12	3.06–47.90
Medium	1	11.22	4.26–29.53
	2	12.59	3.11–50.93
Low	1	7.94	3.89–16.21
	2	10.00	3.72–26.88

Survival rates observed after 4, 8, 15, 22, and 29 (final) d are shown in Figure 1. Final survival after 29 d is expressed as a percentage of animals successfully emerged and molted from the subimago to the imago stage. The results showed that the chronic effect of the short-term exposure occurs at concentrations approximately three orders of magnitude lower than the acute effect (Fig. 1). This difference between the acute and chronic effects was already obvious at 22 d following exposure—that is, before emergence. However, the main effect could be seen during the later stages of development (Fig. 1).

Food deficiency in the LFT resulted in increased larval mortality in control series. Final mortality in this food regime at 1 μg/L also was significantly lower than that at the same exposure concentration in the HFT and MFT (Fig. 1). In contrast, substantial increase in survival at 0.001 and 0.01 μg/L in the LFT was found compared to the respective control (Fig. 1).

Body weight and female fecundity

Adult females of *C. dipterum* are considerably heavier than males. The male to female ratio in the various experimental groups ranged from 0.7 to 1.25. No significant influence of esfenvalerate on adult body weight was found in the present study (not shown).

Food shortage caused a considerable decrease of female fecundity (Fig. 2). Calculation of egg quantity in the female abdomens revealed statistically significant differences between the HFT, MFT, and LFT series ($p < 0.001$, Scheffe's test). Significant differences also were found within the LFT series: The number of eggs was lower at concentrations of 0.1 and 0.01 μg/L compared to the number at 0.001 μg/L and in control treatments ($p < 0.001$, Scheffe's test) (Fig. 2).

DISCUSSION

Larval survival

The median LC50s for a 1-h exposure and 96-h observation found in the present study are comparable with previous results for Trichoptera larvae [23–25]. The LC50 for a 1-h exposure and 24-h observation for fenvalerate found with *Limnephilus lunatus*, 22.62 μg/L (95% confidence interval 15.15–31.62) [23], does not differ statistically from the LC50s for a 1-h exposure and 96-h observation derived here for *C. dipterum* except for the lowest value found for series 1 of the LFT (Table 2).

Acute toxicity data are believed to be important in the risk assessment of pyrethroids [26], but a notable chronic effect of short-term contamination also was documented [12,23]. In the present study, a significant chronic effect of a 1-h contamination with esfenvalerate was found at concentrations ranging from 0.01 to 10 μg/L (Fig. 1). This effect is similar to those observed in comparable studies on *L. lunatus*, in which the lowest effective concentration on survival was 0.01 μg/L [23]. Emergence as well as molting from the subimago to the imago stage appears to be the most sensitive part of the life span of *C. dipterum*, as was found previously for *L. lunatus* [23].

The notable decrease in survival in control series of the LFT evidently was caused by the limitation in food. High mortality at the initial stage of the experiments with 0.001 μg/L in the LFT (observed after 8 and 15 d) may have resulted in a more favorable food supply for surviving individuals. That could explain the significantly higher survival at a later stage with 0.001 μg/L in the LFT (observed after 29 d).

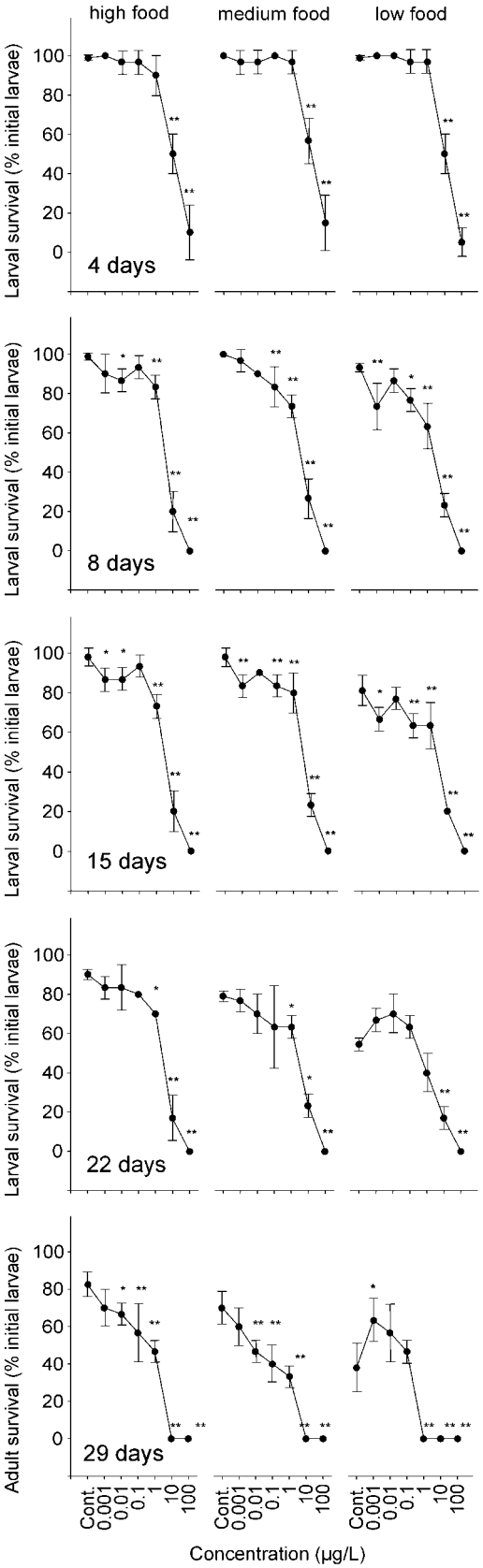

Fig. 1. Mean (± standard deviation) larval and adult survival of *Cloeon dipterum* (% initial larvae) after 4, 8, 22, and 29 d of observation following short-term (1-h) exposure to esfenvalerate and subsequent maintenance at different levels of food supply. Asterisks indicate significant (analysis of variance, Dunnett's test: $*p < 0.05$, $**p < 0.01$) differences from the control (Cont.) series.

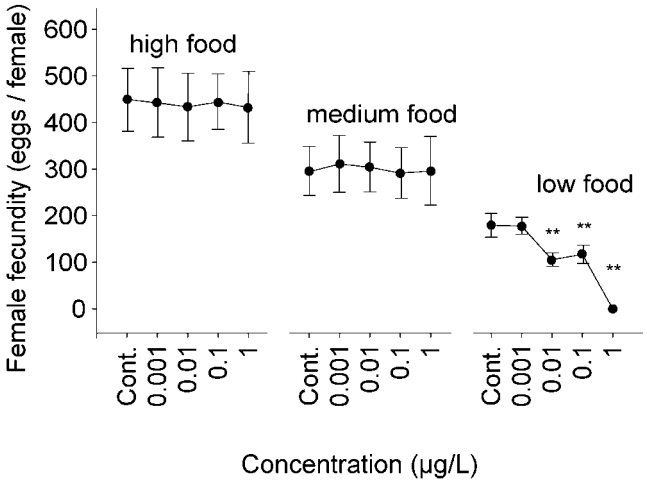

Fig. 2. Mean (± standard deviation) female fecundity of *Cloeon dipterum* (eggs/female) after short-term (1-h) exposure to esfenvalerate and subsequent maintenance at different levels of food supply. Asterisks indicate significant (analysis of variance, Scheffe's test: $**p < 0.01$) differences from the control (Cont.) series.

Food limitation increased medium-term (observed after 8 and 15 d) sensitivity of the tested organisms to the toxicant, but when the chronic effect was assessed, it resulted in compensation in survival (Fig. 1). Thus, after 8 d, survival at 0.001 µg/L in the LFT was significantly lower than in both the HFT and MFT at the same exposure concentration ($p = 0.0024$ and 0.0003, respectively; least-significant-difference test, two-way analysis of variance). The analogous comparison for 15 d revealed similar differences ($p = 0.0001$ and 0.0015, respectively; least-significant-difference test, two-way analysis of variance).

In contrast to the chronic effect at low concentrations, the combination of food limitation and insecticide contamination at high concentrations, close to the acute LC50 at 1 µg/L in the LFT, reveals increased chronic effects of esfenvalerate. Similar results were documented for the polychaete *Capitella* sp. [13]. Interactions between fluoranthene toxic influence and density of the polychaete were compensatory (antagonistic) at low toxicant exposure but synergistic at the highest exposure.

Body weight and female fecundity

Food quantity may have a substantial effect on the growth, body weight, and fecundity of an organism. This is especially so during the larval stages of mayflies, the adults of which are ephemeral and do not feed [1,3,4,27–30]. Many authors reported strong correlations between food quantity and size and weight of aquatic invertebrates, including mayflies [3,31]. In contrast, Cianciara [1] has shown that quantity of food does not affect the weight of adult *Cloeon dipterum* but, rather, causes a temporal shift in development. An influence of food on adult body weight of mayflies becomes apparent through correlation between fecundity and body weight in females [32]. In the present study, we found female fecundity to be correlated with imago weight ($r = 0.82$, $p < 0.001$) when the total data set (all food treatments) was analyzed. However, within the HFT and MFT, no correlation between body weight and fecundity was found. Nevertheless, in the LFT, such a correlation was almost significant ($r = 0.87$, $p = 0.052$).

Reproduction in insects also is related very closely to nutritional factors, the qualitative and quantitative aspects of which have a considerable impact on fecundity [30,33]. A

significant difference in female fecundity of *Cloeon* sp. fed different types of food has been shown [3]. Results of the present study reveal that both qualitative and quantitative food limitation significantly affect female fecundity of *C. dipterum* (Fig. 2).

The effect of fenvalerate on fecundity has been reported previously for *Daphnia galeata* [34]. Sublethal concentrations of the pyrethroid reduced the brood size of this cladoceran species. In the present study, contamination with esfenvalerate had no effect on female fecundity in either the HFT or MFT series, but the influence of the insecticide at exposure concentrations of 0.1 and 0.01 μg/L in the LFT resulted in a significant decrease in egg production by the surviving females (Fig. 2). Perhaps the more favorable food supply in the HFT and MFT compensated for the possible influence of the insecticide on fecundity.

Obviously, this sublethal reduction of growth in terms of female fecundity in the LFT could be an additional cause for the compensation found regarding survival (Fig. 1). Lethal effect as discussed above (mortality at the initial stage) could not explain the compensation entirely, because survival at 0.01 μg/L in the LFT did not decrease either after 8 or 15 d of observation (Fig. 1). However, reduction in terms of fecundity at this concentration was significant (Fig. 2), which could result in compensation regarding the number of individuals.

CONCLUSION

The present study suggests that the concentration–response relationship of populations exposed to esfenvalerate differs according to the degree of competition for food between individuals. In this example, when strong food limitation was present, toxicant effect on survival was reduced over a wide range of concentrations. However, the toxicant effect on female fecundity was present only with food limitation. Hence, for some endpoints, the direct effects of the toxicant may be compensated for, in part, by an indirect reduction of intraspecific competition in comparison to the control. Accordingly, the response to toxicants at the population level, when competition is present, can differ from that at the individual level. Nevertheless, whether these effects will be present in cases where the main form of competition between individuals is interspecific competition remains to be investigated.

Acknowledgement—The authors are grateful to V.A. Chirkov for kind support of the experimental work and to U. Noack for the invaluable support that made possible the discussion of the results at the 14th SETAC Europe Annual Meeting. The authors also thank S. Reynaldi for important help with statistical analyses.

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