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EFFECTS OF DIETARY ESFENVALERATE EXPOSURES ON THREE AQUATIC INSECT SPECIES REPRESENTING DIFFERENT FUNCTIONAL FEEDING GROUPS

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Abstract—Given the chemical properties of synthetic pyrethroids, it is probable that compounds, including esfenvalerate, that enter surface waters may become incorporated into aquatic insect food sources. We examined the effect of dietary esfenvalerate uptake in aquatic insects representing different functional feeding groups. We used three field-collected aquatic insect species: A grazing scraper, *Cinygmula reticulata* McDunnough (Ephemeroptera: Heptageniidae); an omnivorous filter feeder, *Brachycentrus americanus* Banks (Trichoptera: Brachycentridae); and a predator, *Hesperoperla pacifica* Banks (Plecoptera: Perlidae). Laboratory-cultured algae were preexposed for 24 h to esfenvalerate concentrations of 0, 0.025, 0.05, and 0.1 μg/L and provided to two *C. reticulata* age classes (small and final-instar nymphs). Reduction in small nymph growth was observed following three weeks of feeding on algae exposed to 0.05 and 0.1 μg/L of esfenvalerate, and the highest dietary exposure reduced egg production in final-instar nymphs. The diet for *B. americanus* and *H. pacifica* consisted of dead third-instar *Chironomus tentans* larvae preexposed for 24 h to esfenvalerate concentrations ranging between 0.1 and 1.0 μg/L. Consumption of larvae exposed to 0.5 to 1.0 μg/L of esfenvalerate caused case abandonment and mortality in *B. americanus* caddisfly larvae. Although *H. pacifica* nymphs readily consumed esfenvalerate-exposed larvae, no adverse effects were observed during the present study. Furthermore, no evidence of esfenvalerate-induced feeding deterrence was found in any of the species tested, suggesting that aquatic insects may not be able to distinguish between pyrethroid-contaminated and uncontaminated food sources. These findings indicate that feeding deterrence is not a factor in regulating aquatic insect dietary exposures to synthetic pyrethroids.

Keywords—Synthetic pyrethroids Aquatic insects Dietary exposure Functional feeding group

INTRODUCTION

Within the past 15 years, both agricultural and urban uses of pyrethroid compounds have increased, and these compounds are frequently being used as replacements for organophosphate insecticides. Esfenvalerate has been detected in agriculturally associated streams in California (USA) at concentrations ranging between 0.1 and 0.8 μg/L [1]. Significant sublethal effects also have been observed in aquatic insect species following exposures of less than 1 μg/L [2,3], and the pyrethroid mode of action has been shown to impair crucial insect physiological functions, including that of the neurological and endocrine systems [4–6]. Consequently, concern exists that synthetic pyrethroid contamination of surface waters could significantly damage nontarget aquatic insect communities and alter stream ecosystem function.

Esfenvalerate's low water solubility (2 μg/L) and high octanol–water partition coefficient (log K_{OW} = 7.2) suggests partitioning onto lipophilic surfaces (i.e., detritus, dissolved organic matter, and sediment) [7]. Because a significant portion of esfenvalerate in aquatic systems may be sorbed, dietary exposure pathways are of interest. Field sampling in agricultural headwater streams revealed particulate-associated fenvalerate contamination of up to 302 μg/kg following rain events [8]. Adsorption to solids may decrease the fraction of pyrethroids that are bioavailable for cuticular uptake by aquatic invertebrates. Addition of 200 mg/L of suspended solids into the water column was shown to increase medial lethal concentrations for pyrethroid by 2.5- to 13-fold over that in sediment-free water for *Ceriodaphnia dubia* bioassays [9]. Uptake of radiolabeled bifenthrin and permethrin by *Daphnia magna* also was significantly decreased following the addition of suspended solids into the water column.

In addition to examining the impacts of aquatic exposure, it is important to consider other high-probability exposure pathways that may be enabled by the hydrophobic properties of the pyrethroids. Although unavailable for cuticular uptake, particulate-associated pyrethroids may be available for dietary uptake. The introduction of fenvalerate-spiked sediments into microcosms induced invertebrate drift responses similar to those observed following aquatic insecticide contamination [10]. Similarly, decreases in blackfly larval populations were observed following the introduction of particulate-associated DDT and temephos [11,12]. Because blackfly larvae feed on particulate matter filtered from the water column, these findings suggest that synthetic pyrethroids might be toxicologically active even when adsorbed to organic matter.

Feeding studies performed with terrestrial insects demonstrate that pyrethroid insecticides are trophically bioavailable to a number of different insect species, and consumption can impair fitness significantly. *Drosophila melanogaster* larvae reared on a diet containing cypermethrin exhibited decreased reproductive output at lower dietary doses and decreased survival in the highest diet treatment [13]. In particular, the accessory gland of the adult male flies exhibited extensive damage following larval growth on a cypermethrin-laced diet, and elevated heat shock protein gene expression was detected in trophically exposed males and females. Similarly, consumption of cyfluthrin-laced diet reduced weight gain and growth

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in Egyptian cotton leafworm (*Spodoptera littoralis*) larvae and induced several behavioral symptoms of pyrethroid poisoning, including knockdown [14]. The author theorized that feeding on contaminated food decreased the efficiency of energy conversion, potentially as a result of diversion of energy to detoxification processes.

Although particulate-bound pyrethroids may be bioactive following consumption by insects, each aquatic species may react differently to contamination of food sources. Field studies with zinc-contaminated periphyton revealed that aquatic insect colonization rates of zinc-contaminated biofilm varied by species, with the most sensitive species being found in the mayfly family Ephemerellidae and the dipteran families Taeniopterygidae and Simuliidae [15]. The pyrethroids themselves exhibit repellant properties, and a number of terrestrial arthropods have been shown to avoid areas contaminated with pyrethroid insecticides and to forage preferentially in nonexposed areas [16–18]. Aquatic insects therefore may display a range of sensitivities and modes of response to contaminated food sources, including those that contain pyrethroids.

Three aquatic insect species representing different functional feeding groups were used during the course of these experiments: *Cingymula reticulata* (Ephemeroptera: Heptageniidae), a grazing mayfly; *Hesperoperla pacifica* (Plecoptera: Perlidae), a predaceous stonefly; and *Brachycentrus americanus* (Trichoptera: Brachycentridae), an omnivorous filter feeder. Using these insects, we determined whether membership within a particular functional feeding group impacted the extent of dietary esfenvalerate exposure. We also determined the effect of dietary esfenvalerate exposure on *C. reticulata* growth and fecundity and on *B. americanus* and *H. pacifica* behavior.

MATERIALS AND METHODS

Chemicals

Analytical-grade esfenvalerate (ChemService, West Chester, PA, USA) was used throughout the present study; stock solutions of 10, 1, and 0.1 μg esfenvalerate/ml acetone were prepared using pesticide-grade acetone (Fisher Scientific, Pittsburgh, PA, USA). Previous research concerning the affinity of pyrethroid insecticides for macrophytes indicates that the partition coefficients for plant material (K_p) were extremely high, and absorption was determined to be almost irreversible [19]. Consequently, food items were exposed in water samples containing different esfenvalerate concentrations. Methods of dilution to obtain experimental concentrations have been described previously by Johnson et al. [2], as have verification methodologies for nominal concentrations. Each esfenvalerate concentration used during the course of these experiments was verified using this methodology, with duplicate samples analyzed for each treatment concentration. The grand mean of percentages of esfenvalerate recovered during analysis was 71.43% (standard deviation, 12.9%).

Dietary esfenvalerate exposures of H. pacifica *and* B. americanus

To ascertain whether predaceous aquatic insects alter their feeding habits in response to esfenvalerate-contaminated diet, field-collected *H. pacifica* nymphs and *B. americanus* larvae were laboratory-reared on a diet that had been exposed to esfenvalerate. Both were fed a diet of dead third-instar *Chironomus tentans* larvae (San Francisco Bay Brand, Newark, CA, USA). Previous work with these insects indicated that they readily consume freshly killed larvae as well as thawed dead larvae. Dietary exposures were carried out by the following methods.

Twenty thawed *C. tentans* larvae (dry wt, ~30 mg) were each transferred with forceps to 500-ml flasks containing 500 ml of chilled well water at each treatment concentration. Larvae were exposed at esfenvalerate concentrations of 0, 0.1, 0.2, 0.5, and 1.0 μg/L. The flasks were then sealed with aluminum foil and placed in a cold room (10 ± 2°C, mean ± standard deviation throughout). Aeration (supplied by disposable glass pipettes) provided systems with agitation and mixing. Larvae were exposed for 24 h, then removed from esfenvalerate solutions and soaked in clean water on ice for less than 1 h before use in bioassays.

Small *H. pacifica* nymphs (head width, 3 mm; body length, 9 mm) and fourth-instar *B. americanus* larvae were collected from the Metolius River (OR, USA) in April 2007 using a custom-made Surber sampler fitted with a 500-μm filter. Insects were field-identified during collection. Insects were held in chilled and aerated river water during transportation to the laboratory, where they were transferred to circulating tanks containing chilled (10–13°C), aerated well water from the Sinnhuber Aquatic Research Laboratory near Corvallis (OR, USA). Test organisms were allowed to acclimate in laboratory tanks for at least 24 h before bioassays.

The stonefly (*H. pacifica*) nymphs were individually transferred to 100 × 50 mm crystallizing dishes containing pebble substrate (width, ~0.7–1.0 mm) and chilled well water. The nymphs were starved for 3 d before feeding with contaminated *C. tentans* larvae. Feeding was undertaken by presenting dead larvae ad libitum with forceps in front of the nymph's mouthparts, whereupon they would attack and consume the offered prey items until satiated. Three stonefly nymphs were exposed to each dietary esfenvalerate concentration, and these were fed every 72 h for 14 d.

Twelve *B. americanus* larvae were transferred to each of 24 plastic rectangular containers (width, 10.4 cm; length, 14.5 cm; depth, 6.0 cm) containing 250 ml of chilled well water. These containers were placed in chilled water baths (10–12°C), and a circulating water flow was created by carefully placing air bubblers next to a rock placed in the center of each container. Four replicate containers of *B. americanus* larvae were exposed at each of the six dietary esfenvalerate treatment concentrations. These caddisfly larvae would attack and consume the prey items when these were draped over their outstretched legs. Larvae were fed every 72 h for 14 d, and the number of *C. tentans* larvae consumed per replicate was recorded. Container water was changed 12 h after every feeding.

Both the *H. pacifica* and *B americanus* test insects were observed while feeding to note whether exposed food was rejected during the feeding process. Rejection rates of *C. tentans* larvae and the total number of larvae consumed per dietary treatment concentration were used as indicators for avoidance of the esfenvalerate-contaminated diet. Insects were observed 24 h after feeding for behavioral impacts (e.g., incoordination, leg tremors, and protruding mouthparts) and mortality caused by consumption of esfenvalerate-contaminated prey.

Mean numbers of chironomid larvae consumed by *H. pacifica* nymphs and *B. americanus* larvae within each dietary esfenvalerate concentration were analyzed using an analysis of variance (ANOVA) procedure followed by a Tukey–Kramer

posttest (GraphPad Prism, Ver 5.00; GraphPad Software, San Diego, CA, USA; http://www.graphpad.com). Cumulative *B. americanus* case-abandonment and mortality was averaged for each dietary exposure concentration and compared using ANOVA followed by a Tukey–Kramer posttest (GraphPad Prism).

Avoidance of esfenvalerate-contaminated diet by C. reticulata *nymphs*

To determine whether esfenvalerate acts as a feeding deterrent for *C. reticulata* mayflies, medium-sized nymphs (head width, 2.5 ± 0.03 mm; body length, 8 ± 0.24 mm) were collected from the Metolius River in September 2006 using a Surber sampler fitted with a 500-μm filter. They were field-identified during collection, and identification was later verified in the laboratory. Test organisms were allowed to acclimate in laboratory tanks for at least 24 h before bioassays.

The periphyton fed to *C. reticulata* nymphs was cultured in the laboratory and was seeded from algal samples collected from the Metolius River. Unglazed ceramic tiles (2.54 × 2.54 cm) were placed in large, plastic containers of well water. Nutrients (Miracle-Gro® Liquid All Purpose Houseplant Food; Scotts Miracle-Gro, Marysville, OH, USA) were added (10 drops/L water) to support algal growth. Collected algal samples were then added to the water and placed near a window to receive sunlight. The experimental periphyton layer was grown as a thin covering on the tile surfaces, yielding approximately 25 mg dry weight of algae per tile after 20 d.

To expose the periphyton to esfenvalerate, four algae-covered tiles were transferred to 1-L beakers containing 500 ml of chilled well water (10–12°C). Appropriate volumes of esfenvalerate stock solutions were added to beakers to create the desired esfenvalerate concentrations, and total acetone volume was 0.02%. The beakers were covered with aluminum foil and placed in a cold room (10 ± 2°C) for 24 h. Following this exposure period, algal tiles were removed from the beakers using forceps and dipped twice in clean water before immediate use in bioassays.

To determine whether *C. reticulata* nymphs avoid esfenvalerate-contaminated periphyton, algae-covered tiles were exposed to 0 and 0.1 μg/L of esfenvalerate. Two beakers (each containing four tiles) were exposed at each treatment concentration. Ten nymphs each were transferred to each of four 100 × 50 mm crystallizing dishes containing 250 ml of chilled well water. Each dish was supplied with two nonexposed algal tiles and two tiles from the 0.1 μg/L of esfenvalerate treatment. Insect activity was observed daily for 30 min over a 7-d feeding period to determine whether nymphs were using esfenvalerate-exposed tiles. Nymph tile preference (exposed vs nonexposed) was used as an indicator of algae preference (exposed vs nonexposed).

Small C. reticulata *nymph assay: Effects of dietary exposures on growth*

Early instar nymphs (n = 200; length, 2.1 ± 0.15 mm) were used to determine the impact of esfenvalerate-contaminated periphyton consumption on *C. reticulata* growth rates. Growth rates are more rapid during earlier developmental stages, and these insects were better suited for investigation of the growth end point. Insects were collected from the Metolius River in October 2006 and allowed to acclimate in laboratory tanks for at least 24 h before feeding bioassay.

Nymph length was measured using a Leica MZ12 dissecting scope (Leica Microsystems, Wetzlar, Germany) fitted with an ocular micrometer, and species identification was again verified at this time. This experiment was first run once with three replicate dishes per algal treatment concentration and then repeated with two replicate dishes per treatment concentration, for a total of five replicates per concentration. Dietary esfenvalerate treatment concentrations were 0, 0.25, 0.5, and 0.1 μg/L. In preliminary studies, *C. reticulata* consumption of 0.2 μg/L of esfenvalerate-exposed algae resulted in greater than 90% mortality. Each dish, containing 10 insects, was provided with one esfenvalerate-exposed algal tile and held in a chilled water bath (10 ± 2°C), with aeration provided by disposable, 23-cm pipettes connected to air pumps. Algal tiles and water were replaced every 5 d for the duration of the experiment. During the course of this bioassay, gut contents of the nymphs were observed to ensure that feeding was occurring at all dietary treatment concentrations. After 21 d, insects were removed from dishes, and insect length was measured again.

Mean insect lengths as a percentage of initial length were calculated for each replicate dish, and these values were used to provide treatment means for analysis. The results were analyzed using ANOVA followed by a Tukey–Kramer multiple-comparison test (GraphPad Prism).

Final-instar C. reticulata *assay: Emergence and fecundity following dietary exposures*

To determine whether consumption of esfenvalerate-exposed algae affects *C. reticulata* fecundity, final-instar nymphs were reared on an exposure diet for 10 d. Insects were field-collected from the Metolius River in the spring of 2007 as described above. Proximity to emergence was gauged by visual inspection of wing pad length and darkness of body coloration during field collection. Length of feeding bioassay was limited to 10 d; nymphs that did not emerge during this time were discarded. This ensured that insects used in this experiment were of similar age.

Following a 24-h acclimation period in circulating laboratory tanks, 10 insects were transferred to each of twelve 100 × 50 mm crystallizing dishes containing chilled water. Three replicate dishes were run for each algal esfenvalerate treatment concentration of 0, 0.025, 0.05, and 0.1 μg/L. Algal tiles were exposed to the different insecticide concentrations as described above, with two beakers containing four tiles each being prepared at each esfenvalerate treatment concentration. The nymphs were provided with two treated tiles (or untreated control) per dish. Dishes were then placed in a chilled water bath (10 ± 2°C), provided with constant aeration, and covered with screens to prevent the escape of emerged adults. The water was changed every 2 d, and the contaminated algal tiles were replaced every 5 d. Emerged adults were removed every 2 d, and adult wing, thoracic, and abdomen sizes were measured (±0.0005 cm). Female insects were dissected and the numbers of eggs counted. Eggs from emerged females were randomly selected, and egg length was measured (±0.00005 mm).

The *C. reticulata* nymphs exhibited staggered emergence, and emerged females had fed on a contaminated diet for a variable number of days before emergence. Consequently, female egg count was regressed against days feeding within each algal treatment concentration (GraphPad Prism). Egg length was averaged by diet and emergence date and analyzed by ANOVA followed by a Tukey–Kramer posttest to distinguish between treatment groups (GraphPad Prism).

Table 1. Average percentage of available esfenvalerate-exposed food items consumed by *Hesperoperla pacifica* nymphs and *Brachycentrus americanus* larvae[a]

	0.0 μg/L	0.1 μg/L	0.2 μg/L	0.5 μg/L	0.75 μg/L	1.0 μg/L
H. pacifica	66.67 (28.9)	58.33 (14.4)	75 (25)	66.67 (14.4)	66.67 (14.4)	71.67 (14.0)
B. americanus	73.96 (2.69)	60.42 (7.01)	67.38 (5.55)	70.21 (8.80)	67.48 (3.28)	62.74 (12.74)

[a] Standard deviations are reported in parentheses.

RESULTS

Effects of H. pacifica *stonefly consumption of esfenvalerate-exposed diet*

We found no evidence that *H. pacifica* nymphs were more likely to reject prey items that had been preexposed to esfenvalerate. Nymphs fed at all dietary treatments and consumed similar numbers of exposed *C. tentans* larvae (ANOVA, $p > 0.05$) (Table 1). Additionally, we found no evidence of toxicity arising from the four successive dietary exposures. Previous experiments indicated that *H. pacifica* feeding behavior was the most sensitive behavioral end point affected by waterborne esfenvalerate exposures (K.R. Palmquist, unpublished data). Difficulty in feeding appeared to result from an abnormal protrusion of stonefly maxillary and labial palps, which possibly prevented coordinated prey attacks. Neither mouthpart incoordination nor a decrease in feeding following initial dietary exposures, however, was observed in these bioassays.

Effects of B. americanus *caddisfly consumption of esfenvalerate-exposed diet*

Analysis of diet consumed by fourth-instar *B. americanus* larvae indicates no treatment concentration difference in the numbers of esfenvalerate-exposed *C. tentans* larvae consumed (ANOVA, $p > 0.05$) (Table 1). This suggests that esfenvalerate had no feeding deterrence action in *B. americanus* larvae. We also found no difference in the numbers of prey items consumed per *B. americanus* larva over the course of the two-week bioassay. Consequently, no indication of an esfenvalerate-induced feeding inhibition following initial dietary exposures was observed.

Both strong behavioral effects and mortality were observed in the test larvae following dietary esfenvalerate exposures. Case-abandonment was observed following consumption of *C. tentans* larvae exposed to 0.2, 0.5, 0.75, and 1.0 μg/L of esfenvalerate (Fig. 1). This pronounced behavioral response also has been observed following waterborne esfenvalerate exposures [2]. Mortality occurred only at the highest dietary treatment concentration and constituted approximately 25% of the total *B. americanus* response at this treatment. Neither case-abandonment nor mortality was recorded for insects fed the unexposed diet. Total cumulative effect (the sum of case-abandonment and mortality) observed during the two-week bioassay was significantly increased in the 0.75 and 1.0 μg/L diet treatments compared with controls (ANOVA, $p < 0.01$) (Fig. 1). Interestingly, maximum response levels were attained following the second dietary exposure, and the two additional feedings did not significantly increase response rates.

Effects of C. reticulata *mayfly consumption of esfenvalerate-exposed diet*

When given the choice between an esfenvalerate-exposed diet and an unexposed diet, nymphs were observed spending equal time feeding on both diets and did not exhibit behavioral avoidance of preexposed algal tiles. Furthermore, no qualitative differences in gut contents were observed in the small nymphs used for the growth rate bioassay. Hence, no evidence was found that *C. reticulata* nymphs could detect esfenvalerate-exposed algae.

Consumption of an esfenvalerate-exposed diet inhibited the growth of small *C. reticulata* nymphs. Insects reared on the unexposed diet exhibited a 17% increase in length over the 21-d dietary exposure period. This was decreased to 8 and 4% for insects that were reared on an algal diet preexposed to 0.05 and 0.1 μg/L of esfenvalerate, respectively (ANOVA, $p < 0.05$) (Fig. 2). Nymphs reared on 0.025 μg/L of esfenvalerate exhibited an 11% increase in length, which although less than the control insect growth was not statistically different (ANOVA, $p > 0.05$). No significant trend for insect mortality was found among dietary treatment concentrations. No more than 30% mortality occurred in replicates used for statistical analysis. Mortality averaged 22% over the three-week bioassay period.

Dietary esfenvalerate exposure also negatively impacted egg production in final-instar *C. reticulata* nymphs. Because female emergence occurred over a 10-d period, we performed a series of linear regressions to determine whether the duration of dietary esfenvalerate exposure affected the level of reduction in egg production (Fig. 3). Significant deviation from linearity was identified only at the highest dietary exposure concentration ($r^2 = 0.7089$, $p = 0.0011$).

The length of eggs produced by emerged females also varied significantly with dietary esfenvalerate exposure (Fig. 4). Egg dissected from females reared on an esfenvalerate-contaminated diet for 10 d were significantly smaller than those

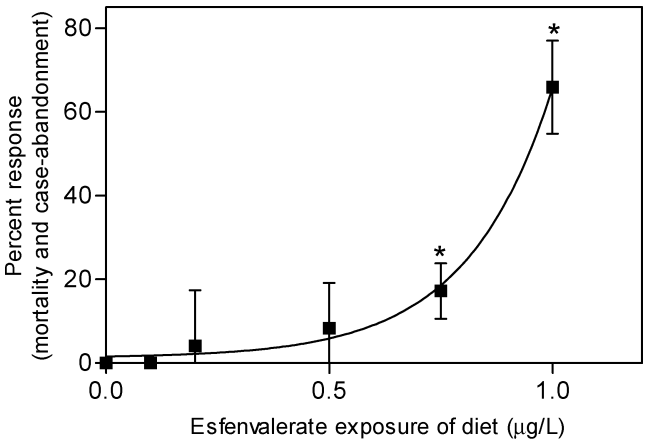

Fig. 1. Total *Brachycentrus americanus* larval responses to two-week dietary exposure to esfenvalerate. The line is the variable-slope dose–response curve fitted by GraphPad Prism software (Ver 5.00; GraphPad Software, San Diego, CA, USA). Error bars represent 95% confidence intervals of the means. Asterisks identify significant differences from control (analysis of variance, $p < 0.05$).

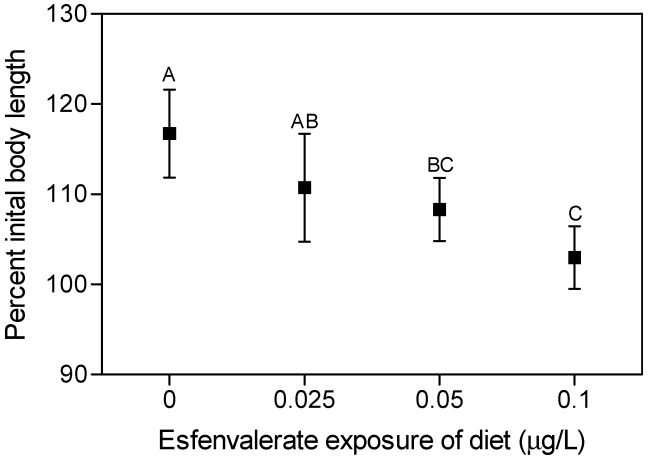

Fig. 2. Increase in *Cinygmula reticulata* mayfly length over a three-week dietary exposure to esfenvalerate-exposed algae. Significant differences in growth are designated by letters (analysis of variance, $p < 0.05$). Error bars represent 95% confidence intervals of the means.

removed from insects reared on the control diet for the same amount of time (ANOVA, $p < 0.05$). This is indicative of further impacts on *C. reticulata* fitness, because egg size is positively correlated with offspring survivorship and fitness [20,21].

DISCUSSION

The extent of pesticide trophic uptake will be impacted by the complex and variable responses of different aquatic taxa to diet quality. Grazing herbivores, including scraping mayflies, rely on poor-quality food sources with low caloric and nutritional content, and they exhibit high rates of food consumption [22]. Predators and omnivores, including filter feeders, however, exhibit decreased ingestion when confronted with food of low quality and increased ingestion rates when food quality improves [23]. Foraging, predatory stonefly larvae also can maximize energy consumption by selectively preying on the most energetically profitable food items [24].

Aquatic insect food quality has been directly linked with fitness and survival parameters, such as adult body weight, length, and egg production [20,24,25]. Decreased larval weight correlated with reductions in *C. tentans* adult weight and fecundity [26], and smaller individuals were more likely to succumb to disease and experience decreased fecundity [25]. Mayflies reared on high-quality periphyton exhibited larger sizes, higher fecundity rates, earlier emergence dates, and larger eggs [20]. Toxic contamination, however, may decrease the quality of aquatic insect food sources, negatively impacting growth and fitness.

Given the chemical properties of synthetic pyrethroid insecticides, large proportions of these compounds will be found adsorbed to particulate and organic matter within aquatic systems [7,27,28]. Whereas particulate-associated pyrethroids are unavailable for cuticular uptake, evidence exists that adsorbed compounds still impact aquatic insect behavior [3,8,10]. Consumption of particulate-associated DDT was shown to be toxic to blackfly larvae [11], and the similar chemical properties of organochlorine and pyrethroid insecticides suggests that particulate-adsorbed pyrethroids also may be toxic. Unlike DDT, however, synthetic pyrethroids show no tendency to bioaccumulate or biomagnify within aquatic systems [29]. Dietary pyrethroid exposure most likely would result in direct toxicity

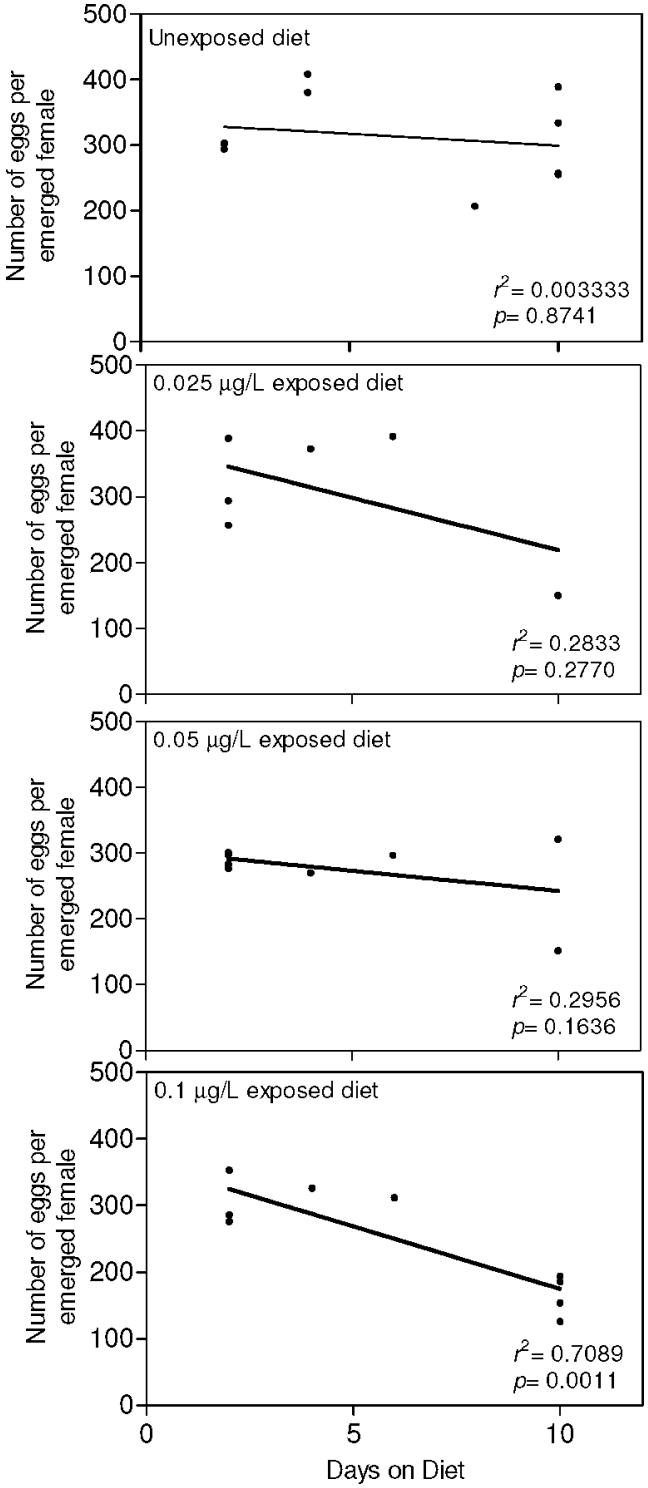

Fig. 3. Effect of final-instar *Cinygmula reticulata* dietary esfenvalerate exposures on female egg production. Lines are the linear regressions calculated using GraphPad software (Ver 5.00; GraphPad Software, San Diego, CA, USA). A significant impact on egg production was observed only in those insects reared on 0.1 µg/L of esfenvalerate-exposed algae.

to the insect consumer rather than extensive food web–level effects.

Results from these feeding bioassays indicate that dietary esfenvalerate is bioavailable to these species of aquatic insects. None of the species used during these experiments, however, rejected esfenvalerate-exposed food. This suggests that despite

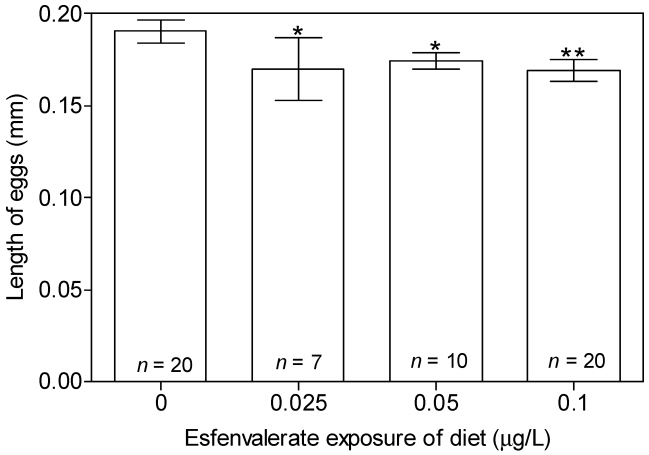

Fig. 4. Average length of eggs dissected from emerged *Cinygmula reticulata* females reared on esfenvalerate-exposed algae for 10 d. Asterisks designate significant difference from control (analysis of variance, $*p < 0.05$, $**p < 0.01$). Error bars are 95% confidence intervals of the means.

the evidence for feeding deterrence in terrestrial insect species [30], aquatic insects are unlikely to selectively reject pyrethroid-contaminated food. Considering pyrethroid chemical behavior and the lack of evidence for feeding deterrence, dietary exposure may be a significant route of pyrethroid exposure for aquatic insects.

Toxic effects observed in both *B. americanus* larvae and *C. reticulata* nymphs indicate that adsorbed pyrethroid compounds are biologically active following dietary exposure. Impacts on both *C. reticulata* growth and fecundity suggest that the consumption of an esfenvalerate-contaminated diet altered energy uptake and allocation. A similar alteration in the scope for growth observed in *S. littoralis* larvae reared on a cyfluthrin-laced diet was determined to be a result of toxic effects following consumption [14]. Those authors reported that changes in growth rates were caused by a pyrethroid-induced decrease in proficiency of food conversion into biomass.

Alterations in aquatic insect energy budgets immediately before metamorphosis may significantly change adult morphology and decrease fecundity. Inducing silk production in fifth-instar *Odontocerum alicorne* caddisfly larvae decreased the energy reserves carried into pupation. Emerged adults exhibited smaller wings and thoracies [31]. Similarly, the limited resources available for egg production are accumulated during the feeding juvenile stages [32]. Consuming esfenvalerate-contaminated food sources appears to reduce the energy available for both growth and reproduction [30].

The observed effects of dietary esfenvalerate exposure on *C. reticulata* egg production lend further support to the theory that energy allocation is impacted by such exposures. The correlation of reduction in fecundity with length of dietary exposure indicates that the length of exposure also is crucial. Similarly, the significantly smaller eggs produced following 10-d dietary esfenvalerate exposure also implies a reduction in available energy reserves. For mayflies, egg size has been shown to positively correlate with female fertility [20]. Hence, the decrease in *C. reticulata* egg size may be indicative of suppressed fertility and reduced survival of offspring. Furthermore, larger and heavier eggs have been shown to sink faster following oviposition, which reduces the risk of egg predation [21]. Dietary esfenvalerate exposure could, potentially, impact fitness into the next generation.

Behavioral effects following dietary esfenvalerate exposure were much more pronounced for *B. americanus* larvae. Interestingly, the case-abandonment response observed during this experiment also was observed in response to waterborne esfenvalerate exposure [2]. Furthermore, the total *B. americanus* response peaked after the second feeding, and the two subsequent feedings did not significantly increase the total response. This lends further evidence that pyrethroids do not accumulate in biological systems, and it suggests that a proportion of the *B. americanus* population may be able to metabolize and eliminate consumed esfenvalerate. Similarly, the lack of response in *H. pacifica* nymphs at all levels of dietary esfenvalerate exposures implies that this species also may have a high metabolic capacity for dietary esfenvalerate.

It is important to note that the *B. americanus* and *H. pacifica* feeding experiments involved the use of several acute dietary exposures applied in succession, whereas *C. reticulata* dietary exposures were more appropriately characterized as chronic. This design was intentional. Establishing the long-term feeding rates of the mayfly nymphs was accommodated by the visibility of gut contents. Because gut contents are not observable in either the *H. pacifica* stonefly nymphs or the *B. americanus* larvae, quantifying the amount of exposed food consumed or rejected over the two-week bioassay was used as a determination of feeding deterrence. This was easily accomplished using chironomid larvae as prey items. Furthermore, both *B. americanus* and *H. pacifica* growth rates are slow and, in the case of the caddisfly larvae, difficult to measure. The impacts of chronic dietary esfenvalerate exposure on *C. reticulata* growth and fecundity, however, may suggest that similar negative effects might be observed for *B. americanus* and *H. pacifica* insects experiencing chronic exposures.

In a field setting, the risk of dietary pyrethroid exposure probably will be greater for those insects whose diet is more likely to contain particulate matter. Scraper insects inhabiting headwater streams commonly consume detrital and particulate matter that has become associated with the periphyton [33]. Filter-feeders, including blackfly larvae and several caddisfly species, extract and consume organic debris from the water column. Increases of *B. americanus* larval filtering activity correlated with increases in the concentration of particulate organic matter in the water column [34]. Furthermore, the food ingestion rate of filter feeders has been shown to correlate positively with water velocity [35], such as those reported during runoff events, potentially as a result of the corresponding influx of allocthonous food items. Consequently, both scraper and filter-feeder insects would be more at risk of dietary pyrethroid exposures resulting from particulate-associated pyrethroid contamination.

Understanding the impact of dietary pyrethroid consumption on aquatic insect species is essential considering that a large percentage of pyrethroid contamination in surface waters will become sorbed to particulate and organic material. Sediment samples obtained from agriculturally associated streams in California were found to contain pyrethroid concentrations that were determined to be toxic to the sediment-dwelling invertebrates *Hyalella azteca* and *C. tentans* [36]. Whereas most sensitive aquatic insect species are not sediment dwellers, many regularly consume potentially contaminated particulate matter during feeding [33,37]. Dietary uptake therefore may be an important route of exposure for water-column aquatic insects, and both chronic and acute dietary pyrethroid expo-

sures have the potential to significantly reduce the populations of exposed insects through a number of pathways.

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