

ESFENVALERATE-INDUCED CASE-ABANDONMENT IN THE LARVAE OF THE CADDISFLY (*BRACHYCENTRUS AMERICANUS*)

KATHERINE R. JOHNSON,*† PAUL C. JEPSON,†‡ and JEFFREY J. JENKINS†

†Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, USA

‡Integrated Plant Protection Center, Oregon State University, Corvallis, Oregon 97331, USA

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Abstract—Field-collected *Brachycentrus americanus* Banks (Trichoptera: Brachycentridae) larvae were used to investigate the relationship between esfenvalerate exposure and case-abandonment response, determine larval ability to construct a new case, and measure the change in predation risk to insects in rebuilt cases. We evaluated case-abandonment following four environmentally relevant esfenvalerate exposures, 0.05, 0.1, 0.2, and 0.4 $\mu\text{g/L}$; 48-h exposures to 0.2 and 0.4 $\mu\text{g/L}$ (nominal) esfenvalerate both resulted in over 60% of larvae abandoning cases and were statistically indistinguishable. Propensity to engage in building behaviors was significantly diminished in 0.2 and 0.4 $\mu\text{g/L}$ esfenvalerate-exposed insects that had abandoned cases, with less than 20% of exposed insects producing cases. Cases built by intoxicated larvae were characterized by a disorganized composition, and required half the pressure to crush versus cases built by nonexposed larvae. Pre-exposing case-building material to 1 $\mu\text{g/L}$ esfenvalerate also reduced the physical strength of rebuilt cases. Larvae inhabiting weaker rebuilt cases and larvae without cases were significantly more susceptible to predation by second year *Hesperoperla pacifica* Banks (Plecoptera: Perlidae) stonefly nymphs than those in original cases. Overall, we concluded that small behavioral responses can have profound consequences for survival of species and reveal susceptible stages in life-cycles that can be overlooked by conventional approaches to ecological risk assessment.

Keywords—Sublethal effects Synthetic pyrethroid Trichoptera Case-abandonment recovery

INTRODUCTION

Caddisflies (order: Trichoptera) are animal architects and, like nesting birds, web-spinning spiders, and burrowing moles [1], they defend themselves from predation and environmental extremes through the construction of a protective enclosure [2]. Many caddisfly species construct hard cases that protect them against predation and physical damage and improve respiration capacity [3,4]. If they are deprived of the case or the ability to construct one, fitness and ultimately survival are likely to be impaired [1]. Given that exposure to certain neurotoxic insecticides can impact behavior, animal builders, including case-building caddisflies, may be uniquely susceptible to low levels of environmental contaminants if deprived of the ability to build or maintain the specialized structures that protect them. An apparently minor, sublethal pollutant impact that critically limits the effectiveness of construction, may therefore have significant consequences for the builder. It is necessary to understand the consequences of pollutant exposure for this important endpoint. This in turn could result in better characterization of the potential for sublethal pollutant concentrations to affect population dynamics and community structure and function [5].

Case-abandonment has been documented as a natural reaction to a number of abiotic stressors; however, this is an extreme escape response, employed only when the larva is severely threatened [6]. Several nonchemical stimuli, including low dissolved oxygen, drought [7], extreme temperatures, and burial in sediment [6], have been shown to provoke larvae

into wriggling out of their cases. Additionally, case-abandonment as a symptom of synthetic pyrethroid poisoning has been previously observed [8], although not thoroughly investigated as a sublethal response to pyrethroid exposure. Further, because case-building behaviors are neurologically, not hormonally, controlled [9], exposure to a synthetic pyrethroid may also impact the ability of caddisflies to rebuild a case.

Synthetic pyrethroids, like esfenvalerate, elicit toxic action by binding to voltage-activated sodium channels, preventing channel inactivation [10]. Nerve activity is prolonged, resulting in excitatory behavioral responses including twitching, muscle spasms, ataxia, and burning or itching sensations [11]. Use of these compounds is increasing in both agricultural and urban settings, and there is concern that this may pose a risk to nontarget aquatic organisms [12,13]. Esfenvalerate concentrations ranging between 0.1 and 0.8 $\mu\text{g/L}$ have been detected in agricultural runoff [14]; 0.54 $\mu\text{g/L}$ fenvalerate was directly lethal to a variety of aquatic insects in experimental mesocosms [15]. Considering the increase in pyrethroid use and its high toxicity to aquatic insects, exposure to sublethal concentrations could induce behavioral responses that may lead to case-abandonment by caddisfly.

Once deprived of a case, a caddisfly larva can rebuild an entirely new case, but this is an energetically costly process. Adults reared from larvae that were forced to rebuild cases exhibited smaller wings, abdomens, and thoraces when compared with adults reared from larvae with no rebuilding energy expenditures [16]. This suggests that larval case-rebuilding depletes energy reserves, adversely impacting adult reproductive fitness and longevity.

In order to minimize the time and energy expenditure required to rebuild cases, caddisflies that have lost their original cases build rapidly and preferentially select softer materials

* To whom correspondence may be addressed (kjohnson@exponent.com). The current address of K.R. Johnson is Exponent Consulting, 15375 SE 30th Place, Bellevue, WA 98005, USA.

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for ease of shaping and cutting, usually resulting in poorly constructed, weaker cases [17]. Inhabiting a weaker case increases the vulnerability of the larva to predators; for example, *Limnephilus frijole* Banks (Trichoptera: Limnephilidae) larvae with weaker cases were more likely to be consumed by a number of predators [18].

Given that pyrethroid exposure can induce case-abandonment in *Brachycentrus americanus* Banks (Trichoptera: Brachycentridae) larvae and that neurological impact may impair recovery efforts, we examined insecticide-mediated case-abandonment and its consequences for fifth-instar *B. americanus* larvae exposed to the synthetic pyrethroid esfenvalerate. We determined whether case-abandonment is a behavioral response to sublethal esfenvalerate exposure, and whether or not it occurs in a concentration-dependent manner. We determined whether exposed larvae were less likely to engage in case-rebuilding behavior after transfer to a clean system, and we measured the force required to crush cases built by exposed and control insects as an index of susceptibility to predation. In addition, we determined whether pre-exposure of case-rebuilding material to esfenvalerate would impact both likelihood and efficacy of case-rebuilding, and whether or not caseless larvae and larvae with rebuilt cases would be more vulnerable to predation.

MATERIALS AND METHODS

Chemicals

Analytical-grade esfenvalerate (ChemService, West Chester, PA, USA) was used during the course of the present study. Stock solutions of 10, 1, and 0.1 μg esfenvalerate/ml acetone were prepared and diluted to test concentrations of 0.05, 0.1, 0.2, and 0.4 $\mu\text{g/L}$ esfenvalerate. Preliminary studies indicated that a 48-h exposure to 0.4 $\mu\text{g/L}$ esfenvalerate resulted in approximately 10% mortality for *B. americanus* larvae.

Nominal concentrations of esfenvalerate in test solutions (0.05, 0.1, 0.2, and 0.4 $\mu\text{g/L}$) were verified by gas chromatography/mass spectrometry electron ionization using the method described in Runes et al. [19]. In order to obtain sufficient sample for analysis of esfenvalerate at ppt levels, we prepared 33.25 L fortified at concentrations of 0.4, 0.2, and 0.1 $\mu\text{g/L}$ esfenvalerate, and 35.25 L at 0.05 $\mu\text{g/L}$ esfenvalerate. At concentrations of 0.4, 0.2, and 0.1 $\mu\text{g/L}$ esfenvalerate, 2 L each was used for analysis and 31.25 L each for bioassays; for 0.05 $\mu\text{g/L}$ esfenvalerate, 4 L was used in analysis and 31.25 L for bioassays. Analysis volume was doubled for the 0.05 $\mu\text{g/L}$ concentration to ensure sufficient sample for analysis. At each concentration, duplicate samples were analyzed. The 1-L samples of the test water were fortified with analytical-grade esfenvalerate in 2 ml acetone in an amber bottle with a Teflon[®]-lined lid. Water samples (containing 1% methanol) were extracted using solid-phase extraction C18 cartridges (500 mg, 6-ml polypropylene reservoir; J.T. Baker, Union City, CA, USA). Solid-phase extraction cartridges were conditioned, and samples were passed through the solid-phase extraction cartridges followed by elution with ethyl acetate. Samples were evaporated using a nitrogen evaporator sample concentrator (Organomation, Berlin, MA, USA) to 400 μl followed by gas chromatography/mass spectrometry analysis.

Analysis of esfenvalerate was performed using a Hewlett-Packard 6890 gas chromatographer and a 5972 mass-selective detector (Hewlett-Packard/Agilent, Wilmington, DE, USA) that was run in selected ion monitoring mode. Operating conditions were as follows: ionization voltage (70 eV), electron

multiplier 2,100 V, and capillary interface at 280°C. Separation of the compounds was accomplished with a fused-silica capillary column of (5%-phenyl) methylpolysiloxane of 0.25- μm film thickness, 30-m length, and 0.25-mm interior diameter (J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 0.9 ml/min. The column temperature was set to 85°C for 1 min and then ramped at 25°C/min to 140°C. Further temperature ramps following this took place at 12°C/min to 260°C, 2°C/min to 280°C, and finally 18°C/min to 310°C. The injector temperature was 280°C. The retention times for the two esfenvalerate peaks (first peak contains *S, S* and *R, S* isomers, and the second peak contains the *S, R* and *R, R* isomers) were 19.50 and 19.93 min, respectively. Quantification, as the sum of the isomer pair base peaks, was based on the response of the 264 ion of the internal standard, benzo[*k*]fluoranthene-d12, whose retention time was 17.48 min. The ions monitored were 264 (base ion), 265, and 132 for benzo[*k*]fluoranthene-d12, and 167 (base ion), 169, and 225 for esfenvalerate. Recovery of esfenvalerate in bioassay solutions averaged 76, 85, 78, and 65% at nominal esfenvalerate concentrations of 0.4, 0.2, 0.1, and 0.05 $\mu\text{g/L}$, respectively.

Case-leaving bioassay

Field-collected *B. americanus* larvae were selected as test organisms because of their abundance in northwest U.S. streams and their important contribution to stream processes and nutrient cycling [20,21]. Fifth-instar larvae (case length of ~15mm) were collected between January and August 2005 from a pristine site in the Metolious River (Camp Sherman, OR, USA) using a custom-made Surber sampler with a 500- μm filter. Individuals were identified in the field using case characteristics; *B. americanus* larvae cut and arrange plant material into a striated, tapered, chimney-like case, with a square cross-section [22,23].

The insects were held in chilled and aerated river water during transport to the laboratory, where they were transferred to circulating tanks containing chilled (10–13°C), aerated groundwater. Test organisms were allowed to acclimate in laboratory tanks for at least 36 h prior to bioassays.

The water used in the course of these experiments (both bioassays and holding tanks) was obtained from two local groundwater sources: one located at the U.S. Environmental Protection Agency Willamette Research Station (Corvallis, OR), and one located at the Sinnhuber Aquatic Research Laboratory (Corvallis, OR, USA).

The flask bioassay system developed by Peterson et al. [24] was modified to study case-leaving. A series of 250-ml Erlenmeyer flasks containing 249 ml groundwater was chilled to $11 \pm 1^\circ\text{C}$ in a cold room. Flasks were aerated using 23-cm disposable Pasteur pipettes connected to aquarium pumps operating at 150 L/h. The risk of chemical loss via this airflow was considered negligible because of the low test concentrations and low volatility of esfenvalerate. Teflon boiling chips were added to each flask to provide substrate for the larvae.

Each of the four test treatments (plus control) involved the use of five replicates, with 10 caddisflies per replicate flask. The experiment was repeated five times, and replicates pooled for run. The mean of the pooled run results is reported here. A total of 250 insects were used at each treatment level. At the start of each experiment, larvae were removed from holding tanks and distributed randomly among all treatment flasks. The number of larvae out of case was recorded at 2, 4, 8, 16, and 32 h after initial exposure and again at the end of the

experiment (48 h after exposure began). Because the abandonment reaction could occur slowly over a couple hours, only those larvae completely out of their cases were counted as having left them.

Data were analyzed by an analysis of variance procedure followed by a Tukey–Kramer multiple-comparison procedure to determine differences between treatment groups (GraphPad Prism, Ver 4.00, GraphPad Software, San Diego, CA, USA, www.graphpad.com). Statistics were performed for each assessment time to determine whether significant differences existed between treatment groups during the progress of the bioassay.

Case-rebuilding assay

To gauge the ability of insects from different treatment groups to successfully rebuild cases after esfenvalerate exposure, caseless larvae were placed in a clean system and supplied with leaf detritus as rebuilding material. Three treatment levels of larvae were used (0, 0.2, and 0.4 $\mu\text{g/L}$), along with two exposure levels for the detritus (0 and 1 $\mu\text{g/L}$ esfenvalerate), resulting in a 3×2 factorial design.

Insects used in the case rebuilding study were provided with thawed whole *Chironomus tentans* larvae (San Francisco Bay Brand, Newark, CA, USA) in excess as food during the 36-h acclimation period to prevent hunger-induced stress. Control insects were removed from cases by gentle prodding with larval forceps; insects that had abandoned their cases in the 0.2 $\mu\text{g/L}$ and 0.4 $\mu\text{g/L}$ esfenvalerate treatments were used in the rebuilding assay.

To determine the effect of prior exposure of the case-building materials to pesticides on case rebuilding, detritus used for rebuilding was pre-exposed to esfenvalerate. Leaf detritus was generated from whole black cottonwood (*Populus trichocarpa*) leaves collected from the forest floor along a pristine stream, Rock Creek (Corvallis, OR, USA) in February 2005. Leaves were frozen prior to use. Whole leaves were blended in groundwater for 20 s, the excess water was squeezed out, and the leaf detritus weighed to yield 10.5 g wet weight of detritus per 250-ml long neck flask that contained 200 ml groundwater.

Control detritus was treated with 0.2 ml acetone alone. To contaminate the case building material, flasks containing the appropriate pesticide or solvent solution with the detritus were placed in a Burrell Wrist Action Shaker (Model 75, Burrell Scientific, Pittsburgh, PA, USA) for 24 h at 22°C. A static renewal procedure was performed 12 h into this mixing period. The detritus was then strained using a 500- μm stainless steel mesh and placed in 1-L beakers containing 500 ml aerated, chilled well water.

Caseless insects were distributed randomly to appropriate beakers, with 10 insects per beaker. Six replicates each were run for unexposed larvae/unexposed detritus, unexposed larvae/exposed detritus, and 0.2 $\mu\text{g/L}$ exposed larvae/unexposed detritus treatment groups; eight replicates were run for each of the remaining treatment groups. The larvae were allowed 96 h to rebuild cases; they then were separated into two groups, those with and without rebuilt cases, and counted. The counts were converted to proportion of rebuilt cases per beaker. Proportions of rebuilt cases were analyzed by an analysis of variance (ANOVA) followed by a Tukey–Kramer multiple-comparison procedure to determine differences in the rates of case-rebuilding among insect treatment groups and between detritus treatments (GraphPad Prism).

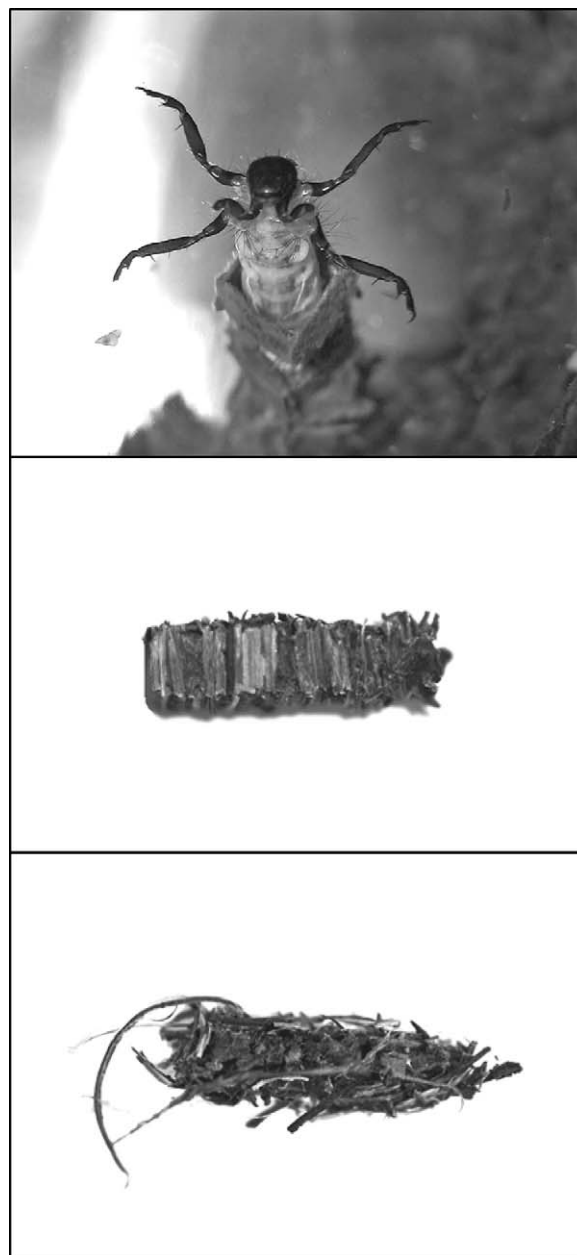


Fig. 1. A nonexposed *Brachycentrus americanus* larva during the rebuilding assay (top). Case (length = 9.8 mm) built by a nonexposed larva (middle). Case (length = 11.1 mm) built by 0.4 $\mu\text{g/L}$ esfenvalerate-exposed insect (bottom).

Impacts on case strength

Because the caddisfly case provides essential protection against predation, we calculated the pressure necessary to crush the case, as an index of potential future predation risk. Following the case-rebuilding assay, larvae were removed from rebuilt cases, and cases were air-dried for 24 h. At this time, they were weighed, and width, length, and ordered length measured. Ordered length refers to the portion of the case in which the striated detritus patterning was clearly visible (Fig. 1). Forty-three cases were recovered from the unexposed larvae/unexposed detritus treatment group; 24 were recovered from the unexposed larvae/exposed detritus treatment group; 13 from the 0.2 $\mu\text{g/L}$ exposed larvae/unexposed detritus treatment group; five from 0.2 $\mu\text{g/L}$ exposed larvae/exposed detritus treatment group; 10 from the 0.4 $\mu\text{g/L}$ exposed larvae/

unexposed detritus treatment group; and seven from the 0.4 $\mu\text{g/L}$ exposed larvae/exposed detritus treatment group.

Rebuilt cases were tested for structural integrity using a 1,000-g capacity small-fruit penetrometer (University of California Firmness Tester, Western Industrial Supply, San Francisco, CA, USA) which measures the weight needed to crush a small object. Cases were uniformly crushed along the horizontal axis, so that the sides collapsed outwards. Case strength was gauged in grams of crushing weight and then converted to kilopascals to correct for slight variations in case dimensions.

The significance of differences in case strength across caddisfly and detritus treatment groups was determined by ANOVA and a Tukey–Kramer procedure (GraphPad Prism). The amount of ordered length within the rebuilt cases was analyzed using the same procedure to differentiate building efficacy between treatment groups.

Predation risk bioassay

To estimate the increased vulnerability to predation resulting from a weakened case, the ability to obstruct predation attempts was examined for stream-built cases and rebuilt cases and for larvae with no cases. Stream-built cases are those that the insect forms slowly over its natural lifetime in the stream environment; rebuilt cases were those produced by larvae extracted from their cases and rebuilt with *P. trichocarpa* leaf material over a 96-h period, following methods used for the case-rebuilding assay.

Predation risk to the three larval case classes was determined using second-year *Hesperoperla pacifica* Banks (Plecoptera: Perlodidae) stonefly nymphs as predators. These occur in the same stream habitats as the *B. americanus* larvae and are readily collected. One stonefly nymph was placed in each of thirty 100 \times 50 mm crystallizing dishes containing Teflon substrate and 200 ml of untreated well water. Dishes were kept in a chilled water bath ($11 \pm 2^\circ\text{C}$) and aeration was provided using disposable 22.9-cm Pasteur pipettes. Stoneflies were starved for 4 d prior to experiment. Eleven predator nymphs were each provided with five caddisfly larvae in stream-built cases; seven nymphs with five caddisfly larvae in rebuilt cases; and 12 with five larvae without their cases. Stonefly nymphs were allowed to feed for 5 d. Numbers of remaining larvae were recorded at 1, 4, 24, 48, 72, 96, and 120 h.

Data from each assessment time were analyzed by ANOVA and a Tukey–Kramer multiple comparison procedure to determine the significance of any difference in predation risk among treatments (GraphPad Prism).

RESULTS

Case-leaving bioassay

A concentration–response relationship was observed for case-abandonment in the esfenvalerate treatments (Fig. 2). The maximum rate of case-leaving was 60 to 65% of the treated insects. This rate was observed at esfenvalerate concentrations of 0.2 and 0.4 $\mu\text{g/L}$, which were statistically similar. At the highest concentration, about 10% of the treated insects died within their cases. None of the out-of-case larvae died; all the dead larvae that were observed were entombed within their cases. Rates of case-abandonment differed significantly and in a dose-dependent manner ($p < 0.01$: multiple-comparison ANOVA) between the control and the three lowest treatments. Case-abandonment behavior occurred soon after exposure be-

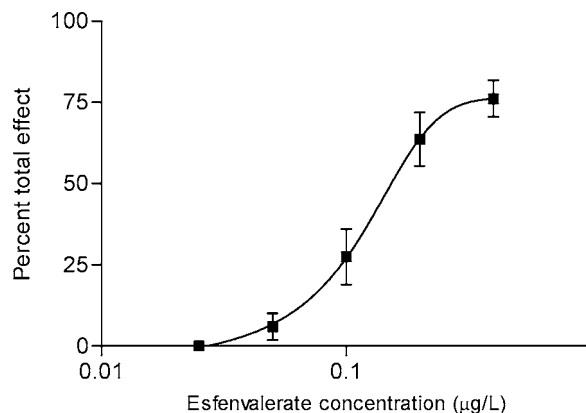


Fig. 2. Total effect (case-abandonment and mortality) of 48-h esfenvalerate exposures on *Brachycentrus americanus* larvae. Mortality (10.71%) was observed only at the highest exposure (0.4 $\mu\text{g/L}$) and occurred solely among insects that remained inside their cases. Computer-fit curve is a variable slope sigmoidal dose–response curve (GraphPad Prism, Ver 4.00, GraphPad Software, San Diego, CA, USA). Error bars represent 95% confidence intervals around the mean.

gan, and increased with exposure time and with increasing esfenvalerate concentration. The response occurred during the first 16 h of exposure in all treatments (Fig. 3).

Case-rebuilding assay

Once out of their cases, larvae exposed to 0.2 and 0.4 $\mu\text{g/L}$ esfenvalerate built 70 to 75% fewer cases compared with control insects ($p < 0.01$: multiple-comparison ANOVA, Fig. 4). No significant difference existed between in the numbers of cases built by exposed insects. Although exposed larvae remained alive throughout the case-rebuilding assay, very few attempted to construct a new case and many exhibited impaired coordination.

Cases built by intoxicated larvae exhibited less ordering than those built by control insects, based on the amount of visible ordered striation of case-building material, which is characteristic of normal building for this species (Fig. 1). Control insects produced cases that were 39% ordered, whereas intoxicated insects (both 0.2 and 0.4 $\mu\text{g/L}$ esfenvalerate treat-

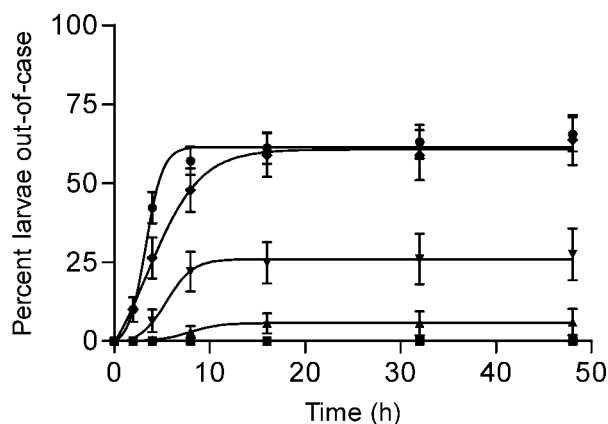


Fig. 3. Timing of the *Brachycentrus americanus* case-abandonment response over the 48-h exposure period. Only those larvae completely out of the case were counted at each time interval. Five esfenvalerate exposure concentrations were tested: 0 (■), 0.05 (▲), 0.1 (▼), 0.2 (◆), and 0.4 (●) $\mu\text{g/L}$. Variable-slope sigmoidal dose–response curve fitted by computer (GraphPad Prism, Ver 4.00, GraphPad Software, San Diego, CA, USA). Error bars are 95% confidence intervals around the mean.

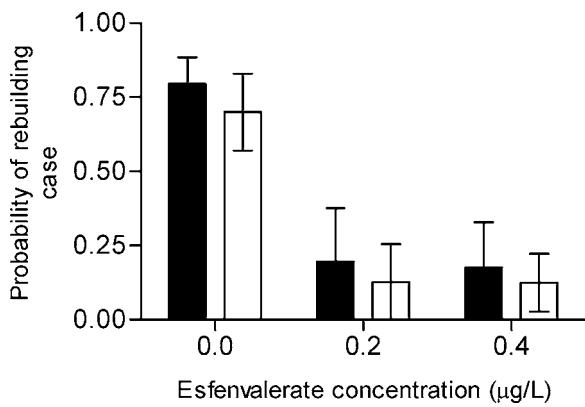


Fig. 4. Effects of esfenvalerate concentration on the capability of *Brachycentrus americanus* larvae to rebuild a case during the 96-h recovery period. Detritus provided for case-building was also exposed to esfenvalerate; (■), unexposed detritus (□), 1 µg/L exposed detritus. Any detrital structure built and inhabited by a larva was included in the count of cases. Error bars represent 95% confidence intervals around the mean.

ments) built cases with 16.6 and 13.4% ordering respectively ($p < 0.05$, ANOVA).

Case resistance to crushing as an index of predation risk

All rebuilt cases required about 70% less weight to crush them compared with stream-built cases, indicating that replacement cases do not offer the same level of protection against predation, as do stream-built cases. More significantly, the cases built by the esfenvalerate-exposed larvae withstood only half the weight of control-built cases ($p < 0.01$: ANOVA), and the pressure required to crush the cases decreased with increasing esfenvalerate exposure concentrations ($p > 0.05$, Fig. 5). Crushing data indicate no significant difference in rebuilt case strength within the 0.2 and 0.4 µg/L exposure groups. Additionally, cases rebuilt with esfenvalerate-exposed detritus required slightly less weight to crush, although this difference was not statistically significant.

The weights, lengths, or widths of rebuilt cases did not differ significantly, indicating that the amount of material used for rebuilding cases was similar for all treatment groups. The disparity in case integrity between treatments may have arisen from differences in the placement and ordering of plant ma-

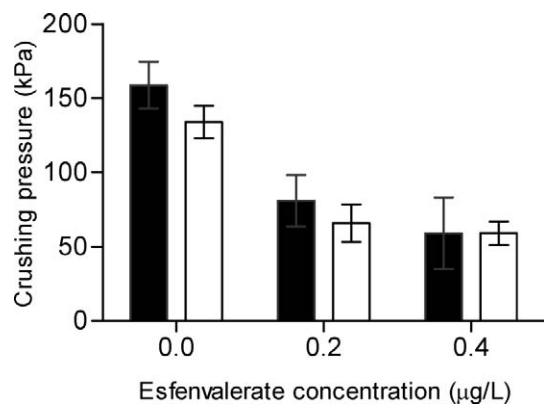


Fig. 5. Effect of insect and detritus esfenvalerate concentration on the strength of rebuilt cases; (■), unexposed detritus (□), 1 µg/L esfenvalerate exposed detritus strength was measured as grams of weight necessary to collapse the case, and then converted to kilopascals. Error bars represent 95% confidence intervals around the mean.

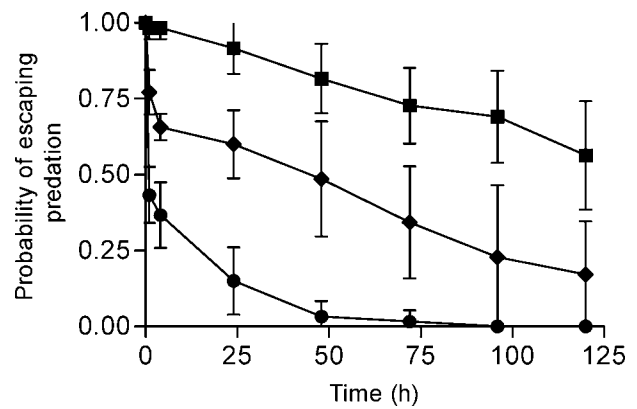


Fig. 6. Probability of *Brachycentrus americanus* larvae survival when exposed to *Hesperoperla pacifica* nymph predation with three levels of case strength/protection: stream-built case (■), rebuilt case (◆), and no case (●). Error bars represent 95% confidence intervals around the mean.

terial, and from observed differences in the patterns of silk that the caddis larvae produce to hold the plant material together. Silk patterns produced by larvae exposed to esfenvalerate displayed larger gaps and contained fewer strands than those produced by control insects.

Predation risk bioassay

Compared with larvae in stream-built cases, larvae without cases were extremely vulnerable to stonefly predation. At each assessment time, stoneflies had consumed significantly more caseless larvae versus those with cases, and larvae in rebuilt cases had been preyed upon in greater numbers than those within stream-built cases ($p < 0.01$: multiple-comparison ANOVA, Fig. 6).

Time to 50% predation-caused mortality was used as an index of the vulnerability of larvae to stoneflies. This time was less than one hour for larvae without cases, approximately 47 h for the larvae with rebuilt cases, and more than 120 h for caddisfly larvae inhabiting stream-built cases. The substrate in the system consisted of white Teflon chips, and it is assumed that larvae in and out of cases were equally visible and equally available to predators, and therefore, that the difference in predation rate was not a result of variability in camouflaging abilities.

Visual observations were made for the first 2 h of the predation bioassay. The disparity in predation rates appeared to result from the differences in prey handling times between larval case categories. Stoneflies effortlessly consumed caseless larvae, eating several during the initial distribution process (which took place over a 5 min period). Cases, both stream-built and rebuilt, provided a physical barrier against stonefly predation. Although stoneflies located these larvae during the distribution process, predation efforts were thwarted by the cases. Stonefly nymphs attacked the cases with their mandibles, working along the length of the case and eventually tearing a hole through which the larvae could be extracted. Stream-built cases appeared to require more effort and time to tear apart than did the rebuilt cases.

DISCUSSION

Agricultural use of synthetic pyrethroids in California, USA, a state with pesticide-use reporting, nearly doubled between 1991 and 2002, and nonagricultural usage increased

fivefold in the same period [25,26]. In addition, newer, more toxic pyrethroid compounds are becoming increasingly popular, making up a greater percentage of total agricultural pyrethroid usage [25]. However, extensive data gaps exist concerning the sublethal impacts of synthetic pyrethroid contamination on nontarget, native aquatic insect species [26].

Without its case, a caddis larva is exposed to predators, adverse environmental conditions, and injury. By undulating their abdomens, the insects force water through the case at an increased rate (versus stream flow), thereby improving respiration in low oxygen water [3]. In addition to enhancing oxygen uptake, cases of caddisflies prevent physical damage to their soft abdomens and provide camouflage to shield the insect from potential predators [4]. Because the caddis case imparts diverse fitness benefits, loss of the case could severely impact the insect's chance of survival.

Although few experiments have been performed on caddisfly case-abandonment behaviors, this behavior has been recorded in the literature. Previous research indicates that this behavior results in response to extreme environmental conditions. Dobson et al. [6] observed *Potamophylax cingulatus* Steph. (Trichoptera: Limnephilidae) larvae abandoning cases after the insects were buried in substrate, and speculated that this abandonment would improve the larvae's chances of digging themselves out of the silt. Otto [7] reported case-abandonment as a response to both extreme drought and to freezing temperatures, in response to very low levels of dissolved oxygen, and in response to simulated bird attacks. Further, there is some previous evidence that case-abandonment occurs as a reaction to exposure to sublethal concentrations of synthetic pyrethroids [8].

The similarities between the case-abandonment behaviors described in the literature and those described here raises two possibilities: Sublethal insecticide concentrations cause physically stressful environmental conditions similar to the ones described above, and case-abandonment is a result of the insect's attempts to escape, or the esfenvalerate mode of action causes a specific neurological dysfunction, inducing a case-abandonment response. The second explanation is more likely: if this were representative of a general reaction to exposure to low concentrations of chemical pollutants, this phenomenon would be observed for other neuroactive insecticides. However, we did not observe case-abandonment following sublethal exposures to two organophosphates and a carbamate, and this phenomenon may be unique to synthetic pyrethroids (K. Johnson, unpublished data).

Experiments performed on the nervous systems of several different Trichoptera species indicate that case-building is neurologically, not hormonally controlled. Whereas injection of juvenile hormone had no effect on case production, severing the connections between the larval thoracic and subesophageal ganglia interfered with material selection or placement and silking behaviors [9,27]. Similarly, the removal of external sensory appendages (e.g., anal hooks and thoracic hairs) resulted in cases that were twice as long as normal and cases constructed of ill-fitting materials [28]. While such studies involved the incision or removal of segments of the insect's nervous system, it is probable that other forms of incapacitation to the nervous system (i.e., through exposure to certain neurotoxins) could produce a similar effect. These results lend credence to the theory that a behavior such as case-rebuilding could be adversely impacted by exposure to neurotoxic insecticides.

Case-rebuilding is a time- and energy-intensive behavior, and larvae forced to rebuild a case preferentially select softer, more pliable materials that require less energy to cut and shape [17]. Rebuilt cases also contain less silk at pupation compared with those built over the entire larval period [16], also suggesting that a conservative use of silk may help preserve some energy stores. Both the selection of soft materials and the sparing use of silk should allow the larva to rebuild at a faster rate, but this also results in a weaker case with the increased predation risk that we recorded.

Despite the evidence for compensatory behaviors, case-rebuilding constitutes an energetically costly task, and depletions of larval energy resources can impact future adult morphology. Stevens et al. [16,29] demonstrated that inducing fifth-instar *Glyptotendipes pellucidus* Retzius (Trichoptera: Limnephilidae) larvae to rebuild cases resulted in reduced wing, thorax, and abdomen size.

Pyrethroid exposure appears to exacerbate the case abnormalities caused by the rebuilding process. The detoxification processes a larva must perform to rid itself of the pyrethroid are likely to be energy intensive, as is the case-rebuilding process, and the combination of the two stressors may increase the magnitude of case irregularities described above. This has been previously described: Wendt-Raisch et al. [30] found that exposing net-spinning caddisfly larvae *Hydropsyche siltala* Dohler (Trichoptera: Hydropsychidae) to sublethal fenvalerate concentrations results in altered silk patterns similar to those that we observed. We observed that esfenvalerate-exposed larvae exhibited further diminished capacities for material selection, resulting in cases comprised of ill-fitting plant materials compared with those constructed by nonexposed larvae. Energy depletions caused by case-rebuilding and pyrethroid metabolism and excretion, together with pyrethroid-induced alterations in silking patterns and material selection may explain why esfenvalerate-exposed larvae build weaker cases than nonexposed larvae.

Once extracted from their cases, caddisfly larvae were extremely vulnerable to predation. Cased insects utilized their cases as a portable refuge from stonefly nymphs and predation rate was reduced, because the stonefly nymphs had to break apart the case before being able to feed. After encountering a cased insect, predator nymphs squeezed and worked the posterior portion of the case with their mandibles until breaking it apart. Often, nymphs lost their grip or evidently became disinterested before reaching this point.

Previous studies indicate that case strength is directly related to predation risk. A number of aquatic predators, including the dragonfly nymph *Oplonaeschna armata* Hagen (Odonata: Aeshnidae) and the predaceous minnow *Gila pandora* Cope (Cypriniformes: Cyprinidae) were more likely to consume caddisflies with weaker cases [18]. Although predators utilized different tactics to separate a larva from its case, in both instances insects with stronger cases were more likely to escape predation. Additionally, Limnephilidae larvae with stronger and wider cases were more likely to survive encounters with predators.

Ultimately, although a caddis larva may be able to recover and rebuild a case after pyrethroid-induced case-leaving, the new case will be softer and will not afford the same protection as the original case. Additionally, there is strong evidence that a reduction in larval energy resources (e.g., through energetically costly case-rebuilding) results in altered adult morphology. Depending on the life history of the caddisfly species,

adults with reduced larval energy resources exhibit decreases in thorax or abdomen size, potentially reducing flight ability and fecundity [29]. In addition, sublethal fenvalerate concentrations were shown to delay and suppress normal *Limnephilus lunatus* caddisfly emergence up to 12 weeks after exposure [31]. Consequently, sublethal pyrethroid exposure may have long-lasting effects on caddis fitness and survival, even into the adult stage. Additionally, the responses reported here occurred following short-term exposures; chronic exposure to synthetic pyrethroids may have an even greater impact on larval survival.

Considering that the survival of case-making caddisfly larvae and other animal builders depends on maintaining their ability to construct protective microhabitats, exposure to sublethal concentrations of behavior-altering neurotoxins that cause case abandonment may lead to population declines and shifts in community structure and function. Preventing, altering, or arresting habitat construction critically damages individual's abilities to resist predation and a number of environmental stresses and animal builders may be uniquely susceptible to low concentrations of these environmental contaminants.

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