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### Authors

Renick, Violet Compton
Anderson, Todd W
Morgan, Steven G
et al.



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# Interactive effects of pesticide exposure and habitat structure on behavior and predation of a marine larval fish

**Violet Compton Renick · Todd W. Anderson · Steven G. Morgan · Gary N. Cherr**

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**Abstract** Coastal development has generated multiple stressors in marine and estuarine ecosystems, including habitat degradation and pollutant exposure, but the effects of these stressors on the ecology of fishes remain poorly understood. We studied the separate and combined effects of an acute 4 h sublethal exposure of the pyrethroid pesticide esfenvalerate and structural habitat complexity on behavior and predation risk of larval topsmelt (*Atherinops affinis*). Larvae were exposed to four nominal esfenvalerate concentrations (control, 0.12, 0.59, 1.18 μg/L), before placement into 12 L mesocosms with a three-spine stickleback (*Gasterosteus aculeatus*) predator. Five treatments of artificial eelgrass included a (1) uniform and (2) patchy distribution of eelgrass at a low density (500 shoots per $m^2$), a (3) uniform and (4) patchy distribution of eelgrass at a high density (1,000 shoots per $m^2$), and (5) the absence of eelgrass. The capture success of predators and aggregative behavior of prey were observed in each mesocosm for 10 min of each trial, and mortality of prey was recorded after 60 min. Exposure to esfenvalerate increased the proportion of larvae with swimming abnormalities. Surprisingly, prey mortality did not increase linearly with pesticide exposure but increased with habitat structure (density of eelgrass), which may have been a consequence of compensating predator behavior. The degree of prey aggregation decreased with both habitat structure and pesticide exposure, suggesting that anti-predator behaviors by prey may have been hampered by the interactive effects of both of these factors.

**Keywords** Esfenvalerate · Pyrethroid · Topsmelt · Behavior · Predation · Habitat

V. C. Renick (✉) · T. W. Anderson
Department of Biology and Coastal and Marine Institute, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-4614, USA
e-mail: violetrenick@gmail.com

V. C. Renick · S. G. Morgan · G. N. Cherr
Bodega Marine Laboratory, University of California Davis, P.O. Box 247, 2099 Westside Rd., Bodega Bay, CA 94923, USA

## Introduction

The diverse faunal assemblages of many coastal and marine ecosystems are currently threatened by chemical stressors such as organic pollutants (Dachs and Méjanelle 2010) in addition to the multiple physical and biological stressors that accompany coastal development (Lotze et al. 2006). The application of pesticides has become a growing concern due to their adverse effects on a variety of non-targeted organisms. Adjacent urban cities and agricultural areas generate run-off containing pesticides and other contaminants that flow into nearby watersheds, where some accumulate in the sediments and biota of estuaries, marshes, and bays (McGourty et al. 2009). Larval and juvenile stages of resident organisms are at particular risk in that they may be exposed during their most vulnerable developmental stages while using these habitats as nursery grounds (Beck et al. 2003; Courrat et al. 2009). Ultimately, the impacts of pesticides in aquatic environments can cascade from individuals to populations (Baldwin et al. 2009), and subsequently decrease biodiversity (Beketov et al. 2013) and alter community structure (Fleeger et al. 2003; Rohr et al. 2006; Macneale et al. 2010), although the mechanisms behind these impacts can be varied and complex.

Pyrethroid pesticides have become increasingly prevalent in urban estuarine environments where they now

replace traditional organophosphate pesticides that are particularly toxic to humans and wildlife (Department of Pesticide Regulation 2005; Brady et al. 2006; Weston and Lydy 2010; van den Berg et al. 2012). Pyrethroids are synthetic broad-spectrum pesticides that are particularly toxic to fishes and invertebrates, even in relatively low concentrations that are commonly found in the environment (Teh et al. 2005). Esfenvalerate has become one of the most commonly applied pyrethroids in California (Brady et al. 2006; Ensminger et al. 2011). Sub-lethal exposure to esfenvalerate can reduce growth and reproductive success in aquatic organisms (Tanner and Knuth 1996), alter behavior by increasing erratic swimming (Little et al. 1993; Floyd et al. 2008) and increase mortality (Teh et al. 2005). Pyrethroid pesticides such as esfenvalerate exert their neurotoxic effects by blocking the inactivation of voltage-gated sodium channels, inhibiting normal neural synapse transmission and resulting in repetitive neural firing (Bradbury and Coats 1989; Maund et al. 2012).

Despite the neurotoxicity of esfenvalerate and other pesticides to fishes and invertebrates, their effects on behavior are rarely investigated relative to other sub-lethal endpoints (Weis et al. 2001; Scott and Sloman 2004; Zala and Penn 2004; Robinson 2009; Sloman and McNeil 2012). The expression of behavior is dependent upon a properly functioning nervous system. Consequently, any chemical stressor which disrupts nervous system functioning may result in severe behavioral modifications. Numerous behaviors may be altered by contaminants, including locomotion and activity, social interactions, feeding activity and rate, and escape and avoidance of predators (see reviews by Fleeger et al. 2003; Scott and Sloman 2004; Weis and Candelmo 2012). Importantly, the population ecology of organisms often depends on behavioral outcomes (Weis et al. 1999; Murphy et al. 2008; Relyea and Edwards 2010). Behavior can thus be a powerful tool to link the sub-organismal effects of sublethal pesticide exposure to species interactions and population-level processes (Weis et al. 2001; Baldwin et al. 2009; Weis and Candelmo 2012).

In addition to the effects of chemical stressors such as pesticides, an organism's behavior may also be influenced by environmental or physical factors such as refuge availability or habitat quality. The presence of habitat such as vegetative structure or complex rocky bottom substrata can provide aquatic prey a refuge while inhibiting the foraging ability and success of predators (Orth et al. 1984; Gotceitas and Colgan 1989; Savino and Stein 1989). Aquatic organisms may display adaptive swimming behavior or activity depending on the presence of predators as well as differential habitat structure (Savino and Stein 1982; Dupuch et al. 2009; Tait and Hovel 2012). Although greater structural habitat complexity may be an important variable in influencing predator–prey interactions, the distribution of habitat (e.g. patchy vs. uniform) may also be important (Orth et al. 1984).

Despite the influence of habitat structure in determining the outcome of ecological interactions, its effects are not often considered in ecotoxicology studies (but see Davis et al. 2012). The presence and distribution of habitat may mediate predator–prey interactions, particularly if swimming behavior or predator detection and avoidance of prey are compromised by pesticide exposure. Such potential interactive effects are environmentally relevant because many urbanized coastal environments are often compromised by chemical, physical, and biological stressors, including both pollution and habitat degradation (Lotze et al. 2006). Many degraded estuarine systems display a range of structural habitat complexity, from a lack of vegetation in highly disturbed areas to highly fragmented or dense stands of aquatic vegetation (Hovel and Lipcius 2001). Yet the interactive effects of habitat structure and pesticides in coastal and estuarine systems are unclear, along with the likely consequences for aquatic fauna (Davis et al. 2012).

The goal of this study was to understand the interactive effects of sublethal pesticide exposure and the structural complexity of simulated eelgrass habitat on the behavior and predation of larvae of a common marine fish *Atherinops affinis* (topsmelt). First, we sought to determine the effects of acute 4 h sublethal exposures to esfenvalerate on the swimming behavior of topsmelt. Second, we aimed to determine whether 4 h esfenvalerate exposures resulted in increased predation risk for larval topsmelt. Finally, we aimed to determine the separate and interactive effects of pesticide exposure and habitat structure in determining larval predation risk. Our approach included observations of prey and predators during experiments to detect the behavioral mechanisms resulting in prey survival.

## Materials and methods

### Study species

Topsmelt are an abundant forage fish in California coastal systems, migrating from near shore waters to upper bays and estuaries to spawn in spring and summer. Topsmelt have been used extensively as a model species in ecotoxicology studies due to their relative sensitivity to contaminants (Anderson et al. 1991; Middaugh et al. 1992), their abundance in estuaries and bays (Allen and Horn 1975), and their trophic importance as prey for piscivorous fish and birds, such as the California least tern, *Sterna antillarum browni* (Atwood and Kelly 1984). Topsmelt

larvae (12–15 days post-hatch, ~10 mm standard length [SL]) were obtained from Aquatic BioSystems, Fort Collins, Colorado. Larvae were transferred to a 38 L aquarium and gradually acclimated to local 0.45 μm filtered seawater (Bodega Marine Reserve) over a period of 48 h before being used in experimental treatments. Daily water quality measurements were made to ensure appropriate acclimation conditions (20 ± 1 °C, salinity: ~34 ppt). Larvae were fed newly hatched brine shrimp (*Artemia*) nauplii ad libitum twice daily.

Three-spine sticklebacks (*Gasterosteus aculeatus*) were used as predators due to their shared use of euryhaline estuarine habitats with larval topsmelt in northern California (Chamberlain and Barnhart 1993). Three-spine sticklebacks are common in freshwater lakes and rivers as well as coastal bays and estuaries (Wootton and Wootton 1984). They are visual predators that rely on movement (Wootton and Wootton 1984) and olfactory cues in cases of high turbidity (Johannesen et al. 2012) for prey detection. The three-spine stickleback is also a model species in behavioral ecology (Huntingford and Ruiz-Gomez 2009) and has been used commonly in experiments as a predator (Floyd et al. 2008). Juvenile three-spine sticklebacks were captured from nearby estuarine habitats using seines and large dip nets. The mean total length of sticklebacks was 39.4 mm ± 0.33 mm (SD). Individuals were acclimated to laboratory conditions and local filtered seawater over 5–7 days and fed frozen brine shrimp once daily until 24 h prior to their use in trials. Prey and predators were held in a temperature controlled room (20 ± 1 °C) with a 14:10 h light:dark photoperiod.

## Esfenvalerate exposures and behavioral observations

Test solutions were made from a single stock solution of esfenvalerate (Pestanal®, Sigma-Aldrich, $C_{26}H_{24}ClNO_2$, 97 % purity) dissolved in laboratory grade methanol (1 mg/mL). A 24 h LC50 range-finding experiment was first conducted to determine experimental sublethal concentrations appropriate for subsequent experiments on swimming behavior and predation risk. The following nominal concentrations were tested: seawater control and solvent control (125 μg/L methanol), 0.01, 0.05, 0.25, 1.25, and 6.25 μg/L esfenvalerate. No detrimental effects of the solvent control on experimental larvae were observed, therefore this treatment was chosen as a control for further experiments. Results from the range-finding experiment were used to determine nominal concentrations for subsequent sublethal exposures: solvent control, 0.12, 0.59, and 1.18 μg/L esfenvalerate. An aliquot of methanol was added to all experimental test vessels so that each vessel received a maximum dose of 0.04 % of the total exposure water volume. While analytical chemistry was not performed on experimental samples, analysis of nominal range-finding concentrations determined an average loss of esfenvalerate of approximately 5 % in filtered seawater solutions. Thus, nominal esfenvalerate experimental concentrations were deemed to reflect actual experimental concentrations.

For range-finding and sublethal exposure experiments, the solvent and test chemical were added to 3 L of filtered seawater, which was vortexed on a stir plate. Test solutions were shaken vigorously and used to rinse 1 L Pyrex glass beaker test vessels. All test vessels were filled with 300 mL of test solution and their placement on the bench was randomized. Test vessels were each stocked with 10 topsmelt larvae chosen haphazardly using a wide bore glass pipette. Larvae were fed approximately 40 brine shrimp nauplii each at least 2 h prior to the test. Behavioral observations of larvae were made after the 4 h exposure and prior to predation trials. Behavioral observations were made on three randomized test vessels from each concentration across 6 replicates. Observational data was not conducted on 3 replicates, and data from seven vessels were lost and excluded from analysis. Each test vessel was observed for 30 s and the number of larvae with significant swimming abnormalities (i.e., twitching/convulsions, loss of orientation, lethargy) was recorded.

## Predation trials

Following 4 h pesticide exposures, larval topsmelt were gently removed from exposure vessels via a wide-bore glass pipette and pooled by concentration exposure. Larvae were placed into 500 mL glass pans containing filtered seawater to remove any remaining pesticide residue. Ten larvae from each concentration were removed using a wide-bore glass pipette and placed into 12 L mesocosms (28.5 cm × 35 cm; height: 14 cm). Larvae were separated from the stickleback predator in the center of the mesocosm by a clear circular barrier which was gently removed at the start of the trial to minimize disturbance to prey and predators. Both prey and predators were given 1 h to acclimate to the experimental arenas before beginning predation trials. Stickleback predators were not exposed to esfenvalerate based on the results of a preliminary study that indicated no substantial effect of the pesticide on their foraging behavior, swimming behavior, or survival within the range of exposure concentrations used in this study.

Observations of prey and predator behavior were made from behind a large blind to minimize the influence of observer presence on fish behavior. Each trial began with a 10 min observation period in which predator strikes (unsuccessful and successful) and the degree of aggregative prey behavior were quantified. The aggregative behavior of prey was visually quantified by observers using a

categorical scale from 1 to 3. Tightly aggregating larvae where the majority of larvae (>5) were within one body length of another were assigned a score of 1, moderately dispersed larvae where the majority of individuals were within one to three body lengths from one another were assigned a score of 2, and highly dispersed larvae where the majority of individuals were more than three body lengths from one another were assigned a score of 3. The number of dead and missing larvae were counted and recorded after 60 min. Missing larvae were presumed to have been eaten, and the dead larvae were considered to have died from attacks, resulting in overall mortality from predation referred to here as "prey mortality". Although it is possible that some larvae may have died due to pesticide exposure, we concluded that this was a rare occurrence based on visual observations of prey.

Experimental mesocosms differed in their habitat treatment via varying levels of density (none, low, high) combined with distribution (patchy or uniform). Artificial seagrass units (ASUs) were constructed to simulate vegetative habitat. ASUs consisted of a plastic mesh base covered in gravel, with attached green laminated ribbon (to simulate seagrass shoots; 14 cm height) at varying densities in a patchy or uniform pattern. Treatments without habitat ("none") consisted solely of the Vexar base covered with gravel, "low" habitat consisted of a density of 50 seagrass shoots (500 shoots per $m^2$), and "high" habitat consisted of a density of 100 seagrass shoots (1,000 shoots per $m^2$). Densities were chosen to simulate a range of natural densities comparable to previous studies (Orth et al. 1984; Hovel and Lipcius 2001; Jones et al. 2013) and to allow behavioral observations on small larval fish ($\sim$10 mm standard length) that are difficult to track visually. Vegetative shoots were either placed uniformly throughout the tank or in patches of five shoots to simulate different distributions of habitat while controlling for density. In total, there were 20 treatments in a fully crossed factorial design of pesticide exposure and habitat structure (five levels, four that combine the density and distribution of seagrass and one with no seagrass), each replicated nine times.

### Statistical analyses

All data were first graphed and analyzed for normality and homogeneity of variances before conducting Kolmogorov–Smirnov goodness-of-fit tests. Data that did not conform to these assumptions were either square-root transformed or arcsin square-root transformed to meet the assumptions for parametric tests. A two-way analysis of variance was used to determine the effects of pesticide exposure and habitat structure on each response variable: prey mortality after 60 min, the proportion of successful predator strikes, and the total number of predator strikes. When differences between treatments were significant, a Tukey's pairwise multiple comparison test was used to determine differences among treatments. Statistical tests were based on treatment means calculated for each response variable from 9 replicates for a total sample size of 180. Despite the relatively small range in predator size, predator length was significantly related to prey mortality and was thus included as a covariate in all analyses related to predation risk and mortality. Chi square tests were used to determine the separate and combined effects of pesticide exposure and habitat structure on the degree of categorical aggregative prey behavior. All statistical tests were conducted using Systat (ver. 12).

## Results

### Mortality of larvae exposed to esfenvalerate

Exposure of topsmelt to esfenvalerate for 24 h resulted in a LC50 of 1.18 μg/L (95 % CI 0.82–1.72 μg/L). Toxicity values from this range-finding experiment were used to determine a range of environmentally realistic 4 h exposure concentrations that larval topsmelt may encounter in typical estuarine habitats. The LC50 at 24 h (1.18 μg/L) was selected as the highest concentration for the subsequent 4 h exposures, the medium concentration was 50 % of this value (0.59 μg/L), and the low concentration was 10 % of the LC50 (0.12 μg/L).

### Effect of esfenvalerate and prey swimming behavior

Larvae exposed to the pesticide for 4 h displayed abnormal swimming behavior characterized primarily by erratic twitching and convulsions. The proportion of larvae in experimental vessels with these abnormal behaviors increased significantly with pesticide concentration ($F_{3,61} = 49.822$, $p < 0.001$; Fig. 1). Larvae in the medium and high concentrations of esfenvalerate exhibited abnormal behaviors significantly above the solvent control, but there was no difference between the "low" concentration of esfenvalerate and the solvent control. No larvae exhibited signs of loss of orientation or lethargy. Mortality of larvae exposed for 4 h was consistently less than 5 % of the total population.

### Effects of esfenvalerate and habitat structure on predation

Despite increasing behavioral abnormalities of larvae exposed to the pesticide, predation after 60 min was not significantly affected by increasing pesticide concentration (Table 1; Fig. 2a). Surprisingly, predation increased significantly with increasing habitat structural complexity (Table 1; Fig. 2b). A Tukey's pairwise comparison test

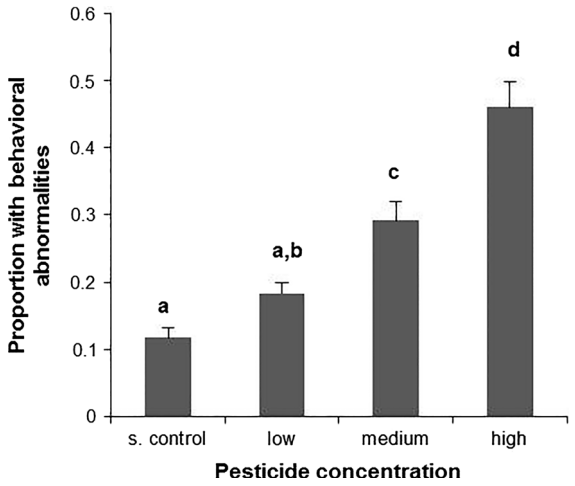


**Fig. 1** The proportion of larvae (mean + SE) with behavioral swimming abnormalities at the end of a 4 h exposure to esfenvalerate (n = 65). Pesticide nominal concentrations: solvent control, low (0.12 µg/L), medium (0.59 µg/L), high (1.18 µg/L). Abnormal behaviors were characterized primarily by twitching and convulsions. Letters denote significant pairwise differences among treatments. Behavioral observations were made on three random test vessels (each with 10 larvae) from each concentration across 6 replicates for a sample size of 65

**Table 1** Two-factor analysis of variance on the effects of pesticide concentration and habitat structure on prey mortality at 60 min

| Source | SS | df | MS | F-ratio | p value |
|---|---|---|---|---|---|
| Habitat | 109.321 | 4 | 27.330 | 2.478 | **0.046** |
| Pesticide | 49.497 | 3 | 16.499 | 1.496 | 0.218 |
| Habitat × pesticide | 189.106 | 12 | 15.509 | 1.406 | 0.168 |
| Predator size | 123.078 | 1 | 123.078 | 11.157 | **<0.001** |
| Error | 1,753.930 | 159 | 11.031 | | |

Predator length was significantly related to prey mortality and was thus included as a covariate. Significant p values are indicated in bold ($p < 0.05$, n = 180)

demonstrated that predation differed between the no-habitat and high-density patchy habitat treatments.

## Effects of esfenvalerate and habitat structure on prey escape and aggregative behavior

Behavioral data during the 10 min observation period revealed that the proportion of successful strikes on topsmelt larvae increased significantly with pesticide concentration, indicating that predators were most successful at the highest pesticide concentration or that prey were less successful in escaping predators (Table 2; Fig. 3). Interestingly the total number of predator strikes decreased significantly with pesticide concentration. However, there was a significant interaction between pesticide concentration and habitat treatment in both cases (Table 2; Fig. 3). Predators made a greater number of total strikes (successful and unsuccessful) on prey in the control and low pesticide concentration, and this pattern was variable across habitat density treatments. The total number of strikes, however, decreased on prey exposed to the medium and high pesticide concentrations. The relative number of unsuccessful strikes decreased with habitat density in the highest pesticide concentration.

The degree of aggregative behavior among larval prey during the first 10 min of a predation trial decreased significantly with increasing esfenvalerate concentration and the presence of habitat structure (Table 3; Fig. 4a–d). Separate Chi square tests were conducted to test whether aggregative behavior differed by habitat treatment within each pesticide concentration, indicating that it was marginally reduced with increasing habitat for prey exposed to low concentrations (Fig. 4b) and significantly reduced for prey exposed to the medium concentration (Fig. 4c). Aggregative behavior was not significantly different across habitat treatments for prey exposed to the highest concentration of pesticide (Fig. 4d), presumably because prey exposed to this concentration displayed equally reduced aggregation regardless of habitat structure.

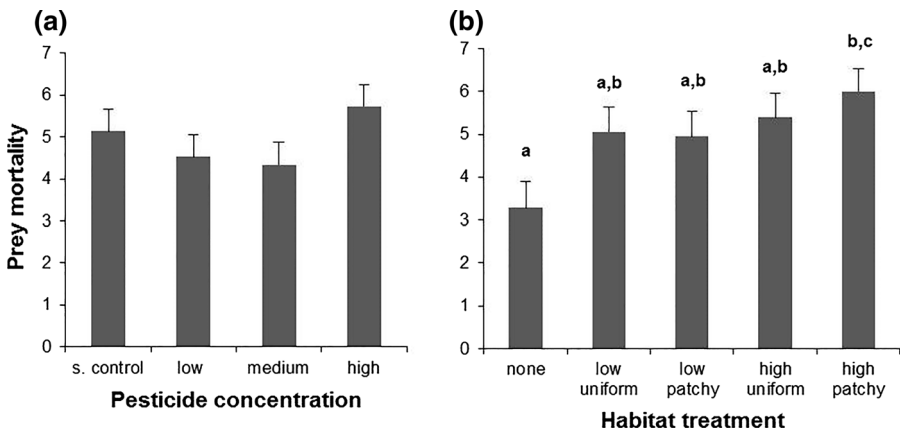


**Fig. 2** Predation on topsmelt larvae (mean + SE) after 60 min: **a** effect of nominal pesticide concentrations on predation: solvent control, low (0.12 µg/L), medium (0.59 µg/L), high (1.18 µg/L), **b** effect of habitat structure on predation. *Letters* denote significant pairwise differences among treatments (n = 180)

**Table 2** Two-factor analysis of variance on the effects of pesticide concentration and habitat structure on the proportion of successful predator strikes and the total number of strikes after 10 min

| Factor | SS | df | MS | F-ratio | p value |
|---|---|---|---|---|---|
| Proportion of successful strikes | | | | | |
| Habitat | 0.320 | 4 | 0.080 | 0.914 | 0.457 |
| Pesticide | 1.151 | 3 | 0.384 | 4.375 | **0.005** |
| Habitat × pesticide | 0.761 | 12 | 0.063 | 0.724 | 0.727 |
| Predator size | 0.723 | 1 | 0.723 | 8.248 | **0.005** |
| Error | 13.938 | 159 | 0.088 | | |
| Total number of strikes | | | | | |
| Habitat | 152.003 | 4 | 38.001 | 1.213 | 0.308 |
| Pesticide | 387.217 | 3 | 129.072 | 4.119 | **0.008** |
| Habitat × pesticide | 769.566 | 12 | 64.130 | 2.047 | **0.023** |
| Predator size | 236.500 | 1 | 236.500 | 7.548 | **0.007** |
| Error | 4,981.944 | 159 | 31.333 | | |

Predator length was significantly related to prey mortality and was thus included as a covariate. Significant p values are indicated in bold ($p < 0.05$, $n = 180$)

**Table 3** Chi square tests comparing the degree of aggregative prey behavior by pesticide concentration and habitat treatment

| Factor | $\chi^2$ | df | p value |
|---|---|---|---|
| Pesticide | 37.349 | 6 | **<0.001** |
| Habitat | 33.388 | 8 | **<0.001** |
| Habitat with in pesticide treatment | | | |
| Control | 9.150 | 8 | 0.330 |
| Low | 15.020 | 8 | 0.059 |
| Medium | 19.250 | 8 | **0.014** |
| High | 3.749 | 8 | 0.879 |

A separate Chi square test was conducted to compare the degree of aggregation by habitat treatment within each level of pesticide treatment. Significant p values are indicated in bold ($p < 0.05$, $n = 180$)

## Discussion

Our study provides insight into the complex ways that pesticide exposure and habitat structure interactively affect behavior and predation of a marine fish. An acute 4 h exposure to environmentally relevant concentrations of the pyrethroid pesticide esfenvalerate (Werner et al. 2004; Floyd et al. 2008) altered the behavior of topsmelt larvae, primarily by inducing extreme and uncontrollable twitching and convulsions. The 4 h exposure simulated the short peak exposure of aquatic organisms to storm water pollution during a rainstorm. These results are in agreement with earlier studies that found significant behavioral impairment and reduction in spawning of juvenile and adult bluegill (*Lepomis macrochirus*) exposed to esfenvalerate (Little et al. 1993; Tanner and Knuth 1996). Our behavioral findings are also supported by a recent study that demonstrated a 4 h exposure of fathead minnows (*Pimephales promelas*) to esfenvalerate that inhibited swimming performance, reduced schooling, and increased the visibility to

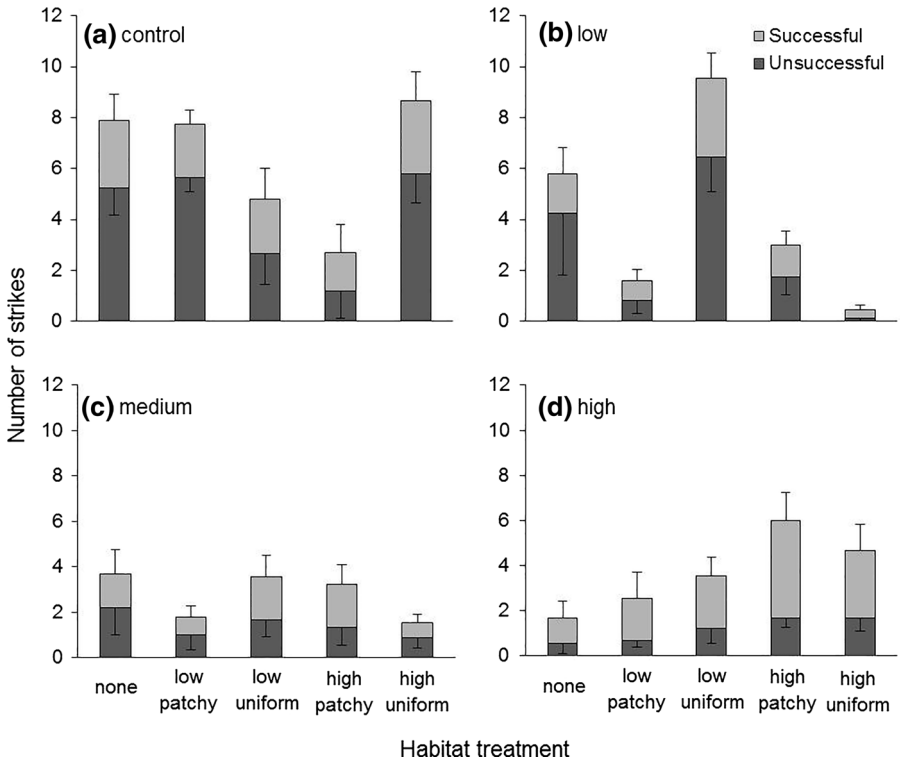


**Fig. 3** Nominal pesticide concentration and habitat structure versus the number of successful (*light gray*) and unsuccessful (*dark gray*) predator strikes which sum to the total number of strikes (mean + SE): **a** solvent control, **b** low (0.12 μg/L), **c** medium (0.59 μg/L), **d** and high (1.18 μg/L) pesticide concentrations ($n = 180$)

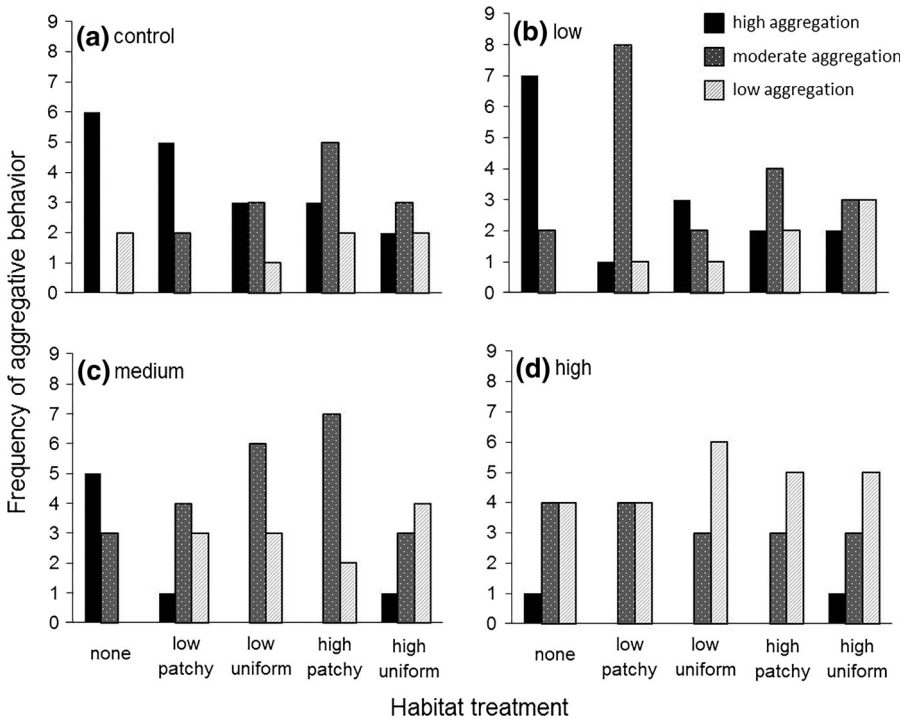


**Fig. 4** Frequency of aggregative prey behavior by pesticide concentration and habitat structure. Degree of aggregation was categorized as high degree of aggregation (1), moderate aggregation (2), and low aggregation (3) (see Materials and Methods). Nominal pesticide concentrations: solvent control, low (0.12 μg/L), medium (0.59 μg/L), high (1.18 μg/L; n = 180)

visual predators (Floyd et al. 2008). However, the consequences for predation differed in the two studies. Floyd and colleagues found that swimming abnormalities increased vulnerability to predation whereas we found an overall increase in prey mortality due to habitat density rather than pesticide exposure.

We suggest that the patterns of prey mortality observed here are due primarily to the compensating behavior of predators in this system in addition to the behavioral responses of prey to habitat structure. Examination of behavioral data within the 10 min observation period supports this hypothesis. In general, predator efficiency, as measured by the ratio of successful to unsuccessful predator strikes, was highest for prey exposed to the highest concentration of pesticide (1.18 μg/L) and lowest for prey exposed to the control and low (0.12 μg/L) concentrations. Predator efficiency did not appear to be influenced by habitat structure. However, when the total number of predator strikes was further examined, an interaction between habitat structure and pesticide concentration revealed that predators made fewer total strikes with increasing pesticide concentration but were more successful with greater habitat structure at the highest pesticide treatment. This resulted in greater overall mortality of prey at the control and low concentrations relative to prey exposed to the higher pesticide concentrations. We conclude that this compensating behavior dampened the effect of the pesticide on mortality, resulting in the observed parabolic pattern in mortality.

The degree of prey aggregative behavior was significantly reduced both by pesticide exposure and habitat structure in this study, which likely contributed to our observed patterns of prey mortality. Prey exposed to the medium and high concentrations of pesticide concentration aggregated more loosely, which is consistent with the highly abnormal swimming behavior observed at higher pesticide concentrations. Previous studies have demonstrated similar decreases in social behavior of juvenile and adult fish due to contaminant exposure (Weis and Weis 1974; Scott and Sloman 2004; Ward et al. 2008). A decline in aggregative behavior with habitat structure was not expected initially, but given that this species often occupies the water column, schooling may have been less effective overall in an area with habitat structure (Savino and Stein 1982). Many species of forage fish, including topsmelt, will aggregate or school in response to increased or perceived predation threat (Miller and Gerlai 2012), yet habitat structure may reduce the amount of space necessary for responsive and effective schooling. This study represents a rare exception whereby prey do not necessarily benefit from greater habitat structure. Interestingly, a recent study by Davis et al. (2012) found a similar decrease in tadpole prey survival by adult crayfish predators with increasing habitat structure demonstrating that this pattern may be present in other systems as well.

Previous studies have found that increasing habitat structure can reduce predation risk, although the responses are frequently species-specific and variable for pelagic

versus benthic species (Savino and Stein 1989; Christensen and Persson 1993). Habitat structure can provide a refuge for prey in aquatic habitats and can improve the survival and escape from predators (Heck and Thoman 1981; Savino and Stein 1982; Orth et al. 1984). However, there is often a critical threshold of vegetative density that must be reached before predator efficiency drops significantly (Orth et al. 1984). For example, Savino and Stein (1982) found that the success of largemouth bass (*Micropterus salmoides*) in capturing bluegill (*Lepomis macrochirus*) prey was significantly reduced at higher densities of artificial vegetation (250 and 1,000 stems per $m^2$) relative to lower densities (0 and 50 stems per $m^2$) whereby predator efficiency increased. However, the density of our habitat treatments (500 and 1,000 shoots per $m^2$) may have been too low to significantly inhibit visually feeding stickleback predators. It is also likely that habitat structure contributed to the compensating behavior of predators because predators in treatments without habitat structure were noticeably less active and foraged less than in other treatments. Their minimal activity levels and foraging may reflect their preferred use of habitat as a refuge from larger piscivorous predators in estuarine and marine habitats (Wootton and Wootton 1984).

The effects of pesticides may override the effects of habitat, depending on the degree of habitat degradation. Habitats composed of dense stands of vegetative structure may still provide a refuge for prey that are behaviorally compromised due to contaminant exposure. Regardless, it is important to examine these relationships and potentially interactive effects more thoroughly, particularly because patterns of behavior and survival in environments with high structural habitat complexity are often species-specific (Baber and Babbitt 2004). The presence of contaminants also can affect the habitat selection of both prey and predators in aquatic communities, changing predator–prey encounter rates and the degree of predation risk (Vonesh and Kraus 2009). Hunting strategy also plays an important role in determining the outcome of predator–prey interactions and will dictate the success of predators in environments with higher structural complexity (Christensen and Persson 1993; Babbitt and Tanner 1998; Baber and Babbitt 2004). Further studies are needed to determine whether habitat structure increases or decreases predator–prey encounter rates and mediates the negative behavioral effects of pollutants on prey survival.

Taken together, the multiple behavioral measures in this study demonstrate the importance and sensitivity of behavior to pesticide exposure and its complex relationship with environmental variables, such as structural habitat complexity. We observed significant behavioral impairments of larval prey topsmelt exposed to concentrations well below those that cause mortality during a short 4 h exposure period. These results indicate that organisms exposed to environmentally realistic pulses of pyrethroid pesticides may experience detrimental effects that may indirectly cause mortality. However, altered behavior does not always translate directly into clear ecological consequences and this will likely differ based on the study system and species involved. For instance, we found that mortality did not increase linearly with esfenvalerate exposure, yet predators did experience higher capture success, particularly for the treatment of high habitat density. Ultimately, the observed behavioral impairments may lead to increased predation of larval prey in pesticide-exposed habitats, particularly where habitat density is high enough to inhibit schooling as an anti-predator response. A limited number of studies have shown that similar changes in behavior can impact population-level endpoints (Murphy et al. 2008), with possible cascading effects on community structure (Fleeger et al. 2003). Thus, risk assessment studies and environmental management plans should consider examining behavioral endpoints to better understand the potential indirect impacts of sublethal pesticides on populations and communities in degraded habitats.

Without directly observing the behavior of both prey and predators, we may have concluded that larval prey would not have heightened predation risk following a 4 h exposure to this pesticide. In our study system, there appears to be an interactive effect of predator and prey behavior on prey mortality, and this relationship may be modified by the presence of habitat structure. These findings highlight the sensitivity and complexity of behavior in evaluating contaminant effects in an ecological context. In the context of risk assessment studies, these results demonstrate the need to include behavioral endpoints as well as environmental context to fully understand contaminant effects on wildlife (Robinson 2009). Thus, it is important to incorporate behavioral endpoints and ecological processes into ecotoxicology studies to assess the complex mechanisms influencing the survival and ecology of species in degraded habitats.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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