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Effect of Bifenthrin and Reduced Salinity Exposure on Larval Sheepshead Minnows (*Cyprinodon variegatus*) and Grass Shrimp (*Palaemon pugio*)

Breanne Yachiyo Hanson[1], Katy W. Chung[2]*, Emily C. Pisarski[2] and Marie E. DeLorenzo[2]

[1]Graduate Program in Marine Biology, University of Charleston, USA

[2]NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Charleston, SC, USA

Corresponding author: Katy W. Chung, NOAA, National Ocean Service, National Centers for Coastal Ocean Science, 331 Fort Johnson Road, Charleston, SC 29412, USA; **Tel:** 843-560-9820.

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Abstract

In 2016 America's coastal counties were home to more than 40 percent of the total population despite accounting for less than 10 percent of the country's landmass. Large-scale changes in land use lead to proportional increases in impervious ground cover, ultimately resulting in increased input of stormwater runoff into adjacent waterways. Stormwater runoff reduces salinity and increases contaminant loads as rainwater washes pollutants, including pesticides such as bifenthrin, into receiving waters. The present study examined bifenthrin toxicity and the potential combined effect of reduced salinity for larval sheepshead minnows (*Cyprinodon variegatus*) and grass shrimp (*Palaemon pugio*). LC50 values were established in salinities of 20, 10, and 5 psu as 0.431, 0.415, 0.377 µg/L and 0.00650, 0.00640, 0.000109 µg/L for larval *C. variegatus* and *P. pugio*, respectively. Salinity did not significantly affect bifenthrin toxicity to larval *C. variegatus*, but mortality rates increased to 90% when larval *P. pugio* were exposed to 0.0015 µg/L of bifenthrin in 5 psu compared to 20 psu. Given that stormwater input is increasing as a result of increasing impervious cover, it is critical to understand how exposure to bifenthrin in low-salinity regimes affects estuarine organisms.

Keywords: *Pesticides, Bifenthrin, Fish, Crustaceans, Toxicity, Salinity, Tidal creeks, Runoff*

Introduction

Impervious surface, a result of urbanization, reduces rainwater infiltration and promotes runoff which accelerates input of sediments, microplastics, metals, pesticides, fertilizers, and bacteria into surface waters [1-3]. A study from 2001 found that pesticides are pervasive in waterways nationwide, with at least one pesticide found in more than 95% of streams sampled and in about 85% of fish sampled [4]. The influx of contaminants such as pesticides often result in habitat degradation and impaired ecosystem functions [5,6]. Impervious cover has also been directly linked with ecosystem effects including changes to community structure, a decline in density, as well as reduced species diversity [2,5,7,8].

Pyrethroids currently account for more than 25% of world-wide insecticide use and are widely applied to crops, turf, golf courses, lawns and home gardens in the U.S. [9,10]. Bifenthrin ((2-methyl-1, 1-biphenyl-3-yl)-methyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl)-2,2-dimethylcycloprpanecarboxylate) is a fourth generation synthetic pyrethroid insecticide. The use of bifenthrin has increased in the last 20 years as a result of bans on pesticides such as DDT, the establishment of the Federal Clean Water Act, which mandates the reduced use of organophosphate insecticides, as well as the increased effectiveness and stability of this new insecticide [9]. However, with greater photostability and insecticidal efficacy than previous generations of pyrethroids, bifenthrin has the potential to be more toxic to non-target species [11,12].

More than one million pounds of bifenthrin was used agriculturally in the U.S. in 2016, mainly applied to corn, soy, cotton, and orchards [13]. There has also been an increasing trend in urban applications of bifenthrin [14]. Urban applications of bifenthrin in the Central Valley of California were reported at 45,000 pounds, over double that of agricultural applications at 20,000 pounds, in California in 2005 [15]. Additionally, pesticide use by acre on golf courses has been reported as equivalent as or greater than use on agricultural crops [16].

Bifenthrin is currently one of the most frequently detected contaminants in California surface waters in areas of urban and agricultural land development [17,18]. Reported surface water concentrations of bifenthrin ranged from 0.005 to 3.79 µg/L (parts per billion - ppb) and bottom and suspended sediment concentrations have been reported in the range of 1.2 to 437 ng/g (ppb) dry weight. The 96-hour LC50 (estimated concentration in which fifty percent of the test organisms die) for rainbow trout, bluegill sunfish, sheepshead minnow, and mysid was 0.15, 0.35, 17.8, and 0.00397 µg/L (ppb), respectively [19-21].

In general, pyrethroid pesticides, like bifenthrin, have octanol/water partition coefficient (log Kow) values of 5 to 7 and, therefore,

partition into the organic carbon fraction of sediments. Although sediment sequestration may lead to confinement in areas of application, pyrethroids are often transported into surface waters via runoff, moving with suspended sediments and dissolved organic matter [22,23]. Additionally, these hydrophobic compounds can become stored in creek-bed sediments to later become resuspended as runoff increases turbidity [18]. Beyond entering waterways via runoff, spray drift, and release of agricultural tailwater also contribute to pyrethroid contamination [22,24,25].

The primary mechanism of pyrethroid toxicity is interference with sodium channel polarization in synaptic nerve terminals [26]. Effectively, this interaction simulates neurotransmission when there is none, causing spastic activity followed by paralysis [20,26]. Additionally, pyrethroids have been shown to inhibit ATPase enzyme production [27]. As pyrethroids impede the ATPase enzyme the critical concentration gradient built to maintain ionic balance and osmoregulation is degraded [27]. Sublethal toxicity has also been reported in organisms exposed to pyrethroids, including altered behavior, reduced growth, immune system effects, endocrine/reproductive effects, histopathological effects, as well as biochemical responses [28-30].

Beyond increased contamination, stormwater runoff as a result of increased impervious cover can also unpredictably and rapidly reduce salinity, with reported drops greater than 26 psu in less than 6 hours [31,32]. While estuarine species have mechanisms to cope with predictable environmental variability (such as tidal phases and seasonal conditions) the rapid and unpredictable effects of stormwater runoff can have serious implications for the biological communities of receiving waterways [1,33]. Numerous studies have demonstrated that reduced salinity can have vastly negative impacts on planktonic larval organisms, including decreased rates of yolk sac absorption, growth, development of feeding apparatus, respiratory tissues, and mortality [1,34-37].

Chemical toxicity may intensify salinity stress, causing an increase in sublethal and lethal effects. In fact, chemical toxicity in combination with salinity stress has been attributed to decreased physiological functions, specifically in the physiological pathways that are responsible for contaminant metabolism and detoxification [38]. Salinity has also been shown to affect biotransformation rates and toxicity for several classes of chemicals [38]. It has been reported that salinity generally decreases water solubility and increases Kow values for pesticides [39]. The water solubility of bifenthrin has been reported as <1 mg/L, with reported log Kow in deionized water as 6.27 ± 0.16, and with 6.78 ± 0.04 in seawater (35 psu) [12,20]. These data suggest that salinity should be considered when bifenthrin toxicity estimates are made for estuarine organisms.

Two abundant, widely distributed, euryhaline, common estuarine test organisms, the grass shrimp, *Palaemon pugio*, and the sheepshead minnow, *Cyprinodon variegatus*, in their larval stages, were selected for the current study [40]. Grass shrimp are generalist foragers that may feed as primary or secondary consumers and play a key role in coastal nutrient cycling as detritivores [40]. Additionally, *P. pugio* are an important prey item for numerous commercially and recreationally important estuarine species [40,41]. Larval *P. pugio* are approximately 2.6 mm at hatching and subsequently undergo a multistage larval development period that can take from 11 days to several months [42]. Planktonic larvae feed on other zooplankton, phytoplankton, and detritus [40]. South Carolina grass shrimp tolerate salinities from nearly freshwater to full strength seawater, and those collected from low salinity sites were of smaller size than those from higher salinity waters. Ultimately, salinities between 20 and 25 psu are optimal for larval development [40].

C. variegatus can live in ambient salinities ranging from 0 psu to greater than 140 psu but a preference for salinities near or less than 20 psu has been documented [43,44]. They feed mostly on plant matter, algae, detritus, mosquito larvae, and smaller fish [45,46]. Sheepshead minnows serve as an important link in the food chain as a source of nutrient cycling and as prey for larger commercially and recreationally important species such as the spotted sea trout, red drum, Atlantic croaker, turtles, and wading birds [41]. Fertilized *C. variegatus* eggs incubate for 4 to 12 days before planktonic larvae of ~4 mm hatch [45]. Larval *C. variegatus* feed on other zooplankton, phytoplankton, and detritus [45,46].

To mitigate negative effects of bifenthrin on natural communities, it is imperative to examine how bifenthrin affects survival rates of important estuarine species. Standard toxicity bioassays, conducted under laboratory conditions, may not be predictive of how changing environmental conditions such as salinity may alter the chemical toxicity of these compounds. The current study sought to evaluate the impacts of acute salinity reduction in combination with bifenthrin exposure on larval estuarine species by using *C. variegatus* and *P. pugio* as model species.

As part of a SC Department of Natural Resources study examining the relationship between urbanization and salinity profiles in estuarine tidal creeks, sediment samples were collected from the same tidal creek sites and analyzed for bifenthrin contamination. The measured field concentrations are reported herein and compared to the laboratory-derived toxicity thresholds to assess relative risk to larval estuarine organisms.

Materials and methods

Animal Acquisition and Holding

Sheepshead minnows, *Cyprinodon variegatus*, collected from a tidal pond on the Fort Johnson campus (32° 44' 53.60" N; 79° 54' 4.45" W) were acclimated and maintained in laboratory conditions of recirculating filtered, aerated seawater at 25°C, 20 psu salinity, and a 16-hour light: 8-hour dark photoperiod. Brooding groups of 2-3 males and 4-6 females were placed in spawning chambers within 75-L aquaria. Adult fish were fed once daily with TetraminVR flake food. Fish eggs collected every day were placed in 20 psu aerated seawater, examined on a light box for hatching events, and hatched larvae were counted, separated, held in aerated 20 psu seawater, and fed daily with Artemia.

Adult grass shrimp, *Palaemon pugio*, were collected from Leadenwah Creek, Wadmalaw Island, South Carolina (32° 38' 51.00"

N; 80° 13' 18.05" W). Shrimp were acclimated and maintained in 75-L aquaria with aerated, 25°C, 20 psu seawater, and a 16-hour light: 8-hour dark photoperiod. Adult shrimp were fed once daily with TetraminVR flake food. Gravid female shrimp were separated, held in hatching chambers containing 20 psu aerated seawater, and examined daily for hatching events. Chambers were removed after hatching event. Larvae were counted, separated, held in aerated 20 psu seawater and fed daily with Artemia.

Seawater Processing

Seawater collected from Charleston Harbor (32° 45' 11.52" N; 79° 53' 58.31" W) was allowed to settle, polished via a sand filtration unit, UV sterilized, and filtered again with 5 µm nominal filtration. The polished seawater was subsequently pumped through a 10 µm carbon filtration before being diluted to 20 psu with deionized water. All water used for testing was additionally pumped through a sterile 0.22-µm filter.

Larval Aqueous Assay

Range finder tests were conducted with both species to determine the definitive test concentrations. A 96-hour aqueous static-renewal toxicity test was conducted to determine the LC50 of bifenthrin in 20, 10, and 5 psu filtered seawater for *C. variegatus* and *P. pugio*. All testing started within 48 hours of hatching. Fish and shrimp larvae were fed Artemia prior to testing and at each 24-hour renewal during the 96-hour test. Stock solutions of bifenthrin were made using pesticide-grade acetone and the final acetone concentration in all treatments and the seawater control was 0.1%. Each species was tested using 5 nominal concentrations of bifenthrin plus a control (0.00 µg/L); 0.17, 0.25, 0.37, 0.56, 0.84 µg/L for the *C. variegatus* assay, and 0.0015, 0.0025, 0.0045, 0.0065, and 0.016 µg/L in the *P. pugio* assay; at each of the three salinities (20, 10, and 5 psu). Three replicate beakers were loaded with 400 mL of seawater dosed with bifenthrin at the target exposure concentration. Larvae (24-48 h old) were taken through a step-wise reduction in salinity. Individuals were allowed to acclimate in a glass finger bowl containing 20 psu water for 90 minutes before being transferred to 15 psu seawater. This process of 90-minute acclimation followed by a transfer into filtered seawater reduced by 5 psu was repeated until the desired exposure concentration was reached (20, 10 or 5 psu). All larvae were transferred each time regardless of salinity reduction to account for any handling stress. After salinity acclimation was complete, 10 larvae were added to each replicated beaker. Beakers were covered with clean foil and aerated through a hole in the foil using a sterile glass pipette tip. Beakers were randomly distributed in an incubator maintained at 25°C and a16-hour light: 8-hour dark photoperiod. Every 24 hours the water was renewed in the same process as above. Temperature, salinity, pH, and dissolved oxygen values were recorded, and each individual was assessed for survival and survivors were transferred to the renewed beaker.

Tidal Creek Sediment Sampling

Tidal creeks in the greater Charleston area were analyzed in ArcGIS Pro using National Land Cover Data, NOAA Coastal Change Analysis Program land use data, and digital elevation models. Four tidal creeks, Guerin Creek, Seaside Creek, Toomer Creek, and Dupont-Wappoo Creek, with similar watershed area and creek volume but representing a range of development and impervious cover within the watershed were selected. Guerin Creek is the most natural creek with less than 1% impervious cover and development, Seaside and Toomer Creek watersheds have roughly 11% impervious cover and 25.9% and 30.2% of land development respectively. Dupont-Wappoo Creek watershed is the most urbanized with about 64% of the watershed developed and around 36% impervious cover. Within each creek four sampling sites were distributed along the length of the creek, from headwaters to the mouth, with the exception of Guerin Creek, where a site in each branch of the headwaters was selected (Figure 1). In late September, bottom sediment samples were collected at each site using a pre-cleaned stainless steel 0.04 m2 Young grab. A sample was also collected from the Leadenwah Creek control site where *P. pugio* were collected for testing. The surficial sediment (top ~3 cm) was homogenized and placed in a pre-cleaned container and stored on ice while in the field. Samples were stored at -40°C until analytical chemistry was conducted.

Analytical Chemistry

Sediment samples collected from each segment of the study site tidal creeks and one sample collected from the reference creek (Leadenwah Creek) were analyzed for a suite of pyrethroid insecticides, including bifenthrin, using accelerated solvent extraction. Sediment samples were weighed (~10 g) and mixed with anhydrous sodium sulfate in a mortar bowl to remove water from the sample. The samples (including method blanks and reference spikes) were transferred to stainless steel ASE cells, spiked with a suite of isotopically labeled internal standards, and extracted with 100% dichloromethane (DCM). Sulfur was removed from the sample extracts using coiled copper strands activated with ~10% hydrochloric acid. Residual water was removed by filtering the sample extract through additional anhydrous sodium sulfate after which the sample was then concentrated to 0.5 mL using TurboVap II Concentration Workstation (40°C, nitrogen at 14 psi). Concentrated samples were cleaned up using activated carbon and alumina solid phase extraction. Eluents were concentrated to 0.5 mL and solvent-exchanged to hexane. Samples were run on an Agilent 6890/5973 gas chromatograph mass spectrometer using a programmable temperature vaporizer inlet connected to a DB-XLB analytical column (30 m x 0.25 mm x 0.25 µm). The mass spectrometer was operated using electron impact and selected ion monitoring modes. Sample chromatograms were analyzed using MSD Chemstation software (ver. E.02.02.1431). An eight-point calibration curve (0. 5-100 ng) was run prior to running samples; r2 values for all analytes of interest were at least 0.995. The method detection limit (MDL) was calculated according to Ragland et al. [47] detectable concentrations of bifenthrin were reported in ng/g dry weight concentrations.

Data Analysis

Two-way analysis of variance with interaction was used to determine significant differences among treatments (RStudio, PBC, Boston, MA). Dunnett's test was used to assess treatment differences from the control and to determine no observable effects concentration

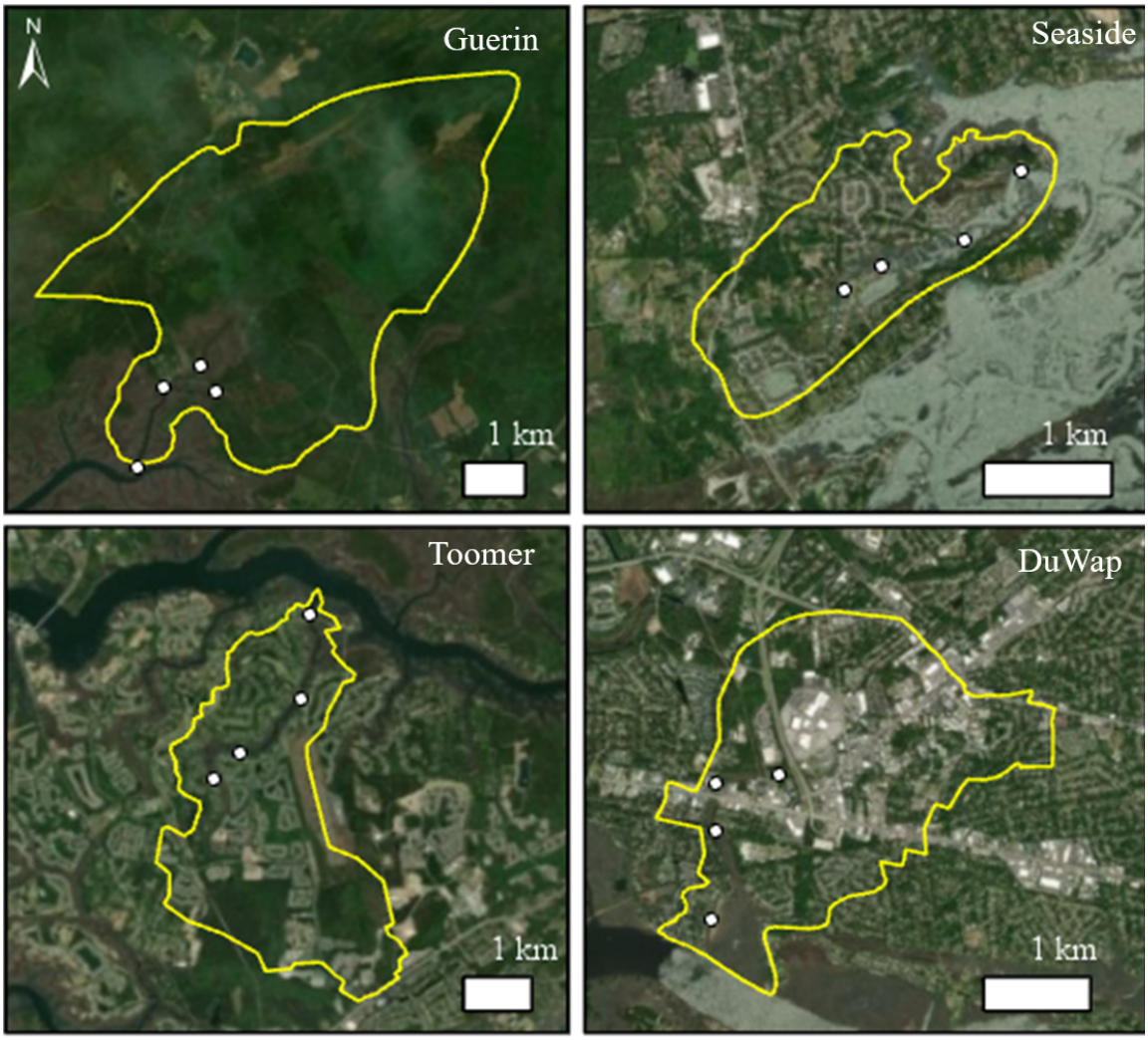

Figure 1: Satellite image of creeks sampled with watershed boundaries and collection sites marked.

(NOEC) and lowest observable effects concentration (LOEC) values. Median lethal concentration (LC50) values with a 95% confidence interval were determined using nominal chemical concentrations (SAS Probit Analysis, PROC PROBIT, SAS V.9.1.3, Cary, NC). Significant differences (α=0.05) in toxicity thresholds among salinity treatments and between species was determined using the LC50 ratio test [47].

Results and discussion

Cyprinodon variegatus Aqueous Assay

Changes in salinity alone did not significantly affect *C. variegatus* survival (p value=0.1633) (Figure 2). Results from a two-way analysis of variance with interaction revealed that bifenthrin concentration was the only factor that significantly affected mortality (Table 1). Less than 10% mortality was observed in the 5, 10, and 20 psu control treatments. The LOEC was 0.37 µg/L bifenthrin at all salinity exposures (Table 2). The NOEC was 0.25 µg/L bifenthrin at all salinity exposures. There was 100% mortality in the highest bifenthrin treatment of 0.84 µg/L for all salinity treatments. The 96-hour LC50 values for the 20, 10, and 5 psu salinity treatments were determined to be 0.432 µg/L

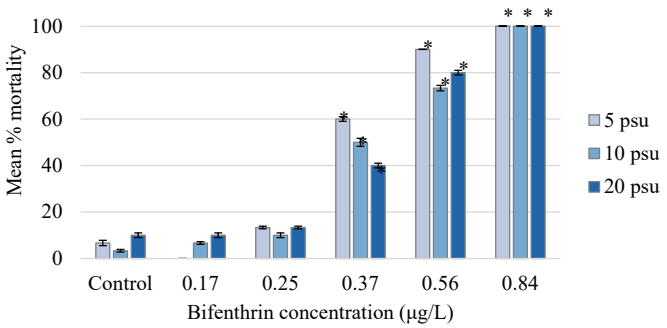

Figure 2: *Cyprinodon variegatus* mean percent mortality at each bifenthrin treatment and salinity exposure. For each salinity exposure, significant differences in percent mortality from the respective control (p value <0.05) are indicated with an asterisk.

Table 1: *Cyprinodon variegatus* two-way analysis of variance with interaction.

Response: Mortality rate	Sum squared	Df	F value	Pr (>F)
Salinity	226	2	1.906	0.1633
Bifenthrin Dose	77009	5	259.906	< 0.0001*
Salinity: Bifenthrin Dose	1019	10	1.7187	0.1140
Residuals	2133	36		

Table 2: *C. variegatus* median lethal concentration (LC50), 95% confidence intervals, lowest observable effects concentration (LOEC), no observable effects concentration (NOEC). There were no significant differences in LC50 values at the different test salinities (LC50 ratio test p>0.05).

Salinity	LC_{50} (µg/L)	95% CI (µg/L)	LOEC (µg/L)	NOEC (µg/L)
20 psu	0.431	0.381-0.484	0.37	0.27
10 psu	0.415	0.367-0.463	0.37	0.27
5 psu	0.377	0.337-0.414	0.37	0.27

(95% CI=0.381-0.484); 0.415 µg/L (95% CI=0.367-0.463), and 0.377 µg/L (95% CI=0.337-0.414), respectively (Table 2). These values are in the range of a previously reported LC50 of 0.47 µg/L for larval sheepshead minnows [48]. Previously published bifenthrin LC50 values for adult sheepshead minnows include 17.8 µg/L and 19.8 µg/L [21,30,49-51]. Greater larval sensitivity is often attributed to higher surface-area-to-volume ratio, underdeveloped fat stores that could sequester lipophilic compounds, and immature immune systems and organs that are important for detoxification and elimination of toxicants [52]. The threshold of toxicity and lack of significant effect due to reduced salinity is not surprising for *C. variegatus*. This species has been reported as a notably hardy organism able to survive in salinities ranging from 0 to 140 psu [43,44,53].

Palaemon pugio Aqueous Assay

There was no mortality observed in the 5, 10, and 20 psu control treatments for the *P. pugio* 96-hour assay (Figure 3). However, 90% mortality was observed in the lowest bifenthrin treatment of 0.0015 µg/L in 5 psu. Results from a two-way analysis of variance with interaction revealed that salinity and bifenthrin dose both significantly affected mortality (p-values <0.0001) and there was a significant interaction between the two variables (Table 3). LOEC values were 0.0065 µg/L at 20 psu, 0.0045 µg/L at 10 psu, and 0.0015 µg/L at 5 psu. NOEC values were 0.0045 µg/L for 20 psu, 0.0025 for 10 psu, and <0.0015 at 5 psu (Table 4). There was 100% mortality in the highest bifenthrin treatment of 0.016 µg/L for all salinity treatments (Figure 3). The 96-hour LC50 values for the 20, 10, and 5 psu salinity treatments were determined to be 0.00650 µg/L (95% CI=0.00637-0.00664); 0.00646 µg/L (95% CI=0.00639-0.00652), and 0.000109 µg/L, respectively (Table 4). Due to the high mortality rates in every bifenthrin concentration tested at 5 psu, 95% confidence intervals could not be calculated. A ratio test comparing the LC50 values revealed no statistically significant difference between 20 and 10 salinity treatments (p=0.9660). Although the lack of confidence intervals in the 5 psu treatment prohibited running a ratio test, salinity clearly affected the mortality rates for larval *P. pugio*, with the LC50 at 20 psu calculated to be 65 times higher than at 5 psu. The 96-hour LC50 of 0.0065 µg/L in 20 psu found in this study was lower than a previously published 96-hour LC50 value of 0.013 µg/L for larval *P. pugio* [30] but was consistent with the LC50 of 0.0056 µg/L reported by [48]. While there was no significant difference in mortality between the 20 psu and 10 psu treatment, a marked increase in grass shrimp mortality occurred in the 5 psu treatments. In fact, there was over 90% mortality in the lowest bifenthrin concentration of 0.0015 µg/L at 5 psu compared to less than 10% mortality at the same concentration in the 20 psu salinity exposure.

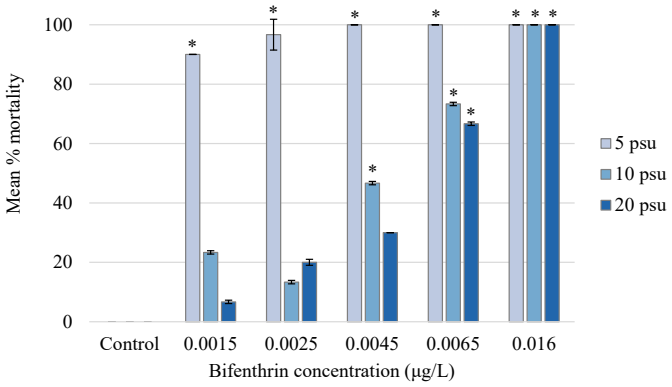

Figure 3: *Palaemon pugio* mean percent mortality at each bifenthrin treatment and salinity exposure. For each salinity exposure, significant differences in percent mortality from the respective control (p value <0.05) are indicated with an asterisk.

Table 3: *P. pugio* two-way analysis of variance with interaction.

Response: Mortality rate	Sum squared	Df	F value	Pr (>F)
Salinity	16411	2	49.233	<0.0001*
Bifenthrin Dose	56622	5	67.947	<0.0001*
Salinity: Bifenthrin Dose	10900	10	6.54	<0.0001*
Residuals	6000	36		

Table 4: *P. pugio* median lethal concentration (LC50), 95% confidence intervals, lowest observable effects concentration (LOEC), no observable effects concentration (NOEC). A ratio test comparing the LC_{50} values revealed no statistically significant difference between 20 and 10 salinity treatments (p=0.966). Although the lack of confidence intervals in the 5 psu treatment prohibited running a ratio test, salinity clearly affected the mortality rates for larval *P. pugio*, with the LC50 at 20 psu calculated to be 65 times higher than at 5 psu.

Salinity	LC_{50} (µg/L)	95% CI (µg/L)	LOEC (µg/L)	NOEC (µg/L)
20 psu	0.006500	0.006367-0.006635	0.0065	0.0045
10 psu	0.006459	0.006395-0.006523	0.0045	0.0025
5 psu	0.000109	*	0.0015	<0.0015

*Unable to calculate confidence interval.

Larval grass shrimp were two orders of magnitude more sensitive than the larval sheepshead minnows to bifenthrin, an expected result based on previously reported LC50 values for these organisms [30,48]. While salinity did not significantly affect bifenthrin toxicity in the sheepshead minnow, the grass shrimp LC50 value for bifenthrin decreased 65-fold for shrimp tested at 20 psu compared to 5 psu. These findings clearly suggest species-specific interactions, increased toxicity with reduced salinity for grass shrimp, and potential sublethal effects due to combined salinity and chemical stress.

Tidal Creek Pyrethroid Contamination

Detectable levels of bifenthrin were found only in the two watersheds; these watersheds had the highest levels of impervious cover. Dupont Wappoo Creek, within the most developed watershed sampled, returned dry mass concentrations of 16.79, 2.23, 1.39, and 2.40 ng/g bifenthrin, at collection site, 1, 2, 3, and 4, respectively, decreasing in concentration from headwater to mouth of the creek (Figure 4). These concentrations are within the range of previously measured bifenthrin concentrations from sediments sampled in an agriculturally influenced creek in California, reporting values from 1.2

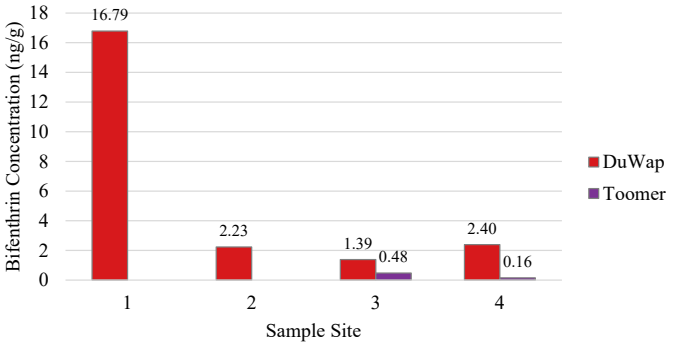

Figure 4: Bifenthrin concentrations (ng/g dry mass) measured in Charleston area tidal creek sediments. Each creek was sampled along a transect from headwater (site 1) to mouth (site 4). Bifenthrin was not detected (<MDL of 0.06-0.17 ng/g) in Guerin Creek or Seaside Creek.

ng/g to 437 ng/g [54]. Sediment from sites 3 and 4 in Toomer Creek had bifenthrin concentrations of 0.479 and 0.155 ng/g, respectively (Figure 4). No pesticides were detected in the sediment sampled from the reference site where the shrimp were collected.

Evaluation of Risk

Given the toxic effect of bifenthrin on aquatic organisms, and the lack of studies examining the interaction between salinity and bifenthrin, ecological impacts of bifenthrin have likely been underestimated. This is the first ecotoxicology study to examine salinity impacts on bifenthrin toxicity in larvae of estuarine ecosystems. This study, among others, demonstrated that the larval stages of two important estuarine species, *C. variegatus* and *P. pugio*, are sensitive to acute bifenthrin exposure. Field measurements of aquatic bifenthrin concentrations reported in California exceed previously and currently reported LC50 values [10,15,55]. Current estimated environmental concentrations for bifenthrin range from 0.005 µg/L to 19.5 µg/L in water samples and 0.155 ng/g to 437 ng/g in sediment samples, and concentration may fluctuate based on localized application patterns and impervious ground cover [55,56]. The LC50 values determined for larval *C. variegatus* and *P. pugio* at the standard test salinity of 20 psu were 40x and 2500x lower, respectively, than the maximum sediment concentration measured in this study. Compared to the reported surface water concentrations that range from 0.01 to 3.79 µg/L for bifenthrin, the LC50 value determined for larval *P. pugio* at the standard test salinity of 20 psu was below published concentrations, and the LC50 value determined for larval *C. variegatus* at the standard test salinity of 20 psu was below three published values [21,57-60]. This indicates significant risk to larval fish and shrimp from bifenthrin at environmentally relevant concentrations. The additional decrease in toxicity thresholds established for grass shrimp at lower salinities further increases their risk for bifenthrin-related mortality during storm water runoff events.

Conclusion

The present study found that bifenthrin was toxic to larval *C. variegatus*, and larval *P. pugio* with laboratory 96-hour aqueous LC50 values, in the standard testing salinity of 20 psu, of 0.431 µg/L and 0.0065 µg/L, respectively. Also noting a statically significant increase in mortality was observed in all bifenthrin concentrations in 5 psu for larval *P. pugio*. However, salinity did not significantly affect toxicity of bifenthrin to *C. variegatus*. These findings suggest that the toxicity of bifenthrin and the influence of combined salinity stress may vary significantly by species and life stage.

Additionally, bifenthrin concentrations ranging from 0.155 to 0.479 ng/g (dry wt.) were measured in South Carolina tidal creek sediments. Bifenthrin concentrations were highest in sediments with the highest level of anthropogenic development near the creek.

Further it must be considered that salinity drops greater than 26 psu in 24 hours have been recorded in South Carolina tidal creeks after rain events. This freshwater inundation induces salinity stress on the organisms in the receiving waters and reduces salinity to potentially lethal ranges for larval *P. pugio*. Simultaneously, the salinization of fresh inland waters, as a result of anthropogenic pressures, has been increasingly reported [61-64]. The present study substantiates the need to take salinity into account when performing toxicity assays and conducting pesticide risk assessments.

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Statements and Declarations

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Competing Interests

The authors report there are no competing interests to declare.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Breanne Y. Hanson, Katy W. Chung and Emily C. Pisarski. The first draft of the manuscript was written by Breanne Y. Hanson and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author (Katy W. Chung) upon reasonable request.

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SUPPORTING MATERIALS

Table 1S: GPS coordinates of sediment sampling locations.

Creek	Site	Latitude	Longitude
Dupont-Wappoo	1	32.793639°	-80.035015°
Dupont-Wappoo	2	32.792957°	-80.041405°
Dupont-Wappoo	3	32.788916°	-80.041523°
Dupont-Wappoo	4	32.781220°	-80.042046°
Guerin	1B	32.95435°	-79.758131°
Guerin	1A	32.958306°	-79.760658°
Guerin	2	32.955044°	-79.767050°
Guerin	3	32.943294°	-79.771861°
Seaside	1	32.702762°	-79.956773°
Seaside	2	32.704363°	-79.953591°
Seaside	3	32.706250°	-79.946664°
Seaside	4	32.711073°	-79.941892°
Toomer	1	32.902279°	-79.799385°
Toomer	2	32.905704°	-79.795374°
Toomer	3	32.912549°	-79.785576°
Toomer	4	32.923973°	-79.784284°

Table 2S: Bifenthrin concentrations reported in surface water samples.

Location	Concentration (µg/L)	Reference
Reclamation Ditch Alisal Creek, CA	0.0165	Kelley and Starner, 2004
Del Puerto Creek, CA	0.00504-0.0554	Kelley and Starner, 2004
New River Outlet, CA	0.0069	LeBlanc *et al.*, 2004
Alamo River, CA	0.0095	LeBlanc *et al.*, 2004
Little Topashaw Creek, MS	0.7 (±2.88)	Smith Jr *et al.*, 2006
Alisal Creek, CA	0.01	Hunt *et al.*, 2006
Oso Flaco Creek, CA	0.025	Hunt *et al.*, 2006
Hines Channel, CA	0.319-3.79	Siepmann and Holm, 2000
Central Irvine Channel, CA	0.249-1.67	Siepmann and Holm, 2000