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Comparative behavioral ecotoxicology of Inland Silverside larvae exposed to pyrethroids across a salinity gradient

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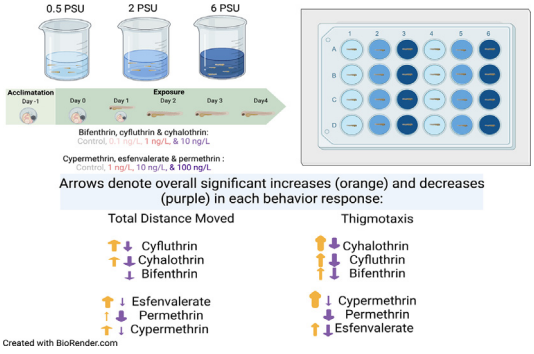
HIGHLIGHTS

- Behavior is altered following exposure to pyrethroids at concentrations as low as 0.1 ng/L.
- At the lowest salinity, inland silversides exposed to cypermethrin had increased anxiety like behavior.
- Larvae exposed to permethrin were hypoactive and displayed decreased thigmotaxis.
- Bifenthrin caused the fewest behavioral changes.
- This is the largest comparison of pyrethroid toxicity across altered abiotic conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Pyrethroids, a class of commonly used insecticides, are frequently detected in aquatic environments, including estuaries. The influence that salinity has on organism physiology and the partitioning of hydrophobic chemicals, such as pyrethroids, has driven interest in how toxicity changes in saltwater compared to freshwater. Early life exposures in fish to pyrethroids cause toxicity at environmentally relevant concentrations, which can alter behavior. Behavior is a highly sensitive endpoint that influences overall organism fitness and can be used to detect toxicity of environmentally relevant concentrations of aquatic pollutants. Inland Silversides (*Menidia beryllina*), a commonly used euryhaline model fish species, were exposed from 5 days post fertilization (~1-day pre-hatch) for 96 h to six pyrethroids: bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, esfenvalerate and permethrin. Exposures were conducted at three salinities relevant to brackish, estuarine habitat (0.5, 2, and 6 PSU) and across 3 concentrations, either 0.1, 1, 10, and/or 100 ng/L, plus a control. After exposure, Inland Silversides underwent a behavioral assay in which larval fish were subjected to a dark and light cycle stimuli to determine behavioral toxicity. Assessment of total distanced moved and thigmotaxis (wall hugging), used to measure hyper/hypoactivity and anxiety like behavior, respectively, demonstrate that even at the lowest concentration of 0.1 ng/L pyrethroids can induce behavioral changes at all salinities. We found that toxicity decreased as salinity increased for all pyrethroids except permethrin. Additionally, we found evidence to suggest that the relationship between log K_{OW} and thigmotaxis is altered between the lower and highest salinities.

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1. Introduction

Pyrethroids are a class of synthetic insecticides, developed from the natural, less toxic pyrethrin that is derived from the flower *Chrysanthemum cinerariifolium* (Hitmi et al., 2000). Pyrethroid insecticides are estimated to make up 25 % of all insecticide applications globally (Aznar-Alemany and Eljarrat, 2020). Pyrethroids have replaced organophosphates in many instances, as they are often touted as less persistent and safer for application due to having low mammalian toxicity (Brander et al., 2016a). However pyrethroids have been shown to exhibit lethal and sublethal toxicity in non-target organisms at concentrations as low as the ng/L range (Hladik and Kuivila, 2009; Weston et al., 2019). Although pyrethroids have low water solubility and high octanol-water partition coefficient (log K_{OW}), which may make them more likely to sorb to soil or lipids in aquatic biota, they are frequently detected in surface waters, including in estuaries.

Global climate change is expected to cause changes in precipitation patterns that will lead to altered freshwater inputs into estuarine ecosystems, causing salinity intrusion (Herbold et al., 2022). These shifts will have a substantial effect on estuarine salinity gradients. For example, the Sacramento-San Joaquin Delta/San Francisco Bay (SFBD), California, USA is predicted to see an increase of up to 9 practical salinity units (PSU) during the spring and summer seasons by 2090 due to sea level rise and altered precipitation patterns (Knowles and Cayan, 2002). Drought is already suspected to be contributing to declining fish populations in the SFBD. The combined effects of drought and sea level rise are likely to complicate management and conservation efforts of native fishes to the SFBD (Cloern et al., 2011; Mahardja et al., 2021). Many estuarine species, including fishes, are dependent upon healthy estuaries for critical habitat, such as nurseries and breeding grounds (Méjanelle et al., 2020). Pyrethroid applications often occur in the spring and can overlap with the spawning seasons of some fishes as well as the beginning of the dry season in many regions. An additional consequence of climate change is a predicted increase in insecticide application (Taylor et al., 2018; Hasenbein et al., 2019). The Central Valley in California is a large agricultural region that relies heavily on pyrethroids; the region drains into the SFBD, and increased insecticide use may have direct implications on pyrethroid load in the SFBD. As species habitat ranges change and regions become warmer, some pest species will hatch sooner and/or expand to new regions causing earlier and more frequent insecticide applications (Delcour et al., 2015). The potential for increased salinity and insecticide use emphasizes the need to understand toxicity in a range of concentrations across a salinity gradient.

Salinity can cause differential toxicity with hydrophobic chemicals, including pesticides like pyrethroids, which may be due in part to the changes in their partitioning behavior and the physiology of the organism. The partitioning of pyrethroids in the water column are altered with changes in salinity. Both bifenthrin and cypermethrin, two commonly used pyrethroids, were evaluated under freshwater and saltwater conditions where it was found that the log K_{OW} increased and water solubility decreased in the saltwater treatments (Saranjampour et al., 2017). Salinity alters osmoregulation of euryhaline fishes, wherein they absorb more water as salinity increases to maintain osmotic balance potentially increasing ingestion and absorption of toxicants (Kültz, 2015). This is in comparison to freshwater fish that absorb little water, which could limit ingestion of toxins. Differences in physiology and chemical partitioning likely work together to cause differential toxicity across a salinity gradient.

Since pyrethroids are neurotoxicants, behavior is an informative sublethal endpoint to use in the study of their effects (Farag et al., 2021). Pyrethroids are typically classified into two types: type I pyrethroids contain a carboxylic ester of cyclopropane and have two chirality centers while type II pyrethroids have a cyano-group in the α position and three chirality centers (Lawrence and Casida, 1982; Soderlund et al., 2002). The different pyrethroid types have been found to induce differential toxicity, for example Zebra Danio (*Danio rerio*), commonly referred to as Zebrafish, had altered gene expression and varying dose response relationships dependent upon pyrethroid type (Soderlund et al., 2002; Awoyemi et al., 2019). Pyrethroids induce toxicity primarily by altering the firing of voltage-gated sodium ion channels. As pyrethroids bind to sodium gates and prevent their closure, the ions fire continually causing neuron excitability which can lead to seizers, paralysis, and mortality. Due to the high sensitivity of behavioral endpoints, studies can identify ecologically relevant, sublethal effects of contaminants at environmentally relevant concentrations (Segarra et al., 2021). For example, exposure to bifenthrin toxicity can alter predator avoidance behavior in Inland Silversides (*Menidia beryllina*) at concentrations as low as 3 ng/L (Frank et al., 2019). Sublethal effects are important for studies on non-target organisms because concentrations in the aquatic environment rarely reach concentrations high enough to elicited acute lethality.

Given the high usage of pyrethroids and their potential risk to estuarine organisms, we have studied six pyrethroids at three salinities relevant to brackish waters to determine their relative toxicity in early life stage Inland Silversides. Inland Silversides are an euryhaline, model fish species commonly used in research and approved for regulatory work. The species is native to the eastern and southern coasts of the United States and were introduced to the SFBD (Middaugh and Hemmer, 1992). Adverse effects have been noted in Inland Silversides following exposure to pyrethroids at environmentally relevant concentrations (Brander et al., 2012; Brander et al., 2016b; DeCourten and Brander, 2017; DeCourten et al., 2020). Findings from studies with Inland Silversides can provide information on the toxicity of compounds to at-risk species, such as the Delta Smelt (*Hypomesus transpacificus*) (Lawrence et al., 2021), an endangered osmerid species endemic to the SFBD (Hobbs et al., 2019). Inland Silversides are found at salinities ranging from 0 to 35 PSU, making them an ideal euryhaline species to use for testing across salinity gradients. In a sister study to the one presented here, the behavior of Delta Smelt larvae was altered when exposed to bifenthrin and permethrin at the same concentrations and salinities used in the current study (Segarra et al., 2021). An assessment of these pyrethroids between two species, one endangered while the other a non-native, model organism, allows for better informed risk assessment of the Delta Smelt because Inland Silversides are frequently used as a surrogate for the more sensitive species found in the SFBD (Lawrence et al., 2021).

Here, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, esfenvalerate, and permethrin were investigated for behavioral toxicity at 0.5, 2, and 6 PSU. These compounds were chosen due to recent regulatory standards in the SFBD in which a Total Maximum Daily Load (TMDL) is set to be implemented for these six pyrethroids (California Regional Water Quality Control Board Central Valley Region, 2017). The goal of this study was to assess the relative toxicity of the six pyrethroids on larval behavior, across the salinities of interest. One of the goals of the larger project that includes this study was to assess the comparative toxicity between Inland Silverside and Delta Smelt. By curating this large toxicity dataset, comparisons can be made between the relative toxicity of these pyrethroids in the Inland Silverside and in the Delta Smelt from our sister study (Segarra et al., 2021). This study expands the estuarine and marine ecotoxicology dataset for pyrethroids and provides further information on how salinity can alter toxicity at ecologically relevant endpoints. This assessment of the six pyrethroids at three salinities demonstrates the largest assessment of pyrethroid toxicity under different abiotic conditions to date.

2. Methods

2.1. Chemicals

Bifenthrin (part #: N-11203; CAS: 82657-04-3), cyfluthrin (part #: N-11130; CAS: 68359-37-5), cyhalothrin (part #: N-12307; CAS: 91465-08-6), cypermethrin (part #: N-11545; CAS: 52315-07-8), esfenvalerate (part #: N-11102; CAS: 66230-04-4), and permethrin (part #: N-12848; CAS: 52645-53-1) were purchased from Chem Service (West Chester, PA, USA). HPLC-grade methanol used to make stock solutions was purchased from Fisher Scientific (Waltham, MA, USA).

2.2. Organisms and husbandry

Adult Inland Silverside broodstock were housed at the Oregon State University (OSU), Hatfield Marine Science Center under Animal Care and

Use Program (ACUP) protocol #4999 and maintained at 10–20 PSU and 23 °C on a 14:10 light cycle. Adult fish were fed a combination of Hikari tropical micro pellets (Kyorin Food Industries Ltd., Kasai City, Japan), Hikari freeze-dried tubifex worms (Kyorin Food Industries Ltd.), Hikari frozen mysid shrimp (Kyorin Food Industries Ltd., Kasai City, Japan), and live *Artemia* nauplii hatched from Brine Shrimp Eggs (Brine Shrimp Direct, Ogden, UT, USA) supplemented with Selcon™ (American Marine Inc., Ridgefield, CT, USA). Brood fish were approximately 1.5–2 years old at the time of spawning. The spawning protocol was adapted from Middaugh et al. (1986) and occurred as described in Hutton et al. (2021) (Middaugh et al., 1986; Hutton et al., 2021). Briefly, substrate was added to adult broodstock tanks for 16–20 h. Spawning substrate and embryos were subsequently transported to OSU main campus, placed in 2 PSU artificial seawater (ASW) created with Instant Ocean and reverse osmosis water, and allowed to develop on the substrate until 4 days post fertilization (dpf). At 4 dpf, embryos were gently removed from the spawning substrate using forceps, placed into ASW made to their respective exposure salinities, rinsed, and assessed for development using a VWR VistaVision Dissecting Scope (VWR International, Radnor, PA, USA). Next, 16–20 embryos were then placed into 250 mL beakers with 50 mL of either 0.5, 2.0, or 6.0 PSU ASW for a 24-hour acclimation period. All experiments were conducted under OSU Institutional Animal Care and Use Committee (IACUC) protocol #0035.

2.3. Experimental design

Following the acclimation period, 50 mL of concentrated exposure solution was poured into beakers to achieve nominal concentrations and a final volume of 100 mL/beaker. All exposures replicates (including controls) contained 0.01 % methanol. Organisms were exposed to three salinities, 0.5, 2.0, and 6.0 PSU and four concentrations including the control. Bifenthrin, cyfluthrin and cyhalothrin exposures were conducted at concentrations of 0.0, 0.1, 1.0, and 10.0 ng/L, and cypermethrin, esfenvalerate and permethrin exposures were conducted at concentrations of 0.0, 1.0, 10.0, and 100.0 ng/L. These concentrations are environmentally relevant and were chosen based on their known occurrences in the SFBD (Oros and Werner, 2005; Woudneh and Oros, 2006; Weston et al., 2015). There were four replicates per treatment combination and 16–20 organisms per replicate. Exposures were conducted for 96 h using semi-static conditions. New exposure solutions were made daily followed by a 50 % water change. At this time survival was assessed and debris were removed. Following experiment maintenance organisms were fed Gemma Microdiet ad libitum (Skretting, Westbrook, Maine). Organisms hatched into exposure solutions between days 1 and 2 of exposure. There were no hatching differences between controls and pyrethroid exposed embryo's hatching rates ($p > 0.05$, ANOVA, Dunnett's post hoc test). pH, dissolved oxygen, salinity, temperature, and ammonia were recorded daily (Table S1). Organisms were maintained on a 14:10 light cycle.

2.4. Behavioral assay

At the end of the 96-hour exposure period, a behavioral assay modified from Segarra et al. (2021), was performed. Twenty-four well, polystyrene plates were loaded randomly with one fish and 1 mL of solution per well. Fish were acclimated to the plate for at least 45 min and then placed into a DanioVision Observation Chamber (Noldus, Wageningen, the Netherlands) maintained at 23 °C using a PULACO Mini Submersible Water Pump (Amazon, Inc.) and JEBO Stainless Steel Aquarium Tank Heating Rod (Walmart, Inc.). An additional 15 min of acclimation occurred inside the observation chamber in the dark followed by an alternating dark: light cycle with three 10-minute periods of dark interspersed with two 5-minute periods of light (Segarra et al., 2021). The dark and light cycles are referred to as dark1, Dark2, and Dark3, and the light cycles are referred to as Light1 and Light2 respectively. A total of 3–5 pseudo replicates (individual fish) from each replicate were analyzed. Behavioral tracking was conducted between 07:00 and 19:00 h, which encompassed the standard light period of the exposures. Behavior was recorded and tracked using a Basler Gen 1 Camera using Ethovision® XT15 software, 1280 × 960 resolution, 10,000 lx of light, and a 25/s frame rate.

The endpoints analyzed in this study were selected due to their use in other behavioral studies and ecological relevance. We assessed total distance moved (TDM) and thigmotaxis (wall hugging) of Inland Silversides. TDM is a well-established behavioral endpoint that informs on both hypo- and hyperactivity (Steenbergen et al., 2011; Steele et al., 2018). Thigmotaxis is often used as a measure of anxiety and fear in fish following exposure to toxicants (Hamilton et al., 2021). Increased movement towards the edge of the well and a tendency to stay close to the wall demonstrates an increase in thigmotaxis and indicates anxiety like behavior. A decrease in thigmotaxis corresponds to a decrease in anxiety like behavior which may increase an organism's risk of predation as they spend less time hiding. Time spent bursting (speed > 20 mm/s), cruising (speed > 0.5 mm/s and <20 mm/s) or freezing (speed < 0.5 mm/s), meander °/s, and velocity (mm/s) were also measured (see supplemental for further information).

2.5. Growth index

Following behavioral assessment, organisms were collected, euthanized with an overdose of buffered MS222 at 200 mg/L, placed in 3 % paraformaldehyde, and stored at 4 °C until analysis. For growth index, 2–3 individuals from each replicate and treatment were imaged using an Olympus SZ61 Stereo Microscope and Olympus DP23 Microscope Digital Camera (Olympys Corporation, Tokyo, Japan), and length and width were measured using cellSens Imaging Software (Olympys Corporation, Tokyo, Japan). Growth index was calculated as described in Siddiqui et al. with the following equation:

$$\frac{W}{L} \times d$$

where W is width, L is length, and d is the number of exposure days (Siddiqui et al., 2022).

2.6. Analytical chemistry

To confirm that pyrethroid stock solutions were made correctly, the highest concentration from each salinity and pyrethroid were collected during experimental maintenance on day three and stored at −20 °C until they were shipped to the USGS Organic Chemistry Research Laboratory (Sacramento, CA). Due to analytical limitations, only the highest concentrations were assessed; however, verification of this concentration provides confidence that the other concentrations were also nominal. Pyrethroid concentrations were confirmed as described in Segarra et al. (2021). Briefly, one-liter water samples were collected in amber glass bottles; pyrethroids concentrations were measured using solid-phase extraction followed by gas chromatography–mass spectrometry (Hladik and Kuivila, 2009; Hladik and McWayne, 2012).

2.7. Statistical analysis

Statistical analysis was performed in R software version 4.0.3 (Vienna, Austria) and R Studio version 1.3.1093 (Boston, MA, USA). Dark and light cycles were compared within stimuli. Different behavioral responses due to stimuli change were assessed by calculating the difference between the response value after the stimuli change compared to the value before the change. For statistical analysis the behavioral data were normalized between 0 and 1 using the following equation:

$$\frac{x - \min(x)}{\max(x) - \min(x)}$$

where x is the value.

Behavioral data were not normally distributed (confirmed by Q-Q plot) and were therefore analyzed using a Kruskal-Wallis non-parametric test

followed by a Dunn's test with a Bonferonni correction for multiple comparison. For heat map visualization of behavioral results, the z-score was calculated from the normalized behavioral data which distributes the results above and below zero. Growth and survival data were normally distributed and were analyzed by one-way ANOVA followed by Dunnett's test to compare treatment vs. control. Dose response analysis was performed on TDM using the *drc* package where 4 parameter log-logistic dose response models were fit to each pyrethroid, salinity, and stimuli combination (Ritz et al., 2015). To determine the effect of salinity, physicochemical properties of each pyrethroid (type and log K_{OW}), and concentration on behavioral, the thigmotaxis data were analyzed using a generalized linear model (GLM) with a quasibinomial distribution. Log K_{OW} values used in analysis were from (Laskowski, 2002). Results were considered statistically significant at an alpha < 0.05.

3. Results

3.1. Analytical chemistry

Analytical results are summarized in Table S2; as the nominal and measured concentrations are consistent, all concentrations are referred to as nominal throughout.

3.2. Survival and growth

There were no significant differences in survival across all compounds, concentrations, and salinity combinations except for cypermethrin 100 ng/L at 2 PSU exposures which had significantly lower survival (89.4 %) relative to the control (97.5 %) ($p < 0.05$, Dunnett's Test) (Fig. S1).

Growth was significantly decreased relative to the control in the 1 and 100 ng/L concentrations of cypermethrin at 0.5 PSU, and the 100 ng/L of esfenvalerate at 2 PSU was significantly increased relative to the control ($p < 0.05$; Dunnett's Test). All other compounds, concentrations, and salinity combinations did not exhibit significant differences in growth in any treatments relative to the control (Fig. S2).

3.3. Behavioral changes during dark: light cycle

Overall, Inland Silversides showed behavioral changes following exposure to all six pyrethroids in all three salinities and concentrations. Inland Silversides experience hyperactivity in the light compared to the dark (Fig. S4). However, there were no differences in the exposed fish's response to the initial light stimuli change relative to the controls ($p > 0.05$; Dunn's Test). For brevity and due to the high correlation of velocity, meander, and time spent bursting, cruising, and freezing to TDM (>0.6, Spearman's Correlation) they are not discussed further, however results can be found in Figs. S5–S9.

3.3.1. Hyper- and hypoactivity

An increase in TDM relative to the control corresponds to hyperactivity and a decrease in TDM relative to the control corresponds to hypoactivity. Overall, we found that in the highest salinity exposure there were fewer effects on TDM relative to the lower salinity exposures. There were 32, 33, and 19 significant differences across all treatments in the 0.5, 2, and 6 PSU exposures respectively. In order of least to most significant differences in TDM the pyrethroids at the studied concentrations are ranked as bifenthrin < cyhalothrin < cyfluthrin and cypermethrin < permethrin < esfenvalerate. More significant differences occurred during the dark than the light cycles (Fig. 1).

3.3.1.1. Bifenthrin. Bifenthrin exposure caused the fewest changes in TDM relative to the other pyrethroids regardless of salinity, followed by cypermethrin (Fig. 2). Here, Inland Silversides exposed to bifenthrin only demonstrated effects on TDM in the dark (Figs. 1 and 2) ($p < 0.05$, Dunn's test). Hypoactivity was found in both the 0.5 and 2 PSU bifenthrin exposures, however, we found that at 6 PSU TDM was increased in the 10 ng/L concentration (Fig. 2) ($p < 0.05$, Dunn's test), which indicates there may have been a decrease in hypoactivity as salinity increased. Additionally, in the lower two salinities bifenthrin demonstrates evidence of non-monotonic responses as the highest concentration had no significant effect on TDM.

3.3.1.2. Cyfluthrin. Cyfluthrin had similar effects on TDM at both 0.5 and 2 PSU wherein it induced significant hypoactivity in the dark at 0.1 and 10 ng/L and hyperactivity in the light in the 1 ng/L exposure (Fig. 2) ($p < 0.05$, Dunn's test). At 0.5 PSU there was also one instance of increased TDM following exposure at 1 ng/L during the first dark cycle. At 6 PSU, TDM was decreased in the 1 ng/L but increased in the 10 ng/L exposures at 6 PSU during the dark cycles (Fig. 2) ($p < 0.05$, Dunn's test).

3.3.1.3. Cyhalothrin. Cyhalothrin induced more significant hypoactivity in the 0.5 PSU exposure compared to the 2 and 6 PSU exposures. At 0.5 PSU hyperactivity only occurred in the 1 ng/L exposure during the first dark cycle. In the other two salinities, the most significant differences found were hyperactive effects; there was one instance of significant hypoactivity in the 6 PSU 0.1 ng/L first dark cycle (Fig. 2) ($p < 0.05$, Dunn's test).

3.3.1.4. Cypermethrin. Cypermethrin induced hyperactivity in all three salinities; however, as salinity increased, the number of significant, hyperactive changes decreased (Fig. 1) ($p < 0.05$, Dunn's test). In the 2 PSU exposures cypermethrin appears to induce a non-monotonic response on hyperactivity, wherein the responses are only in the lower two concentrations and not the highest. Significant hypoactivity was only found in the 0.5 PSU exposure at 1 ng/L during the first light cycle (Fig. 2) ($p < 0.05$, Dunn's test).

3.3.1.5. Esfenvalerate. Esfenvalerate exposure induced significant hyperactivity at all salinities (Fig. 2) ($p < 0.05$, Dunn's test). Inland Silversides displayed only one instance of hypoactivity in the 0.5 PSU 1 ng/L exposure during the second dark cycle (Fig. 2) ($p < 0.05$, Dunn's test). Almost all the effects from esfenvalerate occurred during dark cycles. The only significant difference in TDM during a light cycle occurred in the 2 PSU 10 ng/L exposure (Fig. 2) ($p < 0.05$, Dunn's test).

3.3.1.6. Permethrin. Permethrin was the only pyrethroid studied here to have more significant differences in TDM as salinity increased (Figs. 1 and 2) ($p < 0.05$, Dunn's test). Permethrin induced hypoactivity primarily during the dark periods (Figs. 1 and 2) ($p < 0.05$, Dunn's test). In the 0.5 PSU exposure, only hypoactivity was found. At 2 PSU, there was both hyper and hypoactive effects. At 6 PSU, there was a clear non-monotonic response in which the 1 ng/L exposure induced significant hyperactivity in the light, the 10 ng/L exposure induced strong hypoactive effects in all dark and light cycles, and the 100 ng/L exposure had no significant effects. ($p < 0.05$, Dunn's test).

3.3.2. Thigmotaxis and anti-thigmotaxis behavior

A positive value indicates thigmotaxis behavior (wall-hugging), whereas a negative value indicates decreased thigmotaxis, referred to as anti-thigmotaxis behavior (more time spent in the center of the well, away from the wall). As with TDM, significant effects on thigmotaxis across all pyrethroids, overall, decreased as salinity increased from 33, 27, to 25 significant differences at 0.5, 2.0 and 6.0 PSU, respectively (Fig. 3) ($p < 0.05$, Dunn's test). In order of least to most significant differences in thigmotaxis and anti-thigmotaxis behavior the pyrethroids at the studied concentrations are ranked as bifenthrin < cyfluthrin < cyhalothrin and esfenvalerate < permethrin < cypermethrin.

3.3.2.1. Bifenthrin. Following exposure to bifenthrin anti-thigmotaxis behavior was primarily seen in the 0.5 and 2 PSU exposures ($p < 0.05$, Dunn's test). There was one instance of thigmotaxis at 0.5 PSU in the 10 ng/L second light cycle ($p < 0.05$, Dunn's test). In the 2 PSU exposure, anti-thigmotaxis behavior was detected at 1 ng/L during both light cycles

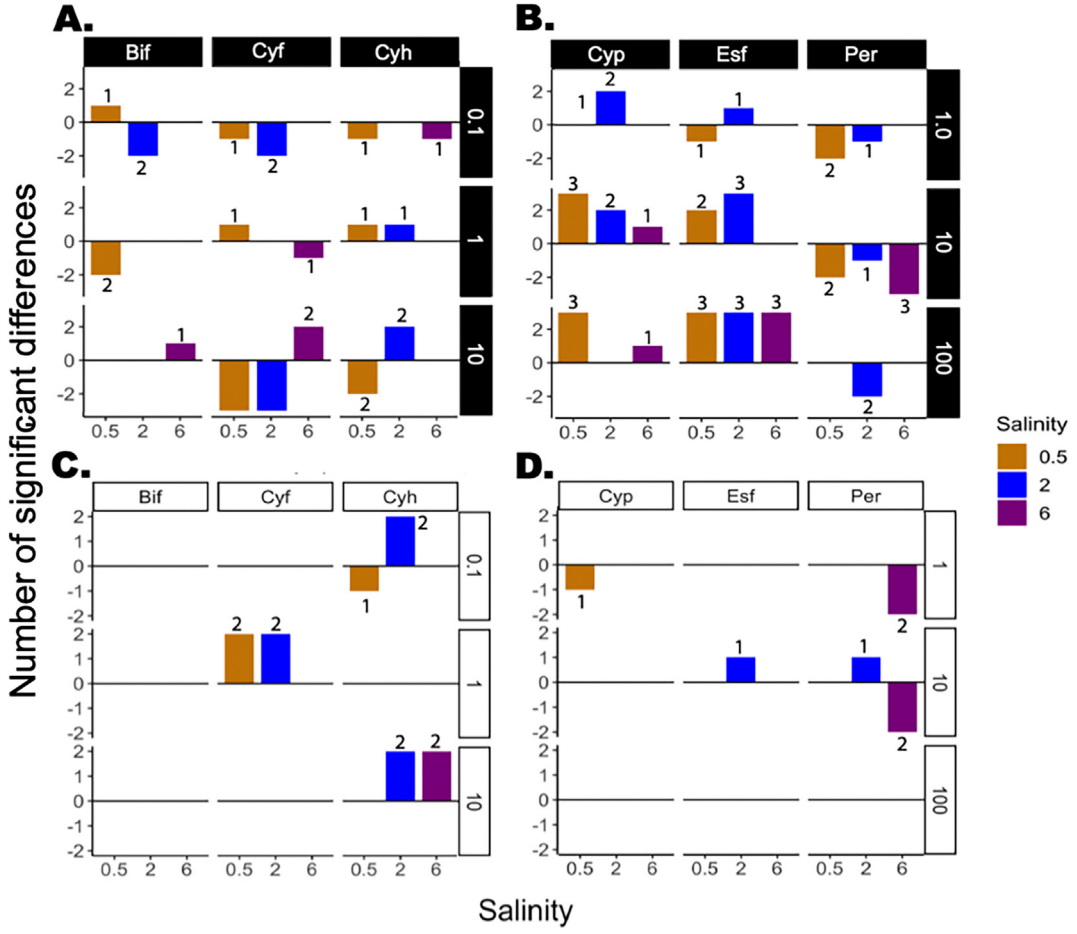

Fig. 1. Number of significant differences found for each pyrethroid, salinity, and concentration (0.1, 1, 10, and/or 100 ng/L, right vertical labels) for total distance moved (TDM) relative to the respective control ($p < 0.05$; Dunn's test). Effects within each dark and light cycle were summarized together. A number below zero signifies significance found corresponding to decreased TDM (hypoactivity), a number above zero signifies the significance found corresponding to increased TDM (hyperactivity). A) Dark cycle for bifenthrin, cyfluthrin, and cyhalothrin. B) Dark cycle for cypermethrin, esfenvalerate, and permethrin. C) Light cycle for bifenthrin, cyfluthrin, and cyhalothrin. D) Light cycle for cypermethrin, esfenvalerate, and permethrin. The dark boarders represent data from the dark cycles and the light boarders represent data from the light cycles. Concentrations (ng/L) are listed on the right of each plot. Bif = bifenthrin, Cyf = cyfluthrin, Cyh = cyhalothrin, Cyp = cypermethrin, Esf = esfenvalerate, and Per = permethrin.

(Fig. 4). In the 6 PSU exposure, thigmotaxis was found during the dark cycles at 10 ng/L ($p < 0.05$, Dunn's test) but no anti-thigmotaxis behavior was detected at this salinity (Fig. 4).

3.3.2.2. Cyfluthrin. In the cyfluthrin exposures anti-thigmotaxis behavior was found in both the 0.5 and 2 PSU during the dark cycles at 10 ng/L and 0.1 and 1 ng/L respectively (Fig. 4) ($p < 0.05$, Dunn's test). The 6 PSU exposure resulted in one instance of anti-thigmotaxis in the second light cycle in the 0.1 ng/L exposure. A significant increase in thigmotaxis behavior was found in the first dark cycle at 0.1 ng/L and the light and dark cycles at 10 ng/L (Fig. 4) ($p < 0.05$, Dunn's test).

3.3.2.3. Cyhalothrin. All three salinity exposures had instances of both thigmotaxis and anti-thigmotaxis behavior. Anti-thigmotaxis behavior was observed during dark cycles in the 0.5 PSU 10 ng/L exposure and dark cycles in the 0.1 ng/L exposures at 2 and 6 PSU (Fig. 4). Thigmotaxis behavior was observed in all three salinities at various concentrations (Fig. 4). Overall, there was a slight increase in thigmotaxis and decrease in anti-thigmotaxis behavior as salinity increased (Fig. 4).

3.3.2.4. Cypermethrin. Cypermethrin had a significant effect on increased thigmotaxis behavior in almost all dark and light cycles and concentrations at 0.5 PSU. At 2 PSU, a strong thigmotaxis response was also observed in dark cycles, but only the 1 ng/L exposure had an effect during the light cycles. The 6 PSU exposure exhibited two instances of anti-thigmotaxis in the 1 and 10 ng/L exposure and one instance of increased thigmotaxis in the 10 ng/L exposure, all during dark cycles ($p < 0.05$, Dunn's test).

3.3.2.5. Esfenvalerate. Esfenvalerate resulted in significant anti-thigmotaxis behavior in the 1 and 10 ng/L 0.5 PSU exposure during dark cycles and significant increased thigmotaxis behavior at 100 ng/L 0.5 PSU exposure also during dark1. At 2 PSU, significant anti-thigmotaxis was observed in the 1 ng/L exposure in dark cycles and thigmotaxis behavior was observed in the 10 ng/L exposure during light cycles. Only thigmotaxis behavior was observed at 6 PSU in the high concentration during the first dark cycle and both light cycles (Fig. 4) ($p < 0.05$, Dunn's test).

3.3.2.6. Permethrin. Permethrin caused significant anti-thigmotaxis behavior in all salinities. Thigmotaxis was decreased in dark and light cycles in the 0.5 and 6 PSU exposure and in dark cycles at 2 PSU ($p < 0.05$, Dunn's test). Similar to the TDM results, permethrin demonstrates a non-monotonic response in the 6 PSU, where the most significant decreases in thigmotaxis are found at 10 ng/L and no effects are found at 100 ng/L in any salinity ($p < 0.05$, Dunn's test) (Fig. 4).

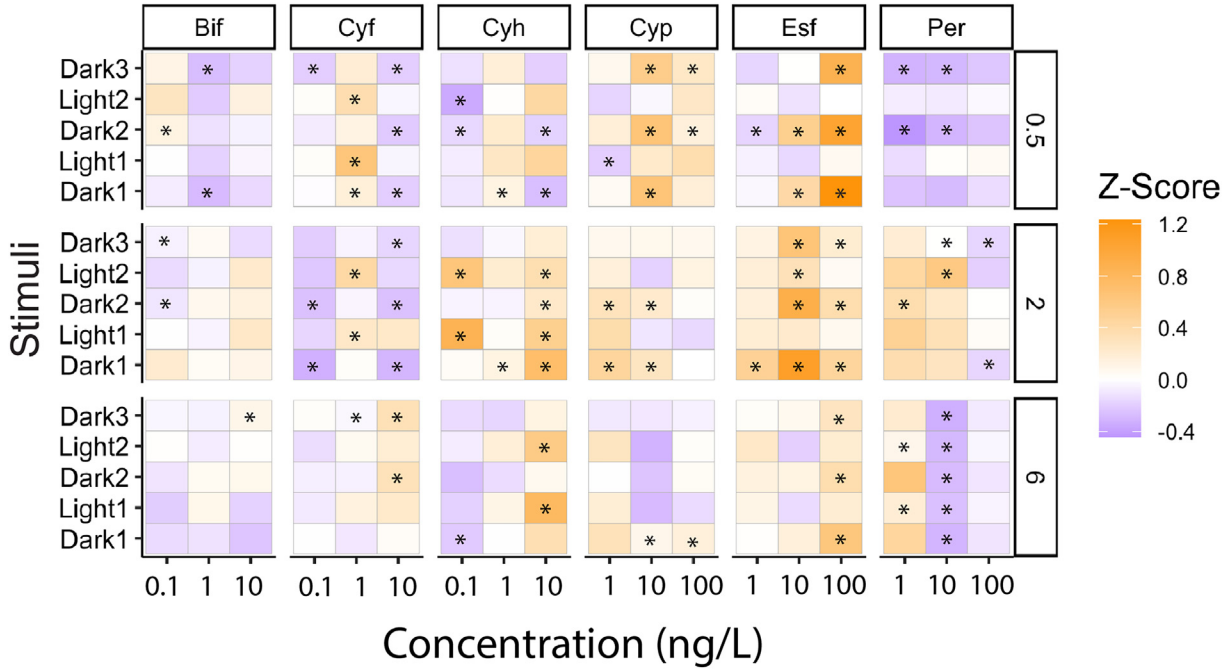

Fig. 2. Total distance moved (TDM) results relative to controls for individual pyrethroids (top horizontal labels), stimuli, concentrations, and salinity combinations (0.5, 2, and 6 PSU; right vertical labels). Behavioral data were binned by minute and normalized between 0 and 1 as described in the methods prior to analysis. *Z*-score was calculated from the normalized data and is used for visualization purposes only; a negative z-score (purple) corresponds to decreased TDM (hypoactivity) and a positive z-score (orange) corresponds to increased TDM (hyperactivity). * denotes significance, $p < 0.05$ (Dunn's Test). Bif = bifenthrin, Cyf = cyfluthrin, Cyh = cyhalothrin, Cyp = cypermethrin, Esf = esfenvalerate, and Per = permethrin.

3.4. Relationship between log K_{OW} and behavior

To determine whether the pyrethroid properties (log K_{OW} and pyrethroid type) influenced toxicity we ran quasibinomial GLMs for all salinity and concentration combinations compared to thigmotaxis behavior for each individual pyrethroid. We found a significant positive relation between log K_{OW} and pyrethroid type for all concentrations in the 0.5 PSU exposures (Fig. 5, Table S2). In the 2 PSU exposure, thigmotaxis and log K_{OW} had a positive correlation in the 1 and 100 ng/L concentrations. Type had a significant negative relationship with thigmotaxis for the 0.1 ng/L exposures and a positive relationship for the 1 and 100 ng/L exposures (Fig. 5, Table S3). In the 6 PSU exposures, there was a negative correlation between thigmotaxis and log K_{OW} at 10 ng/L and 100 ng/L, and pyrethroid type had a positive relationship with thigmotaxis at 10 ng/L (Fig. 5, Table S3).

4. Discussion

Behavior has been used extensively as an endpoint to determine neurological and developmental effects of environmental contaminants in fish species, including pyrethroids (Heintz et al., 2015; Frank et al., 2019; Mundy et al., 2021; Segarra et al., 2021; Siddiqui et al., 2022). Behavior is a high level, sensitive measurement that can indicate the relative toxicity of contaminants (Bownik and Wlodkowic, 2021). Because behavior is a relatively high throughput sub-lethal endpoint, behavioral assays can be used to determine the effects of several pyrethroids across multiple variables.

4.1. Pyrethroids induce neurotoxicity

Behavior is one of the highest-level endpoint to be affected by neurotoxicity. While the main mechanism of pyrethroids toxicity is through overstimulation of the sodium ion channels, studies have shown other pathways can be disrupted (Bownik and Wlodkowic, 2021). Pyrethroids have been shown in both rats and fish to decrease acetylcholinesterase (AChE) activity and impact learning and behavior (Marinowic et al., 2012, Syed et al., 2016, Verma et al., 2021.). In the spotted snakehead (*Channa punctata*) it was found that AChE activity was inhibited following exposure to cyhalothrin and cypermethrin, with greater effects in the cyhalothrin-exposed fish. In our study, cyhalothrin had slightly higher effects on TDM than cypermethrin, but cypermethrin induced more thigmotaxis behavior than cyhalothrin. Deltamethrin, another type II pyrethroid, significantly increased thigmotaxis and anxiety-like behavior in Zebra Rerio, which is consistent with the response to cypermethrin at the lower salinities in Inland Silversides (Li et al., 2019). Cypermethrin also inhibited AChE and induced hyperactivity in the stinging catfish (*Heteropneustes fossilis*) (Tiwari et al., 2019). Inhibition of AChE leads to increased acetylcholine (ACh) buildup which can desensitize the nicotinice ACh receptors, leading to increased muscle weakness (Giniatullin and Magazanik, 1998; Ullah et al., 2019a). Altered AChE activity and subsequent ACh accumulation may contribute to altered swimming behavior via this mechanism (Ullah et al., 2019b). While there are a few studies that assess one or two pyrethroids (Kumar et al., 2009; Tu et al., 2012; Ensibi et al., 2014; Tiwari et al., 2019), the relative differences in AChE activity between multiple pyrethroids or different abiotic conditions is an area of research that could be explored further.

Zebra Rerio exposed to esfenvalerate at 0.02–8 μg/L exhibited hypoactivity and decreased expression of dopamine active transporter gene at 0.2 μg/L, which could influence neurological function and subsequently behavior (Wang et al., 2020). Bifenthrin has additionally been shown to decrease the expression of dopamine receptor 1 gene (Bertotto et al., 2018). The increased dopamine expression in Zebra Rerio exposed to esfenvalerate was associated with boldness, whereas shyness was associated with a decrease in dopamine expression (Thörnqvist et al., 2019). Boldness could be correlated to decreased anxiety (as seen with anti-thigmotaxis) and shyness could be correlated to increased anxiety (like thigmotaxis behavior). Here, esfenvalerate induced anti-thigmotaxis at the lowest salinity (0.5 PSU) but had increased thigmotaxis behavior in the higher two salinities (2 and 6 PSU), and neurological mechanisms

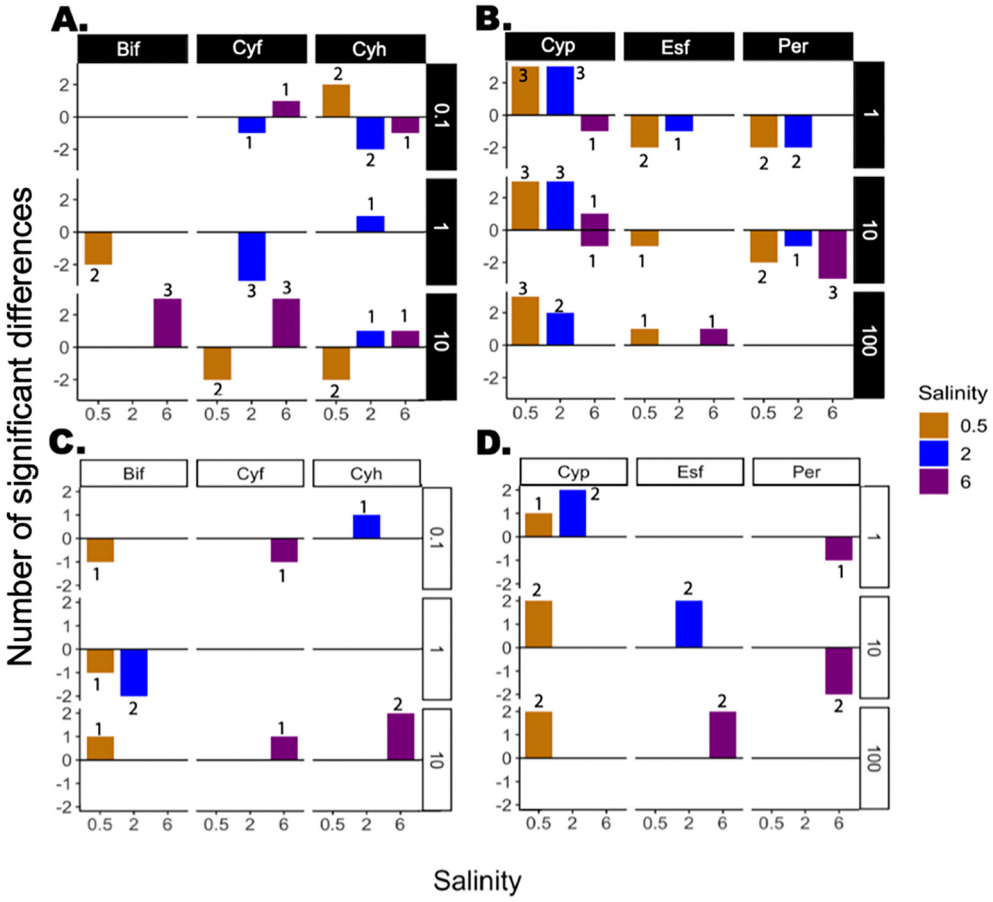

Fig. 3. Number of significant differences found for each pyrethroid, salinity, and concentration (0.1, 1, 10, and/or 100 ng/L, right vertical labels) for thigmotaxis relative to the respective control ($p < 0.05$; Dunn's test). Effects within each dark and light cycle were summarized together. A number below zero corresponds to significant anti-thigmotaxis behavior, a number above zero corresponds to significant thigmotaxis behavior. A) Dark cycle for bifenthrin, cyfluthrin, and cyhalothrin. B) Dark cycle for cypermethrin, esfenvalerate, and permethrin. C) Light cycle for bifenthrin, cyfluthrin, and cyhalothrin. D) Light cycle for cypermethrin, esfenvalerate, and permethrin. The dark boarders represent data from the dark cycles and the light boarders represent data from the light cycles. Bif = bifenthrin, Cyf = cyfluthrin, Cyh = cyhalothrin, Cyp = cypermethrin, Esf = esfenvalerate, and Per = permethrin.

may be leading to altered behavior are impacted by an increase in salinity. In Delta Smelt, esfenvalerate induced a concentration-dependent effect on swimming behavior that was correlated with downregulation of the enzyme aspartoacylase (Connon et al., 2009). Downregulation of aspartoacylase has been linked to changes in myelin, the sheaths that insulate axons and help regulate electrical impulses in the brain, which leads to changes in neuroactivity and cognitive function (Wang et al., 2009), which could also explain the resulting behavioral effects. Studies of Inland Silversides and other teleost species that further explore dopamine and aspartoacylase in connection to behavior would likely improve understanding of the relationship between neurological pathways and behavior.

In addition to changes in sodium channel opening, dopamine activity, and AChE inhibition, another mechanism through which pyrethroids may induce neurotoxicity and disrupt swimming behavior could be through interference with ryanodine receptors (RYR) and mechanistic target of rapamycin (mTOR) signaling pathways. Both of these pathways are involved in muscle contraction olfactory, visual, and neural development and are shown to be disrupted following pyrethroid exposure (Ma et al., 2015; Skalecka et al., 2016; Frank et al., 2018; Stinson et al., 2022). Both Zebra Rerio and Inland Silversides exposed to bifenthrin had altered expression of genes associated with RYR and mTOR and effected behavior (Frank et al., 2018; Frank et al., 2019). These effects may be partially responsible for the behavioral response seen in Inland Silverside after bifenthrin exposure.

Cyfluthrin has been shown to cause DNA damage in fish as well as changes in blood serum levels and liver and gill histology, but studies on the neurotoxic effects of cyfluthrin in fish are scarce (Sepici-Dinçel et al., 2009; Marinowic et al., 2012). Studies in 6–8-week-old mice have demonstrated that cyfluthrin exposure increased expression of reactive oxygen species (ROS), altered the structural morphology of the hippocampus, and reduced learning ability (Verma et al., 2021). Cyfluthrin also caused decrease AChE activity in rats, as seen in other pyrethroids for fish (Marinowic et al., 2012, Syed et al., 2016.). Future mechanistic studies could screen a range of pyrethroids to obtain clearer understanding of the similarities and differences in the affected neurological pathways. Additionally, studying multiple abiotic conditions will continue to be important as climate change alters ecosystems.

4.2. Pyrethroid toxicity across salinity

One hypothesis is that toxicity may differ across a salinity gradient through changes in partitioning behavior of pyrethroids in salt water compared to fresh water. Previous studies have found that bioconcentration factor, log K_{OW}, and water solubility are altered in salt water which may contribute to different bioconcentration and toxicity (Saranjampour et al., 2017). The pyrethroids used in this present study were assessed using the same salinity conditions used by Hladik (2020) to determine how sorption to the beaker may change during the different salinity treatments. Increased salinity increased the fraction of pyrethroid in the water compared to that sorbed to the glass beaker, which may have implications for bioavailability of the pyrethroids in a laboratory beaker setting (Hladik, 2020). In contrast

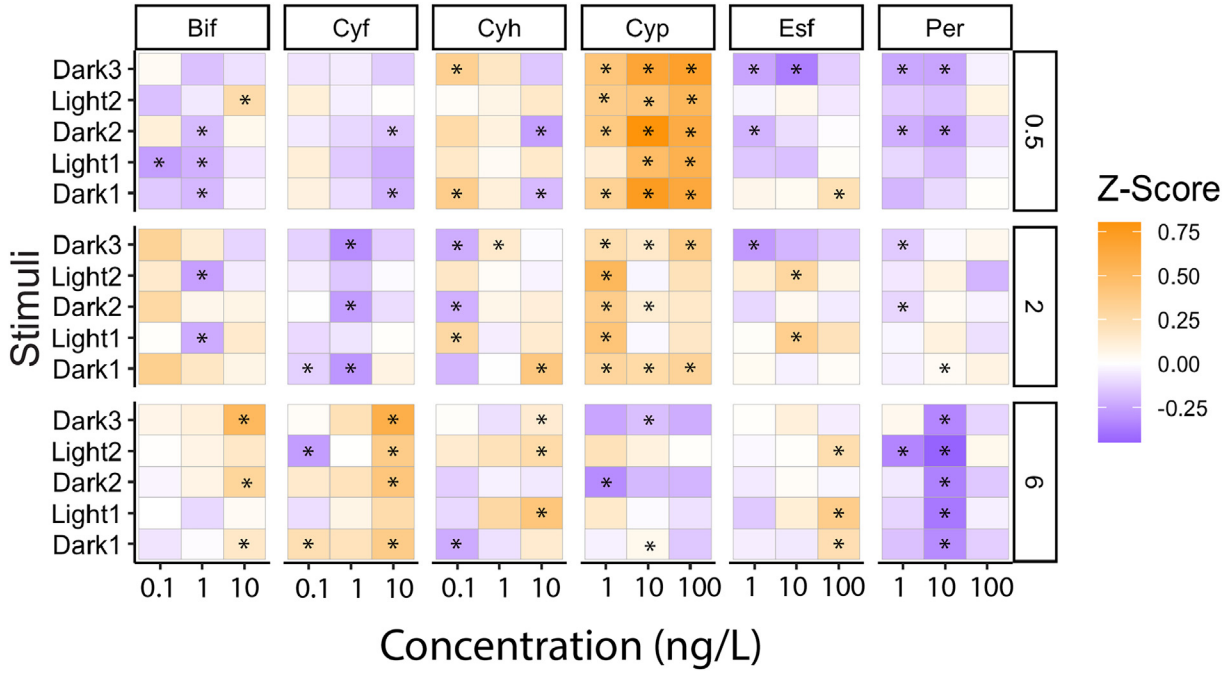

Fig. 4. Thigmotaxis and anti-thigmotaxis behavior relative to controls for individual pyrethroids, stimuli, concentrations, and salinity (0.5, 2, and 6 PSU, right verticle labels). Behavioral data were binned by minute and normalized between 0 and 1 prior to analysis. *Z*-score was calculated from the normalized data and is used for visualization purposes only, a negative z-score (purple) corresponds to decreased wall hugging (anti-thigmotaxis) and a positive z-score (orange) corresponds to increased wall hugging (thigmotaxis). * denotes significance, $p < 0.05$ (Dunn's Test). Bif = bifenthrin, Cyf = cyfluthrin, Cyh = cyhalothrin, Cyp = cypermethrin, Esf = esfenvalerate, and Per = permethrin.

to what was found by Hladik (2020), the salting out effect would predict that the pyrethroids would sorb more to the beaker, not the water, at the higher salinities (Schwarzenbach et al., 2002). The salting out effect explains that a lower portion of the compound would be present in the water at higher salinities because it would be pushed either to the walls of the beaker or into the organism by the increased rigidity of the saltwater (Schwarzenbach et al., 2002). The Hladik (2020) experiments were conducted using deionized water, and when no suspended solids are present in the water, the pyrethroids may preferentially sorb to the beaker. The presence of salt ions may have had the opposite effect as originally hypothesized; instead of pushing the pyrethroids out of the water, the salt ions may have disrupted the pyrethroid molecules sorbing to the beaker, causing a higher fraction to stay in the water column at the higher salinities. Our experiments were similarly conducted without suspended solids in the water which could imply a similar phenomenon may have occurred in our test beakers. This is especially interesting since toxicity was overall decreased at the higher salinities, given the increased concentration of pyrethroid in the water at 6 PSU increased toxicity would be initially implied. However, changes in biotic factors could also impact toxicity which may explain why we saw an overall decrease in toxicity as salinity increased.

Our data suggest that as salinity increased there was a change in the direction and strength of the relationship between chemical partitioning and Inland Silverside thigmotaxis behavior. It is possible that the change in partitioning was not substantial enough between the 0.5 and 2 PSU exposures to completely shift the direction of the relationship. However, the 0.5 and 6 PSU exposures may have large enough differences in ionic strength to alter the relationship between log K_{OW} and thigmotaxis. This could be explained by the potential change in log K_{OW} and water solubility that occurs between fresh and salt water which can alter the bioavailability of the pyrethroids (Saranjampour et al., 2017). Additionally, we saw that the relationship between thigmotaxis behavior and pyrethroid type was only significant in one concentration at the highest salinity but was significant in all the concentrations at the lowest salinity. Others have found pyrethroid type to be a significant factor in the type of toxicity that occurs (Soderlund et al., 2002; Awoyemi et al., 2019). Possibly this relationship is not as strong when salinity is involved since the compounds and organisms physiology change in the new environment.

Another hypothesis is that toxicity is altered across salinity through biotic changes in the organism. Changes in chemical metabolism across a salinity gradient may explain why Inland Silversides exhibit a decrease in behavioral response in the 6 PSU exposures. Enzymes responsible for metabolizing chemicals have been shown to be altered in saline conditions (Zheng et al., 2019). Increased metabolism could explain why less toxicity was observed because the organisms would eliminate the pyrethroids more efficiently. A study of permethrin in Inland Silverside found that bioaccumulation of the parent compound was decreased in higher salinities (13 and 20 PSU) compared to the lower salinity (6 PSU) (Derby et al., 2021). However, in a contradiction to this there were no significant differences in biotransformation of permethrin between the three salinities, although these measurements were done as part of a feeding experiment which may yield different results that water exposures (Derby et al., 2021). Pyrethroid metabolism can also differ between species (Ji et al., 2021), which may account for some of the observed differences between Delta Smelt and Inland Silversides.

Osmoregulation has also been postulated as one reason toxicity increases in studies because fish in saline water have increased energetic demands and metabolic rate (Brooks et al., 2012). However, these studies are more commonly performed on freshwater species exposed to low levels of salinity, not euryhaline fish like the Inland Silverside. Euryhaline fish likely have better ability to respond to chemical exposure during saline conditions than fish less tolerant to saline conditions. In Delta Smelt exposed to bifenthrin, a positive correlation between anti-thigmotaxis in the light cycles and salinity was found, and bifenthrin had increased toxicity at the highest salinity; meanwhile, permethrin toxicity had no correlation to salinity (Segarra et al., 2021). Delta Smelt live in a lower salinity range and spawn at lower salinities compared to Inland Silverside, which can spawn at and tolerate salinities from 0 to 35 PSU. The species differences in salinity tolerance may explain some of the different responses to pyrethroids between the two species.

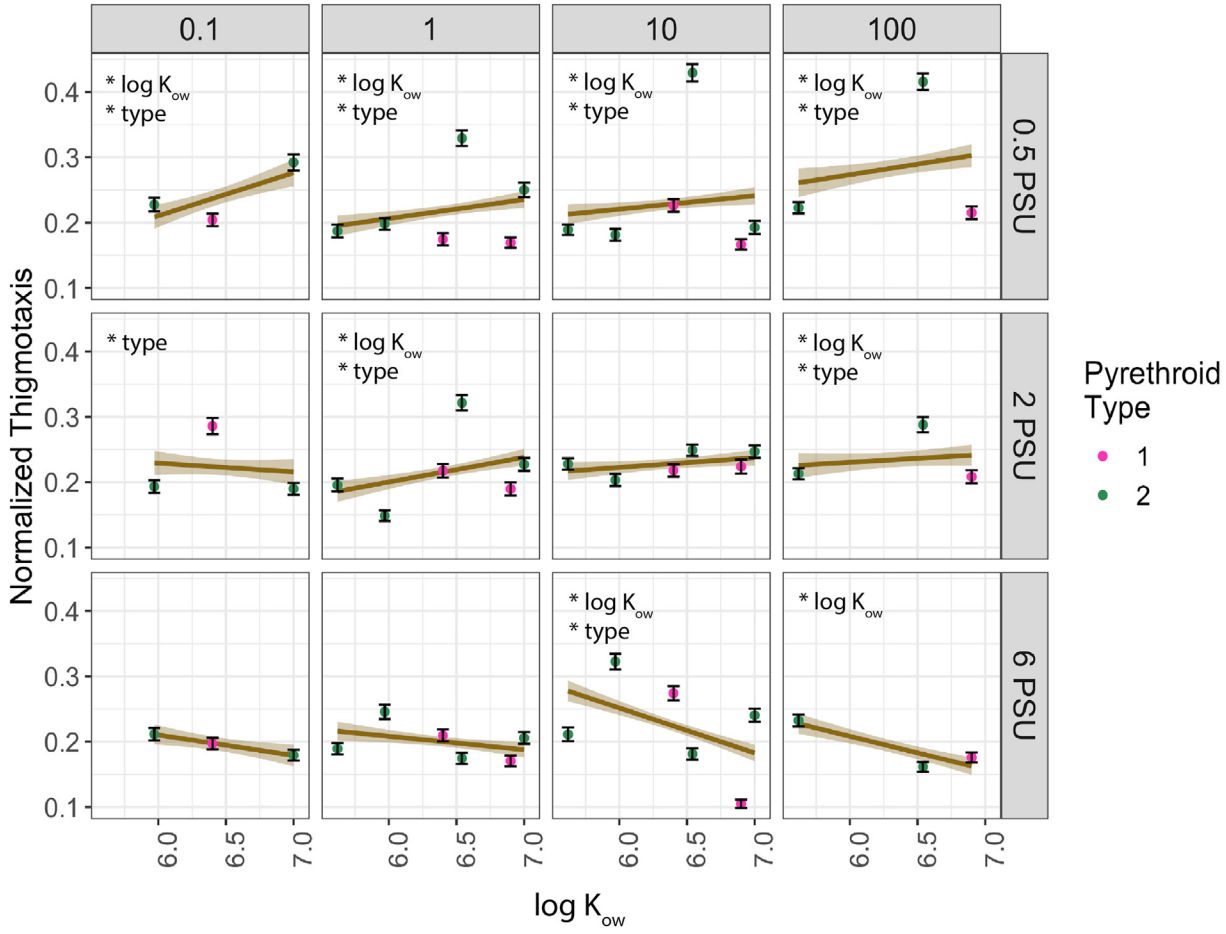

Fig. 5. Logistic regression of log K_{OW} (Table S2) and thigmotaxis behavior for all salinity and concentrations (ng/L). Pyrethroid type was also tested. The error bars denote standard error of thigmotaxis response. Quasibinomial GLM output can be found in Table S3. * denotes $p < 0.05$ (generalized linear model).

4.3. Behavior in risk assessment and environmental management

Exposure to pyrethroids can also have long-lasting effects past the exposure period, even on indirectly and unexposed generations, putting further pressure on sensitive populations under stress (Blanc et al., 2020; DeCourten et al., 2020; Major et al., 2020). Esfenvalerate increased predation risk in fathead minnows following only a short, four-hour exposure window with effects continuing after a 20 h recovery period (Floyd et al., 2008). Permethrin was found to induce hypoactivity in Zebra Rerio into the F2 generation, which suggests the neurological toxicity of pyrethroids can be transferred down the germline (Li et al., 2019). When Zebra Rerio were exposed to bifenthrin, no significant differences were found after a 96-hour exposure, but hyperactivity was detected following a 14-day recovery period in clean water, demonstrating the potential for delayed effects (Frank et al., 2018). Bifenthrin also induces transgenerational toxicity in Inland Silversides at the molecular level (DeCourten et al., 2020, Major et al., 2020); however, assessments of behavioral effects from bifenthrin have not been done on subsequent generations to date.

At low concentrations, pyrethroids commonly display non-monotonic responses, especially in endpoints related to cellular mechanisms and endocrine function. Here, we found examples of non-monotonic responses in Inland Silverside behavioral response data. These responses commonly appear near or below the no-observed-adverse-effect-level (NOAEL) which is a value commonly used in risk assessments. This demonstrates a discrepancy between traditional acute toxicity and more sensitive sublethal tests which may be more predictive of long-term impacts (Jeffries et al., 2015). Similar to concentrations tested here, pyrethroids can be found in the SFBD at concentrations as high as 150 ng/L, indicating that the responses seen from our laboratory study may be occurring in the wild as well (Weston et al., 2019).

Traditional ecological risk assessment primarily relies on three main apical endpoints: growth, reproduction, and mortality. However, concentrations of contaminants in the environment are rarely high enough to elicit these types of responses. The concentrations used here, which are representative of those found in the SFBD, induced very little effects on growth but caused significant behavioral effects across all pyrethroids and salinities. Zebra Rerio exposed to 200 ng/L esfenvalerate similarly had no effects on growth but did demonstrate changes in behavior (Wang et al., 2020). Sub-lethal effects that contribute indirectly to population decline, such as behavior, may be overlooked since these endpoints are less frequently incorporated into risk assessments, even though experts in the field of ecotoxicology agree it is an important endpoint to consider in ecological conservation (Ford et al., 2021). Given the large backlog of risk assessments needing to be conducted and the high throughput nature of behavioral assays, behavioral assay could be standardized for risk assessment, but hesitation remains around their reliability (Bownik and Wlodkowic, 2021; Ford et al., 2021).

As the use of behavioral assays increases, examples of consistent behavioral effects in fish following exposure to a pollutant are emerging that can be linked to population and community effects. For example, permethrin has been demonstrated to repeatedly result in anti-thigmotaxis behavior. In Delta Smelt, permethrin caused an overall decrease in thigmotaxis behavior at all salinities, which is consistent with our findings (Segarra et al., 2021). Permethrin was also found to decrease thigmotaxis in Zebra

Rerio following only a 24-hour exposure. The effects from permethrin exposure in the Zebra Rerio persisted into adulthood, where increased aggression, which is attributed to decreased fear and anxiety link thigmotaxis, was observed (Gerlai, 2010; Nunes et al., 2020). Decreases in anxiety-like behavior may lead to greater risk taking and an increased chance of predation, which contribute to population decline (Gerlai, 2010).

5. Conclusion

Here we compare the behavioral toxicity of Inland Silversides exposed to six pyrethroids across three salinities. To our knowledge, this is the largest number of pyrethroids compared across different salinities in any single study and organism. Overall, we found that increased salinity resulted in a decrease in the number of behavioral effects that the pyrethroids, except for permethrin, had on Inland Silversides. This study accompanies similar work conducted with Delta Smelt and informs risk assessors and managers in the SFBD on how the toxicity of these common pyrethroids differs between the more sensitive species, like Delta Smelt, and the less sensitive model fish, Inland Silverside. Since Inland Silversides are frequently used as a surrogate for Delta Smelt, understanding where uncertainty may exist in these assessments could help in applications of the study results by risk assessors. For example, bifenthrin was more toxic to Delta Smelt than permethrin; however, permethrin was more toxic to Inland Silverside than bifenthrin. Therefore, data from studies using Inland Silversides cannot be assumed to directly apply to the more sensitive fish in the SFBD; thresholds safe for Inland Silversides may not be safe for more sensitive species. Future work focused on pyrethroid toxicity in estuaries, not just the SFBD, could assess how bioaccumulation and metabolism of pyrethroids change across salinities to determine if one is more important in altering toxicity across a gradient. This information could be incorporated into risk assessments and risk modeling to predict how toxicity changes throughout an estuary.

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Institutional review board statement

This study was approved by the Oregon State University Institutional Animal Care and Use Committee (IACUC) protocol #0035, approved 19 October 2019. Adult brood stock was housed and spawned at the Oregon State University Hatfield Marine Science Center under the Animal Care and Use Program (ACUP) protocol #4999.

Credit authorship contribution statement

Sara J. Hutton: Data curation; Formal analysis; Investigation; Visualization; Roles/Writing – original draft. **Samreen Siddiqui:** Formal analysis; Investigation; Visualization; Writing – review & editing. **Emily I. Pedersen:** Data curation; Investigation. **Christopher Y. Markgraf:** Data curation; Investigation. **Amelie Segarra:** Methodology, Writing – review & editing. **Michelle L. Hladik:** Funding acquisition; Methodology; Validation; Writing – review & editing. **Richard E. Connon:** Methodology; Funding acquisition; Writing – review & editing. **Susanne M. Brander:** Methodology; Funding acquisition; Resources; Writing – review & editing.

Data availability

All data generated in this study and R code used for statistical analysis has been made publicly available and can be found at https://github.com/Brander-Harper/STOTEN_6_pyrethroid.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159398.

References

Awoyemi, O.M., Kumar, N., Schmitt, C., Subbiah, S., Crago, J., 2019. Behavioral, molecular and physiological responses of embryo-larval zebrafish exposed to types I and II pyrethroids. Chemosphere 219, 526–537.

Aznar-Alemany, Ò., Eljarrat, E., 2020. Introduction to pyrethroid insecticides: chemical structures, properties, mode of action and use. Pyrethroid Insecticides 1–16.

Bertotto, L.B., Richards, J., Gan, J., Volz, D.C., Schlenk, D., 2018. Effects of bifenthrin exposure on the estrogenic and dopaminergic pathways in zebrafish embryos and juveniles. Environ. Toxicol. Chem. 37, 236–246.

Blanc, M., Cormier, B., Hyötyläinen, T., Krauss, M., Scherbak, N., Cousin, X., Keiter, S.H., 2020. Multi-and transgenerational effects following early-life exposure of zebrafish to permethrin and coumarin 47: impact on growth, fertility, behavior and lipid metabolism. Ecotoxicol. Environ. Saf. 205, 111348.

Bownik, A., Wlodkowic, D., 2021. Applications of advanced neuro-behavioral analysis strategies in aquatic ecotoxicology. Sci. Total Environ. 772, 145577.

Brander, S.M., Gabler, M.K., Fowler, N.L., Connon, R.E., Schlenk, D., 2016a. Pyrethroid pesticides as endocrine disruptors: molecular mechanisms in vertebrates with a focus on fishes. Environ. Sci. Technol. 50, 8977–8992.

Brander, S.M., He, G., Smalling, K.L., Denison, M.S., Cherr, G.N., 2012. The in vivo estrogenic and in vitro anti-estrogenic activity of permethrin and bifenthrin. Environ. Toxicol. Chem. 31, 2848–2855.

Brander, S.M., Jeffries, K.M., Cole, B.J., DeCourten, B.M., White, J.W., Hasenbein, S., Fangue, N.A., Connon, R.E., 2016b. Transcriptomic changes underlie altered egg protein production and reduced fecundity in an estuarine model fish exposed to bifenthrin. Aquat. Toxicol. 174, 247–260.

Brooks, M.L., Fleishman, E., Brown, L.R., Lehman, P.W., Werner, I., Scholz, N., Mitchelmore, C., Lovvorn, J.R., Johnson, M.L., Schlenk, D., 2012. Life histories, salinity zones, and sublethal contributions of contaminants to pelagic fish declines illustrated with a case study of San Francisco estuary, California, USA. Estuar. Coasts 35, 603–621.

California Regional Water Quality Control Board Central Valley Region, 2017. Amendment to the water quality control plan doe the Sacramento River and San Joaquin River basins for the control of pyrethroid exposure. Resolution R5-2017-0057.

Cloern, J.E., Knowles, N., Brown, L.R., Cayan, D., Dettinger, M.D., Morgan, T.L., Schoellhamer, D.H., Stacey, M.T., Van der Wegen, M., Wagner, R.W., 2011. Projected evolution of California's San Francisco Bay-Delta-River system in a century of climate change. PloS one 6, e24465.

Connon, R.E., Geist, J., Pfeiff, J., Loguinov, A.V., D'Abronzo, L.S., Wintz, H., Vulpe, C.D., Werner, I., 2009. Linking mechanistic and behavioral responses to sublethal esfenvalerate exposure in the endangered delta smelt; Hypomesus transpacificus (Fam. Osmeridae). BMC Genomics 10, 1–18.

DeCourten, B.M., Brander, S.M., 2017. Combined effects of increased temperature and endocrine disrupting pollutants on sex determination, survival, and development across generations. Sci. Rep. 7, 9310.

DeCourten, B.M., Forbes, J.P., Roark, H.K., Burns, N.P., Major, K.M., White, J.W., Li, J., Mehinto, A.C., Connon, R.E., Brander, S.M., 2020. Multigenerational and transgenerational effects of environmentally relevant concentrations of endocrine disruptors in an estuarine fish model. Environ. Sci. Technol. 54, 13849–13860.

Delcour, I., Spanoghe, P., Uyttendaele, M., 2015. Literature review: impact of climate change on pesticide use. Food Res. Int. 68, 7–15.

Derby, A.P., Fuller, N.W., Hartz, K.E.H., Segarra, A., Connon, R.E., Brander, S.M., Lydy, M.J., 2021. Trophic transfer, bioaccumulation and transcriptomic effects of permethrin in inland silversides, Menidia beryllina, under future climate scenarios. Environ. Pollut. 275, 116545.

Ensibi, C., Hernández-Moreno, D., Míguez Santiyán, M.P., Daly Yahya, M.N., Rodríguez, F.S., Pérez-López, M., 2014. Effects of carbofuran and deltamethrin on acetylcholinesterase activity in brain and muscle of the common carp. Environ. Toxicol. 29, 386–393.

Farag, M.R., Alagawany, M., Bilal, R.M., Gewida, A.G., Dhama, K., Abdel-Latif, H.M., Amer, M.S., Rivero-Perez, N., Zaragoza-Bastida, A., Binnaser, Y.S., 2021. An overview on the potential hazards of pyrethroid insecticides in fish, with special emphasis on cypermethrin toxicity. Animals 11, 1880.

Floyd, E.Y., Geist, J.P., Werner, I., 2008. Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 27, 1780–1787.

Ford, A.T., Ågerstrand, M., Brooks, B.W., Allen, J., Bertram, M.G., Brodin, T., Dang, Z., Duquesne, S., Sahm, R., Hoffmann, F., 2021. The role of behavioral ecotoxicology in environmental protection. Environ. Sci. Technol. 55, 5620–5628.

Frank, D.F., Brander, S.M., Hasenbein, S., Harvey, D.J., Lein, P.J., Geist, J., Connon, R.E., 2019. Developmental exposure to environmentally relevant concentrations of bifenthrin alters transcription of mTOR and ryanodine receptor-dependent signaling molecules and impairs predator avoidance behavior across early life stages in inland silversides (Menidia beryllina). Aquat. Toxicol. 206, 1–13.

Frank, D.F., Miller, G.W., Harvey, D.J., Brander, S.M., Geist, J., Connon, R.E., Lein, P.J., 2018. Bifenthrin causes transcriptomic alterations in mTOR and ryanodine receptor-dependent signaling and delayed hyperactivity in developing zebrafish (Danio rerio). Aquat. Toxicol. 200, 50–61.

Gerlai, R., 2010. Zebrafish antipredatory responses: a future for translational research? Behav. Brain Res. 207, 223–231.

Giniatullin, R., Magazanik, L., 1998. Desensitization of the post-synaptic membrane of neuromuscular synapses induced by spontaneous quantum secretion of mediator. Neurosci. Behav. Physiol. 28, 438–442.

Hamilton, T.J., Szaszkiewicz, J., Krook, J., Burggren, W., 2021. Analysis of the potential behavioral impact of methanol when used as a solvent: dataset from zebrafish (Danio rerio) behavioral research. Data Brief 36, 107018.

Hasenbein, S., Holland, E.B., Connon, R.E., 2019. Eyes to the future: approaches to assess pesticide impact on surface waters in a changing climate. Pesticides in Surface Water: Monitoring, Modeling, Risk Assessment, and Management. ACS Publications, pp. 189–214.

Heintz, M.M., Brander, S.M., White, J.W., 2015. Endocrine disrupting compounds alter risk-taking behavior in guppies (Poecilia reticulata). Ethology 121, 480–491.

Herbold, B., Bush, E., Castillo, G., Colombano, D., Hartman, R., Lehman, P., Mahardja, B., Sommer, T., 2022. Climate change impacts on San Francisco estuary aquatic ecosystems: a review. San Francisco estuary and watershed. Science 20.

Hitmi, A., Coudret, A., Barthomeuf, C., 2000. The production of pyrethrins by plant cell and tissue cultures of chrysanthemum cinerariaefolium and tagetes species. Crit. Rev. Plant Sci. 19, 69–89.

Hladik, M.L., 2020. Partitioning of Six Pyrethroid Insecticides at Varying Salinities. U.S. Geological Survey data release. https://doi.org/10.5066/P9LQTT8D.

Hladik, M.L., Kuivila, K.M., 2009. Assessing the occurrence and distribution of pyrethroids in water and suspended sediments. J. Agric. Food Chem. 57, 9079–9085.

Hladik, M.L., McWayne, M.M., 2012. Methods of Analysis, Determination of Pesticides in Sediment Using Gas Chromatography/mass Spectrometry. U.S. Geological Survey Techniques and Methods Report 5-C3. http://pubs.usgs.gov/tm/tm5c3.

Hobbs, J.A., Lewis, L.S., Willmes, M., Denney, C., Bush, E., 2019. Complex life histories discovered in a critically endangered fish. Sci. Rep. 9, 16772.

Hutton, S.J., St Romain, S.J., Pedersen, E.I., Siddiqui, S., Chappell, P.E., White, J.W., Armbrust, K.L., Brander, S.M., 2021. Salinity alters toxicity of commonly used pesticides in a model euryhaline fish species (Menidia beryllina). Toxics 9, 114.

Jeffries, K.M., Komoroske, L.M., Truong, J., Werner, I., Hasenbein, M., Hasenbein, S., Fangue, N.A., Connon, R.E., 2015. The transcriptome-wide effects of exposure to a pyrethroid pesticide on the critically endangered delta smelt Hypomesus transpacificus. Endanger. Species Res. 28, 43–60.

Ji, C., Tanabe, P., Shi, Q., Qian, L., McGruer, V., Magnuson, J.T., Wang, X., Gan, J., Gadepalli, R.S., Rimoldi, J., 2021. Stage dependent enantioselective metabolism of bifenthrin in embryos of zebrafish (Danio rerio) and japanese medaka (Oryzias latipes). Environ. Sci. Technol. 55, 9087–9096.

Knowles, N., Cayan, D.R., 2002. Potential effects of global warming on the Sacramento/San Joaquin watershed and the San Francisco estuary. Geophysical Research Letters 29, 38-31–38-34.

Kültz, D., 2015. Physiological mechanisms used by fish to cope with salinity stress. J. Exp. Biol. 218, 1907–1914.

Kumar, A., Rai, D.K., Sharma, B., Pandey, R.S., 2009. λ-cyhalothrin and cypermethrin induced in vivo alterations in the activity of acetylcholinesterase in a freshwater fish, Channa punctatus (Bloch). Pestic. Biochem. Physiol. 93, 96–99.

Laskowski, D.A., 2002. Physical and chemical properties of pyrethroids. Reviews of environmental contamination and toxicology 49–170.

Lawrence, E.J., Elmstrom, S.R., Sharpe, E.E., Landis, W.G., 2021. Technical Memo: Incorporating Mixture Toxicity Into Bayesian Networks to Calculate Risk to Pesticides in the Upper San Francisco Estuary.

Lawrence, L.J., Casida, J.E., 1982. Pyrethroid toxicology: mouse intracerebral structure-toxicity relationships. Pestic. Biochem. Physiol. 18, 9–14.

Li, M., Liu, X., Feng, X., 2019. Cardiovascular toxicity and anxiety-like behavior induced by deltamethrin in zebrafish (Danio rerio) larvae. Chemosphere 219, 155–164.

Ma, S., Venkatesh, A., Langellotto, F., Le, Y.Z., Hall, M.N., Rüegg, M.A., Punzo, C., 2015. Loss of mTOR signaling affects cone function, cone structure and expression of cone specific proteins without affecting cone survival. Exp. Eye Res. 135, 1–13.

Mahardja, B., Tobias, V., Khanna, S., Mitchell, L., Lehman, P., Sommer, T., Brown, L., Culberson, S., Conrad, J.L., 2021. Resistance and resilience of pelagic and littoral fishes to drought in the San Francisco estuary. Ecol. Appl. 31.

Major, K.M., DeCourten, B.M., Li, J., Britton, M., Settles, M.L., Mehinto, A.C., Connon, R.E., Brander, S.M., 2020. Early life exposure to environmentally relevant levels of endocrine disruptors drive multigenerational and transgenerational epigenetic changes in a fish model. Frontiers in Marine Science 7.

Marinowic, D.R., Mergener, M., Pollo, T.A., Maluf, S.W., da Silva, L.B., 2012. In vivo genotoxicity of the pyrethroid pesticide β-cyfluthrin using the comet assay in the fish Bryconamericus iheringii. Z. Naturforsch. C 67, 308–311.

Méjanelle, L., Jara, B., Dachs, J., 2020. Fate of Pyrethroids in Freshwater and Marine Environments.

Middaugh, D.P., Hemmer, M.J., 1992. Reproductive ecology of the inland silverside, Menidia beryllina,(Pisces: Atherinidae) from Blackwater Bay, Florida. Copeia 53–61.

Middaugh, D.P., Hemmer, M.J., Lamadrid-Rose, Y., 1986. Laboratory spawning cues in Menidia beryllina, and M. peninsulae (Pisces, Atherinidae) with notes on survival and growth of larvae at different salinities. Environ. Biol. Fish 15, 107–117.

Mundy, P.C., Hartz, K.E.H., Fulton, C.A., Lydy, M.J., Brander, S.M., Hung, T.-C., Fangue, N., Connon, R.E., 2021. Exposure to permethrin or chlorpyrifos causes differential dose- and time-dependent behavioral effects at early larval stages of an endangered teleost species. Endanger. Species Res. 44, 89–103.

Nunes, M., Schimith, L., Costa-Silva, D., Leandro, L., Martins, I., De Mello, R., Nunes, F., Santer, M., Vieira, P., Posser, T., 2020. Acute embryonic exposure of zebrafish to permethrin induces behavioral changes related to anxiety and aggressiveness in adulthood. J. Psychiatr. Res. 121, 91–100.

Oros, D.R., Werner, I., 2005. PyrethroidInsecticides: An Analysis of Use Patterns, Distributions, Potential Toxicity andFate in the Sacramento-San Joaquin Delta and Central Valley. Citeseer.

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PloS one 10, e0146021.

Saranjampour, P., Vebrosky, E.N., Armbrust, K.L., 2017. Salinity impacts on water solubility and n-octanol/water partition coefficients of selected pesticides and oil constituents. Environ. Toxicol. Chem. 36, 2274–2280.

Schwarzenbach, R.P.G., Philip, M., Imboden, Dieter M., 2002. Activity coefficient and solubility in water. Environmental Organic Chemistry, pp. 133–180.

Segarra, A., Mauduit, F., Amer, N.R., Biefel, F., Hladik, M.L., Connon, R.E., Brander, S.M., 2021. Salinity changes the dynamics of pyrethroid toxicity in terms of behavioral effects on newly hatched delta smelt larvae. Toxics 9, 40.

Sepici-Dinçel, A., Benli, A.Ç.K., Selvi, M., Sarıkaya, R., Şahin, D., Özkul, I.A., Erkoç, F., 2009. Sublethal cyfluthrin toxicity to carp (Cyprinus Carpio L.) fingerlings: biochemical, hematological, histopathological alterations. Ecotoxicol. Environ. Saf. 72, 1433–1439.

Siddiqui, S., Dickens, J., Cunningham, B., Hutton, S., Pedersen, E., Harper, B., Harper, S., Brander, S., 2022. Internalization, reduced growth, and behavioral effects following exposure to micro and nano tire particles in two estuarine indicator species. Chemosphere 296, 133934.

Skalecka, A., Liszewska, E., Bilinski, R., Gkogkas, C., Khoutorsky, A., Malik, A.R., Sonenberg, N., Jaworski, J., 2016. mTOR kinase is needed for the development and stabilization of dendritic arbors in newly born olfactory bulb neurons. Dev. Neurobiol. 76, 1308–1327.

Soderlund, D.M., Clark, J.M., Sheets, L.P., Mullin, L.S., Piccirillo, V.J., Sargent, D., Stevens, J.T., Weiner, M.L., 2002. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology 171, 3–59.

Steele, W.B., Kristofco, L.A., Corrales, J., Saari, G.N., Haddad, S.P., Gallagher, E.P., Kavanagh, T.J., Kostal, J., Zimmerman, J.B., Voutchkova-Kostal, A., 2018. Comparative behavioral toxicology with two common larval fish models: exploring relationships among modes of action and locomotor responses. Sci. Total Environ. 640, 1587–1600.

Steenbergen, P.J., Richardson, M.K., Champagne, D.L., 2011. The use of the zebrafish model in stress research. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 1432–1451.

Stinson, S.A., Hasenbein, S., Connon, R.E., Deng, X., Alejo, J.S., Lawler, S.P., Holland, E.B., 2022. Agricultural surface water, imidacloprid, and chlorantraniliprole result in altered gene expression and receptor activation in Pimephales promelas. Sci. Total Environ. 806, 150920.

Syed, F., Chandravanshi, L.P., Khanna, V.K., Soni, I., 2016. Beta-cyfluthrin induced neurobehavioral impairments in adult rats. Chem. Biol. Interact. 243, 19–28.

Taylor, R., Herms, D.A., Cardina, J., Moore, R.H., 2018. Climate change and pest management: unanticipated consequences of trophic dislocation. Agronomy 8, 7.

Thörnqvist, P.-O., McCarrick, S., Ericsson, M., Roman, E., Winberg, S., 2019. Bold zebrafish (Danio rerio) express higher levels of delta opioid and dopamine D2 receptors in the brain compared to shy fish. Behav. Brain Res. 359, 927–934.

Tiwari, R.K., Singh, S., Pandey, R.S., 2019. Assessment of the acute toxicity of chlorpyrifos and cypermethrin to Heteropneustes fossilis and their impact on acetylcholinesterase activity. Drug Chem. Toxicol. 42, 463–470.

Tu, H.T., Silvestre, F., De Meulder, B., Thome, J.-P., Phuong, N.T., Kestemont, P., 2012. Combined effects of deltamethrin, temperature and salinity on oxidative stress biomarkers and acetylcholinesterase activity in the black tiger shrimp (Penaeus monodon). Chemosphere 86, 83–91.

Ullah, S., Li, Z., Arifeen, M.Z.U., Khan, S.U., Fahad, S., 2019a. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp (Hypophthalmichthys molitrix). Chemosphere 214, 519–533.

Ullah, S., Li, Z., Zuberi, A., Arifeen, M.Z.U., Baig, M.M.F.A., 2019b. Biomarkers of pyrethroid toxicity in fish. Environ. Chem. Lett. 17, 945–973.

Verma, R., Rajawat, N.K., Awasthi, K.K., Syed, F., John, P., Soni, I., 2021. ROS dependent neurotoxicity, genotoxicity & histopathological alterations triggered by β-cyfluthrin, a synthetic pyrethroid. Mater. Today: Proc. 42, 1737–1743.

Wang, J., Leone, P., Wu, G., Francis, J.S., Li, H., Jain, M.R., Serikawa, T., Ledeen, R.W., 2009. Myelin lipid abnormalities in the aspartoacylase-deficient tremor rat. Neurochem. Res. 34, 138–148.

Wang, X.H., Souders II, C.L., Xavier, P., Li, X.Y., Yan, B., Martyniuk, C.J., 2020. The pyrethroid esfenvalerate induces hypoactivity and decreases dopamine transporter expression in embryonic/larval zebrafish (Danio rerio). Chemosphere 243, 125416.

Weston, D., Moschet, C., Young, T., Johanif, N., Poynton, H., Major, K., Connon, R., Hasenbein, S., 2019. Chemical and toxicological impacts to cache Slough following storm-driven contaminant inputs. San Francisco estuary and watershed. Science 17.

Weston, D.P., Chen, D., Lydy, M.J., 2015. Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California. Sci. Total Environ. 527, 18–25.

Woudneh, M.B., Oros, D.R., 2006. Pyrethroids, pyrethrins, and piperonyl butoxide in sediments by high-resolution gas chromatography/high-resolution mass spectrometry. J. Chromatogr. A 1135, 71–77.

Zheng, J., Yu, Y., Feng, W., Li, J., Liu, J., Zhang, C., Dong, Y., Pessah, I.N., Cao, Z., 2019. Influence of nanomolar deltamethrin on the hallmarks of primary cultured cortical neuronal network and the role of ryanodine receptors. Environ. Health Perspect. 127, 067003.