Title
Understanding the Extent of Hyperactivity and Related Behavioral Alterations in Delta Smelt and Longfin Smelt

Author
Patullo, Caitlyn Elaine

Publication Date
2022

Peer reviewed|Thesis/dissertation

Understanding the Extent of Hyperactivity and Related Behavioral Alterations in Delta Smelt and Longfin Smelt

By

CAITLYN ELAINE PATULLO

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Pharmacology and Toxicology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA DAVIS

Approved:

Richard Connon, Chair

Sascha Nicklisch

Anne Todgham

Committee in Charge

2022

Acknowledgements

I thank Dr. Richard Connon for his support and guidance throughout the experimental process. I also thank Drs. Amelie Segarra and Florian Mauduit for their wisdom, support, and direction in challenging times and Dr. Tien-Chieh Hung along with the staff at the Fish Conservation and Culture Laboratory (FCCL) for providing me with Delta smelt and Longfin smelt embryos. I thank my thesis committee members, Drs. Anne Todgham and Sascha Nicklisch for their expertise and help throughout the thesis writing and editing process. I thank my parents Donna and Vince Patullo, my sister Leigh Anne Patullo, and my grandfather Eugene Keller without whom I would not be the person or the scientist I am today. Finally, I thank my late grandmother Muriel Porter Patullo, who kept my spirits up throughout this challenging process, even when her own health was failing. Without her optimism, encouragement, and regular mentioning of how proud she was that I moved to California pursue my dream to her doctors and peers, I would not have made it as far as I have. Even after her passing, she continued to support this dream by gifting me the financial support I needed to remain in Davis and finish my coursework. While all of my family and friends have been an endless source of support and deserve all the recognition in the world, she is the one I would like to dedicate this thesis to.

Abstract

Agricultural and urban contaminants enter aquatic environments at concentrations that can affect a variety of sublethal endpoints, including organismal behavior, which can in turn lead to impacts at the population level. The San Francisco Bay Delta (SFBD) is home to multiple threatened aquatic species, such as Delta smelt (*Hypomesus transpacificus*) and Longfin smelt (*Spirinchus thaleichthys*), with contaminant exposure likely playing a role in widespread population declines. Pesticides induce hyperactive or hypoactive states in these species, though little is known of the extent of hyperactivity that could be elicited by exposure to neurotoxic compounds. My study used pentylenetetrazole (PTZ), a γ-aminobutyrate (GABA) receptor antagonist, to explore hyperactive behavior in Delta smelt and Longfin smelt. I evaluated induced and spontaneous movement in light and dark conditions following exposure to increasing concentrations of PTZ. Delta smelt and Longfin smelt exposed to PTZ experienced changes in behaviors reflective of induced hyperactivity, including distance moved and swimming velocity, as well as spontaneous hyperactivity, such as time and frequency spent freezing, bursting, or entering the center of the arena (anti-thigmotaxis). The maximum hyperactivity for Delta smelt larvae was recorded following exposure to 8mM PTZ and for Longfin smelt larvae to 4mM PTZ. Together, this information not only confirms that PTZ could be used as a positive control in future behavioral toxicology studies for hyperactivity, but also provides for a better understanding of hyperactive behavior in these species of ecological concern.

Table of Contents

1. Introduction

In recent decades, the quality of life of human populations has been greatly improved by the use of pharmaceuticals, personal care products, and agricultural chemicals such as pesticides. However, these chemicals are often either not effectively removed by wastewater treatment methods or are leached into the environment via agricultural or urban surface runoff, which can impact water resources in either their parent form or as biologically active metabolites (Jaffrézic et al., 2017; Wilkinson et al., 2017; Battaglin et al., 2018; Menon et al., 2019; Bradley et al., 2020; Stefanakis and Becker, 2020; Wiles et al., 2020; Calvo et al., 2021; Shen et al., 2021). Pesticides, in particular, can also enter surface waters through runoff, and be hazardous to aquatic ecosystems.

Contaminant exposure effects are broad, with research highlighting sublethal effects including endocrine disruption, impaired growth and development, and changes in organismal behavior (Fong et al., 2016; Battaglin et al., 2018; Menon et al., 2019; Mundy et al., 2020; Stefanakis and Becker, 2020; Wiles et al., 2020; Segarra et al., 2021; Mundy et al., 2022; Magnuson et al., 2022). Such impacts, occurring at the organismal level, can in turn lead to impacts at the population level (Brander et al., 2013; Connon et al., 2019; Jacquin et al., 2020; Stefanakis and Becker, 2020). For example, exposure to pollutants can affect behavioral traits such as swimming activity, boldness, and olfactory recognition, which can influence how effectively fish are able to escape predation (Jacquin et al., 2020). If the ability to avoid predation, for example, is reduced following exposure to neurotoxic compounds, fewer individuals would likely survive to a reproductive age (Jacquin et al., 2020), consequently impacting population abundance.

Fish display a large range of quantifiable behaviors that are reflective of physiological and biochemical processes, which are valuable endpoints for toxicological studies (Kane et al., 2005; Basnet et al., 2019; Dutra Costa et al., 2020). Behavioral endpoints such as swimming velocity or total distance moved have been extensively studied over the last 50 years (Moss and McFarland, 1970; Little and Finger, 1990; Geist et al., 2007; Hernández-Moreno et al., 2011; Schnörr et al., 2012; Peng et al., 2016; Mundy et al., 2020; Segarra et al., 2021; Magnuson et al., 2022; Mundy et al., 2022). More recently, behavioral assays have been designed to investigate more complex behaviors such as predator-prey interactions (Baskerville-Bridges et al., 2004), cognition (Salena et al., 2021), and learning ability (Galhardo et al., 2011). An abundance of studies that use behavioral endpoints have enhanced the field of toxicology as they provide scientists with a sensitive means of evaluating detrimental but sublethal effects on exposed organisms. Behavioral studies are particularly suitable for fish species of conservation concern, including those in the San Francisco Bay Delta (SFBD).

The SFBD is a critical geographic feature for California's population, as its water resources are depended on by both urban populations and agricultural industries since the discovery of gold in California in 1848 (Cloern and Jassby, 2012; USGS, 2016;

MacVean et al., 2018; Stern et al., 2020; Tempel et al., 2021). In the early 2000s, multiple pelagic fish species in the SFBD experienced unprecedented population declines (Cloern and Jassby, 2012; MacVean et al., 2018; Tempel et al., 2021). In 2010, the SFBD was categorized as impaired for aquatic life by the Environmental Protection Agency (EPA) due to the presence of harmful contaminants, including metals, pesticides, and chlorinated compounds (Fong et al., 2016; SWRCB, 2010; Connon et al., 2019). Since this time, toxicological studies have been conducted on multiple species endemic to the San Francisco Bay Delta, including Chinook salmon (*Oncorhynchus tshawytscha*), Delta smelt (*Hypomesus transpacificus*), and Striped bass (*Morone saxatilis*), some of which have incorporated a behavioral perspective (Geist et al., 2007; Segarra et al., 2021; Magnuson et al., 2022; Mundy et al., 2022; Mundy et al., 2020). Data regarding the impacts of contaminants present in the SFBD on fish behavior, particularly during their sensitive early life stages, would inform ongoing conservation efforts.

Two species of conservation concern in the SFBD are the Delta smelt and Longfin smelt (*Spirinchus thaleichthys*). Delta smelt are small (<120mm in length) euryhaline fish endemic to the San Francisco Bay Delta (SFBD) (Lindberg et al., 2013; LaCava et al., 2015; Lessard et al., 2018; Tempel et al., 2021). Populations of Delta smelt have been declining since the 1980s, ultimately leading to the U.S. Fish and Wildlife Service listing them as threatened in 1993 under the U.S. Endangered Species Act (USFWS, 1993). In 2010, the state of California changed this listing to endangered following further population declines (CDFG, 2010). Since then, Delta smelt have become a species of interest in conservation studies as they have been recognized as an indicator species of the overall environmental health of the SFBD, due to observable correlations between environmental conditions, population size, distribution, and behavior (Lessard et al., 2018; Mundy et al., 2020). Longfin smelt are a slightly larger (<150mm) species that were once more abundant than Delta smelt in the SFBD (Tempel et al., 2021; Yanagitsuru et al., 2022). Historically, populations of Longfin smelt have fluctuated, with low numbers recorded in 1979 followed by a recovery in the early 1980s (Tempel et al., 2021). However, due to multiple anthropogenic and environmental factors, Longfin smelt populations have continuously been in decline since the late 1980s, ultimately leading to the state of California listing the species as threatened in 2009 (CDFG, 2009; Yanagitsuru et al., 2022).

In response to these population declines, researchers have established a captive culture program for Delta smelt (Lindberg et al., 2013), and similar efforts are underway for Longfin smelt (Yanagitsuru et al., 2020). This has presented a unique opportunity for researchers to study both species in order to improve conservation efforts by providing a better understanding of sensitivity to environmental stressors in their natural environment. Three studies have evaluated how pesticide exposure can impact behavior in Delta smelt yolk-sac larvae (Mundy et al., 2020; Mundy et al., 2022; Segarra et al., 2021). Thus far a single study has evaluated behavioral responses to pesticide exposure in Longfin smelt larvae (Mauduit et al., in preparation).

Of particular interest are the hyperactive responses that were observed in these studies. Hyperactivity is defined as an increase in the spontaneous or induced movement of an organism (Ogungbemi et al., 2019). This behavior can manifest as either an increase in overall velocity, distance moved, or both (i.e., induced movement), or as intermittent bursts of high-velocity movements which can precede periods of minimal movement (i.e., spontaneous movement) (Ogungbemi et al., 2019; Basnet et al., 2019; Mandralis et al., 2021). While the above studies did detect variations in several specific endpoints related to hyperactivity, there are still gaps in our knowledge of hyperactive behavior that have not been addressed. For example, the implications of hyperactivity relative to organismal capacity or impact (e.g., what is the maximum measurable hyperactivity) are still largely unknown. Additionally, we have yet to identify experimental standards (i.e., positive controls for hyperactivity) with which to compare across studies or clutches of larvae, which would be valuable tools in future investigations.

To this effect, I designed a study that sought to provide a better understanding of hyperactivity in larval Delta and Longfin smelt exposed to pentylenetetrazole (PTZ). PTZ is a γ-aminobutyrate (GABA) receptor antagonist (Figure 1; Huang et al., 2001), which has historically been used in epilepsy studies, as it is able to induce seizures in model organisms, including rats (Klioueva et al., 2001), mice (Hansen et al., 2004), and fish; e.g., Zebrafish, *Danio rerio* (Baraban et al., 2005; Afrikanova et al., 2013; Peng et al., 2016; Bandara et al., 2020; Shaw et al., 2022). Multiple studies on Zebrafish found that at low doses, hyperactive behavior can be induced without being accompanied by seizure-like behavior (Baraban et al., 2005; Peng et al., 2016). I used PTZ to better understand a) whether maximum hyperactivity can be determined following exposure to PTZ, for both Delta smelt and Longfin smelt, and b) how hyperactive behavior manifests in response to visual stimuli in Delta and Longfin smelt. Together, this data would inform if PTZ could be used as experimental standards (e.g., positive controls) for use in future behavioral toxicology studies. I hypothesized that increases in activity would be concentration dependent and that hyperactive behavior would increase the amount of activity achievable in response to light stimuli in larvae exposed to PTZ compared to unexposed controls. To test these hypotheses, I used a photomotor response assay protocol optimized for Delta smelt larvae by Mundy et al. (2020) with which I evaluated behavioral responses in both Delta smelt and Longfin smelt yolk-sac larvae following short-term, acute exposures to PTZ at both 48 and 72 hours post-hatch (hph).

2. Methods and Materials

2.1 Fish source and maintenance

Delta smelt and Longfin smelt embryos were fertilized via strip spawning (2:2 or 1:2 female:male) by the UC Davis Fish Conservation and Culture Laboratory (FCCL), in Byron, CA, according to protocol #19841, which was approved for use by the University of California Institutional Animal Care and Use Committee (IACUC). Delta and Longfin smelt embryos were maintained at FCCL until 7 days post fertilization (dpf) prior to transportation to the UC Davis School of Veterinary Medicine.

Upon arrival at the UC Davis School of Veterinary Medicine, both Delta smelt and Longfin smelt larvae were randomly distributed into glass beakers containing 100mL of filtered ground water (0.2um) sourced from the UC Davis Center for Aquatic Biology and Aquaculture (CABA), in Davis, CA. Larvae were maintained in 24-hr dark conditions at a stocking density of 25 larvae per beaker. Research on early larval stages was conducted according to IACUC protocol #20705.

Water quality was measured twice daily throughout the duration of the experiment, once prior to and once immediately following 50% water replacement from an aerated carboy. Delta smelt larvae were maintained at 16°C ± 2°C and 8.5 ± 0.25 pH. Longfin smelt larvae were maintained at 12°C ± 2°C and 8.5 ± 0.25 pH. Ammonia values were maintained at 0 mg/L and salinity at 0.4 ppt for both species.

At 16°C, Delta smelt larvae are expected to hatch between 8 and 10dpf (Romney et al., 2019) and begin exogenous feeding within the 5-7 days post-hatch (dph), before the yolk-sac is depleted (Baskerville-Bridges et al., 2005; Lessard et al., 2018). Longfin smelt embryo maintained at 12°C typically hatch within 14-16dpf (Mulvaney et al., 2022), with initiation of exogenous feeding also occurring 5-7dph. Experiments on both species were completed at 48 and 72hph, prior to the initiation of feeding behavior. In order to ensure that the age of the larvae was consistent between experimental groups, newly hatched larvae from each species were separated from the remaining embryos. A stocking density of 25 embryos per beaker was maintained as closely as possible during this process for both unhatched embryo and hatched larvae.

2.2 Pentylenetetrazole Exposure

Larvae were exposed to one of the following concentrations of PTZ (Sigma-Aldrich, Burlington, MA, USA; CAS: 54-95-5): 2mM, 4mM, 8mM, 12mM, or 16mM for the duration of the experiment (approx. 40 min) (Figure 2). The concentration range was chosen to represent that which has been shown to induce hyperactivity in zebrafish (Baraban et al., 2005; Peng et al., 2016; Bandara et al., 2020).

Pentylenetetrazole exposures were completed following transfer of the larvae in the 12-well plate into the Daniovision chamber in order to reduce additional stress on the larvae (Figure 2). Prior to exposure, each well in the 24-well plate contained 0.5mL of filtered water and 1 larva (Figure 2). Exposure was achieved by pipetting 0.5mL of dosing solution into the existing 0.5mL of filtered water. As such, dosing solutions were made to be double the experimental concentration (4mM, 8mM, 16mM, 24mM, and 32mM) so that the optimal concentration would be reached following dilution in the plate water. The distribution of each concentration of PTZ in the well plate was randomized for each plate to reduce any potential position bias.

2.3 Photomotor Response Assay

The photomotor response assay used in this study was adapted from the light-dark (LD) cycle behavioral assay developed by Mundy et al. (2020). On the day of the experiment, larvae that had reached either 48 or 72hph were carefully transferred using a 1mL plastic pipette into a non-treated 24-well cell culture plate (Genessee Scientific, Cat #: 25-101, San Diego, CA, USA) so that one larva inhabited each well containing

0.5mL of filtered water. The larvae were then allowed to acclimate to the plate conditions for 1 h prior to transportation into a Noldus DanioVision Observation Chamber (Leesburg, VA, USA; Figure 2). The observation chamber was equipped with an infrared light source that allowed for tracking in dark environments as well as a visible light source which were used as stimuli to induce movement. Further, the chamber was designed to maintain a stable internal environment by blocking out external light and absorbing vibration from nearby movement.

Once in the DanioVision Chamber, larvae were acclimated to the chamber for approximately 5 min before receiving 0.5mL of dosing solution. Distribution of each dosing solution throughout the plate was randomized for all plates to avoid position-related biases. For the duration of the experiment, larvae were filmed from the top of the chamber using a digital IR-sensitive camera. The position and movement of the larvae in each well was tracked using Noldus' Ethovision XT 15 software (Leesburg, VA, USA). Illumination within the chamber was programmed to initiate the following LD cycle at the start of each trial: 10 min dark period (split into two 5 min periods for analysis, Dark 1 and Dark 2), 5 min light period (Light 1), 5 min dark period (Dark 3), 5 min light period (Light 2), and a final 10 min dark period (split into Dark 4 and Dark 5 for analysis) (Figure 2). During each light cycle, the programmable light within the DanioVision chamber was set to 10,000 lx. The temperature of the plate was stabilized at each species' optimal rearing temperature (16°C and 12°C, respectively) using a recirculating water system attached to a chiller (TECO-US, Terrell, TX, USA).

2.4 Behavioral Assessment of Pentylenetetrazole-exposed Larvae

Quantitative data collected from each PTZ-exposed larvae included total distance moved (mm), mean velocity (mm/s), average maximum velocity (mm/s) and total duration in center zone (anti-thigmotaxis) (s) of respective exposure wells. Prior to data acquisition, arenas and zones were defined in the Ethovision software. The diameter of each arena was 16mm, while the diameter of each center zone was 9mm. The measured velocities were grouped by speed into three categories: cruising (5mm/s-20mm/s), bursting (20mm/s-100mm/s), and freezing (less than 5mm/s). Velocity categories were selected to reflect categories from previous studies using Delta smelt (Mundy et al., 2020; Segarra et al., 2021; Mundy et al., 2022). To aid visualization, Z-scores were calculated for use in heatmap plots presenting multiple parameters. Z-scores were calculated using the following equation: Z-score = $(x-\boldsymbol{\mu})/\boldsymbol{\sigma}$, where x = value, $\boldsymbol{\mu}$ = mean, and $\boldsymbol{\sigma}$ = standard deviation.

2.6 Statistical Analyses

Each behavioral endpoint was binned by minute prior to statistical analysis. The statistical computing software R was used to calculate the means of each endpoint per experimental group on a per minute and per photoperiod basis for the purpose of measuring differences in swimming behavior. Photoperiod refers to 5-min periods of dark or light. In the following figures and tables, these photoperiods are titled Dark 1 (0-5 mins), Dark 2 (5-10 mins), Light 1 (10-15 mins), Dark 3 (15-20 mins), Light 2 (20-25 mins), Dark 4 (25-30 mins), and Dark 5 (30-35 mins) (Figure 2). All behavioral endpoints for treated groups were compared to the control group within their own species (Delta smelt or Longfin smelt) and developmental timepoint (48 or 72hph).

All behavioral data was subjected to hypothesis testing for statistical significance through a sequence of four tests in R (version 4.1.3). The normality of the data was tested using the Shapiro-Wilk test (package = “stats” version 4.1.3). Then, the homogeneity of variance for each variable was assessed using the Levene’s test (package = “rstatix” version 0.7.0). The statistical evaluation of differences between treated and untreated experimental groups was performed using the Kruskal-Wallis nonparametric test (package = “rstatix” version 0.7.0). Then, a post hoc analysis was performed via emmeans multiple comparison test (package = “emmeans” version 1.7.4) using the contrast method to compare control larvae to exposed larvae ($\alpha < 0.05$). Following this analysis, the p-value was adjusted using the dunnetx method (Dunnet’s test). It is otherwise important to note that the Dunn’s multiple comparisons test was run at $\alpha < 0.05$ with Bonferroni p-value adjustment as an additional post hoc analysis (package = “rstatix” version 0.7.0).

3. Results

Both Delta smelt and Longfin smelt were significantly impacted by exposure to PTZ within the tested range of concentrations (Figures 3-6). In Delta smelt larvae, exposure to 8mM PTZ resulted in the most significant alterations across multiple measured endpoints in both 48hph and 72hph larvae (Figures 3 and 4). In Longfin smelt, exposure to 4mM PTZ was sufficient to induce significant shifts in behavior across multiple measured endpoints at 48hph, while 16mM resulted in the highest change in activity at 72hph (Figures 5 and 6).

3.1 Delta Smelt

At 48hph, larvae exposed to either 8mM, 12mM, or 16mM PTZ swam a significantly greater distance than control larvae during the first three photoperiods; the first 15 minutes of the behavioral test ($p < 0.05$; Table 1) (Figure 3; Supplementary Table 1). Larvae exposed to 8mM moved an average of 224mm (min = 38mm; max = 896mm) during the first light photoperiod, which was the highest recorded value amongst Delta smelt larvae at 48hph (Supplementary Table 1). At 72hph, larvae exposed to 8mM PTZ also experienced increases in distance moved, statistically significant during two photoperiods: Light 1 and Dark 3 ($p < 0.005$; Table 2) (Figure 3; Supplementary Table 1). Larvae exposed to this concentration at 72hph moved an average of 197mm (min = 29mm; max = 1009mm) during the first light photoperiod, which was the highest recorded value among 72hph Delta smelt larvae (Supplementary Table 1).

Patterns in mean velocity data, as expected, mirrored those of total distance moved. At 48hph, larvae exposed to 8mM, 12mM, or 16mM PTZ swam at significantly higher velocities than control larvae during the first three photoperiods ($p < 0.05$; Supplementary Table 2) (Figure 4). Larvae exposed to 8mM PTZ at 48hph demonstrated a mean swimming velocity of 3.72mm/s (min = 0.64mm/s; 14.93mm/s) during Light 1; the highest value recorded amongst Delta smelt at this developmental time point. At 72hph, larvae exposed to 8mM PTZ swam at significantly higher velocities than controls during Light 1 and Dark 3 ($p < 0.05$; Supplementary Table 2) (Figure 4). Larvae exposed to this concentration at 72hph also demonstrated the highest mean velocity (3.28mm/s; min = 0.49mm/s; max = 16.81mm/s) recorded throughout the experiment across experimental groups (Supplementary Table 2).

Delta smelt larvae also experienced changes in maximum velocity following exposure to PTZ. At 48hph, larvae exposed to either 4mM or 8mM exhibited significantly higher maximum velocities than control larvae during Dark 1 ($p < 0.05$; Supplementary Table 3) (Figure 4). Larvae exposed to 4mM at 48hph demonstrated an average maximum velocity of 63.40mm/s (min = 1.84mm/s; max = 137.79; Supplementary Table 3) during Dark 1, which was the maximum value achieved by larvae at this developmental time point. At 72hph, larvae exposed to 8mM or 12mM demonstrated significantly higher maximum velocities than controls during Dark 1 ($p < 0.05$; Supplementary Table 3) (Figure 4). At this developmental time point, the highest average maximum velocity was 55.69mm/s (min = 1.59mm/s; max = 119.98mm/s; Supplementary Table 3) in larvae exposed to 12mM PTZ.

At 48hph, larvae exposed to all concentrations of PTZ responded with significantly higher freezing times during Dark 1 than control larvae ($p < 0.05$; Supplementary Table 4) (Figure 4). At 72hph, larvae exposed to all concentrations of PTZ demonstrated a significant decrease in freezing frequency compared to control larvae during Dark 1 ($p < 0.001$; Supplementary Table 5) (Figure 4).

In regard to cruising behavior, larvae exposed to 12mM PTZ at either 48hph or 72hph spent significantly more time cruising during Dark 1 when compared to control larvae ($p < 0.001$; Supplementary Table 6) (Figure 4). While no other significant changes in cruising duration were detected in 48hph larvae, those exposed to 2mM PTZ at 72hph spent significantly more time cruising than controls in Dark 4 ($p < 0.005$; Supplementary Table 6) (Figure 4). Cruising frequency also increased in Delta smelt larvae exposed to PTZ at 48hph and 72hph (Figure 4; Supplementary Table 7). At 48hph, larvae exposed to 8mM PTZ exhibited significantly higher cruising frequencies than control larvae during Dark 1, Light 1, and Light 2 ($p < 0.05$; Supplementary Table 7) (Figure 4). Larvae exposed to 4mM and 12mM at 48hph also demonstrated significantly higher cruising frequencies than unexposed larvae, though this was only observed during Dark 1 ($p < 0.05$; Supplementary Table 7) (Figure 4). At 72hph, larvae exposed to 12mM PTZ exhibited significantly higher cruising frequencies compared to controls during Dark 1. Larvae exposed to 2mM at 72hph demonstrated a significant increase in cruising frequency compared to control larvae during Dark 4 ($p < 0.05$; Supplementary Table 7) (Figure 4).

The bursting behavior of both 48hph and 72hph Delta smelt larvae was also altered following exposure to concentrations of PTZ. At 48hph, significant increases in bursting duration were observed in larvae exposed to 2mM in Light 2, 8mM in Light 1, and 12mM in Dark 2 compared to control larvae ($p < 0.05$; Supplementary Table 8) (Figure 4). Delta smelt larvae exposed to 2mM PTZ at 48hph also demonstrated a significant increase in bursting frequency during Light 2 when compared to unexposed larvae ($p < 0.05$; Supplementary Table 9) (Figure 4). In 72hph larvae, bursting duration significantly increased in larvae exposed to 8mM PTZ in Dark 1 and 4mM in Light 1 compared to control larvae ($p < 0.05$; Supplementary Table 8) (Figure 4). Finally, larvae exposed to 4mM at 72hph demonstrated a significant increase in bursting frequency compared to controls in Light 1 ($p < 0.05$; Supplementary Table 9) (Figure 4).

Delta smelt larvae exposed to concentrations of PTZ at 48hph did not demonstrate significant changes in either the duration or frequency of presence in the center of the arena (anti-thigmotaxis) compared to controls (Figure 4; Supplementary Tables 10 and 11). At 72hph, Delta smelt larvae exposed to 16mM PTZ spent significantly more time in the center zone compared to control larvae during Light 2 and Dark 4 ($p < 0.05$; Supplementary Table 10); however, frequency of time spent in the center zone was statistically indistinguishable from controls at this concentration ($p > 0.05$; Supplementary Table 11) (Figure 4). Larvae exposed to 12mM PTZ at 72hph demonstrated significantly increased frequencies in the center zone compared to controls during Dark 1 ($p < 0.05$; Supplementary Table 11) (Figure 4).

In summary, Delta smelt larvae exposed to PTZ resulted in instances of significant changes in behavior characteristic of hyperactivity, across all tested concentrations. Larvae exposed to 8mM PTZ demonstrated instances of significant changes in hyperactivity-related behavioral endpoints during light and dark photoperiods at both 48hph and 72hph (Figures 3 and 4). At 48hph, these changes included increased distance moved, swimming velocity, maximum swimming velocity, freezing duration, cruising frequency, and bursting duration. Larvae exposed to 8mM PTZ at 72hph also demonstrated significant changes in hyperactivity-related behavior (Figures

3 and 4). Specifically, larvae exposed to 8mM PTZ at 72hph experienced significant increases in distance moved, mean velocity, maximum velocity, bursting duration, and bursting frequency. However, larvae exposed to 8mM PTZ showed a significant decrease in freezing frequency at 72hph, which was recorded at the beginning of the experiment (Dark 1) (Figure 2). Of note, at 48hph, larvae were seemingly more sensitive to PTZ exposure than at 72hph.

3.2 Longfin Smelt

Longfin smelt larvae exposed to concentrations of PTZ at 48hph demonstrated significant increases in distance moved compared to controls ($p < 0.05$; Table 2) (Figure 5; Supplementary Table 12). Specifically, larvae exposed to 4mM PTZ at 48hph moved significantly more distance from Dark 1 onward when compared to control larvae ($p < 0.05$; Table 2) (Figure 5; Supplementary Table 12). During the Light 1, larvae exposed to this concentration swam an average of 598mm (min = 106mm; max = 935mm), which was the highest recorded value amongst larvae at this developmental time point (Supplementary Table 12). At 72hph, larvae exposed to 16mM PTZ demonstrated significant increases in distance moved in four dark photoperiods when compared to controls: Dark 1, Dark 2, Dark 3, and Dark 5 ($p < 0.05$; Table 2) (Figure 5; Supplementary Table 12). Longfin smelt larvae exposed to 12mM at 72hph also moved significantly higher distances compared to unexposed larvae, though this was limited to Dark 2 ($p < 0.05$; Table 2) (Figure 5; Supplementary Table 12). Amongst 72hph larvae, those exposed to 16mM exhibited the highest recorded distance moved of 199mm (min = 23mm; max = 1022mm) (Supplementary Table 12).

Velocity data of Longfin smelt larvae was similar to the distance moved data at both 48hph and 72hph. At 48hph, larvae exposed to all concentrations of PTZ exhibited significantly higher velocities than unexposed larvae during multiple photoperiods ($p < 0.05$; Supplementary Tables 13 and 14) (Figure 6). Larvae exposed to 4mM PTZ at 48hph demonstrated significant increases in velocity throughout the duration of the experiment when compared to controls ($p < 0.05$; Supplementary Tables 13 and 14) (Figure 6). Further, larvae exposed to this concentration at 48hph exhibited the highest values in mean velocity (9.97mm/s; min = 1.77mm/s; max = 15.58mm/s) and average maximum velocity (89.74mm/s; min = 53.62mm/s; max = 130.86mm/s) recorded during the experiment (Supplementary Tables 13 and 14). At 72hph, larvae exposed to 16mM PTZ exhibited significantly higher velocities than control larvae during Dark 1, Dark 2, Dark 3, and Dark 5, while those exposed to 12mM demonstrated a significant increase during Dark 2 compared to controls ($p < 0.05$; Supplementary Tables 13 and 14) (Figure 6). Amongst 72hph larvae, those exposed to 16mM exhibited the highest velocity values recorded in terms of both mean velocity (6.67mm/s; min = 0.39mm/s; max = 17.13mm/s) and average maximum velocity (80.84mm/s; min = 1.30mm/s; max = 121.16mm/s) amongst experimental groups (Supplementary Tables 13 and 14).

Larval Longfin smelt exposed to PTZ also experienced significant changes in cruising, bursting, and freezing behavior. However, effects on freezing behavior were limited to one instance of freezing frequency significantly decreasing in 48hph Longfin smelt larvae exposed to 8mM of PTZ ($p < 0.05$; Supplementary Table 16) (Figure 6).

In terms of cruising behavior, larvae exposed to both 2mM and 4mM PTZ at 48hph spent significantly more time cruising in light photoperiods than control larvae ($p < 0.05$; Supplementary Table 17) (Figure 6). Longfin smelt larvae exposed to either 8mM or 16mM at 48hph demonstrated significantly longer cruising durations than controls in all photoperiods ($p < 0.05$; Supplementary Table 17) (Figure 6). Larvae exposed to 12mM PTZ at 48hph also exhibited significant increases in cruising duration compared to unexposed larvae for the majority of the experiment ($p < 0.001$), except during Dark 1 ($p > 0.10$; Supplementary Table 17) (Figure 6). Notably, Longfin smelt larvae exposed to all concentrations of PTZ at 48hph demonstrated significantly higher cruising durations during both light photoperiods compared to control larvae ($p < 0.05$; Supplementary Table 17) (Figure 6). At 72hph, significant changes in cruising duration compared to controls were only detected in larvae exposed to either 12mM or 16mM PTZ (Figure 6; Supplementary Table 17). Specifically, larvae exposed to 12mM at 72hph spent significantly more time cruising during Dark 2 ($p < 0.05$; Supplementary Table 17) (Figure 6). Larvae exposed to 16mM, on the other hand, spent significantly more time cruising compared to control larvae during Dark 1, Dark 2, Dark 3, and Dark 5 ($p < 0.05$; Supplementary Table 17) (Figure 6).

Changes in cruising frequency amongst Longfin smelt larvae were also detected following exposure to PTZ compared to controls (Figure 6; Supplementary Table 18). At 48hph, larvae exposed to all concentrations of PTZ demonstrated at least one instance of significantly increased cruising frequencies compared to control larvae. Larvae exposed to 12mM and 16mM PTZ at 48hph demonstrated significant increases across all photoperiods compared to control larvae ($p < 0.05$; Supplementary Table 18) (Figure 6). Larvae exposed to 8mM at 48hph demonstrated significantly increased cruising frequencies from Dark 2 through Dark 5 ($p < 0.05$; Supplementary Table 18), while larvae exposed to 4mM demonstrated significantly increased cruising frequencies from Light 1 through Dark 5 ($p < 0.05$; Supplementary Table 18) (Figure 6). Finally, larvae exposed to 2mM at 48hph demonstrated significantly higher cruising frequencies during Light 2 and Dark 5 ($p < 0.05$; Supplementary Table 18) (Figure 6). At 72hph, larvae only exhibited significant changes in cruising frequencies compared to controls when exposed to either 12mM or 16mM PTZ (Figure 6; Supplementary Table 18). Specifically, larvae exposed to 12mM at 72hph demonstrated significantly higher cruising frequencies compared to control larvae during Dark 1 and Dark 2 ($p < 0.05$; Supplementary Table 18) (Figure 6). Cruising frequencies of larvae exposed to 16mM of PTZ were significantly higher than unexposed larvae in Dark 1, Dark 2, Dark 3, and Dark 5 ($p < 0.05$; Supplementary Table 18) (Figure 6).

Bursting behavior was also affected following exposure to PTZ in both 48hph and 72hph larvae compared to controls (Figure 6; Supplementary Tables 19 and 20). Larvae exposed to 4mM PTZ at 48hph experienced significantly increased bursting durations during Light 2 ($p < 0.05$; Supplementary Table 19), though bursting frequency was statistically indistinguishable from controls (Figure 6; Supplementary Table 20). Larvae exposed to 12mM PTZ at 48hph demonstrated significant increases in both bursting duration and frequency compared to control larvae during Dark 1, Dark 2, and Dark 3 ($p < 0.05$; Supplementary Tables 19 and 20) (Figure 6). Further, larvae exposed to 16mM at 48hph experienced significant increases in both bursting behaviors during Dark 1 and

Dark 2 ($p < 0.05$; Supplementary Tables 19 and 20). At 72hph, the only observable significant effects on bursting duration and frequency were in larvae exposed to 16mM PTZ ($p < 0.005$; Supplementary Tables 19 and 20) during Dark 1(Figure 6).

Finally, 48hph Longfin smelt thigmotaxis behavior data suggested that this behavior was also influenced following exposure to concentrations of PTZ (Figure 6; Supplementary Tables 21 and 22). For example, larvae exposed to 8mM or 12mM at 48hph spent significantly less time in the center compared to controls in Dark 3 ($p < 0.01$; Supplementary Table 21) (Figure 6). Longfin smelt larvae exposed to 16mM of PTZ at 48hph, on the other hand, spent significantly more time in the center of the arena compared to controls in Light 2 ($p < 0.05$; Supplementary Table 21) (Figure 6). Observations in the frequency data at 48hph were less consistent, with larvae exposed to 8mM exhibiting significantly higher frequencies in the center compared to unexposed larvae during Dark 4 ($p < 0.005$; Supplementary Table 22) and larvae exposed to 16mM demonstrating the same in Light 2 and Dark 4 ($p < 0.05$; Supplementary Table 22) (Figure 6). Further, at 72hph, larvae exposed to the tested range of PTZ concentrations did not demonstrate any significant changes in thigmotaxis behavior when compared to control larvae (Figure 6; Supplementary Table 22).

In summary, as did Delta smelt larvae, Longfin smelt larvae exposed to PTZ demonstrated multiple instances of significant changes in behavior that are characteristic of hyperactivity, across all tested concentrations. At 48hph, larvae exposed to 4mM PTZ exhibited significant changes in multiple hyperactivity-related endpoints in both light and dark conditions (Figures 5 and 6). These changes included increased total distance moved, mean velocity, and maximum velocity. Trends in cruising and bursting data at this endpoint revealed that these larvae experienced increased cruising and bursting behavior (Figure 6). At 72hph, larvae exposed to 16mM PTZ resulted in significant changes in multiple measured behavioral endpoints, though these changes were only recorded in dark conditions (Figures 5 and 6). Specifically, at 72hph, longfin smelt larvae exposed to 16mM demonstrated increased distance moved, mean velocity, cruising duration, and cruising frequency. Significant increases in bursting duration and frequency were also recorded in larvae exposed at this concentration, but only during Dark 1 (Figure 6). Additionally, Longfin smelt larvae were seemingly more sensitive to PTZ exposure at 48hph than at 72hph, since more instances of statistical significance were recorded at this developmental time point.

4. Discussion

In this study, I sought to better understand a) whether maximum hyperactivity can be quantified following exposure to PTZ and b) how hyperactive behavior manifests in response to visual stimuli in Delta smelt and Longfin smelt larvae. Together, this information would help determine if PTZ could be used as an experimental standard (e.g., positive control) in future behavioral toxicology studies. To accomplish this, a Light/Dark photomotor response assay was adapted from Mundy et al., (2020), allowing for the collection of data regarding distance moved, swimming velocity, and positioning within the experimental arena following exposure to multiple concentrations of PTZ. Through this approach, I have shown that Delta smelt and Longfin smelt larvae exposed to PTZ exhibited hyperactive behavior in response to light stimuli to a degree that allowed for quantification of maximum hyperactivity in both species.

The Light/Dark photomotor response assay allows for rapid yet effective characterization of behavioral effects following exposure to contaminants (Steele et al., 2018; Dach et al., 2019), which can be applied to multiple species (Mundy et al., 2020; Segarra et al., 2021; Mundy et al., 2022; Siddiqui et al., 2022; Hutton et al., 2023). This assay provides a stable environment, free from the influence of external light and vibrations. Such an environment is crucial for behavioral studies on species such as Delta smelt and Longfin smelt, which are highly sensitive to external stimuli, as it aids in attributing observed behavioral changes to the chemical of interest rather than unintentional environmental influences.

Data collected in this study consisted of 11 behavioral endpoints to allow for a thorough analysis of hyperactive behaviors, which can include complex behavioral patterns that single endpoints struggle to comprehensively address. I also incorporated a thigmotaxis assay into my analysis, with anti-thigmotaxis usually associated with organismal boldness, which would also provide information regarding irregular movement patterns that sometimes accompany sudden, spontaneous movements.

It is important to note that there are many limitations to studying behavior in fish species, including variability in behavior not only between species, but also between breeding clutches and individuals (Shaw, 2020; Goc et al., 2021). While no existing studies have attempted to quantify such variability in Delta smelt specifically, differences in behavior between batches can be observed when comparing results from this study with previous Delta smelt behavioral studies that used the same experimental system and yolk-sac larvae from the same source (i.e., FCCL). For example, I was unable to observe differences in behavior in unexposed Delta smelt larvae when comparing swimming activity in light and dark conditions (Figures 1 and 2). This contrasts with the findings of previous studies using a similar experimental design, which observed significantly increased activity throughout each light photoperiod when compared to dark photoperiods (Mundy et al., 2020; Segarra et al., 2021; Mundy et al.,2022). These discrepancies could be due to either batch effects (e.g., spawn quality, parental influence) or the overall health of study subjects, as both of these could influence the behavior of an organism.

My study also detected behavioral variability in both species that appeared to increase with development, as behavioral changes observed in 72hph larvae exposed to PTZ were less statistically distinguishable from controls than those of 48hph exposed larvae (Figures 3-6). These differences are likely due to the developmental stage of the organisms. For example, it is possible that the growth experienced by larvae over a 24h period was sufficient to make the organisms less sensitive to exposure, as their yolk-sac, which is susceptible to chemical absorption, is nearly fully absorbed at this developmental time point (i.e., larval Delta smelt and Longfin smelt begin exogenous feeding between 4 and 7 dph) (Baskerville-Bridges et al., 2005; Baskerville-Bridges et al., 2004). Other behavioral studies on Delta smelt have included larvae at different developmental stages (Mundy et al., 2020; Segarra et al., 2021; Mundy et al., 2022), but these studies exposed embryos to the chemical of interest for multiple days prior to behavioral analysis, allowing ample time for potential bioaccumulation to occur, and longer-term impacts on development to be determined.

Both Delta smelt and Longfin smelt demonstrated significant increases in distance moved following exposure to PTZ that often were not photoperiod (dark or light) dependent. In both species, regardless of whether larvae were 48hph and 72hph, increases in distance moved were also accompanied by simultaneous increases in swimming velocity. Therefore, Delta smelt and Longfin smelt exposed to PTZ can display hyperactive behaviors in the form of induced movement. Additionally, following exposure to PTZ, 48hph Longfin smelt demonstrated consistently significant behavioral effects in both distance moved and swimming velocity. Once a behavioral effect was observed it was likely to be maintained throughout the remainder of the experiment. This finding suggests that PTZ can be used as a standard for these behavioral endpoints. However, since the degree of consistency was not observed in both species, nor across both developmental time points tested, further investigation is warranted to better understand what specifically represents a positive control, critical information if PTZ is to be used as a standard component of behavioral assays for these sensitive species. Since other studies have consistently reported hyperactive behavior in Zebrafish exposed to similar concentrations of PTZ (Baraban et al., 2005; Peng et al., 2016), it is highly likely that the threatened species studied herein, have different sensitivity thresholds to that of Zebrafish.

Besides induced movement, my study also revealed behavioral patterns that suggested spontaneous movements were elicited in both Delta smelt and Longfin smelt exposed to PTZ. In Delta smelt, 48hph and 72hph larvae exposed to PTZ demonstrated changes in freezing and bursting behavior compared to control larvae, while 72hph larvae exposed to higher concentrations of PTZ also exhibited changes in thigmotaxis behavior compared to controls. Similar patterns were observed in Longfin smelt, as larvae exposed to various concentrations of PTZ at both 48hph and 72hph demonstrated significant changes in bursting behavior, while larvae exposed to 8mM, 12mM, and 16mM PTZ at 48hph also exhibited increases in anti-thigmotaxis behavior. Of note, these behavioral changes mostly occurred during a single photoperiod that varied by exposure concentration and did not appear to correlate to the type of light conditions present. Regardless of occurring as isolated events, these findings revealed that both Delta smelt and Longfin smelt larvae exposed to various concentrations of PTZ were more likely to exhibit spontaneous behaviors associated with hyperactivity than control larvae. Furthermore, numerous studies have shown that high concentrations of PTZ can induce clonic or tonic seizures in model organisms, including Zebrafish (Huang et al., 2001; Hansen et al., 2004; Baraban et al., 2005; Afrikanova et al., 2013; Peng et al., 2016; Bandara et al., 2020; Shaw et al., 2020). Given the approach's sensitivity to spontaneous hyperactive behaviors that my study has demonstrated, it is feasible that PTZ can also be utilized as a standard to spontaneous movement endpoints, as well as to assess the potential for the occurrence of seizures in fish species of conservation concern when evaluating neurotoxic responses. However, further investigation is necessary to validate seizure occurrence and to determine concentrations that would lead to seizures in both Delta and Longfin smelt.

I hypothesized that PTZ as a hyperactive behavior-inducing compound would further increase the extent of activity achievable by Delta smelt and Longfin smelt when triggered by light stimuli. While Delta smelt larvae were not influenced by light conditions following analysis by photoperiod, both control and exposed larvae demonstrated an observable increase in movement and swimming velocity immediately following the onset of light conditions, with larvae exposed to PTZ achieving observably higher levels of movement and velocity than controls. Longfin smelt larvae exposed to PTZ at both 48hph and 72hph mirrored this response pattern with elevated levels of movement in a concentration-dependent manner. These findings suggest that hyperactive behavior in both Delta smelt and Longfin smelt can increase the level of swimming activity achievable in response to light stimuli.

While it may seem like a benefit for larvae to be more physically active and responsive to changes in their environment, such behavior is not always advantageous. Responding to stressors in the environment is an energy-demanding process (Wendelaar Bonga, 1997; Evans and Kültz, 2019). So, while Delta or Longfin smelt, in a natural state, may be more physically able to avoid predation by increasing activity, e.g., bursting, an induced or spontaneous hyperactive state is likely to impede their capacity to balance energy supply with energy demand, which would have detrimental effects on survival. Studies have highlighted that both Delta smelt and Longfin smelt are particularly vulnerable to predation at early life stages (Schreier et al., 2016; Hobbs et al., 2017). Therefore, understanding energy use and regulation in these species when exposed to contaminants would be valuable information for future conservation efforts.

This study has shown that the maximum hyperactivity achievable by each species is quantifiable following exposure to PTZ. Delta smelt exposed to 8mM PTZ at 48hph achieved the highest values in distance moved, mean velocity, and maximum velocity within this species. Therefore, this concentration is recommended to be used as a standard for observing hyperactive behavior in Delta smelt larvae at the same developmental time point in future studies. For Longfin smelt, however, larvae exposed to 4mM PTZ at 48hph achieved the highest values in the same parameters, implying this concentration is optimal for inducing maximum hyperactivity in this species and would be the most suitable selection for a positive control or comparative standard. Furthermore, given that 8mM is the most suitable standard concentration for Delta smelt and 4mM is optimal for Longfin smelt, it is plausible that Longfin smelt larvae are more sensitive to the neurotoxic effects of PTZ than Delta smelt. Therefore, it is highly recommended that studies on other sensitive fish species investigate a range of concentrations prior to selecting a single standard concentration, as their sensitivity may differ from the species used in this study.

Results of this study not only provide valuable information regarding hyperactivity and related behaviors in Delta smelt and Longfin smelt larvae but also show that PTZ could be a valuable tool in future behavioral toxicology studies for a multitude of reasons. First, larvae exposed to PTZ displayed hyperactive behaviors that are characteristic of both induced and spontaneous movement. This enables PTZ to be used as a comparative standard for other contaminants that affect hyperactivity. Second, the ability of PTZ to be used as a comparative standard is not limited to application on chemicals with the same MOA, as hyperactivity and related behaviors can be induced through multiple neurological pathways. Additionally, exposure of larvae to PTZ allows for the identification and quantification of the maximum hyperactive behavior achievable in the species of interest, which could aid the interpretation of results in terms of relative activity following exposure to other neurotoxic compounds.

5. Conclusion

My study utilized a photomotor response assay to better understand hyperactivity and related behaviors in both Delta smelt and Longfin smelt larvae. Results of this study confirmed that Delta smelt and Longfin smelt larvae display a complex range of hyperactive behaviors following exposure to neurotoxic compounds. These results also provided us with ample information regarding how hyperactivity affects each species' response to light stimuli as well as the maximum hyperactivity achievable by each species under the conditions of this experiment. Specifically, maximum activity was achieved at 8mM PTZ in Delta smelt and 4mM in the more sensitive Longfin smelt yolk-sac larvae. From these findings, it is evident that the use of PTZ as a positive control in future behavioral toxicology studies would allow for a better interpretation of the significance of neurological alterations within ecological risk assessment, enhancing behavioral approaches as valuable tools in the field of ecotoxicology.

Figures

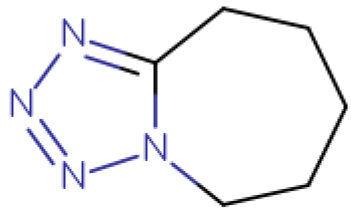

Figure 1. Chemical structure of pentylenetetrazole (PTZ).

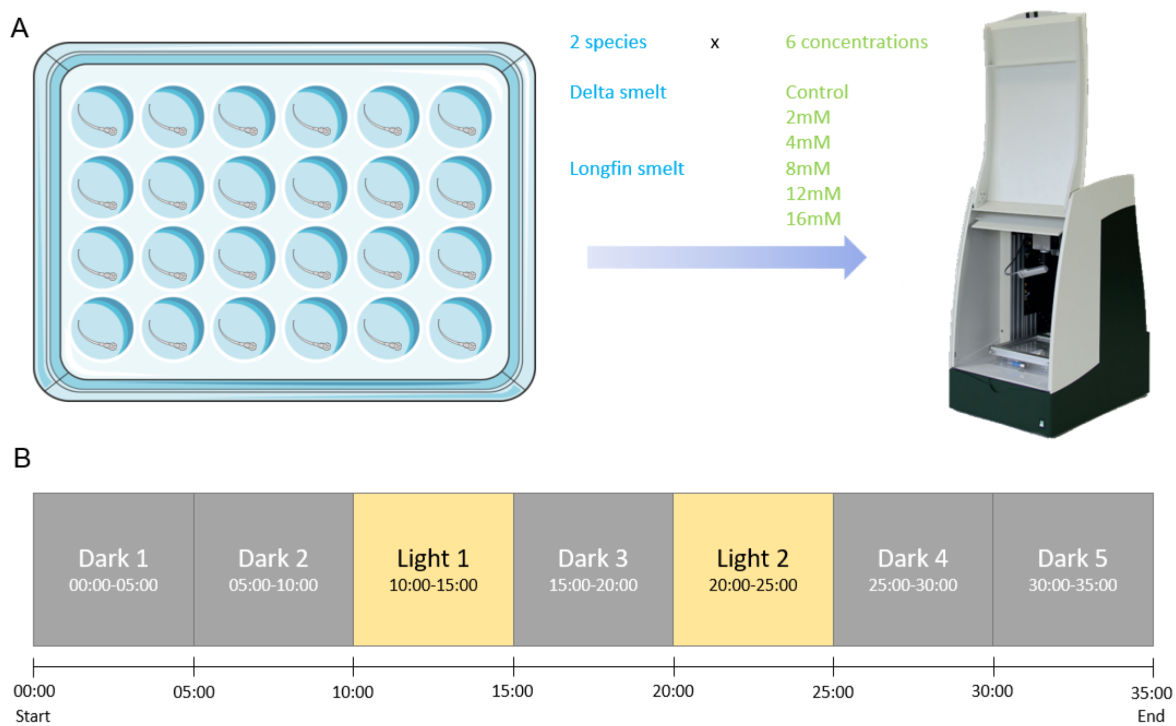

Figure 2. Experimental design for exposure of Delta smelt and Longfin smelt larvae to pentylenetetrazole (PTZ). A) Exposure conditions; including plate setup, species and concentrations used, and Noldus Daniovision chamber used for acquiring tracking data. B) Experimental timeline, including light and dark photoperiods as well as time stamps. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

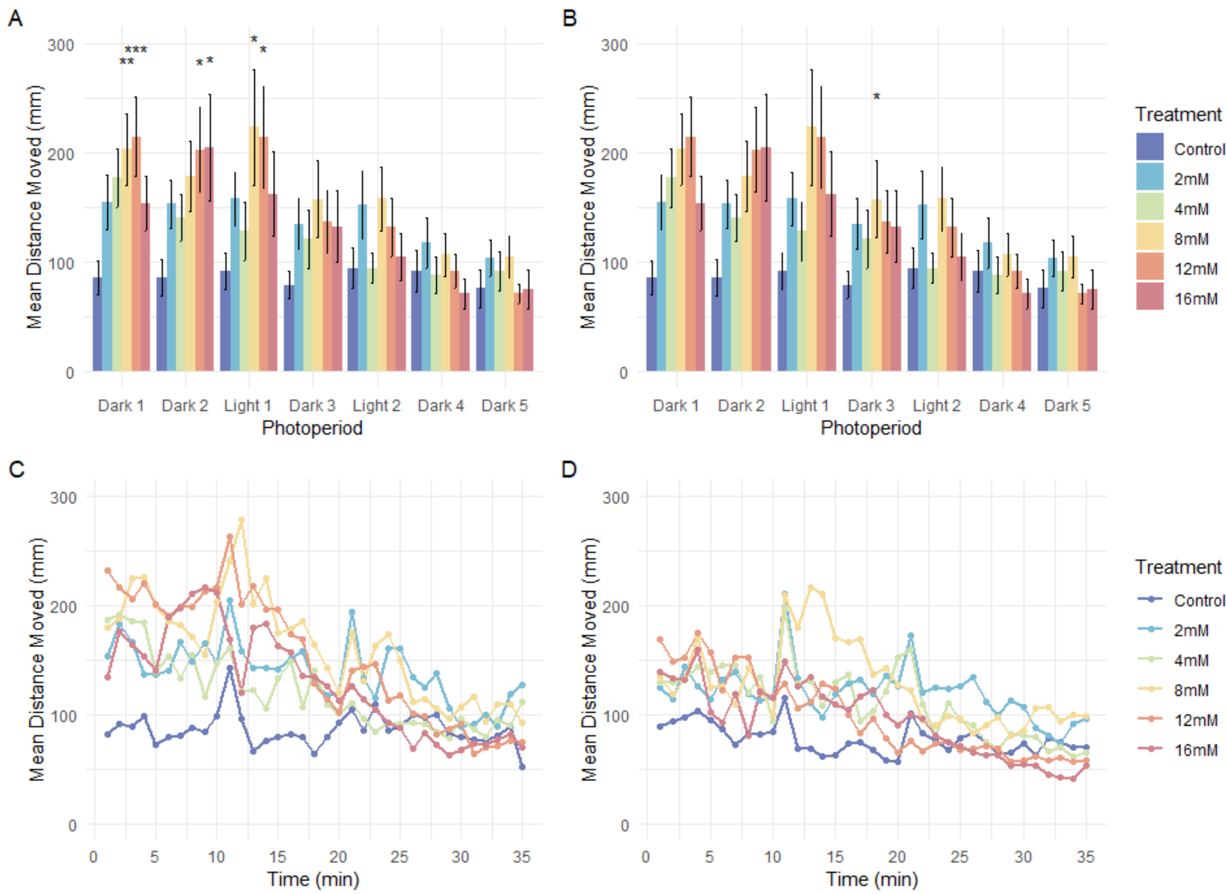

Figure 3. Total distance moved by Delta smelt in the photomotor response assay immediately following exposure to pentylenetetrazole (PTZ). Error bars display standard error to account for variation in sample size between experimental groups. Significance is represented by either * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$), as determined by a Dunnett Test when comparing all experimental doses to the negative control within each cycle. A) Mean total distance moved by 48hph Delta smelt larvae during each light or dark cycle. Each bar represents biological replicates ranging from 16 to 20 larvae. B) Mean total distance moved by 72hph Delta smelt larvae over each cycle. Each bar represents biological replicates ranging from 18 to 20 larvae. C) Mean total distance moved by 48hph Delta smelt larvae at each minute of the experimental period. Each point represents biological replicates ranging from 16 to 20 larvae, depending on experimental dose. D) Mean total distance moved by 72hph Delta smelt larvae at each minute of the experimental period. Each point represents biological replicates ranging from 18 to 20 larvae.

Table 1. Significance of behavioral changes in total distance moved by 48hph and 72hph Delta smelt in the photomotor response assay. Significance is represented by either * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$), as determined by a Dunnett Test when comparing all experimental doses to the negative control within each cycle.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.
2mM	48	0.216	↑	0.399	↑	0.495	↑	0.382	↑	0.214	↑	0.592	↑	0.651	↑
4mM	48	0.056	↑	0.573	↑	0.857	↑	0.627	↑	1.000	≈	0.998	↓	0.949	↑
8mM	**48**	**0.008**	**↑******	0.144	↑	**0.034**	**↑***	0.124	↑	0.160	↑	0.883	↑	0.588	↑
12mM	**48**	**0.002**	**↑*******	**0.031**	**↑***	**0.042**	**↑***	0.326	↑	0.577	↑	1.000	≈	0.979	↓
16mM	**48**	0.227	↑	**0.030**	**↑***	0.424	↑	0.408	↑	0.982	↑	0.758	↓	0.992	↓
2mM	72	0.778	↑	0.671	↑	0.478	↑	0.161	↑	0.086	↑	0.069	↑	0.847	↑
4mM	72	0.578	↑	0.602	↑	0.410	↑	0.249	↑	0.528	↑	0.967	↑	0.999	↑
8mM	**72**	0.537	↑	0.710	↑	**0.017**	**↑***	**0.029**	**↑***	0.799	↑	0.774	↑	0.350	↑
12mM	72	0.142	↑	0.490	↑	0.684	↑	0.915	↑	0.967	↓	0.962	↓	0.901	↓
16mM	72	0.616	↑	0.908	↑	0.591	↑	0.522	↑	0.998	↑	0.868	↓	0.568	↓

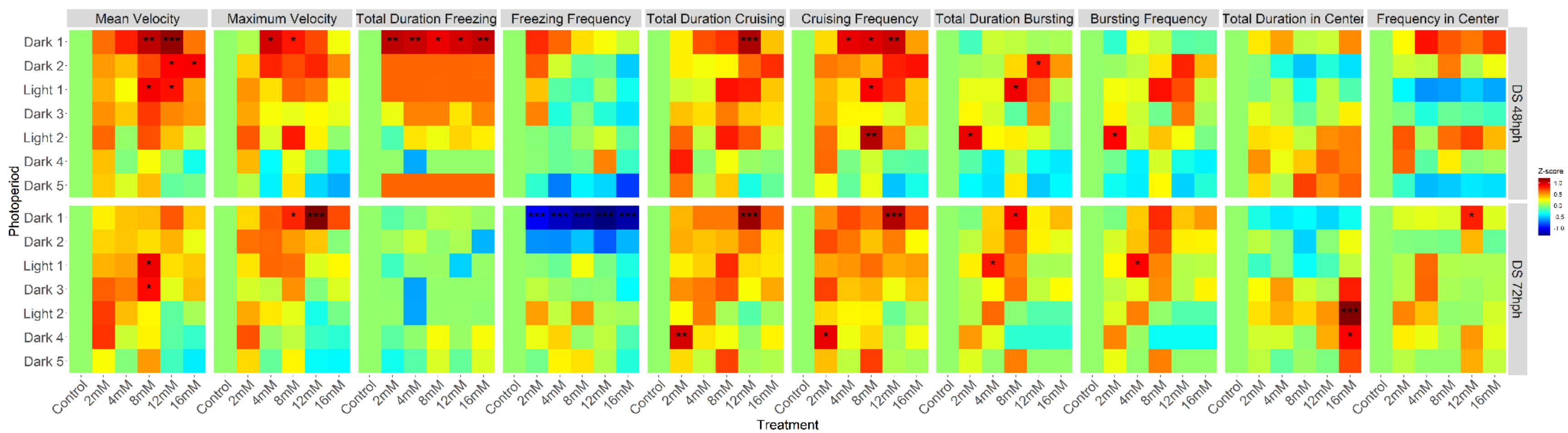

Figure 4. Heatmap representing behavioral changes demonstrated by 48hph and 72hph Delta smelt following exposure to either 2mM, 4mM, 8mM, 12mM or 16mM PTZ. Data is represented as z-scores that have been normalized to those of controls. Bursting velocities are defined as swimming velocities between 20mm/s and 100mm/s, cruising velocities range from 5mm/s to 20mm/s, and freezing velocities are defined as those below 5mm/s. Significance values are represented as either * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$).

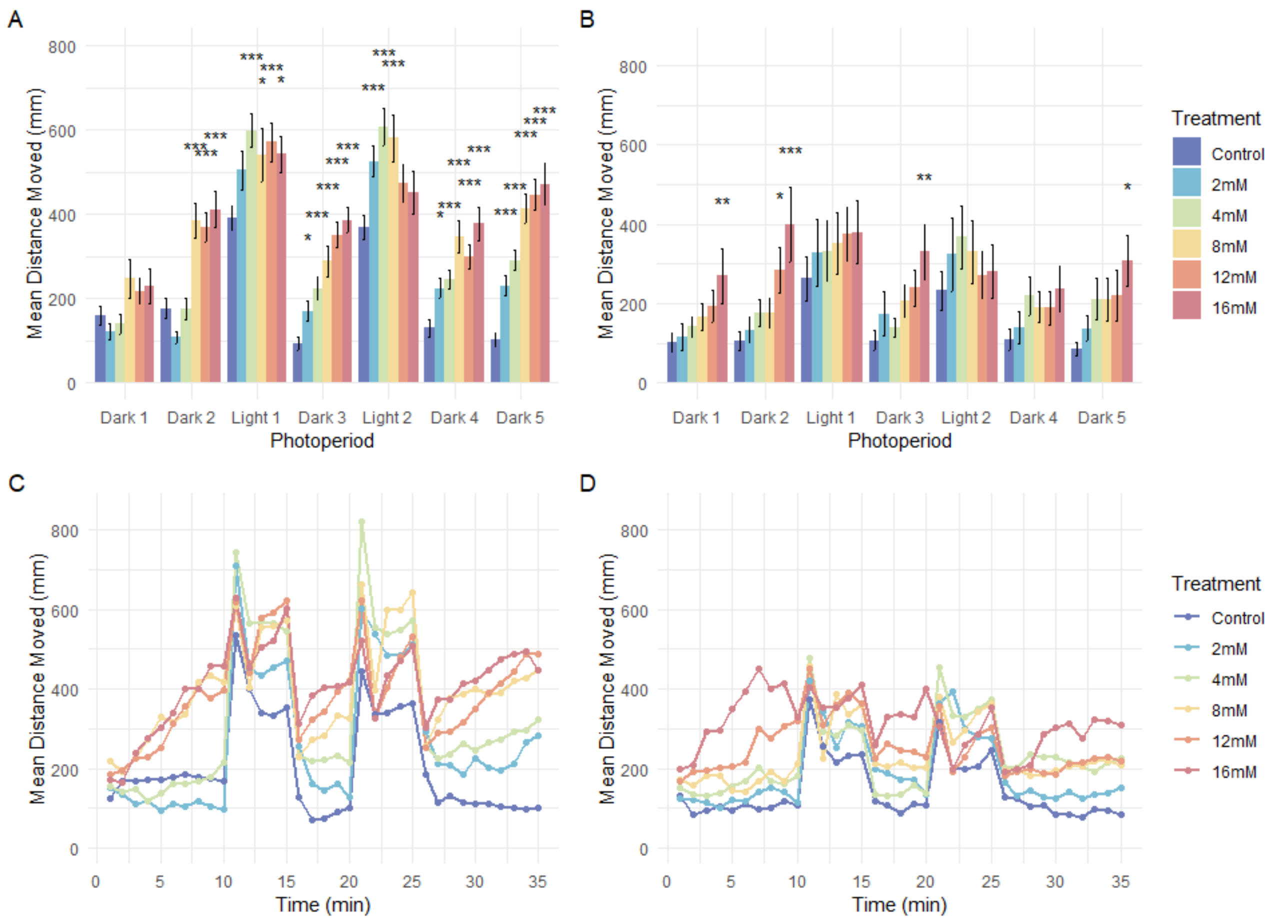

Figure 5. Total distance moved by Longfin smelt in the photomotor response assay immediately following exposure to pentylenetetrazole (PTZ). Error bars display standard error to account for variation in sample size between experimental groups. Significance is represented by either * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$), as determined by a Dunnett Test when comparing all experimental doses to the negative control within each cycle. A) Mean total distance moved by 48hph Longfin smelt larvae during each light or dark cycle. Each bar represents biological replicates ranging from 13 to 20 larvae. B) Mean total distance moved by 72hph Longfin smelt larvae over each cycle. Each bar represents biological replicates ranging from 11 to 15 larvae. C) Mean total distance moved by 48hph Longfin smelt larvae at each minute of the experimental period. Each point represents biological replicates ranging from 13 to 20 larvae, depending on experimental dose. D) Mean total distance moved by 72hph Longfin smelt larvae at each minute of the experimental period. Each point represents biological replicates ranging from 11 to 15 larvae.

Table 2. Significance of behavioral changes in total distance moved by 48hph and 72hph Longfin smelt in the photomotor response assay. Significance is represented by either * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$), as determined by a Dunnett Test when comparing all experimental doses to the negative control within each cycle.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.	P.adj	Sig.
2mM	**48**	0.590	↓	0.120	↓	0.062	↑	**0.044**	↑*	**0.003**	↑***	**0.022**	↑*	**0.004**	↑***
4mM	**48**	0.904	↓	1.000	≈	**<0.001**	↑***	**<0.001**	↑***	**<0.001**	↑***	**0.004**	↑***	**<0.001**	↑***
8mM	**48**	0.074	↑	**<0.001**	↑***	**0.020**	↑*	**<0.001**	↑***	**<0.001**	↑***	**<0.001**	↑***	**<0.001**	↑***
12mM	**48**	0.256	↑	**<0.001**	↑***	**<0.001**	↑***	**<0.001**	↑***	0.079	↑	**<0.001**	↑***	**<0.001**	↑***
16mM	**48**	0.160	↑	**<0.001**	↑***	**0.010**	↑*	**<0.001**	↑***	0.273	↑	**<0.001**	↑***	**<0.001**	↑***
2mM	72	0.987	↑	0.964	↑	0.911	↑	0.691	↑	0.813	↑	0.948	↑	0.878	↑
4mM	72	0.830	↑	0.637	↑	0.896	↑	0.946	↑	0.569	↑	0.231	↑	0.290	↑
8mM	72	0.504	↑	0.628	↑	0.801	↑	0.360	↑	0.761	↑	0.479	↑	0.289	↑
12mM	**72**	0.259	↑	**0.026**	↑*	0.706	↑	0.166	↑	0.980	↑	0.540	↑	0.262	↑
16mM	**72**	**0.009**	↑**	**<0.001**	↑***	0.709	↑	**0.007**	↑**	0.970	↑	0.175	↑	**0.024**	↑*

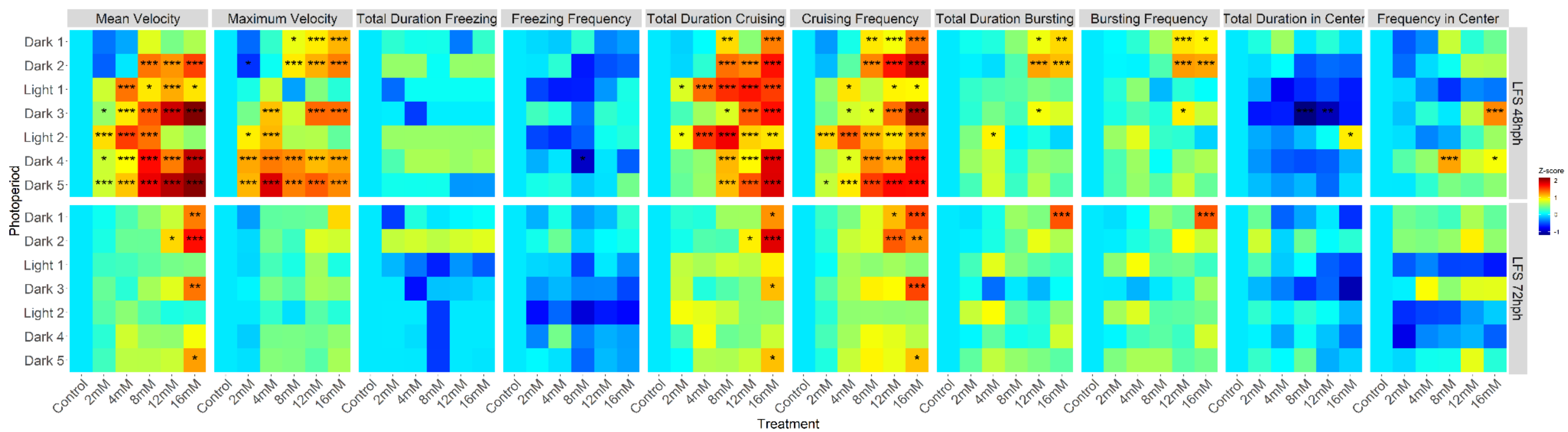

Figure 6. Heatmap representing behavioral changes demonstrated by 48hph and 72hph Longfin smelt following exposure to either 2mM, 4mM, 8mM, 12mM or 16mM PTZ. Data is represented as z-scores that have been normalized to those of controls. Bursting velocities are defined as swimming velocities between 20mm/s and 100mm/s, cruising velocities range from 5mm/s to 20mm/s, and freezing velocities are defined as those below 5mm/s. Significance values are represented as either * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$).

References

Afrikanova, T., A. S. K. Serruys, O. E. M. Buenafe, R. Clinckers, I. Smolders, P. A. M. de Witte, A. D. Crawford, and C. V. Esguerra. 2013. "Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs." *PLoS ONE* 8 (1): e54166. https://doi.org/10.1371/journal.pone.0054166.

Bandara, S. B., D. R. Carty, V. Singh, D. J. Harvey, N. Vasylieva, B. Pressly, H. Wulff, and P. J. Lein. 2020. "Susceptibility of larval zebrafish to the seizurogenic activity of gaba type a receptor antagonists." *NeuroToxicology* 76 (January): 220–34. https://doi.org/10.1016/j.neuro.2019.12.001.

Baraban, S.C., M.R. Taylor, P.A. Castro, and H. Baier. 2005. "Pentylenetetrazole induced changes in zebrafish behavior, neural activity and C-FOS expression." *Neuroscience* 131 (3): 759–68. https://doi.org/10.1016/j.neuroscience.2004.11.031.

Baskerville-Bridges, B., J. C. Lindberg, and S. I. Doroshav. 2004. "The effect of light intensity, alga concentration, and prey density on the feeding behavior of delta smelt larvae." *American Fisheries Society Symposium* 39.

Baskerville-Bridges B, J. C. Lindberg, and S. I. Doroshov. 2005. "Manual for the intensive culture of delta smelt (*Hypomesus transpacificus*)." Report to CALFED Bay-Delta Program. University of California, Davis, Sacramento, CA.

Basnet, R., D. Zizioli, S. Taweedet, D. Finazzi, and M. Memo. 2019. “Zebrafish larvae as a behavioral model in neuropharmacology.” *Biomedicines* 7 (1): 23. https://doi.org/10.3390/biomedicines7010023.

Battaglin, W. A., P. M. Bradley, L. Iwanowicz, C. A. Journey, H. L. Walsh, and V. S. Blazer. 2018. “Pharmaceuticals, hormones, pesticides, and other bioactive contaminants in water, sediment, and tissue from Rocky Mountain National Park, 2012–2013.” *Science of The Total Environment* 643 (December): 651–73. https://doi.org/10.1016/j.scitotenv.2018.06.150.

Bradley, P. M., C. A. Journey, D. T. Button, D. M. Carlisle, B. J. Huffman, S. L. Qi, K. M. Romanok, and P. C. Van Metre. 2020. “Multi-region assessment of pharmaceutical exposures and predicted effects in usa wadeable urban-gradient streams.” *PLOS ONE* 15 (1): e0228214. https://doi.org/10.1371/journal.pone.0228214.

Brander, S. M., R. E. Connon, G. He, J. A. Hobbs, K. L. Smalling, S. J. Teh, J. Wilson White, I. Werner, M. S. Denison, and G. N. Cherr. 2013. “From ‘omics to otoliths: responses of an estuarine fish to endocrine disrupting compounds across biological scales.” *PLoS ONE* 8 (9): e74251. https://doi.org/10.1371/journal.pone.0074251.

California Department of Fish and Game (CDFG). 2009. “California endangered species act incidental take permit no. 2081–2009-001-03" for the Department of Water Resources California State Water Project Delta Facilities and Operations, Yountville, CA, USA.

California Department of Fish and Game (CDFG). 2010. “State and federally listed endangered and threatened animals of California.” *CDFG*, Sacramento, CA, USA.

Calvo, S., S. Romo, J. Soria, and Y. Picó. 2021. “Pesticide contamination in water and sediment of the aquatic systems of the Natural Park of the Albufera of Valencia (Spain) during the rice cultivation period.” *Science of The Total Environment* 774 (June): 145009. https://doi.org/10.1016/j.scitotenv.2021.145009.

Connon, R., S. Hasenbein, S. Brander, H. Poynton, E. Holland, D. Schlenk, J. Orlando, et al. 2019. “Review of and recommendations for monitoring contaminants and their effects in the San Francisco Bay−Delta.” *San Francisco Estuary and Watershed Science* 17 (4): 2. https://doi.org/10.15447/sfews.2019v17iss4art2.

Cloern, J. E. and A. D. Jassby. 2012. “Drivers of change in estuarine-coastal ecosystems: discoveries from four decades of study in San Francisco Bay.” *Reviews of Geophysics* 50 (4): RG4001. https://doi.org/10.1029/2012RG000397.\

Dach, K., B. Yaghoobi, M. R. Schmuck, D. R. Carty, K. M. Morales, and P. J. Lein. 2019. “Teratological and behavioral screening of the National Toxicology Program 91-compound library in Zebrafish (*Danio rerio*).” *Toxicological Sciences* 167 (1): 77–91. https://doi.org/10.1093/toxsci/kfy266.

Dutra Costa, B. P., L. Aquino Moura, S. A. Gomes Pinto, M. Lima-Maximino, and C. Maximino. 2020. “Zebrafish models in neural and behavioral toxicology across the life stages.” *Fishes* 5 (3): 23. https://doi.org/10.3390/fishes5030023.

Evans, T. G., and D. Kültz. 2020. “The cellular stress response in fish exposed to salinity fluctuations.” *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 333 (6): 421–35. https://doi.org/10.1002/jez.2350.

Finger, A. J., B. Mahardja, K. M. Fisch, A. Benjamin, J. Lindberg, L. Ellison, T. Ghebremariam, T.C. Hung, and B. May. 2018. “A conservation hatchery population of delta smelt shows evidence of genetic adaptation to captivity after 9 generations.” *Journal of Heredity* 109 (6): 689–99. https://doi.org/10.1093/jhered/esy035.

Fong, S., S. Louie, I. Werner, and Richard E. Connon. 2016. “Contaminant effects on California Bay–Delta species and human health.” *San Francisco Estuary and Watershed Science* 14 (4). https://doi.org/10.15447/sfews.2016v14iss4art5.

Galhardo, L., J. Vital, and R. F. Oliveira. 2011. “The role of predictability in the stress response of a cichlid fish.” *Physiology & Behavior* 102 (3–4): 367–72. https://doi.org/10.1016/j.physbeh.2010.11.035.

Geist, J., I. Werner, K. J. Eder, and C. M. Leutenegger. 2007. “Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass (*Morone saxatilis*) following treatment with copper and esfenvalerate.” *Aquatic Toxicology* 85 (1): 28–39. https://doi.org/10.1016/j.aquatox.2007.07.011.

Goc, G. L., J. Lafaye, S. Karpenko, V. Bormuth, R. Candelier, and G. Debrégeas. 2021. “Thermal modulation of zebrafish exploratory statistics reveals constraints on individual behavioral variability.” *BMC Biology* 19 (1): 208. https://doi.org/10.1186/s12915-021-01126-w.

Hansen, S. L., B. B. Sperling, and C. Sánchez. 2004. “Anticonvulsant and antiepileptogenic effects of gabaa receptor ligands in pentylenetetrazole-kindled mice.” *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 28 (1): 105–13. https://doi.org/10.1016/j.pnpbp.2003.09.026.

Hernández-Moreno, D., M. Pérez-López, F. Soler, C. Gravato, and L. Guilhermino. 2011. “Effects of carbofuran on the sea bass (*Dicentrarchus labrax*): study of biomarkers and behaviour alterations.” *Ecotoxicology and Environmental Safety* 74 (7): 1905–12. https://doi.org/10.1016/j.ecoenv.2011.07.016.

Huang, R. Q., C. L. Bell-Horner, M. I. Dibas, D. F. Covey, J. A. Drewe, and G. H. Dillon. 2001. “Pentylenetetrazole-induced inhibition of recombinant Gamma-Aminobutyric Acid Type A (GABA(A)) receptors: mechanism and site of action.” *The Journal of Pharmacology and Experimental Therapeutics* 298 (3): 986–95.

Hutton, Sara J., Samreen Siddiqui, Emily I. Pedersen, Christopher Y. Markgraf, Amelie Segarra, Michelle L. Hladik, Richard E. Connon, and Susanne M. Brander. 2023. “Comparative behavioral ecotoxicology of inland silverside larvae exposed to pyrethroids across a salinity gradient.” *Science of The Total Environment* 857 (January): 159398. https://doi.org/10.1016/j.scitotenv.2022.159398.

Jacquin, L., Q. Petitjean, J. Côte, P. Laffaille, and S. Jean. 2020. “Effects of pollution on fish behavior, personality, and cognition: some research perspectives.” *Frontiers in Ecology and Evolution* 8 (April): 86. https://doi.org/10.3389/fevo.2020.00086.

Jaffrézic, A., E. Jardé, A. Soulier, L. Carrera, E. Marengue, A. Cailleau, and B. Le Bot. 2017. “Veterinary pharmaceutical contamination in mixed land use watersheds: from agricultural headwater to water monitoring watershed.” *Science of The Total Environment* 609 (December): 992–1000. https://doi.org/10.1016/j.scitotenv.2017.07.206.

Kane, A. S., J. D. Salierno, and S. K. Brewer. "Fish models in behavioral toxicology: automated techniques, updates and perspectives." *Methods in Aquatic Toxicology* 2 (2005): 559-590.

Klioueva, I.A, E.L.J.M van Luijtelaar, N.E Chepurnova, and S.A Chepurnov. 2001. "PTZ-Induced Seizures in Rats: Effects of Age and Strain." *Physiology & Behavior* 72 (3): 421–26. https://doi.org/10.1016/S0031-9384(00)00425-X.

LaCava, M., K. Fisch, M. Nagel, J. C. Lindberg, B. May, and A. J. Finger. 2015. "Spawning behavior of cultured delta smelt in a conservation hatchery." *North American Journal of Aquaculture* 77 (3): 255–66. https://doi.org/10.1080/15222055.2015.1007192.

Lessard, J., B. Cavallo, P. Anders, T. Sommer, B. Schrier, D. Gille, et al. 2018. "Considerations for the use of captive-reared delta smelt for species recovery and research." *San Francisco Estuary and Watershed Science* 16 (3). https://doi.org/10.15447/sfews.2018v16iss3art3.

Lindberg, J. C., G. Tigan, L. Ellison, T. Rettinghouse, M. M. Nagel, and K. M. Fisch. 2013. "Aquaculture methods for a genetically managed population of endangered delta smelt." *North American Journal of Aquaculture* 75 (2): 186–96. https://doi.org/10.1080/15222055.2012.751942.

Little, E. E., and S. E. Finger. 1990. “Swimming behavior as an indicator of sublethal toxicity in fish.” *Environmental Toxicology and Chemistry* 9 (1): 13–19. https://doi.org/10.1002/etc.5620090103.

MacVean, L. J., S. Thompson, P. Hutton, and M. Sivapalan. 2018. “Reconstructing early hydrologic change in the California Delta and its watersheds.” *Water Resources Research* 54 (10): 7767–90. https://doi.org/10.1029/2017WR021426.

Magnuson, J. T., N. Fuller, K. E. Huff Hartz, S. Anzalone, G. W. Whitledge, S. Acuña, M. J. Lydy, and D. Schlenk. 2022. “Dietary exposure to bifenthrin and fipronil impacts swimming performance in juvenile chinook salmon (*Oncorhynchus tshawytscha*).” *Environmental Science & Technology* 56 (8): 5071–80. https://doi.org/10.1021/acs.est.1c06609.

Mandralis, I., P. Weber, G. Novati, and P. Koumoutsakos. 2021. “Learning swimming escape patterns for larval fish under energy constraints.” *Physical Review Fluids* 6 (9): 093101. https://doi.org/10.1103/PhysRevFluids.6.093101.

Menon, N. G., S. Mohapatra, L. P. Padhye, S. S. V. Tatiparti, and S. Mukherji. 2020. “Review on occurrence and toxicity of pharmaceutical contamination in Southeast Asia.” In *Emerging Issues in the Water Environment during Anthropocene* 63–91. Springer Transactions in Civil and Environmental Engineering. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-32-9771-5_4.

Moss, S. A., and W. N. McFarland. 1970. “The influence of dissolved oxygen and carbon dioxide on fish schooling behavior.” *Marine Biology* 5 (2): 100–107. https://doi.org/10.1007/BF00352592.

Mulvaney, W., M. Rahman, L. S. Lewis, J. Cheng, and T. C. Hung. 2022. “Captive rearing of longfin smelt (*Spirinchus thaleichthys)*: first attempt of weaning cultured juveniles to dry feed.” *Animals* 12 (12): 1478. https://doi.org/10.3390/ani12121478.

Mundy, P. C., M. F. Carte, S. M. Brander, T.C. Hung, N. Fangue, and R. E. Connon. 2020. “Bifenthrin exposure causes hyperactivity in early larval stages of an endangered fish species at concentrations that occur during their hatching season.” *Aquatic Toxicology* 228 (November): 105611. https://doi.org/10.1016/j.aquatox.2020.105611.

Mundy, P. C., K. E. Huff Hartz, C. A. Fulton, M. J. Lydy, S. M. Brander, T. C. Hung, N. Fangue, and R. E. Connon. 2021. “Exposure to permethrin or chlorpyrifos causes differential dose- and time-dependent behavioral effects at early larval stages of an endangered teleost species.” *Endangered Species Research* 44 (February): 89–103. https://doi.org/10.3354/esr01091.

Ogungbemi, A., D. Leuthold, S. Scholz, and E. Küster. 2019. “Hypo- or hyperactivity of zebrafish embryos provoked by neuroactive substances: a review on how experimental parameters impact the predictability of behavior changes.” *Environmental Sciences Europe* 31 (1): 88. https://doi.org/10.1186/s12302-019-0270-5.

Peng, X., J. Lin, Y. Zhu, X. Liu, Y. Zhang, Y. Ji, X. Yang, Y. Zhang, N. Guo, and Q. Li. 2016. “Anxiety-related behavioral responses of pentylenetetrazole-treated zebrafish larvae to light-dark transitions.” *Pharmacology Biochemistry and Behavior* 145 (June): 55–65. https://doi.org/10.1016/j.pbb.2016.03.010.

Romney, A. L. T., Y. R. Yanagitsuru, P. C. Mundy, N. A. Fangue, T. C. Hung, S. M. Brander, and R. E. Connon. 2019. “Developmental staging and salinity tolerance in embryos of the delta smelt, *Hypomesus transpacificus*.” *Aquaculture* 511 (September): 634191. https://doi.org/10.1016/j.aquaculture.2019.06.005.

Salena, M. G., A. J. Turko, A. Singh, A. Pathak, E. Hughes, C. Brown, and S. Balshine. 2021. “Understanding fish cognition: a review and appraisal of current practices.” *Animal Cognition* 24 (3): 395–406. https://doi.org/10.1007/s10071-021-01488-2.

Schnörr, S. J., P. J. Steenbergen, M. K. Richardson, and D. L. Champagne. 2012. “Measuring thigmotaxis in larval zebrafish.” *Behavioural Brain Research* 228 (2): 367–74. https://doi.org/10.1016/j.bbr.2011.12.016.

Schreier, B. M., M. R. Baerwald, J. L. Conrad, G. Schumer, and B. May. 2016. “Examination of predation on early life stage delta smelt in the San Francisco Estuary using DNA diet analysis.” *Transactions of the American Fisheries Society* 145 (4): 723–33. https://doi.org/10.1080/00028487.2016.1152299.

Siddiqui, S., J.M. Dickens, B.E. Cunningham, S.J. Hutton, E.I. Pedersen, B. Harper, S. Harper, and S.M. Brander. 2022. “Internalization, reduced growth, and behavioral effects following exposure to micro and nano tire particles in two estuarine indicator species.” *Chemosphere* 296 (June): 133934. https://doi.org/10.1016/j.chemosphere.2022.133934.

Segarra, A., F. Mauduit, N. Amer, F. Biefel, M. Hladik, R. Connon, and S. Brander. 2021. “Salinity changes the dynamics of pyrethroid toxicity in terms of behavioral effects on newly hatched delta smelt larvae.” *Toxics* 9 (2): 40. https://doi.org/10.3390/toxics9020040.

Shaw, P. A. G., S. K. Panda, A. Stanca, and W. Luyten. 2022. “Optimization of a Locomotion-Based Zebrafish Seizure Model.” *Journal of Neuroscience Methods* 375 (June): 109594. https://doi.org/10.1016/j.jneumeth.2022.109594.

Shen, B., J. Wu, S. Zhan, and M. Jin. 2021. “Residues of organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) in waters of the Ili-Balkhash Basin, arid Central Asia: concentrations and risk assessment.” *Chemosphere* 273 (June): 129705. https://doi.org/10.1016/j.chemosphere.2021.129705.

Steele, B. W., L. A. Kristofco, J. Corrales, G. N. Saari, S. P. Haddad, E. P. Gallagher, T. J. Kavanagh, et al. 2018. "Comparative behavioral toxicology with two common larval fish models: exploring relationships among modes of action and locomotor responses." *Science of The Total Environment* 640–641 (November): 1587–1600. https://doi.org/10.1016/j.scitotenv.2018.05.402.

Stefanakis, A. I., and J. A. Becker. 2020. "A review of emerging contaminants in water: classification, sources, and potential risks." *Waste Management* 177–202. https://doi.org/10.4018/978-1-7998-1210-4.ch008.

Stern, M. A., L. E. Flint, A. L. Flint, N. Knowles, and S. A. Wright. 2020. "The future of sediment transport and streamflow under a changing climate and the implications for long-term resilience of the San Francisco Bay-Delta." *Water Resources Research* 56 (9). https://doi.org/10.1029/2019WR026245.

State Water Resources Control Board (SWRCB). 2010. Transmittal of the 2010 Integrated Report [Clean Water Act Section 303(d) and Section 305(b)]. Letter to Alexis Strauss, USEPA, and four CDs of supporting materials, including the staff report, fact sheets, and responsiveness summary, dated October 11, 2010. [Internet]. [accessed 2020 September 4]. Available from: https://www.epa.gov/tmdl/impaired-waters-and-tmdls-pacific-southwest-region-9

Tempel, T. L., T. D. Malinich, J. Burns, A. Barros, C. E. Burdi, and J. A. Hobbs. 2021. "The value of long-term monitoring of the San Francisco Estuary for delta smelt and longfin smelt." *California Fish and Wildlife Journal*, no. CESA Special Issue (July): 148–71. https://doi.org/10.51492/cfwj.cesasi.7.

U.S. Fish and Wildlife Service (UFWS). 1993. "Endangered and threatened wildlife and plants; determination of threatened status for the delta smelt." *Federal Register* 58 (42): 12854 – 12864.

U.S. Geological Survey (USGS), M. Dettinger, J. Anderson, M. Anderson, L. Brown, et al. 2016. "Climate change and the delta." *San Francisco Estuary and Watershed Science* 14 (3). https://doi.org/10.15447/sfews.2016v14iss3art5.

Wendelaar Bonga, S. E. 1997. "The stress response in fish." *Physiological Reviews* 77 (3): 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.

Wilkinson, J., P. S. Hooda, J. Barker, S. Barton, and J. Swinden. 2017. "Occurrence, fate and transformation of emerging contaminants in water: an overarching review of the field." *Environmental Pollution* 231 (December): 954–70. https://doi.org/10.1016/j.envpol.2017.08.032.

Wiles, S. C., M. G. Bertram, J. M. Martin, H. Tan, T. K. Lehtonen, and B. B. M. Wong. 2020. "Long-term pharmaceutical contamination and temperature stress disrupt fish behavior." *Environmental Science & Technology* 54 (13): 8072–82. https://doi.org/10.1021/acs.est.0c01625.

Yanagitsuru, Y. R., M. A. Main, L. S. Lewis, J. A. Hobbs, T. C. Hung, R. E. Connon, and N. A. Fangue. 2021. "Effects of temperature on hatching and growth performance of embryos and yolk-sac larvae of a threatened estuarine fish: longfin smelt (*Spirinchus thaleichthys*)." *Aquaculture* 537 (May): 736502. https://doi.org/10.1016/j.aquaculture.2021.736502.

Yanagitsuru, Y. R., I. Y. Daza, L. S. Lewis, J. A. Hobbs, T. C. Hung, R. E. Connon, and N. A. Fangue. 2022. "Growth, osmoregulation and ionoregulation of longfin smelt (*Spirinchus thaleichthys*) yolk-sac larvae at different salinities." *Conservation Physiology* 10 (1): coac041. https://doi.org/10.1093/conphys/coac041.

Appendix 1. Supplementary Tables

Supplementary Table 1. Total distance moved data for 48hph and 72hph Delta smelt.

Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig	Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig
Control	48	Dark 1	86.4	27.8	274	n/a	n/a	Control	72	Dark 1	95.7	24.2	252	n/a	n/a
2mM	48	Dark 1	155	8.9	600	0.216	ns	2mM	72	Dark 1	125	25.9	489	0.778	ns
4mM	48	Dark 1	177	25	493	0.056	ns	4mM	72	Dark 1	135	24.9	431	0.578	ns
8mM	**48**	**Dark 1**	**203**	**28.7**	**584**	**0.008**	**	8mM	72	Dark 1	136	26.5	472	0.537	ns
12mM	**48**	**Dark 1**	**215**	**0.524**	**639**	**0.002**	***	12mM	72	Dark 1	160	18.8	534	0.142	ns
16mM	48	Dark 1	153	31	478	0.227	ns	16mM	72	Dark 1	133	27	514	0.616	ns
Control	48	Dark 2	86.1	27.1	285	n/a	n/a	Control	72	Dark 2	81.3	24.1	293	n/a	n/a
2mM	48	Dark 2	153	15.6	448	0.399	ns	2mM	72	Dark 2	123	25.7	581	0.671	ns
4mM	48	Dark 2	141	31.3	434	0.573	ns	4mM	72	Dark 2	127	26.2	485	0.602	ns
8mM	48	Dark 2	179	28.5	665	0.144	ns	8mM	72	Dark 2	120	27.5	868	0.710	ns
12mM	**48**	**Dark 2**	**203**	**28.9**	**998**	**0.031**	*	12mM	72	Dark 2	133	28	822	0.490	ns
16mM	**48**	**Dark 2**	**205**	**29.2**	**1020**	**0.030**	*	16mM	72	Dark 2	106	26.6	912	0.908	ns
Control	48	Light 1	92	32.5	365	n/a	n/a	Control	72	Light 1	75.3	24.8	238	n/a	n/a
2mM	48	Light 1	158	33.2	421	0.495	ns	2mM	72	Light 1	134	19.8	576	0.478	ns
4mM	48	Light 1	129	36.1	577	0.857	ns	4mM	72	Light 1	139	24.1	754	0.410	ns
8mM	**48**	**Light 1**	**224**	**38.4**	**896**	**0.034**	*	**8mM**	**72**	**Light 1**	**197**	**29.4**	**1010**	**0.017**	*
12mM	**48**	**Light 1**	**215**	**29.9**	**848**	**0.042**	*	12mM	72	Light 1	119	23.1	676	0.684	ns
16mM	48	Light 1	163	29.5	813	0.424	ns	16mM	72	Light 1	127	21.4	581	0.591	ns
Control	48	Dark 3	79.5	23.4	262	n/a	n/a	Control	72	Dark 3	66	27.8	225	n/a	n/a
2mM	48	Dark 3	135	26.2	452	0.382	ns	2mM	72	Dark 3	128	25.7	453	0.161	ns
4mM	48	Dark 3	121	30.4	574	0.627	ns	4mM	72	Dark 3	121	0.282	530	0.249	ns
8mM	48	Dark 3	158	32	653	0.124	ns	**8mM**	**72**	**Dark 3**	**148**	**28.4**	**536**	**0.029**	*
12mM	48	Dark 3	137	28.2	594	0.326	ns	12mM	72	Dark 3	84.3	27.3	432	0.915	ns
16mM	48	Dark 3	133	30.9	625	0.408	ns	16mM	72	Dark 3	106	21.6	591	0.522	ns
Control	48	Light 2	94.7	28.8	299	n/a	n/a	Control	72	Light 2	81.1	27.1	486	n/a	n/a
2mM	48	Light 2	153	32.9	717	0.214	ns	2mM	72	Light 2	133	31.1	399	0.086	ns
4mM	48	Light 2	95	29	325	1.000	ns	4mM	72	Light 2	111	0.136	589	0.528	ns
8mM	48	Light 2	158	28.5	716	0.160	ns	8mM	72	Light 2	101	32.5	373	0.799	ns
12mM	48	Light 2	132	32.6	578	0.577	ns	12mM	72	Light 2	71.7	27.6	307	0.967	ns
16mM	48	Light 2	105	1.66	363	0.982	ns	16mM	72	Light 2	84.6	24	403	0.998	ns
Control	48	Dark 4	91.6	0.925	348	n/a	n/a	Control	72	Dark 4	72.2	29.3	291	n/a	n/a
2mM	48	Dark 4	118	35	571	0.592	ns	2mM	72	Dark 4	113	0.0527	447	0.069	ns
4mM	48	Dark 4	88.3	12.2	489	0.998	ns	4mM	72	Dark 4	79.5	27.8	340	0.967	ns
8mM	48	Dark 4	107	31.1	384	0.883	ns	8mM	72	Dark 4	87.6	11.8	269	0.774	ns
12mM	48	Dark 4	91.8	31.6	364	1.000	ns	12mM	72	Dark 4	64.8	22	262	0.962	ns
16mM	48	Dark 4	71.2	1.36	438	0.758	ns	16mM	72	Dark 4	59.8	29.7	195	0.868	ns

Control	48	Dark 5	76.1	0.12	353	n/a	n/a	Control	72	Dark 5	70.9	27.5	259	n/a	n/a
2mM	48	Dark 5	104	34.7	341	0.651	ns	2mM	72	Dark 5	85.8	0.0527	307	0.847	ns
4mM	48	Dark 5	91.8	30	420	0.949	ns	4mM	72	Dark 5	68.6	21.4	222	0.999	ns
8mM	48	Dark 5	105	31.8	353	0.588	ns	8mM	72	Dark 5	101	26.7	524	0.350	ns
12mM	48	Dark 5	71.3	28.9	227	0.979	ns	12mM	72	Dark 5	58.8	20.8	176	0.901	ns
16mM	48	Dark 5	75.1	29.8	448	0.992	ns	16mM	72	Dark 5	47.1	25.2	208	0.568	ns

Supplementary Table 2. Mean Velocity data for 48hph and 72hph Delta smelt.

Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.	Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.
Control	48	Dark 1	1.44	0.463	4.56	n/a	n/a	Control	72	Dark 1	1.60	0.403	4.19	n/a	n/a
2mM	48	Dark 1	2.59	0.148	10.00	0.216	ns	2mM	72	Dark 1	2.08	0.432	8.15	0.778	ns
4mM	48	Dark 1	2.95	0.416	8.22	0.056	ns	4mM	72	Dark 1	2.25	0.415	7.18	0.578	ns
8mM	**48**	**Dark 1**	**3.39**	**0.479**	**9.73**	**0.008**	******	8mM	72	Dark 1	2.27	0.441	7.87	0.537	ns
12mM	**48**	**Dark 1**	**3.58**	**0.009**	**10.70**	**0.002**	*******	12mM	72	Dark 1	2.67	0.314	8.90	0.142	ns
16mM	48	Dark 1	2.56	0.517	7.96	0.227	ns	16mM	72	Dark 1	2.21	0.450	8.56	0.616	ns
Control	48	Dark 2	1.44	0.451	4.75	n/a	n/a	Control	72	Dark 2	1.35	0.402	4.89	n/a	n/a
2mM	48	Dark 2	2.55	0.260	7.47	0.399	ns	2mM	72	Dark 2	2.06	0.428	9.68	0.671	ns
4mM	48	Dark 2	2.35	0.522	7.24	0.573	ns	4mM	72	Dark 2	2.12	0.436	8.07	0.602	ns
8mM	48	Dark 2	2.99	0.475	11.10	0.144	ns	8mM	72	Dark 2	2.01	0.458	14.50	0.710	ns
12mM	**48**	**Dark 2**	**3.38**	**0.482**	**16.60**	**0.031**	*****	12mM	72	Dark 2	2.21	0.467	13.70	0.490	ns
16mM	**48**	**Dark 2**	**3.42**	**0.487**	**17.10**	**0.030**	*****	16mM	72	Dark 2	1.76	0.443	15.20	0.908	ns
Control	48	Light 1	1.53	0.542	6.09	n/a	n/a	Control	72	Light 1	1.25	0.413	3.96	n/a	n/a
2mM	48	Light 1	2.63	0.553	7.01	0.496	ns	2mM	72	Light 1	2.24	0.330	9.61	0.477	ns
4mM	48	Light 1	2.14	0.602	9.62	0.858	ns	4mM	72	Light 1	2.31	0.402	12.60	0.410	ns
8mM	**48**	**Light 1**	**3.72**	**0.640**	**14.90**	**0.034**	*****	**8mM**	**72**	**Light 1**	**3.28**	**0.490**	**16.80**	**0.017**	*****
12mM	**48**	**Light 1**	**3.58**	**0.499**	**14.10**	**0.042**	*****	12mM	72	Light 1	1.99	0.385	11.30	0.684	ns
16mM	48	Light 1	2.71	0.491	13.60	0.424	ns	16mM	72	Light 1	2.11	0.357	9.68	0.591	ns
Control	48	Dark 3	1.32	0.390	4.37	n/a	n/a	Control	72	Dark 3	1.10	0.463	3.74	n/a	n/a
2mM	48	Dark 3	2.25	0.436	7.53	0.382	ns	2mM	72	Dark 3	2.13	0.429	7.55	0.161	ns
4mM	48	Dark 3	2.02	0.507	9.57	0.627	ns	4mM	72	Dark 3	2.02	0.005	8.83	0.249	ns
8mM	48	Dark 3	2.63	0.534	10.90	0.124	ns	**8mM**	**72**	**Dark 3**	**2.47**	**0.473**	**8.94**	**0.029**	*****
12mM	48	Dark 3	2.29	0.47	9.9	0.326	ns	12mM	72	Dark 3	1.41	0.455	7.20	0.915	ns
16mM	48	Dark 3	2.22	0.514	10.40	0.408	ns	16mM	72	Dark 3	1.77	0.360	9.85	0.522	ns
Control	48	Light 2	1.58	0.480	4.98	n/a	n/a	Control	72	Light 2	1.35	0.452	8.10	n/a	n/a
2mM	48	Light 2	2.55	0.549	11.90	0.214	ns	2mM	72	Light 2	2.22	0.518	6.65	0.086	ns
4mM	48	Light 2	1.58	0.484	5.42	1.000	ns	4mM	72	Light 2	1.85	0.002	9.81	0.528	ns
8mM	48	Light 2	2.63	0.475	11.90	0.160	ns	8mM	72	Light 2	1.68	0.541	6.22	0.799	ns

12mM	48	Light 2	2.20	0.544	9.63	0.578	ns	12mM	72	Light 2	1.19	0.461	5.12	0.967	ns
16mM	48	Light 2	1.75	0.028	6.05	0.982	ns	16mM	72	Light 2	1.41	0.400	6.71	0.998	ns
Control	48	Dark 4	1.53	0.015	5.80	n/a	n/a	Control	72	Dark 4	1.20	0.487	4.85	n/a	n/a
2mM	48	Dark 4	1.97	0.584	9.51	0.593	ns	2mM	72	Dark 4	1.88	<0.001	7.46	0.069	ns
4mM	48	Dark 4	1.48	0.203	8.15	0.999	ns	4mM	72	Dark 4	1.32	0.463	5.67	0.967	ns
8mM	48	Dark 4	1.78	0.518	6.41	0.883	ns	8mM	72	Dark 4	1.46	0.196	4.49	0.774	ns
12mM	48	Dark 4	1.53	0.527	6.07	1.000	ns	12mM	72	Dark 4	1.08	0.368	4.37	0.962	ns
16mM	48	Dark 4	1.19	0.023	7.30	0.758	ns	16mM	72	Dark 4	0.10	0.495	3.25	0.868	ns
Control	48	Dark 5	1.27	0.006	5.89	n/a	n/a	Control	72	Dark 5	1.18	0.458	4.32	n/a	n/a
2mM	48	Dark 5	1.73	0.579	5.68	0.651	ns	2mM	72	Dark 5	1.43	<0.001	5.13	0.847	ns
4mM	48	Dark 5	1.53	0.501	7.00	0.949	ns	4mM	72	Dark 5	1.14	0.357	3.70	0.999	ns
8mM	48	Dark 5	1.75	0.530	5.88	0.588	ns	8mM	72	Dark 5	1.68	0.444	8.74	0.350	ns
12mM	48	Dark 5	1.19	0.482	3.79	0.979	ns	12mM	72	Dark 5	0.98	0.347	2.94	0.901	ns
16mM	48	Dark 5	1.25	0.497	7.46	0.992	ns	16mM	72	Dark 5	0.79	0.420	3.47	0.568	ns

Supplementary Table 3. Maximum Velocity data for 48hph and 72hph Delta smelt.

Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.	Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.
Control	48	Dark 1	37.1	1.6	122	n/a	n/a	Control	72	Dark 1	23.4	1.43	101	n/a	n/a
2mM	48	Dark 1	38.9	1.88	112	0.995	ns	2mM	72	Dark 1	33	1.41	93.9	0.634	ns
4mM	**48**	**Dark 1**	**63.4**	**1.84**	**138**	**0.012**	*****	4mM	72	Dark 1	41.2	1.43	141	0.133	ns
8mM	**48**	**Dark 1**	**59.2**	**1.69**	**118**	**0.050**	*****	**8mM**	**72**	**Dark 1**	**44.9**	**1.47**	**118**	**0.042**	*****
12mM	48	Dark 1	55.9	0.00873	125	0.102	ns	**12mM**	**72**	**Dark 1**	**55.7**	**1.59**	**120**	**<0.001**	*******
16mM	48	Dark 1	43.3	1.9	118	0.871	ns	16mM	72	Dark 1	42.3	1.41	122	0.097	ns
Control	48	Dark 2	34.4	1.61	109	n/a	n/a	Control	72	Dark 2	21.9	1.24	97.9	n/a	n/a
2mM	48	Dark 2	45.4	1.86	127	0.658	ns	2mM	72	Dark 2	38.5	1.5	123	0.218	ns
4mM	48	Dark 2	57.8	1.74	133	0.076	ns	4mM	72	Dark 2	39.2	1.53	147	0.187	ns
8mM	48	Dark 2	55	1.75	128	0.153	ns	8mM	72	Dark 2	35.6	1.48	122	0.368	ns
12mM	48	Dark 2	57.7	1.74	147	0.066	ns	12mM	72	Dark 2	32.9	1.56	119	0.549	ns
16mM	48	Dark 2	50.5	1.83	146	0.328	ns	16mM	72	Dark 2	21	1.52	161	0.999	ns
Control	48	Light 1	36.4	1.84	109	n/a	n/a	Control	72	Light 1	23.9	1.38	107	n/a	n/a
2mM	48	Light 1	52	2.28	179	0.399	ns	2mM	72	Light 1	34.8	1.47	136	0.740	ns
4mM	48	Light 1	46.4	1.97	120	0.744	ns	4mM	72	Light 1	45.4	1.4	236	0.191	ns
8mM	48	Light 1	55.9	2.08	205	0.220	ns	8mM	72	Light 1	44.8	1.72	136	0.205	ns
12mM	48	Light 1	54	1.68	135	0.272	ns	12mM	72	Light 1	30.2	1.44	129	0.927	ns
16mM	48	Light 1	43.4	1.66	139	0.886	ns	16mM	72	Light 1	33.2	1.16	129	0.819	ns
Control	48	Dark 3	30.8	1.55	120	n/a	n/a	Control	72	Dark 3	20.6	1.56	125	n/a	n/a
2mM	48	Dark 3	42.7	1.76	114	0.487	ns	2mM	72	Dark 3	24.9	1.62	129	0.951	ns

4mM	48	Dark 3	36.8	1.73	150	0.882	ns	4mM	72	Dark 3	23.4	0.00549	113	0.984	ns
8mM	48	Dark 3	35.9	1.94	129	0.924	ns	8mM	72	Dark 3	34.5	1.68	136	0.339	ns
12mM	48	Dark 3	37.4	1.88	147	0.847	ns	12mM	72	Dark 3	19.5	1.64	117	0.999	ns
16mM	48	Dark 3	35.8	1.76	117	0.922	ns	16mM	72	Dark 3	25.3	1.44	126	0.940	ns
Control	48	Light 2	29.4	1.88	115	n/a	n/a	Control	72	Light 2	22.4	1.58	119	n/a	n/a
2mM	48	Light 2	47.1	1.82	138	0.165	ns	2mM	72	Light 2	33.3	1.83	112	0.440	ns
4mM	48	Light 2	33.8	1.95	111	0.949	ns	4mM	72	Light 2	29.6	0.00227	131	0.752	ns
8mM	48	Light 2	51.2	1.54	124	0.061	ns	8mM	72	Light 2	27	1.86	114	0.912	ns
12mM	48	Light 2	37.1	2.06	107	0.789	ns	12mM	72	Light 2	15.2	1.65	79.5	0.744	ns
16mM	48	Light 2	29.1	0.191	96.5	1.000	ns	16mM	72	Light 2	20.7	1.63	90.4	0.994	ns
Control	48	Dark 4	29.7	1.66	134	n/a	n/a	Control	72	Dark 4	17.3	1.76	121	n/a	n/a
2mM	48	Dark 4	39.6	2.02	142	0.544	ns	2mM	72	Dark 4	30.2	0.000878	125	0.123	ns
4mM	48	Dark 4	21.5	1.89	95.6	0.685	ns	4mM	72	Dark 4	17.4	1.66	116	1.000	ns
8mM	48	Dark 4	34.7	1.73	128	0.903	ns	8mM	72	Dark 4	19.3	1.63	84.7	0.981	ns
12mM	48	Dark 4	27.8	1.89	126	0.992	ns	12mM	72	Dark 4	13.2	1.33	79.3	0.884	ns
16mM	48	Dark 4	20.3	0.549	99.6	0.574	ns	16mM	72	Dark 4	12.4	1.48	60.8	0.835	ns
Control	48	Dark 5	31.7	0.00634	111	n/a	n/a	Control	72	Dark 5	16.9	1.38	99.7	n/a	n/a
2mM	48	Dark 5	34.5	1.95	108	0.992	ns	2mM	72	Dark 5	23.7	0.000878	124	0.666	ns
4mM	48	Dark 5	23.2	1.58	114	0.622	ns	4mM	72	Dark 5	14.7	1.38	71.9	0.980	ns
8mM	48	Dark 5	39.1	1.9	128	0.742	ns	8mM	72	Dark 5	22.3	1.42	131	0.792	ns
12mM	48	Dark 5	21.8	1.74	73.9	0.480	ns	12mM	72	Dark 5	10.1	1.32	80.4	0.654	ns
16mM	48	Dark 5	19.4	1.53	121	0.300	ns	16mM	72	Dark 5	9.73	1.54	66.7	0.634	ns

Supplementary Table 4. Total duration freezing data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	**48**	**0.007**	**	0.220	ns	0.220	ns	0.910	ns	0.951	ns	1.000	ns	0.220	ns
4mM	**48**	**0.010**	**	0.220	ns	0.220	ns	0.326	ns	0.762	ns	0.322	ns	0.220	ns
8mM	**48**	**0.024**	*	0.231	ns	0.236	ns	0.342	ns	0.909	ns	1.000	ns	0.231	ns
12mM	**48**	**0.010**	*	0.202	ns	0.202	ns	0.747	ns	0.674	ns	1.000	ns	0.202	ns
16mM	**48**	**0.007**	**	0.216	ns	0.211	ns	0.317	ns	0.800	ns	1.000	ns	0.210	ns
2mM	72	0.965	ns	0.999	ns	0.91	ns	1.000	ns	1.000	ns	0.971	ns	0.748	ns
4mM	72	0.999	ns	0.970	ns	1.000	ns	0.262	ns	0.265	ns	1.000	ns	0.962	ns
8mM	72	0.987	ns	1.000	ns	1.000	ns	1.000	ns	1.000	ns	0.865	ns	0.942	ns
12mM	72	0.991	ns	0.996	ns	0.435	ns	1.000	ns	1.000	ns	1.000	ns	0.980	ns
16mM	72	1.000	ns	0.324	ns	1.000	ns	1.000	ns	1.000	ns	0.866	ns	0.944	ns

Supplementary Table 5. Freezing frequency data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.083	ns	0.164	ns	0.611	ns	0.296	ns	1.000	ns	1.000	ns	0.866	ns
4mM	48	0.218	ns	0.958	ns	0.971	ns	0.812	ns	0.986	ns	0.986	ns	0.166	ns
8mM	48	0.734	ns	0.978	ns	0.894	ns	0.999	ns	1.000	ns	0.996	ns	0.651	ns
12mM	48	0.869	ns	0.986	ns	0.999	ns	0.818	ns	0.962	ns	0.297	ns	0.475	ns
16mM	48	0.976	ns	0.414	ns	0.755	ns	0.525	ns	0.982	ns	0.893	ns	0.051	ns
2mM	**72**	**0.004**	*******	0.196	ns	1.000	ns	0.986	ns	0.403	ns	0.900	ns	0.994	ns
4mM	**72**	**0.001**	*******	0.185	ns	0.986	ns	1.000	ns	0.981	ns	0.655	ns	0.803	ns
8mM	**72**	**<0.001**	*******	0.365	ns	0.876	ns	0.999	ns	0.342	ns	1.000	ns	0.713	ns
12mM	**72**	**<0.001**	*******	0.068	ns	0.997	ns	1.000	ns	0.997	ns	0.984	ns	0.994	ns
16mM	**72**	**<0.001**	*******	0.327	ns	0.797	ns	0.663	ns	0.999	ns	0.944	ns	0.821	ns

Supplementary Table 6. Total duration cruising data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.792	ns	0.804	ns	0.970	ns	0.615	ns	0.199	ns	0.063	ns	0.260	ns
4mM	48	0.140	ns	0.881	ns	0.940	ns	0.742	ns	0.982	ns	1.000	ns	0.998	ns
8mM	48	0.091	ns	0.837	ns	0.051	ns	0.325	ns	0.062	ns	0.880	ns	0.636	ns
12mM	**48**	**0.004**	*******	0.202	ns	0.053	ns	0.618	ns	0.144	ns	1.000	ns	0.774	ns
16mM	48	0.563	ns	0.087	ns	0.592	ns	0.734	ns	0.980	ns	1.000	ns	0.982	ns
2mM	**72**	0.480	ns	0.480	ns	0.789	ns	0.329	ns	0.845	ns	**0.010**	******	0.688	ns
4mM	72	0.221	ns	0.581	ns	0.637	ns	0.252	ns	0.265	ns	0.720	ns	0.979	ns
8mM	72	0.208	ns	0.502	ns	0.064	ns	0.136	ns	0.998	ns	0.860	ns	0.094	ns
12mM	**72**	**0.002**	*******	0.208	ns	0.667	ns	0.845	ns	0.953	ns	1.000	ns	0.998	ns
16mM	72	0.166	ns	0.718	ns	0.744	ns	0.445	ns	0.999	ns	0.786	ns	0.994	ns

Supplementary Table 7. Cruising frequency data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.690	ns	0.280	ns	0.531	ns	0.521	ns	0.216	ns	0.228	ns	0.899	ns
4mM	**48**	**0.019**	*****	0.339	ns	0.738	ns	0.835	ns	0.892	ns	0.996	ns	0.982	ns
8mM	**48**	**0.017**	*****	0.543	ns	**0.043**	*****	0.841	ns	**0.007**	******	0.965	ns	0.916	ns
12mM	**48**	**0.008**	******	0.062	ns	0.078	ns	0.949	ns	0.281	ns	0.974	ns	0.401	ns
16mM	48	0.395	ns	0.051	ns	0.578	ns	0.594	ns	0.989	ns	0.966	ns	0.778	ns
2mM	**72**	0.304	ns	0.125	ns	0.443	ns	0.107	ns	0.681	ns	**0.016**	*****	0.546	ns
4mM	72	0.101	ns	0.238	ns	0.365	ns	0.591	ns	0.820	ns	0.886	ns	0.985	ns
8mM	72	0.188	ns	0.556	ns	0.205	ns	0.479	ns	0.803	ns	0.637	ns	0.086	ns
12mM	**72**	**0.003**	*******	0.243	ns	0.435	ns	0.873	ns	0.986	ns	0.999	ns	0.995	ns
16mM	72	0.121	ns	0.833	ns	0.401	ns	0.584	ns	0.996	ns	0.883	ns	0.999	ns

Supplementary Table 8. Total duration bursting data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	**48**	0.933	ns	0.999	ns	0.910	ns	0.830	ns	**0.023**	*****	0.966	ns	0.680	ns
4mM	48	0.974	ns	0.994	ns	0.915	ns	0.955	ns	0.839	ns	0.610	ns	0.560	ns
8mM	**48**	0.988	ns	0.699	ns	**0.030**	*****	0.975	ns	0.796	ns	0.989	ns	0.773	ns
12mM	**48**	0.999	ns	**0.050**	*****	0.190	ns	0.337	ns	0.939	ns	0.723	ns	0.419	ns
16mM	48	0.998	ns	0.380	ns	0.991	ns	1.000	ns	0.999	ns	0.600	ns	0.659	ns
2mM	72	0.997	ns	0.985	ns	0.769	ns	0.993	ns	0.994	ns	0.372	ns	0.934	ns
4mM	**72**	0.623	ns	0.618	ns	**0.040**	*****	0.845	ns	0.188	ns	0.921	ns	1.000	ns
8mM	**72**	**0.022**	*****	0.187	ns	0.261	ns	0.224	ns	1.000	ns	0.839	ns	0.263	ns
12mM	72	0.754	ns	0.785	ns	0.997	ns	0.980	ns	0.951	ns	0.834	ns	1.000	ns
16mM	72	0.510	ns	0.920	ns	0.997	ns	0.845	ns	0.954	ns	0.844	ns	1.000	ns

Supplementary Table 9. Bursting frequency data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	**48**	0.879	ns	1.000	ns	0.881	ns	0.816	ns	**0.037**	*	0.992	ns	0.744	ns
4mM	48	0.915	ns	0.989	ns	0.920	ns	0.973	ns	0.955	ns	0.650	ns	0.616	ns
8mM	48	0.984	ns	0.759	ns	0.053	ns	0.991	ns	0.597	ns	0.961	ns	0.849	ns
12mM	48	0.965	ns	0.063	ns	0.125	ns	0.269	ns	0.870	ns	0.773	ns	0.462	ns
16mM	48	0.975	ns	0.526	ns	0.984	ns	0.998	ns	0.996	ns	0.640	ns	0.832	ns
2mM	72	0.996	ns	0.950	ns	0.800	ns	0.987	ns	0.938	ns	0.271	ns	0.902	ns
4mM	**72**	0.599	ns	0.568	ns	**0.025**	*	0.926	ns	0.273	ns	1.000	ns	1.000	ns
8mM	72	0.056	ns	0.120	ns	0.262	ns	0.334	ns	1.000	ns	0.674	ns	0.264	ns
12mM	72	0.551	ns	0.771	ns	0.991	ns	0.992	ns	0.949	ns	0.665	ns	1.000	ns
16mM	72	0.351	ns	0.798	ns	0.988	ns	0.804	ns	0.953	ns	0.683	ns	1.000	ns

Supplementary Table 10. Total duration in center of the arena data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.776	ns	1.000	ns	0.999	ns	0.930	ns	0.736	ns	0.381	ns	0.694	ns
4mM	48	0.456	ns	0.818	ns	0.993	ns	0.990	ns	0.785	ns	0.910	ns	0.994	ns
8mM	48	0.992	ns	0.453	ns	0.801	ns	0.986	ns	0.987	ns	0.644	ns	0.130	ns
12mM	48	0.975	ns	0.832	ns	0.992	ns	0.992	ns	0.351	ns	0.199	ns	0.340	ns
16mM	48	0.350	ns	0.559	ns	0.953	ns	0.812	ns	0.302	ns	0.305	ns	0.195	ns
2mM	72	0.783	ns	0.986	ns	0.897	ns	0.841	ns	0.691	ns	0.956	ns	0.984	ns
4mM	72	0.571	ns	1.000	ns	1.000	ns	0.364	ns	0.437	ns	0.953	ns	0.978	ns
8mM	72	0.660	ns	0.444	ns	0.701	ns	0.959	ns	0.628	ns	1.000	ns	1.000	ns
12mM	72	0.472	ns	1.000	ns	0.927	ns	0.996	ns	0.304	ns	0.408	ns	0.997	ns
16mM	**72**	0.735	ns	0.884	ns	0.984	ns	0.051	ns	**0.001**	***	**0.024**	*	0.109	ns

Supplementary Table 11. Frequency in center of the arena data for 48hph and 72hph Delta smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.840	ns	0.974	ns	0.683	ns	0.998	ns	0.164	ns	0.216	ns	0.904	ns
4mM	48	0.054	ns	0.987	ns	0.249	ns	0.835	ns	0.999	ns	0.969	ns	0.402	ns
8mM	48	0.181	ns	0.312	ns	0.291	ns	0.988	ns	0.240	ns	0.798	ns	0.474	ns
12mM	48	0.246	ns	0.997	ns	0.455	ns	0.966	ns	0.102	ns	1.000	ns	0.590	ns
16mM	48	0.099	ns	0.897	ns	0.305	ns	0.922	ns	0.528	ns	1.000	ns	0.556	ns
2mM	72	0.938	ns	0.999	ns	0.995	ns	0.993	ns	0.297	ns	0.784	ns	0.988	ns
4mM	72	0.915	ns	0.997	ns	0.208	ns	0.190	ns	0.567	ns	0.904	ns	1.000	ns
8mM	72	0.944	ns	0.994	ns	0.994	ns	0.997	ns	1.000	ns	0.998	ns	1.000	ns
12mM	**72**	**0.044**	*****	0.499	ns	0.996	ns	1.000	ns	1.000	ns	0.355	ns	0.461	ns
16mM	72	0.931	ns	0.983	ns	0.996	ns	0.995	ns	0.954	ns	0.926	ns	0.982	ns

Supplementary Table 12. Total distance moved data for 48hph and 72hph Longfin smelt.

Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.	Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.
Control	48	Dark 1	161	34.7	386	n/a	n/a	Control	72	Dark 1	50.9	23.4	354	n/a	n/a
2mM	48	Dark 1	122	36.7	507	0.590	ns	2mM	72	Dark 1	57.9	23.8	571	0.987	ns
4mM	48	Dark 1	140	33	448	0.904	ns	4mM	72	Dark 1	70.8	21.9	455	0.830	ns
8mM	48	Dark 1	247	22.2	797	0.074	ns	8mM	72	Dark 1	83.5	16.8	661	0.504	ns
12mM	48	Dark 1	217	51.2	685	0.256	ns	12mM	72	Dark 1	96.3	26.8	506	0.259	ns
16mM	48	Dark 1	230	27.4	664	0.160	ns	**16mM**	**72**	**Dark 1**	**135**	**67.9**	**897**	**0.009**	******
Control	48	Dark 2	177	45.4	420	n/a	n/a	Control	72	Dark 2	53.3	15.3	350	n/a	n/a
2mM	48	Dark 2	107	39.7	392	0.120	ns	2mM	72	Dark 2	66.6	18.6	587	0.964	ns
4mM	48	Dark 2	176	36	464	1.000	ns	4mM	72	Dark 2	88.2	0.298	413	0.637	ns
8mM	**48**	**Dark 2**	**385**	**98.3**	**751**	**<0.001**	*******	8mM	72	Dark 2	88.1	27.1	644	0.628	ns
12mM	**48**	**Dark 2**	**369**	**57.7**	**754**	**<0.001**	*******	**12mM**	**72**	**Dark 2**	**142**	**26.2**	**742**	**0.026**	*****
16mM	**48**	**Dark 2**	**412**	**37.7**	**760**	**<0.001**	*******	**16mM**	**72**	**Dark 2**	**199**	**22.7**	**1020**	**<0.001**	*******
Control	48	Light 1	391	170	662	n/a	n/a	Control	72	Light 1	131	21.5	725	n/a	n/a
2mM	48	Light 1	504	42.3	1150	0.063	ns	2mM	72	Light 1	164	22.5	1020	0.911	ns
4mM	**48**	**Light 1**	**598**	**106**	**935**	**<0.001**	*******	4mM	72	Light 1	166	3.45	1030	0.896	ns
8mM	**48**	**Light 1**	**540**	**38.6**	**1030**	**0.020**	*****	8mM	72	Light 1	176	14.6	1020	0.801	ns
12mM	**48**	**Light 1**	**571**	**59.3**	**950**	**<0.001**	*******	12mM	72	Light 1	188	38.9	1030	0.706	ns

16mM	48	Light 1	542	164	928	0.010	*	16mM	72	Light 1	190	36.8	818	0.709	ns
Control	48	Dark 3	93.4	31.4	327	n/a	n/a	Control	72	Dark 3	53.6	17.6	439	n/a	n/a
2mM	48	Dark 3	170	26.5	462	0.044	*	2mM	72	Dark 3	86.8	23.3	842	0.691	ns
4mM	48	Dark 3	224	34.3	505	0.0002	***	4mM	72	Dark 3	69.9	12.7	302	0.946	ns
8mM	48	Dark 3	289	52	654	<0.001	***	8mM	72	Dark 3	103	18.3	583	0.360	ns
12mM	48	Dark 3	351	136	705	<0.001	***	12mM	72	Dark 3	120	30	600	0.166	ns
16mM	48	Dark 3	385	141	691	<0.001	***	16mM	72	Dark 3	165	38.8	768	0.007	**
Control	48	Light 2	369	123	677	n/a	n/a	Control	72	Light 2	116	20	572	n/a	n/a
2mM	48	Light 2	525	101	913	0.003	***	2mM	72	Light 2	162	19.2	1140	0.813	ns
4mM	48	Light 2	607	174	1130	<0.001	***	4mM	72	Light 2	184	30.7	1010	0.569	ns
8mM	48	Light 2	580	191	1060	<0.001	***	8mM	72	Light 2	165	17.4	1020	0.761	ns
12mM	48	Light 2	474	114	855	0.079	ns	12mM	72	Light 2	136	28.5	823	0.980	ns
16mM	48	Light 2	452	35	846	0.273	ns	16mM	72	Light 2	140	29.4	837	0.970	ns
Control	48	Dark 4	131	30.1	407	n/a	n/a	Control	72	Dark 4	54.5	18.1	375	n/a	n/a
2mM	48	Dark 4	224	47.5	634	0.022	*	2mM	72	Dark 4	69.3	25	628	0.948	ns
4mM	48	Dark 4	245	69.9	527	0.004	***	4mM	72	Dark 4	109	27.6	789	0.231	ns
8mM	48	Dark 4	348	85.3	639	<0.001	***	8mM	72	Dark 4	95.7	21.2	498	0.479	ns
12mM	48	Dark 4	300	82.5	607	<0.001	***	12mM	72	Dark 4	94.3	15.9	668	0.540	ns
16mM	48	Dark 4	377	35.2	666	<0.001	***	16mM	72	Dark 4	119	31	742	0.175	ns
Control	48	Dark 5	102	31.1	388	n/a	n/a	Control	72	Dark 5	43.4	17.4	208	n/a	n/a
2mM	48	Dark 5	231	57.8	515	0.004	***	2mM	72	Dark 5	68.6	22.2	455	0.878	ns
4mM	48	Dark 5	291	90.4	522	<0.001	***	4mM	72	Dark 5	105	22.2	830	0.290	ns
8mM	48	Dark 5	414	58.8	694	<0.001	***	8mM	72	Dark 5	106	14.9	758	0.289	ns
12mM	48	Dark 5	446	105	857	<0.001	***	12mM	72	Dark 5	110	21	816	0.262	ns
16mM	48	Dark 5	471	74.8	756	<0.001	***	16mM	72	Dark 5	154	30.5	594	0.024	*

Supplementary Table 13. Mean velocity data for 48hph and 72hph Longfin smelt.

Conc.	Hph	Photoperiod	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.	Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.
Control	48	Dark 1	2.72	0.628	6.42	n/a	n/a	Control	72	Dark 1	1.7	0.39	5.91	n/a	n/a
2mM	48	Dark 1	2.03	0.612	8.47	0.556	ns	2mM	72	Dark 1	1.94	0.396	9.54	0.987	ns
4mM	48	Dark 1	2.33	0.549	7.48	0.887	ns	4mM	72	Dark 1	2.38	0.365	7.67	0.827	ns
8mM	48	Dark 1	4.15	0.722	13.3	0.078	ns	8mM	72	Dark 1	2.8	0.28	11.4	0.502	ns
12mM	48	Dark 1	3.63	0.854	11.4	0.292	ns	12mM	72	Dark 1	3.24	0.447	8.53	0.253	ns
16mM	48	Dark 1	3.85	0.458	11.1	0.178	ns	16mM	72	Dark 1	4.51	1.13	15.1	0.009	**
Control	48	Dark 2	2.95	0.756	6.99	n/a	n/a	Control	72	Dark 2	1.78	0.255	5.84	n/a	n/a
2mM	48	Dark 2	1.79	0.661	6.53	0.120	ns	2mM	72	Dark 2	2.22	0.31	9.78	0.964	ns

4mM	48	Dark 2	2.94	0.6	7.72	1.000	ns	4mM	72	Dark 2	2.96	0.00497	6.9	0.634	ns
8mM	**48**	**Dark 2**	**6.41**	**1.64**	**12.5**	**<0.001**	*******	8mM	72	Dark 2	2.94	0.453	10.7	0.631	ns
12mM	**48**	**Dark 2**	**6.14**	**0.964**	**12.6**	**<0.001**	*******	**12mM**	**72**	**Dark 2**	**4.74**	**0.437**	**12.4**	**0.026**	*****
16mM	**48**	**Dark 2**	**6.86**	**0.629**	**12.7**	**<0.001**	*******	**16mM**	**72**	**Dark 2**	**6.67**	**0.378**	**17.1**	**<0.001**	*******
Control	48	Light 1	6.53	2.83	11	n/a	n/a	Control	72	Light 1	4.4	0.359	12.1	n/a	n/a
2mM	48	Light 1	8.41	0.705	19.2	0.062	ns	2mM	72	Light 1	5.48	0.376	17.1	0.913	ns
4mM	**48**	**Light 1**	**9.97**	**1.77**	**15.6**	**<0.001**	*******	4mM	72	Light 1	5.59	0.0575	17.3	0.894	ns
8mM	**48**	**Light 1**	**9**	**0.643**	**17.3**	**0.020**	*****	8mM	72	Light 1	5.95	0.243	17.3	0.791	ns
12mM	**48**	**Light 1**	**9.51**	**0.989**	**15.8**	**<0.001**	*******	12mM	72	Light 1	6.31	0.651	17.8	0.702	ns
16mM	**48**	**Light 1**	**9.04**	**2.74**	**15.5**	**0.011**	*****	16mM	72	Light 1	6.44	0.613	14.1	0.689	ns
Control	48	Dark 3	1.56	0.524	5.45	n/a	n/a	Control	72	Dark 3	1.79	0.294	7.33	n/a	n/a
2mM	**48**	**Dark 3**	**2.83**	**0.442**	**7.7**	**0.044**	*****	2mM	72	Dark 3	2.9	0.389	14.1	0.693	ns
4mM	**48**	**Dark 3**	**3.74**	**0.573**	**8.42**	**<0.001**	*******	4mM	72	Dark 3	2.34	0.211	5.03	0.946	ns
8mM	**48**	**Dark 3**	**4.82**	**0.867**	**10.9**	**<0.001**	*******	8mM	72	Dark 3	3.47	0.305	10.2	0.353	ns
12mM	**48**	**Dark 3**	**5.84**	**2.27**	**11.7**	**<0.001**	*******	12mM	72	Dark 3	4.02	0.5	10.1	0.168	ns
16mM	**48**	**Dark 3**	**6.42**	**2.35**	**11.5**	**<0.001**	*******	**16mM**	**72**	**Dark 3**	**5.53**	**0.646**	**12.9**	**0.007**	******
Control	48	Light 2	6.15	2.05	11.3	n/a	n/a	Control	72	Light 2	3.89	0.334	9.65	n/a	n/a
2mM	**48**	**Light 2**	**8.75**	**1.68**	**15.2**	**0.003**	*******	2mM	72	Light 2	5.41	0.32	19.2	0.814	ns
4mM	**48**	**Light 2**	**10.1**	**2.89**	**18.9**	**<0.001**	*******	4mM	72	Light 2	6.17	0.511	16.9	0.570	ns
8mM	**48**	**Light 2**	**9.67**	**3.18**	**17.7**	**<0.001**	*******	8mM	72	Light 2	5.57	0.289	17.4	0.753	ns
12mM	48	Light 2	7.9	1.9	14.2	0.080	ns	12mM	72	Light 2	4.56	0.475	13.9	0.980	ns
16mM	48	Light 2	7.53	0.584	14.1	0.274	ns	16mM	72	Light 2	4.72	0.49	14.3	0.968	ns
Control	48	Dark 4	2.18	0.501	6.79	n/a	n/a	Control	72	Dark 4	1.82	0.302	6.26	n/a	n/a
2mM	**48**	**Dark 4**	**3.74**	**0.791**	**10.6**	**0.022**	*****	2mM	72	Dark 4	2.32	0.417	10.5	0.948	ns
4mM	**48**	**Dark 4**	**4.08**	**1.16**	**8.77**	**0.004**	*******	4mM	72	Dark 4	3.65	0.46	13.2	0.233	ns
8mM	**48**	**Dark 4**	**5.8**	**1.42**	**10.7**	**<0.001**	*******	8mM	72	Dark 4	3.19	0.353	8.29	0.482	ns
12mM	**48**	**Dark 4**	**5**	**1.38**	**10.1**	**<0.001**	*******	12mM	72	Dark 4	3.15	0.266	11.2	0.542	ns
16mM	**48**	**Dark 4**	**6.29**	**0.587**	**11.1**	**<0.001**	*******	16mM	72	Dark 4	3.96	0.517	12.4	0.175	ns
Control	48	Dark 5	1.71	0.519	6.47	n/a	n/a	Control	72	Dark 5	1.45	0.289	3.47	n/a	n/a
2mM	**48**	**Dark 5**	**3.84**	**0.963**	**8.59**	**0.004**	*******	2mM	72	Dark 5	2.29	0.37	7.58	0.878	ns
4mM	**48**	**Dark 5**	**4.85**	**1.51**	**8.7**	**<0.001**	*******	4mM	72	Dark 5	3.52	0.371	13.8	0.290	ns
8mM	**48**	**Dark 5**	**6.9**	**0.98**	**11.6**	**<0.001**	*******	8mM	72	Dark 5	3.52	0.248	12.6	0.291	ns
12mM	**48**	**Dark 5**	**7.43**	**1.76**	**14.3**	**<0.001**	*******	12mM	72	Dark 5	3.68	0.35	13.7	0.259	ns
16mM	**48**	**Dark 5**	**7.86**	**1.25**	**12.6**	**<0.001**	*******	**16mM**	**72**	**Dark 5**	**5.16**	**0.509**	**10.4**	**0.023**	*****

Supplementary Table 14. Maximum velocity data for 48hph and 72hph Longfin smelt.

Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.	Conc.	Hph	Photo-period	Mean mm/s	Min mm/s	Max mm/s	P.adj	Sig.
Control	48	Dark 1	161	34.7	386	n/a	n/a	Control	72	Dark 1	49.1	1.44	114	n/a	n/a
2mM	48	Dark 1	122	36.7	507	0.590	ns	2mM	72	Dark 1	41	1.41	104	0.892	ns
4mM	48	Dark 1	140	33	448	0.904	ns	4mM	72	Dark 1	60.8	1.43	174	0.751	ns
8mM	48	Dark 1	247	22.2	797	0.074	ns	8mM	72	Dark 1	61.1	1.34	133	0.724	ns
12mM	48	Dark 1	217	51.2	685	0.256	ns	12mM	72	Dark 1	61.7	1.8	111	0.725	ns
16mM	48	Dark 1	230	27.4	664	0.160	ns	16mM	72	Dark 1	80.8	11.5	366	0.070	ns
Control	48	Dark 2	177	45.4	420	n/a	n/a	Control	72	Dark 2	45.6	0.995	127	n/a	n/a
2mM	48	Dark 2	107	39.7	392	0.120	ns	2mM	72	Dark 2	43.8	1.09	105	0.998	ns
4mM	48	Dark 2	176	36	464	1.000	ns	4mM	72	Dark 2	60.1	0.00497	141	0.667	ns
8mM	**48**	**Dark 2**	**385**	**98.3**	**751**	**<0.001**	*******	8mM	72	Dark 2	54.3	1.4	129	0.897	ns
12mM	**48**	**Dark 2**	**369**	**57.7**	**754**	**<0.001**	*******	12mM	72	Dark 2	71.6	1.61	139	0.210	ns
16mM	**48**	**Dark 2**	**412**	**37.7**	**760**	**<0.001**	*******	16mM	72	Dark 2	70.2	1.3	121	0.292	ns
Control	48	Light 1	391	170	662	n/a	n/a	Control	72	Light 1	58.2	1.2	120	n/a	n/a
2mM	48	Light 1	504	42.3	1150	0.062	ns	2mM	72	Light 1	55.9	1.42	298	0.998	ns
4mM	**48**	**Light 1**	**598**	**106**	**935**	**<0.001**	*******	4mM	72	Light 1	68.9	1.43	175	0.877	ns
8mM	**48**	**Light 1**	**540**	**38.6**	**1030**	**0.020**	*****	8mM	72	Light 1	63.7	1.2	224	0.977	ns
12mM	**48**	**Light 1**	**571**	**59.3**	**950**	**<0.001**	*******	12mM	72	Light 1	79.6	2.46	133	0.486	ns
16mM	**48**	**Light 1**	**542**	**164**	**928**	**0.010**	*****	16mM	72	Light 1	63.7	2.37	121	0.982	ns
Control	48	Dark 3	93.4	31.4	327	n/a	n/a	Control	72	Dark 3	41.6	1.01	108	n/a	n/a
2mM	**48**	**Dark 3**	**170**	**26.5**	**462**	**0.044**	*****	2mM	72	Dark 3	42.9	1.35	121	0.999	ns
4mM	**48**	**Dark 3**	**224**	**34.3**	**505**	**<0.001**	*******	4mM	72	Dark 3	57.2	1.17	102	0.639	ns
8mM	**48**	**Dark 3**	**289**	**52**	**654**	**<0.001**	*******	8mM	72	Dark 3	52.5	1.1	166	0.831	ns
12mM	**48**	**Dark 3**	**351**	**136**	**705**	**<0.001**	*******	12mM	72	Dark 3	61.7	1.73	134	0.461	ns
16mM	**48**	**Dark 3**	**385**	**141**	**691**	**<0.001**	*******	16mM	72	Dark 3	60.1	2.78	135	0.568	ns
Control	48	Light 2	369	123	677	n/a	n/a	Control	72	Light 2	55.8	1.13	118	n/a	n/a
2mM	**48**	**Light 2**	**525**	**101**	**913**	**0.003**	*******	2mM	72	Light 2	55.8	1.12	194	1.000	ns
4mM	**48**	**Light 2**	**607**	**174**	**1130**	**<0.001**	*******	4mM	72	Light 2	64.7	1.58	176	0.939	ns
8mM	**48**	**Light 2**	**580**	**191**	**1060**	**<0.001**	*******	8mM	72	Light 2	56.9	1.24	147	0.999	ns
12mM	48	Light 2	474	114	855	0.079	ns	12mM	72	Light 2	69.5	1.64	154	0.833	ns
16mM	48	Light 2	452	35	846	0.273	ns	16mM	72	Light 2	63.1	2.14	212	0.969	ns
Control	48	Dark 4	131	30.1	407	n/a	n/a	Control	72	Dark 4	39.1	1.15	107	n/a	n/a
2mM	**48**	**Dark 4**	**224**	**47.5**	**634**	**0.022**	*****	2mM	72	Dark 4	36	1.34	148	0.994	ns
4mM	**48**	**Dark 4**	**245**	**69.9**	**527**	**0.004**	*******	4mM	72	Dark 4	54.1	1.35	176	0.665	ns
8mM	**48**	**Dark 4**	**348**	**85.3**	**639**	**<0.001**	*******	8mM	72	Dark 4	54.5	1.24	125	0.649	ns
12mM	**48**	**Dark 4**	**300**	**82.5**	**607**	**<0.001**	*******	12mM	72	Dark 4	53.5	1.23	130	0.716	ns
16mM	**48**	**Dark 4**	**377**	**35.2**	**666**	**<0.001**	*******	16mM	72	Dark 4	57	2.13	121	0.597	ns
Control	48	Dark 5	102	31.1	388	n/a	n/a	Control	72	Dark 5	33.5	1.04	89.3	n/a	n/a
2mM	**48**	**Dark 5**	**231**	**57.8**	**515**	**0.004**	*******	2mM	72	Dark 5	32.4	1.45	126	1.000	ns

4mM	**48**	**Dark 5**	**291**	**90.4**	**522**	**<0.001**	*******	4mM	72	Dark 5	51.1	1.38	117	0.586	ns
8mM	**48**	**Dark 5**	**414**	**58.8**	**694**	**<0.001**	*******	8mM	72	Dark 5	50.3	1.08	112	0.624	ns
12mM	**48**	**Dark 5**	**446**	**105**	**857**	**<0.001**	*******	12mM	72	Dark 5	45.8	1.58	106	0.824	ns
16mM	**48**	**Dark 5**	**471**	**74.8**	**756**	**<0.001**	*******	16mM	72	Dark 5	55.2	1.78	115	0.482	ns

Supplementary Table 15. Total duration freezing data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.905	ns	0.301	ns	0.574	ns	1.000	ns	0.292	ns	0.742	ns	0.977	ns
4mM	48	0.976	ns	0.324	ns	0.990	ns	0.303	ns	0.310	ns	0.225	ns	0.980	ns
8mM	48	0.978	ns	0.992	ns	0.992	ns	1.000	ns	0.386	ns	0.297	ns	0.983	ns
12mM	48	0.659	ns	0.301	ns	0.989	ns	1.000	ns	0.292	ns	0.282	ns	0.828	ns
16mM	48	0.896	ns	0.352	ns	0.990	ns	1.000	ns	0.343	ns	0.250	ns	0.856	ns
2mM	72	0.468	ns	0.273	ns	0.971	ns	1.000	ns	1.000	ns	1.000	ns	1.000	ns
4mM	72	0.970	ns	0.232	ns	0.773	ns	0.264	ns	1.000	ns	0.999	ns	1.000	ns
8mM	72	1.000	ns	0.298	ns	0.334	ns	0.984	ns	0.445	ns	0.444	ns	0.447	ns
12mM	72	0.999	ns	0.253	ns	0.902	ns	0.990	ns	1.000	ns	1.000	ns	1.000	ns
16mM	72	0.996	ns	0.245	ns	0.697	ns	0.999	ns	1.000	ns	1.000	ns	1.000	ns

Supplementary Table 16. Freezing frequency data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.998	ns	1.000	ns	0.301	ns	0.817	ns	0.304	ns	0.923	ns	0.970	ns
4mM	48	0.988	ns	0.911	ns	0.146	ns	0.952	ns	0.211	ns	1.000	ns	0.946	ns
8mM	**48**	0.926	ns	0.200	ns	0.222	ns	0.683	ns	0.553	ns	**0.035**	*	0.989	ns
12mM	48	0.724	ns	0.373	ns	0.820	ns	0.888	ns	0.979	ns	1.000	ns	1.000	ns
16mM	48	0.883	ns	0.536	ns	0.966	ns	0.975	ns	0.939	ns	0.502	ns	0.562	ns
2mM	72	0.962	ns	0.986	ns	0.998	ns	0.881	ns	0.231	ns	0.826	ns	0.999	ns
4mM	72	0.980	ns	0.732	ns	0.995	ns	0.974	ns	0.693	ns	0.665	ns	1.000	ns
8mM	72	0.912	ns	0.996	ns	0.503	ns	0.851	ns	0.108	ns	0.798	ns	0.693	ns
12mM	72	0.982	ns	0.998	ns	1.000	ns	0.875	ns	0.323	ns	0.962	ns	0.979	ns
16mM	72	0.962	ns	0.988	ns	0.910	ns	0.609	ns	0.294	ns	0.934	ns	0.943	ns

Supplementary Table 17. Total duration cruising data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	**48**	**0.990**	ns	0.991	ns	**0.017**	*	0.655	ns	**0.011**	*	0.373	ns	0.381	ns
4mM	**48**	**0.992**	ns	0.806	ns	**<0.001**	***	0.118	ns	**<0.001**	***	0.207	ns	0.136	ns
8mM	**48**	**0.009**	**	**<0.001**	***	**<0.001**	***	**0.040**	*	**<0.001**	***	**0.002**	***	**<0.001**	***
12mM	**48**	0.190	ns	**<0.001**	***	**<0.001**	***	**<0.001**	***	**0.001**	***	**0.005**	***	**<0.001**	***
16mM	**48**	**<0.001**	***	**<0.001**	***	**<0.001**	***	**<0.001**	***	**0.005**	**	**<0.001**	***	**<0.001**	***
2mM	72	0.931	ns	0.976	ns	0.283	ns	0.242	ns	0.116	ns	0.880	ns	0.997	ns
4mM	72	0.962	ns	0.746	ns	0.396	ns	0.964	ns	0.166	ns	0.122	ns	0.321	ns
8mM	72	0.402	ns	0.782	ns	0.234	ns	0.805	ns	0.200	ns	0.714	ns	0.344	ns
12mM	**72**	0.445	ns	**0.036**	*	0.353	ns	0.630	ns	0.639	ns	0.645	ns	0.264	ns
16mM	**72**	**0.017**	*	**<0.001**	***	0.123	ns	**0.045**	*	0.653	ns	0.321	ns	**0.044**	*

Supplementary Table 18. Cruising frequency data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	**48**	0.751	ns	0.827	ns	0.314	ns	0.260	ns	**0.003**	***	0.145	ns	**0.031**	*
4mM	**48**	0.952	ns	0.220	ns	**0.012**	*	**0.019**	*	**<0.001**	***	**0.029**	*	**0.002**	***
8mM	**48**	**0.008**	**	**<0.001**	***	0.182	ns	**0.022**	*	**0.004**	***	**<0.001**	***	**<0.001**	***
12mM	**48**	**0.004**	***	**<0.001**	***	**0.016**	*	**<0.001**	***	**0.005**	***	**<0.001**	***	**<0.001**	***
16mM	**48**	**<0.001**	***	**<0.001**	***	**0.029**	*	**<0.001**	***	**<0.001**	***	**<0.001**	***	**<0.001**	***
2mM	72	0.870	ns	0.938	ns	0.994	ns	0.781	ns	0.955	ns	0.860	ns	0.967	ns
4mM	72	0.552	ns	0.181	ns	0.431	ns	0.333	ns	0.534	ns	0.425	ns	0.235	ns
8mM	72	0.122	ns	0.105	ns	0.532	ns	0.051	ns	0.388	ns	0.123	ns	0.073	ns
12mM	**72**	**0.020**	*	**0.001**	***	0.471	ns	0.087	ns	0.573	ns	0.194	ns	0.145	ns
16mM	**72**	**0.001**	***	**0.007**	**	0.624	ns	**0.002**	***	0.591	ns	0.390	ns	**0.043**	*

Supplementary Table 19. Total duration bursting data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.976	ns	0.999	ns	0.884	ns	0.908	ns	0.395	ns	0.273	ns	0.782	ns
4mM	**48**	0.940	ns	0.999	ns	0.767	ns	0.482	ns	**0.019**	*	0.119	ns	0.099	ns
8mM	48	0.421	ns	0.703	ns	0.755	ns	0.828	ns	0.999	ns	0.955	ns	0.881	ns
12mM	**48**	**0.014**	*	**<0.001**	***	0.448	ns	**0.029**	*	0.952	ns	0.325	ns	0.176	ns
16mM	**48**	**0.010**	**	**0.001**	***	0.634	ns	0.085	ns	0.996	ns	0.437	ns	0.745	ns
2mM	72	1.000	ns	1.000	ns	0.875	ns	0.988	ns	0.251	ns	0.999	ns	0.771	ns
4mM	72	0.978	ns	0.977	ns	0.180	ns	0.741	ns	0.111	ns	0.773	ns	0.307	ns
8mM	72	0.379	ns	0.693	ns	0.584	ns	1.000	ns	0.752	ns	0.986	ns	0.516	ns
12mM	72	0.574	ns	0.173	ns	0.824	ns	0.974	ns	0.953	ns	0.971	ns	0.665	ns
16mM	**72**	**0.002**	***	0.433	ns	0.938	ns	0.997	ns	0.584	ns	0.313	ns	0.836	ns

Supplementary Table 20. Bursting frequency data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.997	ns	0.997	ns	0.924	ns	0.958	ns	0.257	ns	0.750	ns	0.740	ns
4mM	48	0.900	ns	0.999	ns	0.378	ns	0.747	ns	0.058	ns	0.286	ns	0.325	ns
8mM	48	0.277	ns	0.715	ns	0.966	ns	0.951	ns	0.891	ns	0.995	ns	0.761	ns
12mM	**48**	**0.003**	*******	**<0.001**	*******	0.924	ns	**0.013**	*****	0.904	ns	0.328	ns	0.120	Ns+
16mM	**48**	**0.018**	*****	**<0.001**	*******	0.994	ns	0.121	ns	0.994	ns	0.240	ns	0.636	ns
2mM	72	1.000	ns	1.000	ns	0.540	ns	1.000	ns	0.425	ns	0.999	ns	0.617	ns
4mM	72	0.996	ns	0.972	ns	0.122	ns	0.966	ns	0.204	ns	0.885	ns	0.418	ns
8mM	72	0.432	ns	0.839	ns	0.742	ns	1.000	ns	0.773	ns	0.796	ns	0.418	ns
12mM	72	0.694	ns	0.116	ns	0.725	ns	0.960	ns	0.802	ns	0.917	ns	0.817	ns
16mM	**72**	**0.002**	*******	0.317	ns	0.922	ns	0.535	ns	0.744	ns	0.299	ns	0.750	ns

Supplementary Table 21. Total duration in center of the arena data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.951	ns	0.999	ns	0.915	ns	0.102	ns	0.895	ns	0.817	ns	0.966	ns
4mM	48	0.293	ns	0.995	ns	0.064	ns	0.141	ns	0.719	ns	0.488	ns	0.849	ns
8mM	**48**	1.000	ns	0.484	ns	0.267	ns	**0.004**	*******	0.962	ns	0.480	ns	0.963	ns
12mM	**48**	0.886	ns	0.561	ns	0.331	ns	**0.008**	******	0.987	ns	0.474	ns	0.556	ns
16mM	**48**	0.999	ns	0.555	ns	0.170	ns	0.141	ns	**0.016**	*****	0.951	ns	0.998	ns
2mM	72	0.533	ns	0.222	ns	0.818	ns	0.969	ns	0.943	ns	1.000	ns	0.922	ns
4mM	72	0.652	ns	0.997	ns	0.992	ns	0.801	ns	1.000	ns	0.940	ns	0.998	ns
8mM	72	0.930	ns	0.700	ns	0.997	ns	0.409	ns	0.909	ns	0.992	ns	0.735	ns
12mM	72	1.000	ns	0.998	ns	0.672	ns	0.695	ns	0.995	ns	0.988	ns	0.960	ns
16mM	72	0.454	ns	0.959	ns	0.546	ns	0.086	ns	0.978	ns	0.777	ns	0.997	ns

Supplementary Table 22. Frequency in center of the arena data for 48hph and 72hph Longfin smelt.

		Dark 1		Dark 2		Light 1		Dark 3		Light 2		Dark 4		Dark 5	
Conc.	Hph	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.	P.adj.	Sig.
2mM	48	0.377	ns	0.523	ns	0.999	ns	0.967	ns	0.999	ns	0.656	ns	1.000	ns
4mM	48	0.732	ns	0.998	ns	0.791	ns	0.892	ns	0.727	ns	0.307	ns	0.906	ns
8mM	48	0.140	ns	0.987	ns	0.318	ns	1.000	ns	0.925	ns	**0.004**	***	0.550	ns
12mM	48	0.887	ns	0.114	ns	0.757	ns	0.087	ns	0.812	ns	0.065	ns	0.625	ns
16mM	**48**	0.543	ns	0.172	ns	0.684	ns	**<0.001**	***	0.301	ns	**0.037**	*	0.412	ns
2mM	72	0.588	ns	0.562	ns	0.629	ns	1.000	ns	0.397	ns	0.144	ns	0.998	ns
4mM	72	0.790	ns	0.620	ns	0.839	ns	0.097	ns	0.520	ns	0.842	ns	0.988	ns
8mM	72	0.894	ns	0.483	ns	0.508	ns	0.491	ns	0.642	ns	0.915	ns	0.986	ns
12mM	72	0.441	ns	0.106	ns	0.568	ns	0.183	ns	0.919	ns	0.970	ns	0.184	ns
16mM	72	0.806	ns	0.584	ns	0.387	ns	0.204	ns	0.995	ns	0.711	ns	0.965	ns

Appendix 2. R script for statistical analysis

```
############################
# DS_PTZ_48hph
# Total distance Traveled stats
# File 1 of 2
# Elle Patullo
############################

# DS + PTZ 48hph total distance traveled stats
# Method: Hypothesis testing
# Tests: Shapiro-Wilk, Levene's, Kruskal-Wallace, Dunn's, Dunnet's

# Before using r:
# export data from ethovision w/ blank values as "NA"
# verify/simplify column names
# Add column with light/dark photoperiods (e.g. "Dark 1", "Light 1", "Dark 2", etc.)

# packages

library(readr)
library(dplyr)
library(forcats)
library(tidyr)
library(stats)
library(purrr)
library(broom)
library(rstatix)


##### Step 1: Load and prep data #####

# set working directory
setwd("~/Thesis Project/Data/022822_DS_PTZ_48hph")

# upload data and create object
DS_PTZ_48hph <- read_csv("30secbins_EDITED_022822_DS_PTZ_48hph_RETRACK.csv")
View(DS_PTZ_48hph)

# remove NA values from total distance moved column
DS_PTZ_48hph.omit <- DS_PTZ_48hph[!(is.na(DS_PTZ_48hph$total_distance_moved)), ]
View(DS_PTZ_48hph.omit)

# Reorganize/transform
```

```
DM_48h <- DS_PTZ_48hph.omit %>%
  mutate(trial = fct_relevel(trial, "Trial     2", "Trial     3", "Trial     4", "Trial     5", "Trial     6"),
         trial = fct_recode(trial, "Trial1" = "Trial     2",
                            "Trial2" = "Trial     3",
                            "Trial3" = "Trial     4",
                            "Trial4" = "Trial     5",
                            "Trial5" = "Trial     6")) %>%
  unite(col = newID, trial, replicate, sep = "_") %>%
  mutate(treatment = fct_relevel(treatment, "Control", "2mM", "4mM", "8mM", "12mM", "16mM"),
         treatment = fct_recode(treatment, "0" = "Control",
                                "2" = "2mM",
                                "4" = "4mM",
                                "8" = "8mM",
                                "12" = "12mM",
                                "16" = "16mM"),
         photoperiod = fct_relevel(photoperiod, "dark1", "dark2", "light1", "dark3", "light2", "dark4",
"dark5")) %>%
  group_by(treatment, newID, photoperiod) %>%
  summarize(MeanDM = mean(`total_distance_moved`))

View(DM_48h)


#### Step 2: Explore data Visually ####

# create a histogram to look at distribution
hist(DM_48h$MeanDM, main = "Distribution of Mean Distance Moved", xlab = "Mean Distance
Moved")

# create a boxplot to check for outliers
boxplot(MeanDM~treatment, data = DM_48h, main = "Mean distance moved per treatment")


#### Step 3: Shapiro-Wilk test ####

DS_PTZ_48hph_shapiro <- DM_48h %>%
  group_by(treatment, photoperiod) %>%
  nest() %>%
  ungroup() %>%
  mutate(shapiro = map(data, ~tidy(shapiro.test(.x$MeanDM)))) %>%
  unnest(shapiro) %>%
  select(treatment, photoperiod, statistic, p.value, method)
View(DS_PTZ_48hph_shapiro)
```

```
# the code above runs the Shapiro-Wilk test to compare the distance moved between each treatment WITH Photoperiod taken into consideration as well
# a p-value > 0.05 is normally distributed
# a p-value < 0.05 is not normally distributed.


#### Step 4: Levene's Test ####

DS_PTZ_48hph_levene_photoperiod<- DM_48h %>%
  group_by(treatment) %>%
  nest() %>%
  mutate(levene = map(data, ~levene_test(.x, MeanDM ~ photoperiod))) %>%
  unnest(levene) %>%
  select(treatment, df1, df2, statistic, p)
View(DS_PTZ_48hph_levene_photoperiod)

# if p-values < 0.05, the variance among the groups is not equal
# if p-values > 0.05, the variance among groups is equal


DS_PTZ_48hph_levene_treatment<- DM_48h %>%
  group_by(photoperiod) %>%
  nest() %>%
  mutate(levene = map(data, ~levene_test(.x, MeanDM ~ treatment))) %>%
  unnest(levene) %>%
  select(photoperiod, df1, df2, statistic, p)
View(DS_PTZ_48hph_levene_treatment)

# if p-values < 0.05, the variance among the groups is not equal
# if p-values > 0.05, the variance among groups is equal


#### Step 5: Kruskal-Wallis Test ####

DS_PTZ_48hph_KRUSKAL <- DM_48h %>%
  group_by(photoperiod) %>%
  nest() %>%
  mutate(kruskal = map(data, ~kruskal_test(.x, MeanDM ~ treatment))) %>%
  unnest(kruskal)
View(DS_PTZ_48hph_KRUSKAL)


#### Step 6: Dunn's Test ####
```

```
DS_PTZ_48hph_DUNNS <- DM_48h %>%
  group_by(photoperiod) %>%
  nest() %>%
  mutate(dunns = map(data, ~dunn_test(.x, MeanDM ~ treatment, p.adjust.method =
"bonferroni"))) %>%
  unnest(dunns)  %>%
  mutate(significant = case_when(p.adj > 0.05 ~ "ns",
                                 p.adj <= 0.05 ~"*",
                                 p.adj <= 0.01 ~"**"))
View(DS_PTZ_48hph_DUNNS)

# simplify to only show doses compared to control
DS_PTZ_48hph_DUNNS_simp <- DS_PTZ_48hph_DUNNS[-c(6:15, 21:30, 36:45, 51:60,
66:75, 81:90, 96:105), ]
View(DS_PTZ_48hph_DUNNS_simp)

# reorganize and export as .csv
DS_PTZ_48hph_DUNNS_simp_forprint <- DS_PTZ_48hph_DUNNS_simp %>%
  select(-data, -n1, -n2) %>%
  ungroup()
View(DS_PTZ_48hph_DUNNS_simp_forprint)

write.csv(DS_PTZ_48hph_DUNNS_simp_forprint, "DS_PTZ_48h_DUNNS_simp.csv")


#### Step 7: Dunnet's Test ####

PTZ_48h_means_dunnetx <- tidymaster.forstat %>%
  gather(Variable, Value, -treatment, -newID, -photoperiod) %>%
  group_by(Variable, photoperiod) %>%
  nest() %>%
  mutate(dunnetx = map(data,
                 ~tidy(contrast(emmeans((ref_grid(lm(Value ~ treatment, data=.x))),"treatment"),
method="trt.vs.ctrl")))) %>%
  unnest(dunnetx) %>%
  mutate(significant = case_when(adj.p.value > 0.05 ~ "ns",
                                 adj.p.value <= 0.005 ~ "***",
                                 adj.p.value <= 0.01 ~"**",
                                 adj.p.value <= 0.05 ~"*"))
View(PTZ_48h_means_dunnetx)


# reorganize and export as .csv
```

```
PTZ_48h_means_DUNNETforprint <- PTZ_48h_means_dunnetx %>%
  select(-data) %>%
  ungroup()

write.csv(PTZ_48h_means_DUNNETforprint, "DS_PTZ_48h_means_DUNNETforprint.csv")
```