Tissot et al.

Environmental Toxicology

The effects of imidacloprid and polyester microfibers on the larval development of the endangered Sunflower Star

Alexandra G. Tissot,[1,*] Elise F. Granek,[1] Fiona Curliss,[2] Augustin Kalytiak-Davis,[2] Jason Hodin,[2] and Michelle L. Hladik[3]

[1] Department of Environmental Science and Management, Portland State University, Portland, OR, United States

[2] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, United States

[3] U.S. Geological Survey, California Water Science Center, Sacramento, CA, United States

Abstract: Sea star wasting syndrome (SSWS) has affected numerous species of sea star, with populations of *Pycnopodia helianthoides* (Brandt, 1835) left most at risk. As their populations are struggling to recover, it is important to gain a better understanding of the impacts that the multiple stressors in their habitats can have on their populations. Contaminant stressors in particular are of increasing importance, as aquatic organisms can be exposed to a dynamic range of contaminants from nearby anthropogenic activity that may affect their future recovery efforts. This study is the first to quantify the effects of contaminant stressors on the larvae of *P. helianthoides*. We exposed *P. helianthoides* larvae to the neonicotinoid insecticide imidacloprid and polyester microfibers, both individually and in combination, at environmentally relevant concentrations (10 ng/L and 25 fibers/L, respectively) to measure the effects of these contaminants on their early life stages. Imidacloprid exposure resulted in stomach malformation in 10% of larvae and increased mortality during early development ($p<0.001$), and all treatments resulted in increased larval lengths relative to controls ($p<0.001$). During settlement,

imidacloprid resulted in more rapid settlement responses than in the controls ($p<0.01$). These findings highlight the need for further research investigating the effects of contaminant stressors to endangered organisms during reintroduction, as well as a more comprehensive understanding of the effects of pesticides to non-target organisms.

Keywords: mixture toxicology, insecticide, microplastics, invertebrate toxicology, benthic macroinvertebrates

*Corresponding author: Alexandra G. Tissot. Email: tissot@pdx.edu

1. Introduction

Aquatic organisms are exposed to a number of abiotic and biotic stressors in their environments, including fluctuating temperatures and changes in disease and parasite distribution (Altizer et al., 2013; Aalto et al. 2020; Burge et al., 2014; Hewitt et al., 2016). Additionally, changes in contaminant production and release from industrialization expose them to a number of diverse pollutants (Álvarez-Muñoz et al., 2016). Along with the individual effects of each stressor, organisms are vulnerable to interactive effects, leading to potential declines in populations and species distribution (Gissi et al., 2021; Harvell et al., 2019; Macaulay et al., 2021).

In 2013, sea star wasting syndrome (SSWS) impacted populations of numerous sea star species along the West Coast of North America (Dawson et al. 2023). While many species are recovering, populations of the Sunflower Star, *Pycnopodia helianthoides*, remain highly impacted, with the species currently recognized internationally as endangered (Gravem et al. 2021; Harvell et al., 2019). Current efforts are underway to identify and quantify the drivers of SSWS as well as better understand the life cycle of *Pycnopodia* for purposes of captive rearing and potential reintroduction to increase natural populations (Hodin et al., 2021); however,

whether contaminant stressors are one of the factors limiting recovery of *Pycnopodia* is currently unknown. A study by Aalto et al. (2020) explored how environmental stressors may perpetuate SSWS. Furthermore, given the impacts that contaminants can have on organismal development and immune system functions (Kataoka & Kashiwada, 2021), their potential as multiple stressors should not be ignored. Two contaminant classes that are ubiquitous in aquatic systems are pesticides and microplastics. Not only do they individually impact species, but they have the potential to interact with one another, as well as other contaminants and stressors in the environment (Altenburger et al., 2018; Luo et al., 2021; Tissot et al., 2022).

Pesticides commonly reach aquatic environments via run-off, aerial deposition, and bioaccumulation in organisms (Katagi, 2010; Seiber & Kleinschmidt 2010). Contaminants, including but not limited to, pesticides, per- and polyfluoroalkyl substances (PFAS), pharmaceuticals, and microplastics are detected in tidally influenced and estuarine ecosystems (Baechler et al., 2020; Horn et al., 2019; Noland et al., 2022; Tian et al., 2020), yet little research has examined how these compounds may affect nearshore species.

Imidacloprid is a neonicotinoid insecticide that targets acetylcholinesterase receptors (Sheets 2010); it has been banned from outdoor use in the European Union due to its high toxicity to pollinators (Smit et al., 2015). However, it ranks as the second most "popular" pesticide worldwide and is commonly used in the United States, and concentrations detected in surface waters frequently exceed the U.S. Environmental Protection Agency's (EPA) aquatic life benchmark (Batikian et al., 2019; Borsuah et al., 2020; Noland et al., 2022). Additionally, while the chronic aquatic life benchmark for freshwater invertebrate exposure to imidacloprid is 10 ng/L, currently there is no established benchmark for estuarine/marine invertebrates (US EPA, 2017). Imidacloprid was chosen for testing in this study due to its widespread detection along the

West Coast of the US, as well as its persistence in aquatic environments (Heberger et al., 2020; Morrissey et al., 2015; Noland et al., 2022).

Microplastic pollution is ubiquitous in marine ecosystems, exposing organisms both through water and via aerial deposition (Li et al. 2023). The microplastic type most commonly detected in marine ecosystems is synthetic textile microfibers, including polyester (Gago et al., 2018; Mishra et al., 2019). Synthetic microfibers affect organisms due to both the physical impact of fibers as they pass through an organism's digestive system, and the chemicals that adhere to them (Athey et al., 2022; Wright et al., 2013). For this study, polyester microfibers were chosen as the second contaminant stressor.

As conservation and recovery efforts for *Pycnopodia* advance, an understanding of how these contaminant stressors affect various life stages could be key to ensuring their successful rehabilitation. In this study, we asked the following questions: 1. What impact do imidacloprid and polyester microfibers have on *Pycnopodia* larval development, if any? 2. Does the combination of these contaminants produce synergistic, additive, or antagonistic effects? 3. Which stages in larval development are most affected by each contaminant?

2. Methods

2.1 Fertilization and Experimental Set-up

The study was conducted at Friday Harbor Laboratories in San Juan Island, Washington (48°32′46″N 123°00′46″W). Adult *Pycnopodia* were collected and maintained as described by Hodin et al. (2021).

Adult sea stars were spawned on January 26, 2023, using 1-methyladenine injections following methods by Hodin et al. (2021). Sperm and eggs were collected directly from three male and two female stars, respectively, to create independent genetic crosses between each pair.

Sperm and eggs were added to 250 mL beakers to facilitate fertilization. Upon visual confirmation of a fertilization envelope (indication of fertilization success), embryos were transferred to new 250 mL beakers on a sea table at ambient temperature (8 °C) until the early gastrula stage (4 days post fertilization [dpf]). At this stage, larvae from all genetic crosses were combined in equal proportions into one 2 L beaker for equivalent genetic distribution across treatment jars.

Density of larval cultures were assessed, and approximately 600 larvae were added to each of 27 experimental jars, which were placed in a sea table maintained at 14°C. Larvae were allowed to acclimate for 2 days (6 dpf) until complex gut formation, at which point initial feeding and contaminant exposure began (Fig. 1). Larvae were gradually thinned over 2 days through daily water changes to reach a total of 500 larvae/jar (1 larva/mL) at 8 dpf to establish exact counts in each jar.

2.2 General Larval Care

Larvae were maintained at 14 °C in glass jars filled with 500 mL filtered seawater, distributed on a sea table with circulating water; each jar experienced constant stirring using a motor driven stirring apparatus with plexiglass paddles (Strathmann, 2014; Fig. A1). Full water changes were conducted every other day using forward filtering (see Hodin et al., 2019) with mesh filters (77 μm until 14 dpf, then 118 μm until end of experiment) to avoid bacterial and waste buildup in experimental jars, and to maintain consistent food levels. Jars were randomly assigned to treatments and after each water change, the location of each jar on the stir rack was changed within treatments to account for any variation in paddle shape, stirring, and light exposure. Larvae were initially cultured at a concentration of 1 larva/mL until 23 dpf when they were thinned to 1 larva/2 mL, and then again at 28 dpf to 1 larva/4 mL to avoid crowding stress

throughout development (Fig. 1). Contaminant dosing and feeding were administered after each water change. Larvae were fed *Dunaliella tertiolecta* and *Rhodomonas sp.* at 3000 cells/mL and 2500 cells/mL, respectively.

2.3 Contaminant Exposures

2.3a Microfiber Preparation

Microfibers were prepared by cutting a used neon-green polyester hoodie into ≤5 mm strips using fabric shears and then pulsing the strips using an immersion blender in a glass container (Erdle 2022). All materials were first thoroughly rinsed with reverse osmosis water to avoid additional fiber contamination. Fiber length was confirmed using a Nikon Eclipse 50i compound microscope, resulting in fibers of lengths <5 mm (average 2,804.3 µm). The length of microfibers dosed in this experiment were chosen based on environmental detections (Gago et al., 2018) and were cut to the smallest size possible with available equipment.

Fibers were weathered in natural seawater (filtered to 5 µm) using ultraviolet (UV, A and B) exposure and abrasion from a glass stir bar for one week prior to exposure. The UV B lamp was placed 14 cm above the beaker and UV A lamps were placed 7 cm from opposite sides of the beaker. The stirrer was set to 100 revolutions per minute and leachate water was replaced on days three and six. During leachate water changes, fibers were filtered using a clean 200 µm filter and filtered seawater. A dose of 25 microfibers/L was chosen based on environmental detections (Barrows et al., 2018; Gago et al., 2018). Before each water change, microfibers were measured out and added to a clean glass test tube where they were mixed thoroughly in seawater via agitation before being added to the jars to ensure the fibers would not remain suspended in the surface tension of the water. Microfibers were dosed after each water change to ensure a consistent concentration.

2.3b Pesticide Dosing

A concentration of 10 ng/L imidacloprid (PESTANAL, 98% purity; Sigma Aldrich) was chosen based on environmentally realistic detections in the State of Washington by the Washington State Department of Agriculture (WSDA) at tidally influenced sites (Noland et al., 2022). A fresh stock solution was prepared weekly via serial dilutions using fresh seawater to achieve nominal concentrations (listed in Table 1). Jars were dosed individually with 1 mL of concentrated solution until the first thinning event (23 dpf), when the total volume of water varied amongst jars and therefore bulk 2 L stock solutions were created at each water change. Jars were dosed at each water change to maintain pesticide concentrations. Additionally, a separate sample of polyester microfibers was spiked with imidacloprid to quantify any absorbed compound.

2.4 Sampling

Larval counts and sampling for photograph analysis were conducted weekly. For sampling, each jar was individually pulled from the stir rack and slightly agitated to more evenly distribute larvae in the water column. Using a glass turkey baster, larvae were removed from the jar and placed into a small glass bowl where 25 were haphazardly selected. The microscope lenses were adjusted out of focus to avoid selection bias. Ten larvae were haphazardly selected for live photographs of larval length measurements, and 15 were selected and immediately fixed. Live samples were then collected and frozen for pesticide analysis, whereas fixed samples were analyzed for ingested microplastic compounds.

Due to limited space on the water table and to avoid pesticide cross-contamination during water changes, the control treatments were filtered and sampled prior to the microfiber treatments. The control and microfiber jars were then kept on a separate sea table while the

imidacloprid and imidacloprid/microfiber treatments were filtered. This method was changed after 32 dpf as the number of jars were reduced and the separate sea table was not temperature controlled, therefore the larvae from the controls and microfiber treatment were being held at approximately 8 °C for about 6–8 hours without food during each water change day, which likely slowed their development.

2.4a Photography and Measurements

Until 34 dpf, photographs were taken on a Nikon Eclipse 50i compound microscope using a 3-megapixel color mount microscope camera from AmScope. Measurements were taken using ImageJ (151-J8), calibrated at 4x and 10x. After 34 dpf, photographs were taken using an Olympus BH-2 compound scope to allow for light polarization to observe skeletal development during brachiolar stages. Ingested microfibers were analyzed using a ZEISS Axio Observer inverted microscope (Carl Zeiss, White Plains, NY) as per Siddiqui et al. (2022).

2.4b Developmental Scoring

At weeks two and three, the presence or absence of a fully fused anterior coelom was scored in sampled larvae (Fig. 2). During week six sampling, brachiolar scoring was conducted following criteria by Hodin et al. (2021) to rank development of the adhesive disk, brachiolar arms, radial canals, and skeletal plates (Fig. 2).

2.4c Pesticide Confirmation

Organic contaminant (pesticide) analysis was completed at the U.S. Geological Survey Organic Chemistry Research Laboratory in Sacramento, California. The water samples (0.150 to 0.200 L) were concentrated via solid phase extraction and then analyzed using both gas and liquid chromatography with tandem mass spectrometry for 183 pesticides including imidacloprid (Gross et al. 2024). Imidacloprid detection levels were approximately 2.5 ng/L. Sea star samples

were dried and extracted using acetonitrile (Black et al, 2023), no additional matrix removal was needed, and liquid chromatography tandem mass spectrometry was used for analysis (Gross et al., 2024). Detection levels for 0.02 g samples were approximately 25 ng/g for imidacloprid. Laboratory quality assurance and quality control included the addition of imidacloprid-d_4 to each sample prior to extractions (recoveries were within the acceptable range of 70–130%) and each batch (10 samples) had at least one laboratory blank, and one laboratory replicate if there was sufficient sample mass.

2.4d Microfiber Confirmation

Baseline dosing samples and pre-water change samples were collected for microfiber analysis in week three. Samples were immediately frozen until their analysis at Portland State University. Each sample was filtered through a 5 μm polycarbonate filter (Isopore) using a vacuum pump system in a hood with air filtration. Filters were analyzed under a ZEISS Primostar 3 dissecting microscope for fiber counts. A snorkel hood and air fall filters were deployed during microscope observations and pink cotton clothing was worn to quantify contamination from air deposition and the researcher. Fixed larval samples were analyzed under a ZEISS microscope with a polarized lens at Oregon State University for ingested microplastic particles.

Although the lead researcher wore cotton clothing during the experiment, the laboratory was shared and open to other researchers and students, which may have introduced contamination to the jars (Table 2, A1).

2.5 Settlement Experiment

An experiment to observe the effects of imidacloprid and two settlement cues on larval settlement success was attempted with larvae from the previously described culture. However,

due to an unknown issue, the larvae did not settle as expected at 48 dpf despite appearing competent (Hodin et al. 2021). Therefore, an additional settlement experiment was conducted using larvae from a contemporaneous culture of the same fertilization that were raised at 11 °C until 55 dpf. The chosen cues were adult *P. helianthoides* or diatom biofilm and were paired with either control or imidacloprid dosed water. Glass jars (240 mL) for the adult biofilm treatment were placed in a flow-through tank at ambient temperature with adult *P. helianthoides* for one week prior to the experiment. Diatom film (a 50:50 combination of *Navicula salinicola* and *Nitzschia frustulum*) was grown for 48 hours under fluorescent lighting at room temperature (approx. 18 °C) with a modified f/2 culture medium that supports diatom growth (see appendix for details). Prior to the experiment, all jars were lightly sprayed with filtered seawater at each respective cultured temperature to rinse off any non-adherent organisms or particles without disrupting the biofilm, and in the case of the diatom film, to rinse off unadhered diatoms and remove all culture medium. The jars were then allowed to acclimatize to the 14 °C water table for 4 hours prior to the experiment. During acclimatization, jars were filled to 150 mL with clean or imidacloprid dosed filtered seawater in each perspective treatment (Fig. 6, A2). The articulated coralline alga, *Calliarthron tuberculosum*, as per Hodin et al. (2021), was harvested and cleaned to remove any potential organisms, then patted dry and weighed to reach a ratio of 0.1 g *C. tuberculosum:* to 8 mL seawater in each jar (Fig. A2).

Larvae for the experiment were chosen based on the presence of brachiolar structures: fully fused skeletal spicules, formed brachiolar arms with papillae, and an adhesive disk (see Hodin et al., 2021). Larvae that passed the criteria were then placed into one beaker and haphazardly distributed into the dosed jars (10 larvae per jar). After 48 hours, larvae in each jar were observed and settlement position was annotated as *unattached larva, attached larva,*

settling juvenile, or *settled juvenile* (Fig. 6). Note that whereas attachment is reversible, once larvae reach the "settling juvenile" stage they are committed to completing their transformation into a juvenile (Hodin et al., 2021).

2.6 Statistical Analysis

Differences in larval survivorship, length, and coelom presence were analyzed using a generalized linear model (GLM; Bolker et al., 2009). Replicate and time period were set as random effects in each model, with each treatment as a fixed effect. The survivorship and coelom models were assessed with a binomial family, whereas the lengths were assessed with a gamma family. The settlement experiment was analyzed using a GLM with a Poisson family, where the total amount of settled larvae, pesticide treatment, settlement phase, and biofilm type were set as fixed effects, and replicate as a random effect. Statistical significance was established as $p \leq 0.05$. All analyses were conducted using R software (version 2023.06.1 +524; Horton & Kleinman, 2015).

3. Results

3.1 Survivorship

The imidacloprid treatment (10 ng/L) resulted in significantly lower survivorship than the control and microfiber (50 microfibers/L) treatments at week two, but higher survivorship at weeks three and four ($p<0.001$, Fig. 3). Both the microfiber and imidacloprid + microfiber combined treatment resulted in higher survivorship than the control at week 4 ($p<0.001$, Fig. 3). Imidacloprid and microfibers together in the combined treatment resulted in an overall increase in survivorship compared to their individual treatments ($p<0.001$, Fig. 3).

3.2 Length

All treatments resulted in increased larval lengths compared to the control ($p<0.001$, Fig. 3). During week one, larvae in the microfiber treatment were longer than all treatments and the control; however, during weeks three and four, larvae in the combined imidacloprid + microfiber treatment were the longest ($p<0.001$). At weeks five and six larvae in the imidacloprid treatment were longer than all other treatments and the control ($p<0.001$, Fig. 3). Imidacloprid and microfibers in combination had a positive effect on larval lengths in comparison to each individual treatment, with the exception of week six ($p<0.001$, Fig. 3)

3.3 Ingested Contaminants

Neither imidacloprid nor microfibers were detected in sampled larvae. Examined stomachs did not contain synthetic microparticles visible at 100x magnification (Fig. A3).

3.4 Development

Coelom and brachiolar development did not differ across treatments (Fig. 4). However, stomach malformations were observed in 10% of the larvae in the imidacloprid treatment at week two, and in 10% of the larvae in the imidacloprid treatment and 5% of the larvae in the imidacloprid/microfiber combination treatment at week four (Fig. 5).

3.5 Pesticide and Microfiber Detections

Pesticide concentrations are outlined in Table 1. There was no pesticide cross-contamination detected in any jars. Average microfiber concentrations are outlined in Table 2, all detected microparticles with color descriptions are included in Table A1. Average microfiber contamination across all treatments was 6.7 fibers/L. Imidacloprid was not detected in the imidacloprid-spiked microfibers.

3.6 Separate Settlement Experiment

In the adult biofilm treatment, the number of larvae that reached settlement was higher in the presence of imidacloprid compared to the control ($p<0.01$, Fig. 6). Overall, larval settlement was significantly lower in the diatom treatment compared to the adult biofilm treatment, regardless of pesticide dosing ($p<0.001$, Fig. 6).

4. Discussion

This study is the first to quantify effects of contaminant stressors on the larvae of *Pycnopodia helianthoides*. Both lethal and sublethal effects from exposure to environmentally relevant concentrations of a neonicotinoid insecticide, imidacloprid, and polyester microfibers (<5 mm length) were observed. Given the unknown risk of these contaminant stressors to *Pycnopodia,* understanding these stressors is necessary to ensure that *Pycnopodia* cultured in a clean laboratory are able to thrive when released into their natural ecosystem. These findings indicate that *Pycnopodia* larvae can be sensitive to imidacloprid at early developmental stages as well as during settlement, and that both contaminants can affect larval length throughout development.

4.1 Survivorship

The decrease in early life-stage survivorship paired with the malformed stomach structures observed in the imidacloprid exposure treatments at week two indicate that the pesticide may be toxic to Pycnopodia larvae during gut formation. Whereas this study was not designed to identify the specific mechanisms responsible for these observed effects, other studies have observed disruption in cellular activity during larval metamorphosis in both target and non-target insects that resulted in malformation of the midgut (Carneiro et al., 2023; Fernandes et al., 2015; Yasmeen & Amir, 2023). Fernandes et al. (2015) observed malformation of the midgut in a targeted larval mosquito (*Aedes aegypti*) from interference in cell regeneration, and similarly,

Carneiro et al. (2023) observed changes in the midgut of non-targeted larval honeybees (*Apis mellifera*) from increased cell death. In a targeted larval fly (*Chrysomya megacephala* [Fabricius, 1794]), the midgut was also impacted after imidacloprid exposure, caused by modifications in muscle layers and membrane as well as a reduction in proteins and carbohydrates compared to the control (Yasmeen & Amir, 2023). Furthermore, imidacloprid is an acetylcholinesterase (AChE) inhibitor and AChE activity has previously been measured in the pyloric caeca of adult common starfish *Asterias rubens* (Den Besten et al., 2001), in the coelomocytes of adult sea urchins (*Paracentrotus lividus*; Angelini et al., 2003), and in morphogenetic cells in urchin plutei (Pesando et al., 2003), indicating that AChE activity may occur in larval sea star digestive cells during development. Further research is necessary to support this hypothesis. Additionally, given the observed reduction in AChE in adult Pycnopodia affected by SSWS (Fuess et al., 2015), AChE-inhibiting contaminants like imidacloprid need further investigation.

The increases in survivorship that were observed in weeks three and four in all contaminant treatments compared to the control may be explained by shifts in larval densities and algal availability in each treatment. Algal concentrations were calculated based on water volume in each jar, which was not adjusted weekly until the first thinning event after week two. In the case of imidacloprid, the decrease in survivorship during week two with no alteration in algal concentrations meant there were more algal cells available per larva, potentially contributing to the boost in survivorship at week three (Fig. A5). Additionally, the act of thinning itself may have had an effect on survivorship at those timesteps. *Pycnopodia* larvae have been known to clone via fission in response to disturbance from thinning (pers. comm., J. Hodin); therefore, it is possible that the thinning event caused stress to larvae in each treatment. This stress, however, may have been disproportionate across treatments, causing increases in

survivorship for the contaminant treatments. At the start of the experiment, each jar had the same larval density and during the thinning events, larvae were removed from each jar to standardize the density of larvae/mL, accommodating for lower density as they grew bigger. However, due to unequal mortalities in the first two weeks (Fig. 3), the densities leading up to the thinning events became uneven as the total number of larvae decreased in the imidacloprid and combination treatments. Thus in the controls and microfiber treatment, more larvae were being removed and therefore the change in density was much more sizable than the imidacloprid and combined treatments that had lower early survivorship (Fig. A4). While necessary for the long-term health of the larval culture, these thinning events may have had a temporary effect on the survivorship.

4.2 Development

Larval length was consistently longer in contaminant treatments compared to the controls throughout the six-week study (Fig. 3). Similarly, other studies have found that urchin larvae exposed to pesticides develop much more quickly or grow larger/longer than the control organisms (Aluigi et al., 2010; Sanhueza et al., 2018). Rendleman et al. (2018) observed that low-fed larvae were significantly longer compared to high-fed larvae and experienced a decrease in respiration and ingestion rates. Although these variables were outside the scope of this study, future studies would benefit from the quantification of respiration and ingestion measurements. Additionally, the larval length from the combined treatment appeared to be more similar to that of the imidacloprid treatment than that of microfibers until week four and onward, when it was instead more similar to the microfiber treatment (Fig. 3). This may be indicative of differences in effects from the contaminants depending on the larval development stage.

The ontogeny of developmental features, such as coeloms and brachiolar structures, were not significantly affected by contaminant treatment, though it is important to note that the larvae in our study were exposed after their embryonic stage (6 dpf). Other studies that exposed organisms to contaminants in the embryonic stage have found effects on urchin skeletal formation (Aluigi et al., 2010; Pesando et al., 2003). Pesando et al. (2003) observed the inhibition of skeletal formation in larval sea urchins after organophosphate and carbamate pesticide exposure during larval development at the pluteus stage. In another study, exposure to chlorpyrifos during late-stage urchin development resulted in the eventual re-absorption of rudiment structures and death of juvenile urchins (Aluigi et al., 2010). The lack of an observed effect on the brachiolar structures may be due to exposure during post-embryonic development or limited replication.

The limited effect from microfiber exposure in this study may be related to the microfiber size in relation to the study organism. Several larvae were observed with microfibers stuck near their mouths (Fig. 5, fibers were declared stuck after multiple attempts to remove the fiber from the larva with a pipette failed), but they appeared too long to fully ingest. We were unable to track these larvae over time due to the size of the cultures, therefore a side experiment was attempted to observe their development, but this was unsuccessful. Given that microparticles detected in marine environments range greatly in size (Barrows et al., 2018), microfibers smaller than those used in this study are present in marine ecosystems and may be swallowed or cause physical damage to feeding *Pycnopodia* larvae in the wild. Additionally, though the chosen pesticide is a hydrophilic compound and thus did not bind to the microfibers, numerous chemicals do bind to microplastics in the environment and may cause toxicity if ingested at this early life stage (Andrady, 2011; Barboza & Gimenez, 2015; Wang et al., 2024).

Larvae in the combined imidacloprid + microfiber treatment were observed attempting to settle sooner than in the other treatments. These larvae were observed sticking to or settling directly onto the microfibers and could not be removed with a pipette, indicating early settlement. This was not observed in the microfiber treatment alone, indicating an interaction between imidacloprid and the fibers that promoted settlement. This is consistent with previous findings from the Hodin lab that stressed larvae settled earlier and attempted to settle on fibers (Hodin, unpublished data). This may indicate a lack of other materials to settle on as would be found in the wild; therefore further experimentation on settlement preferences between microfibers and other substrate types is needed to explore this hypothesis.

In the separate settlement experiment, imidacloprid exposure resulted in increased settlement when adult biofilm was present. This observation is similar to a previous study, in which the settlement of purple sea urchin larvae (*Strongylocentrotus purpuratus*) was significantly higher upon exposure to a chemical musk compared to control larvae (Hodin 2006). In the current study, imidacloprid only affected settlement in the presence of adult biofilm, whereas both imidacloprid and the control larvae experienced similarly low settlement percentages with the diatom film. The mechanisms responsible for these differences were outside the scope of this study and future research is needed to understand and quantify direct effects from imidacloprid exposure to *Pycnopodia* settlement.

Traditional toxicity testing favors organisms that are representative of multiple ecosystems, more reliable to culture, able to be cultured year-round, and do not require extensive care (Bay et al., 1993; US EPA, 2017). Thus, echinoderm toxicology studies classically focus on sea urchins and sand dollars (ex. *Strongylocentrotus purpuratus*, *Arbacia punctulata*, *Lytechinus pictus*, and *Dendraster excentricus*) as reliable test organisms (Bay et al., 1993). Whereas these

organisms yield more efficient studies, and their results can often be expanded to a number of echinoderms, many ecologically important species go unstudied. In the case of *Pycnopodia helianthoides*, its heightened susceptibility to wasting syndrome, as well as observed differences in bacterial biome (McCracken et al., 2023), sets it apart from fellow asteroid species and necessitates species-specific studies to understand the effects of contaminant stressors. *Pycnopodia* larvae are highly variable, so the level of replication needed to reach statistical power strongly limits the range of experimentation that is feasible. Therefore, toxicity information about *Pycnopodia* does not currently exist, leaving a complete gap in our understanding of which contaminants affect this species and to what degree.

The results of this study lay the groundwork for future studies on the sensitivity of *Pycnopodia* to contaminant stressors and whether contaminant sensitivity is limiting the recovery of the species. As the full suite of drivers of SSWS is still unknown, including contaminant stressors in assessments of factors affecting recovery and in identification of ideal habitat for reintroduction could improve our understanding. *Pycnopodia* larvae experienced sub-lethal effects from imidacloprid at 10 ng/L. Currently there is no EPA aquatic life benchmark for chronic exposure of marine invertebrates to imidacloprid; however, the dosed concentration in this study is equal to the current benchmark for chronic exposure to freshwater invertebrates (US EPA, 2017). Although environmentally relevant, this concentration is often lower than peak levels of exposure detected in waters of the western United States. In the State of Washington, for example, the highest detected imidacloprid concentration was 90 ng/L at a tidally influenced site (Noland et al., 2022) and detections along the West Coast frequently exceed 10 ng/L (Heberger et al. 2020). The observation that imidacloprid has a similar effect on a non-target organism as its intended effect on target species has implications for the management of these

pesticides and their uses, supporting the case for more meticulous analysis of their effects in the environment. Furthermore, marine organisms are exposed to diverse contaminant and environmental stressors that make it difficult to quantify the full range of stressor effects these organisms experience. Although difficult to culture under experimental conditions, toxicity testing of *Pycnopodia* and other non-typical test organisms is critical to understand the threats these contaminants pose to a wide suite of species that are ecologically, culturally, and economically important.

Acknowledgements: We thank G. Lambert and the Charles Lambert Memorial Endowment for providing the funding for this project, and to everyone at Friday Harbor Laboratories for their moral and technical support. A special thank you to P. Combs for their help with shipping and acquiring materials and J. Ullmann for shop guidance. Thank you to K. Brown, K. Plummer, and B. Carlson for their invaluable aid, and to A. Vigue, M. Connor, L. Waite, J. Waite for taking care of Nova. Thank you to the Brander and Harper labs at OSU for use of the fluorescent microscope and training, the Applied Coastal Ecology Lab and A. Kidd for feedback, and W. Lhamo and V. Partida for their help measuring larval photos. Thank you to our reviewers for their valuable feedback and advice. We greatly appreciate the lovely folks at King's Market for the late-night food runs, and J.Á. Osorio Balvín and G. Way for keeping us moving.

Disclaimer: Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Funding Statement: Funding provided by the Charles Lambert Memorial Endowment through the University of Washington.

Ethics Statement: IACUC approval was not required for this study as sea stars are not included in IACUC standards.

Conflict of Interest Statement: None.

Data Availability Statement: Data are available at https://github.com/xandratissot/pycnopodia

Author Contribution Statement: Alexandra G. Tissot: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration, Funding Acquisition **Elise F. Granek**: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition **Fiona Curliss**: Methodology, Investigation, Resources, Writing – Review & Editing **Augustin Kalytiak-Davis**: Methodology, Investigation, Resources, Writing – Review & Editing **Jason Hodin**: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding Acquisition **Michelle L. Hladik**: Methodology, Resources, Writing – Review & Editing

Figure 1. Experiment timeline, first row of numbers indicates days post fertilization (dpf). Downward triangles represent thinning days. In the second row, F represents fertilization day and stars are aligned with their corresponding larva image to represent sample week. The dashed lines connect each star to its weekly sampling day in the dpf timeline. The accompanying images show the larval morphology each week, starting with a fertilized egg and ending with brachiolaria. Scale bars for weeks 0–3 mark 100 µm and, bars for weeks 3–6 mark 500 µm.

Figure 2. Larval development metrics, showing bipinnaria larvae under 4x magnification with A) an unfused coelom and, B) an anteriorly fused coelom. Brachiolar metrics shown at 10x magnification are C) radial canals, D) skeletal rudiment formation, E1) brachiolar arms, and E2) adhesive disk.

Figure 3. Line plots of survivorship (A-E) and box plots of larval length at each sampling week (F). A) total counts per treatment and (B-E) non-cumulative larval survivorship by week

throughout the experiment. Included are B) all of the treatments together with dashed lines representing thinning events, C) the microfiber treatment and the control, D) the imidacloprid treatment and the control, and E) the combined imidacloprid + microfiber treatment and the control. Imid + Mf refers to the imidacloprid and microfiber combined treatment. Error bars indicate standard error, dots represent outliers. Microfiber doses were 15 fibers/L, imidacloprid doses were 10 ng/L. F) Larval length at each sampling week, by treatment. Box plots represent distribution of data with thick black lines representing median values and upper and lower quartiles forming the box. Lines outside of the box represent the range of "normal" lowest and highest values with outliers represented by black dots. Week 1 was omitted due to high sample error.

Figure 4. Developmental measurements of A) percent of larvae with anteriorly fused coelom and B) brachiolar development scoring by treatment. Box plots represent distribution of data with thick black lines representing median values and upper and lower quartiles forming the box. Lines outside of the box represent the range of lowest and highest values for 95% of the measurements with outliers represented by black dots. Boxes missing upper or lower quartile are indicative of median being identical to upper or lower quartile, respectively. Note: C= control, MF= microfibers, IMI= imidacloprid, and IMF and Imid + MF = imidacloprid + microfibers.

Figure 5. Images of (A) larvae with contorted gut structures from the imidacloprid treatment at week 2 versus (B) a control larva, (C) the combined imidacloprid + microplastic treatment at week 4 versus (D) a control larva, and E) larvae with microfibers firmly lodged in their bodies.

Figure 6. Settlement experiment schematics and results. A) Schematic of the settlement experiment treatments, diatom control (DC), diatom imidacloprid (DI), adult control (AC), and adult imidacloprid (AI), each with three replicates. "Adult" refers to adult biofilm. All jars also

contained *C. tuberculosum* at 0.1 g per 8 mL volume. B-D) Settlement stage scoring criteria for the settlement experiment, B) attached, C) settling, and D) settled. E) Larval counts in each settlement stage, separated by biofilm type and adult versus diatom. Box plots represent distribution of data with thick black lines representing median values and upper and lower quartiles forming the box. Lines outside of the box represent the range of lowest and highest values for 95% of the measurements. Boxes missing upper or lower quartile are indicative of median being identical to upper or lower quartile, respectively.

References

Aalto, E.A., Lafferty, K.D., Sokolow, S.H. Grewelle, R.E., Ben-Horin, T., Boch, C.A., Raimondi, P.T., Bograd, S.J., Hazen, E.L., Jacox, M.G., Micheli, F., De Leo, G.A. (2020). Models with environmental drivers offer a plausible mechanism for the rapid spread of infectious disease outbreaks in marine organisms. *Scientific Reports,* 10, 5975. https://doi.org/10.1038/s41598-020-62118-4

Altenburger, R., Scholze, M., Busch, W., Escher, B. I., Jakobs, G., Krauss, M., Krüger, J., Neale, P. A., Ait-Aissa, S., Almeida, A. C., Seiler, T.-B., Brion, F., Hilscherová, K., Hollert, H., Novák, J., Schlichting, R., Serra, H., Shao, Y., Tindall, A., … Kortenkamp, A. (2018). Mixture effects in samples of multiple contaminants – An inter-laboratory study with manifold bioassays. *Environment International*, *114*, 95–106. https://doi.org/10.1016/j.envint.2018.02.013

Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, *341*(6145), 514–519. https://doi.org/10.1126/science.1239401

Aluigi, M. G., Falugi, C., Mugno, M. G., Privitera, D., & Chiantore, M. (2010). Dose-dependent effects of chlorpyriphos, an organophosphate pesticide, on metamorphosis of the sea urchin,

Paracentrotus lividus. *Ecotoxicology*, *19*(3), 520–529. https://doi.org/10.1007/s10646-009-0433-z

Álvarez-Muñoz, D., Llorca, M., Blasco, J., & Barceló, D. (2016). Contaminants in the Marine Environment. In *Marine Ecotoxicology* (pp. 1–34). Elsevier. https://doi.org/10.1016/B978-0-12-803371-5.00001-1

Andrady, A.L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62(8), 1596-1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Angelini, C., Amaroli, A., Falugi, C., Di Bella, G., & Matranga, V. (2003). Acetylcholinesterase activity is affected by stress conditions in Paracentrotus lividus coelomocytes. *Marine Biology*, *143*(4), 623–628. https://doi.org/10.1007/s00227-003-1120-x

Athey, S. N., Carney Almroth, B., Granek, E. F., Hurst, P., Tissot, A. G., & Weis, J. S. (2022). Unraveling Physical and Chemical Effects of Textile Microfibers. *Water*, *14*(23), 3797. https://doi.org/10.3390/w14233797

Baechler, B. R., Granek, E. F., Hunter, M. V., & Conn, K. E. (2020). Microplastic concentrations in two Oregon bivalve species: Spatial, temporal, and species variability. *Limnology and Oceanography Letters*, *5*(1), 54–65. https://doi.org/10.1002/lol2.10124

Barboza, L.G.A. & Gimenez, B.C.G. (2015). Microplastics in the marine environment: current trends and future perspectives. *Marine Pollution Bulletin*, 97(1-2), 5-12. https://doi.org/10.1016/j.marpolbul.2015.06.008

Barrows, A. P. W., Cathey, S. E., & Petersen, C. W. (2018). Marine environment microfiber contamination: Global patterns and the diversity of microparticle origins. *Environmental Pollution*, *237*, 275–284. https://doi.org/10.1016/j.envpol.2018.02.062

Batikian, C. M., Lu, A., Watanabe, K., Pitt, J., & Gersberg, R. M. (2019). Temporal pattern in levels of the neonicotinoid insecticide, imidacloprid, in an urban stream. *Chemosphere*, *223*, 83–90. https://doi.org/10.1016/j.chemosphere.2019.01.165

Bay, S., Burgess, R., & Nacci, D. (1993). Status and applications of echinoid (Phylum Echinodermata) toxicity test methods. ASTM SPECIAL TECHNICAL PUBLICATION, 1179, 281-281.

Black, G. P., Woodward, E. E., Sanders, C. J., Gross, M. S., & Hladik, M. L. (2023). Multiresidue extraction of current-use pesticides from complex solid matrices using energized dispersive guided extraction with analysis by gas and liquid chromatography tandem mass spectroscopy. Chemosphere, 327, 138550.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Borsuah, J. F., Messer, T. L., Snow, D. D., Comfort, S. D., & Mittelstet, A. R. (2020). Literature Review: Global Neonicotinoid Insecticide Occurrence in Aquatic Environments. *Water*, *12*(12), 3388. https://doi.org/10.3390/w12123388

Burge, C. A., Mark Eakin, C., Friedman, C. S., Froelich, B., Hershberger, P. K., Hofmann, E. E., Petes, L. E., Prager, K. C., Weil, E., Willis, B. L., Ford, S. E., & Harvell, C. D. (2014). Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society. *Annual Review of Marine Science*, *6*(1), 249–277. https://doi.org/10.1146/annurev-marine-010213-135029

Carneiro, L. S., Santos, C. G., Resende, M. T. C. S. D., Souza, D. L. L. D., Souza, D. D. S., Souza, A. M. D. C., Motta, J. V. D. O., Nere, P. H. A., Oliveira, A. H. D., & Serrão, J. E. (2023). Effects of the insecticide imidacloprid on the post-embryonic development of the honey bee Apis mellifera (Hymenoptera: Apidae). *Science of The Total Environment*, *905*, 167278. https://doi.org/10.1016/j.scitotenv.2023.167278

Dawson, M.N., Duffin, P.J., Giakoumis, M., Schiebelhut, L.M., Beas-Luna, R., Bosley, K.L., Castilho, R., Ewers=Saucedo, C., Gavenus, K.A., Keller, A., Konar, B., Largier, J.L., Lorda, J., Miner, C.M., Moritsch, M.M., Navarrete, S.A., Tragier, S.B., Turner, M.S., & Wares, J.P. (2023). A Decade of Death and Other Dynamics: Deepening Perspectives on the Diversity and Distribution of Sea Stars and Wasting. *The Biological Bulletin* 244(3), 143-163. https://doi.org/10.1086/727969

Den Besten, P. J., Valk, S., Van Weerlee, E., Nolting, R. F., Postma, J. F., & Everaarts, J. M. (2001). Bioaccumulation and biomarkers in the sea star Asterias rubens (Echinodermata: Asteroidea): a North Sea field study. *Marine Environmental Research*, *51*(4), 365–387. https://doi.org/10.1016/S0141-1136(00)00134-3

Erdle, L. (2022). Microfibers in a freshwater ecosystem: Sources, fate, effects, and mitigation (Doctoral dissertation, University of Toronto (Canada)).

Fernandes, K. M., Gonzaga, W. G., Pascini, T. V., Miranda, F. R., Tomé, H. V. V., Serrão, J. E., & Martins, G. F. (2015). Imidacloprid impairs the post-embryonic development of the midgut in the yellow fever mosquito *S tegomyia aegypti* (= *A edes aegypti*). *Medical and Veterinary Entomology*, *29*(3), 245–254. https://doi.org/10.1111/mve.12122

Fuess, L. E., Eisenlord, M. E., Closek, C. J., Tracy, A. M., Mauntz, R., Gignoux-Wolfsohn, S., Moritsch, M. M., Yoshioka, R., Burge, C. A., Harvell, C. D., Friedman, C. S., Hewson, I.,

Hershberger, P. K., & Roberts, S. B. (2015). Up in Arms: Immune and Nervous System Response to Sea Star Wasting Disease. *PLOS ONE*, *10*(7), e0133053. https://doi.org/10.1371/journal.pone.0133053

Gago, J., Carretero, O., Filgueiras, A. V., & Viñas, L. (2018). Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. *Marine Pollution Bulletin*, *127*, 365–376. https://doi.org/10.1016/j.marpolbul.2017.11.070

Gissi, E., Manea, E., Mazaris, A. D., Fraschetti, S., Almpanidou, V., Bevilacqua, S., Coll, M., Guarnieri, G., Lloret-Lloret, E., Pascual, M., Petza, D., Rilov, G., Schonwald, M., Stelzenmüller, V., & Katsanevakis, S. (2021). A review of the combined effects of climate change and other local human stressors on the marine environment. *Science of The Total Environment*, *755*, 142564. https://doi.org/10.1016/j.scitotenv.2020.142564

Gravem, S. A., Heady, W. N., Saccomanno, V. R., Alvstad, K. F., Gehman, A. L. M., Frierson, T. N., & Hamilton S.L. (2021). Pycnopodia helianthoides. IUCN Red List of Threatened Species.

Gross, M.S., Sanders, C.J., De Parsia, M.D., and Hladik, M.L., 2024, Methods of analysis—Determination of pesticides in filtered water and suspended sediment using liquid chromatography- and gas chromatography-tandem mass spectrometry: U.S. Geological Survey Techniques and Methods, book 5, chap. A12, 33 p., https://doi.org/10.3133/tm5A12

Harvell, C. D., Montecino-Latorre, D., Caldwell, J. M., Burt, J. M., Bosley, K., Keller, A., Heron, S. F., Salomon, A. K., Lee, L., Pontier, O., Pattengill-Semmens, C., & Gaydos, J. K. (2019). Disease epidemic and a marine heat wave are associated with the continental-scale collapse of a pivotal predator (*Pycnopodia helianthoides*). *Science Advances*, *5*(1), eaau7042. https://doi.org/10.1126/sciadv.aau7042

Heberger, M., Sutton, R., Buzby, N., Sun, J., Lin, D., Mendez, M., Hladik, M., Orlando, J., Sanders, C. & Furlong, E.T. (2020). Current-use pesticides, fragrance ingredients, and other emerging contaminants in San Francisco Bay margin sediment and water. San Francisco Estuary Institute. Retrieved June, 15, 2020.

Hewitt, J. E., Ellis, J. I., & Thrush, S. F. (2016). Multiple stressors, nonlinear effects and the implications of climate change impacts on marine coastal ecosystems. *Global Change Biology*, *22*(8), 2665–2675. https://doi.org/10.1111/gcb.13176

Hodin, J. (2006). Expanding networks: Signaling components in and a hypothesis for the evolution of metamorphosis. *Integrative and Comparative Biology*, *46*(6), 719–742. https://doi.org/10.1093/icb/icl038

Hodin, J., Heyland, A., Mercier, A., Pernet, B., Cohen, D. L., Hamel, J.-F., Allen, J. D., McAlister, J. S., Byrne, M., Cisternas, P., & George, S. B. (2019). Culturing echinoderm larvae through metamorphosis. In *Methods in Cell Biology* (Vol. 150, pp. 125–169). Elsevier. https://doi.org/10.1016/bs.mcb.2018.11.004

Hodin, J., Pearson-Lund, A., Anteau, F. P., Kitaeff, P., & Cefalu, S. (2021). Progress Toward Complete Life-Cycle Culturing of the Endangered Sunflower Star, *Pycnopodia helianthoides*. *The Biological Bulletin*, *241*(3), 243–258. https://doi.org/10.1086/716552

Horn, D., Miller, M., Anderson, S., & Steele, C. (2019). Microplastics are ubiquitous on California beaches and enter the coastal food web through consumption by Pacific mole crabs. *Marine Pollution Bulletin*, *139*, 231–237. https://doi.org/10.1016/j.marpolbul.2018.12.039

Horton, N. J., & Kleinman, K. (2015). Using *R* and **RStudio** for Data Management, Statistical Analysis and Graphics (2nd Edition). *Journal of Statistical Software*, *68*(Book Review 4). https://doi.org/10.18637/jss.v068.b04

Katagi, T. (2010). Bioconcentration, Bioaccumulation, and Metabolism of Pesticides in Aquatic Organisms. In D. M. Whitacre (Ed.), *Review of Environmental Contamination and Toxicology Volume 204* (Vol. 204, pp. 1–132). Springer New York. https://doi.org/10.1007/978-1-4419-1440-8_1

Kataoka, C., & Kashiwada, S. (2021). Ecological Risks Due to Immunotoxicological Effects on Aquatic Organisms. *International Journal of Molecular Sciences*, *22*(15), 8305. https://doi.org/10.3390/ijms22158305

Li, J., Shan, E., Zhao, J., Teng, J., & Wang, Q. (2023). The factors influencing the vertical transport of microplastics in marine environment: A review. Science of The Total Environment, 870, 161893.

Luo, T., Weng, Y., Huang, Z., Zhao, Y., & Jin, Y. (2021). Combined hepatotoxicity of imidacloprid and microplastics in adult zebrafish: Endpoints at gene transcription. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 246, 109043.

Macaulay, S. J., Hageman, K. J., Piggott, J. J., Juvigny-Khenafou, N. P. D., & Matthaei, C. D. (2021). Warming and imidacloprid pulses determine macroinvertebrate community dynamics in experimental streams. *Global Change Biology*, *27*(21), 5469–5490. https://doi.org/10.1111/gcb.15856

McCracken, A.R., Christensen, B.M., Munteanu, D., Case, B.K.M., Lloyd, M., Herbert, K.P., Pespeni, M.H. (2023). Microbial dysbiosis precedes signs of sea star wasting disease in wild populations of *Pycnopodia helianthoides*. *Frontiers in Marine Science*, 10, 1130912. https://doi.org/10.3389/fmars.2023.1130912

Mishra, S., Rath, C. C., & Das, A. P. (2019). Marine microfiber pollution: A review on present status and future challenges. *Marine Pollution Bulletin*, *140*, 188–197. https://doi.org/10.1016/j.marpolbul.2019.01.039

Morrissey, C. A., Mineau, P., Devries, J. H., Sanchez-Bayo, F., Liess, M., Cavallaro, M. C., & Liber, K. (2015). Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environment International*, *74*, 291–303. https://doi.org/10.1016/j.envint.2014.10.024

Noland, K., Nickelson, A., Ryan, J., Drennan, M. (2022) Ambient Monitoring for Pesticides in Washington State Surface Water: 2020 Technical Report (102-629 (R/9/22)). Washington State Department of Agriculture. https://agr.wa.gov/forms-and-publications/publications/nras

Pesando, D., Huitorel, P., Dolcini, V., Angelini, C., Guidetti, P., & Falugi, C. (2003). Biological targets of neurotoxic pesticides analysed by alteration of developmental events in the Mediterranean sea urchin, Paracentrotus lividus. *Marine Environmental Research*, *55*(1), 39–57. https://doi.org/10.1016/S0141-1136(02)00215-5

Rendleman, A. J., Rodriguez, J. A., Ohanian, A., & Pace, D. A. (2018). More than morphology: Differences in food ration drive physiological plasticity in echinoid larvae. *Journal of experimental marine biology and ecology*, *501*, 1-15.

Sanhueza, S., Neira, K., Rojas, C., Geneviere, A., & Fernandez, C. (2018). Effects of three pesticides used to control sea lice on the early development of Choromytilus chorus, Sphaerechinus granularis, and Paracentrotus lividus. *Latin American Journal of Aquatic Research*, *46*(5), 969–980. https://doi.org/10.3856/vol46-issue5-fulltext-10

Seiber, J. N., & Kleinschmidt, L. (2010). Environmental transport and fate. In Hayes' Handbook of Pesticide Toxicology (pp. 1219-1227). Academic Press.

Sheets, L. P. (2010). Imidacloprid: a neonicotinoid insecticide. In Hayes' handbook of pesticide toxicology (pp. 2055-2064). Academic Press.

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

Smit, C. E., Posthuma-Doodeman, C. J. A. M., Van Vlaardingen, P. L. A., & De Jong, F. M. W. (2015). Ecotoxicity of Imidacloprid to Aquatic Organisms: Derivation of Water Quality Standards for Peak and Long-Term Exposure. *Human and Ecological Risk Assessment: An International Journal*, *21*(6), 1608–1630. https://doi.org/10.1080/10807039.2014.964071

Strathmann, R. R. (2014). Culturing Larvae of Marine Invertebrates. In D. J. Carroll & S. A. Stricker (Eds.), *Developmental Biology of the Sea Urchin and Other Marine Invertebrates* (Vol. 1128, pp. 1–25). Humana Press. https://doi.org/10.1007/978-1-62703-974-1_1

Tian, Z., Peter, K. T., Gipe, A. D., Zhao, H., Hou, F., Wark, D. A., Khangaonkar, T., Kolodziej, E. P., & James, C. A. (2020). Suspect and Nontarget Screening for Contaminants of Emerging Concern in an Urban Estuary. *Environmental Science & Technology*, *54*(2), 889–901. https://doi.org/10.1021/acs.est.9b06126

Tissot, A. G., Granek, E. F., Thompson, A. W., Hladik, M. L., Moran, P. W., & Scully-Engelmeyer, K. (2022). The silence of the clams: Forestry registered pesticides as multiple stressors on soft-shell clams. *Science of The Total Environment*, *819*, 152053. https://doi.org/10.1016/j.scitotenv.2021.152053

US Environmental Protection Agency; (2017). *Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides.* https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk

Wang, F., Li, S., Peng, L., Wang, F., & Zeng, E. Y. (2024). Sorption of toxic chemicals on microplastics. In Microplastic contamination in aquatic environments (pp. 113-139). Elsevier.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, *178*, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Yasmeen, S., & Amir, M. (2023). Imidacloprid-induced mortality, histopathology and biochemical impairments in the larvae of oriental latrine fly (*Chrysomya megacephala*). *Medical and Veterinary Entomology*, *37*(3), 586–599. https://doi.org/10.1111/mve.12657

Table 1. Average measured imidacloprid (IMI) concentrations (ng/L) in dosed (10 ng/L) jars at sample weeks. MF represents the microfiber treatment and IMF represents the imidacloprid + microfiber treatment. Non-detects indicated by ND, N/A indicates not applicable, "dosed" fibers indicate concentrations of intentionally dosed fibers while "other" indicates unintended contamination.

	Week Sampled			
	0	1	3	6
Control	ND	ND	ND	ND
MF	ND	ND	ND	ND
IMI	11.9	12.4	13.9	13.1
IMF	10.7	11.7	13.6	16.4

Table 2. Average microfiber (MF) concentrations and lengths (22.7 fibers/L and 2804.3 µm, respectively) at sample weeks. IMI represents the imidacloprid treatment and IMF represents the imidacloprid + microfiber treatment. Non-detects indicated by ND, N/A indicates not applicable, "dosed" fibers indicate concentrations of intentionally dosed fibers while "other" indicates unintended contamination.

	Week Sampled							
	0				3			
	Dosed	Length	Other	Length	Dosed	Length	Other	Length
Control	N/A	N/A	5.8	1751.7	N/A	N/A	9.4	3572.3
MF	22.5	2869.5	7.5	3849.9	24.4	3327	10.6	3701
IMI	N/A	N/A	6.1	1669.8	N/A	N/A	6.7	5742.6
IMF	19.4	2823.2	1.1	6099.3	24.4	2197.5	6.1	2852

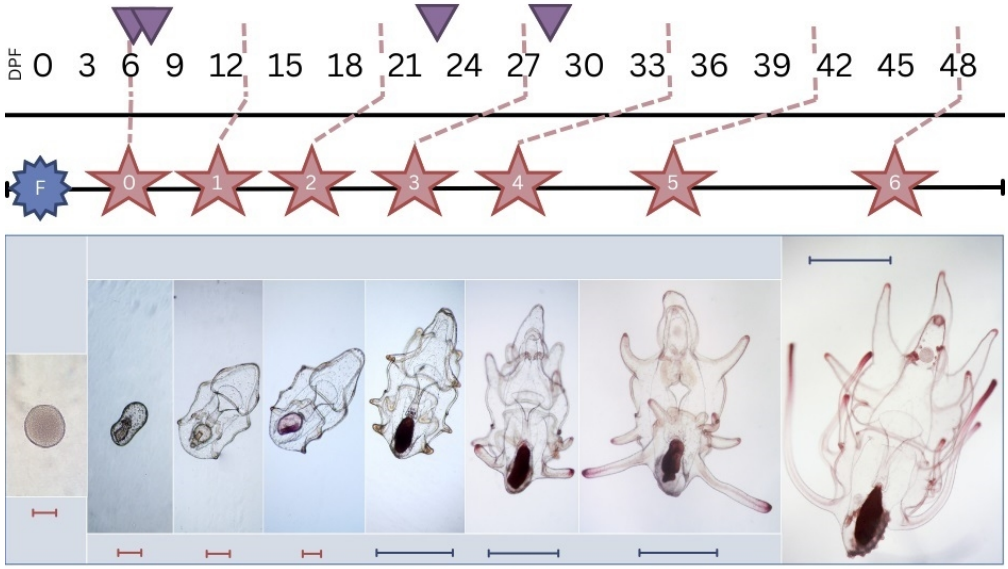

Figure 1

248x145mm (96 x 96 DPI)

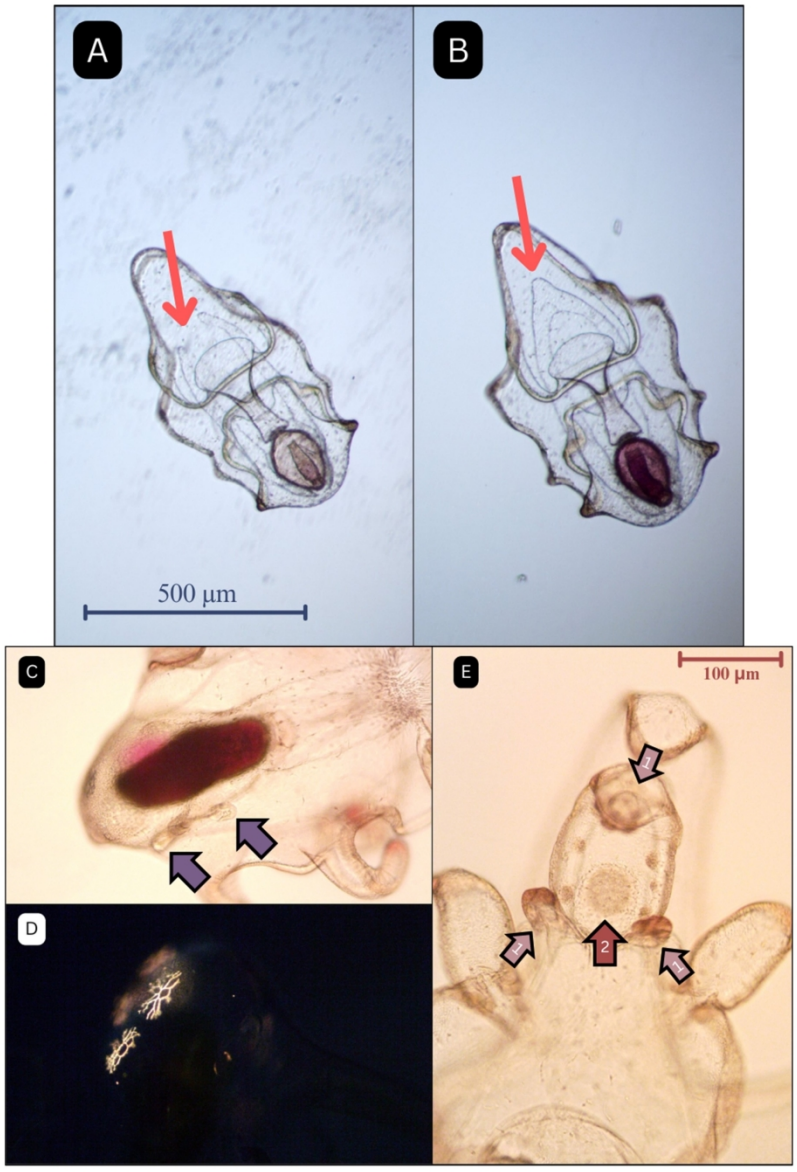

Figure 2

190x278mm (300 x 300 DPI)

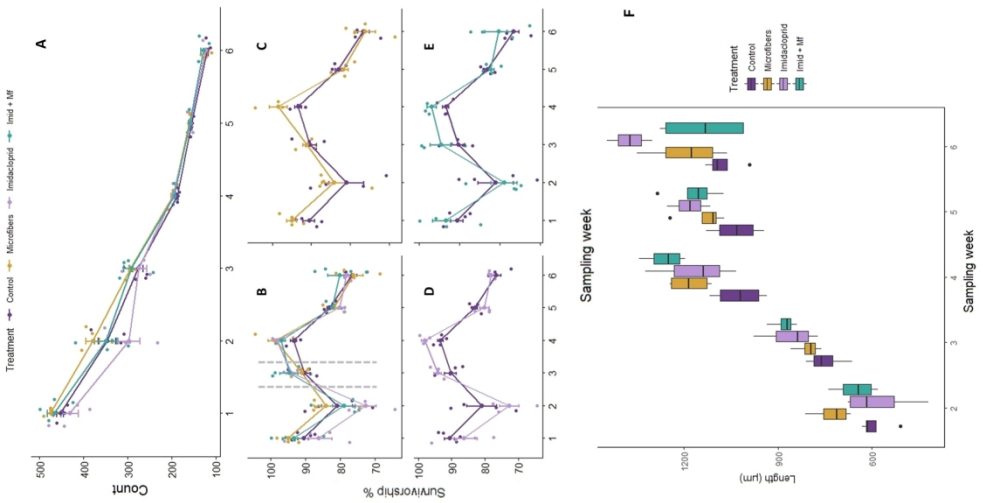

Figure 3

338x190mm (300 x 300 DPI)

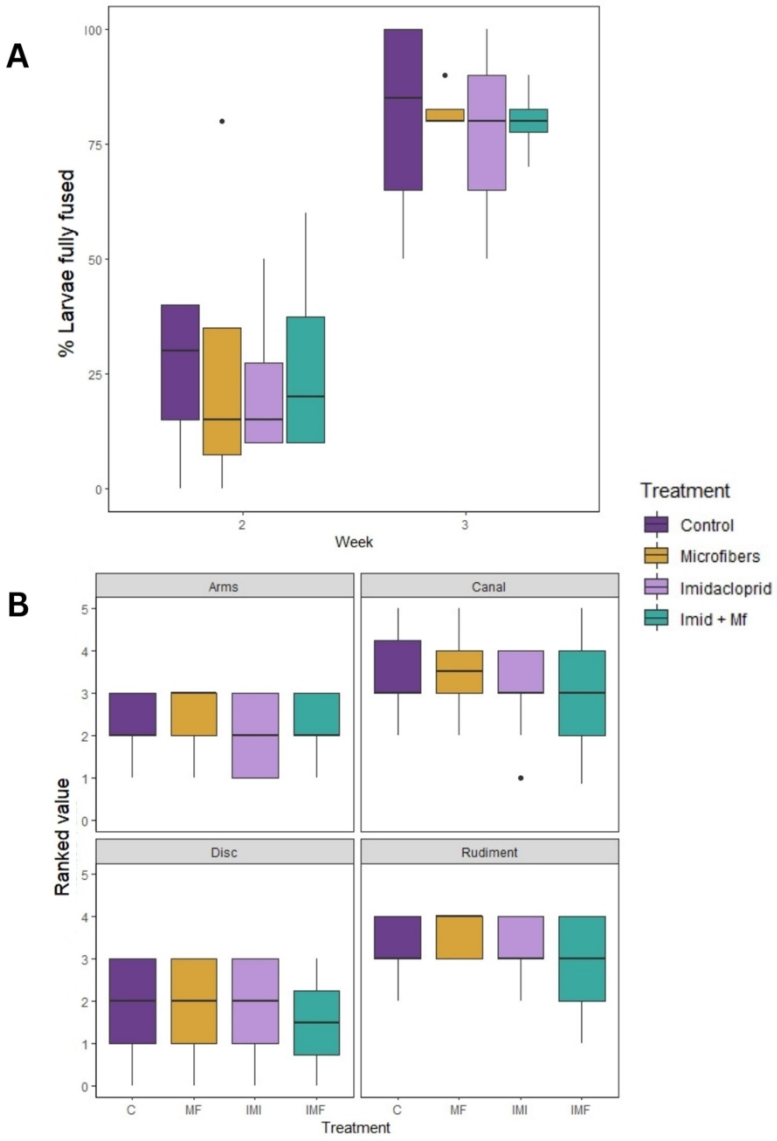

Figure 4

190x280mm (300 x 300 DPI)

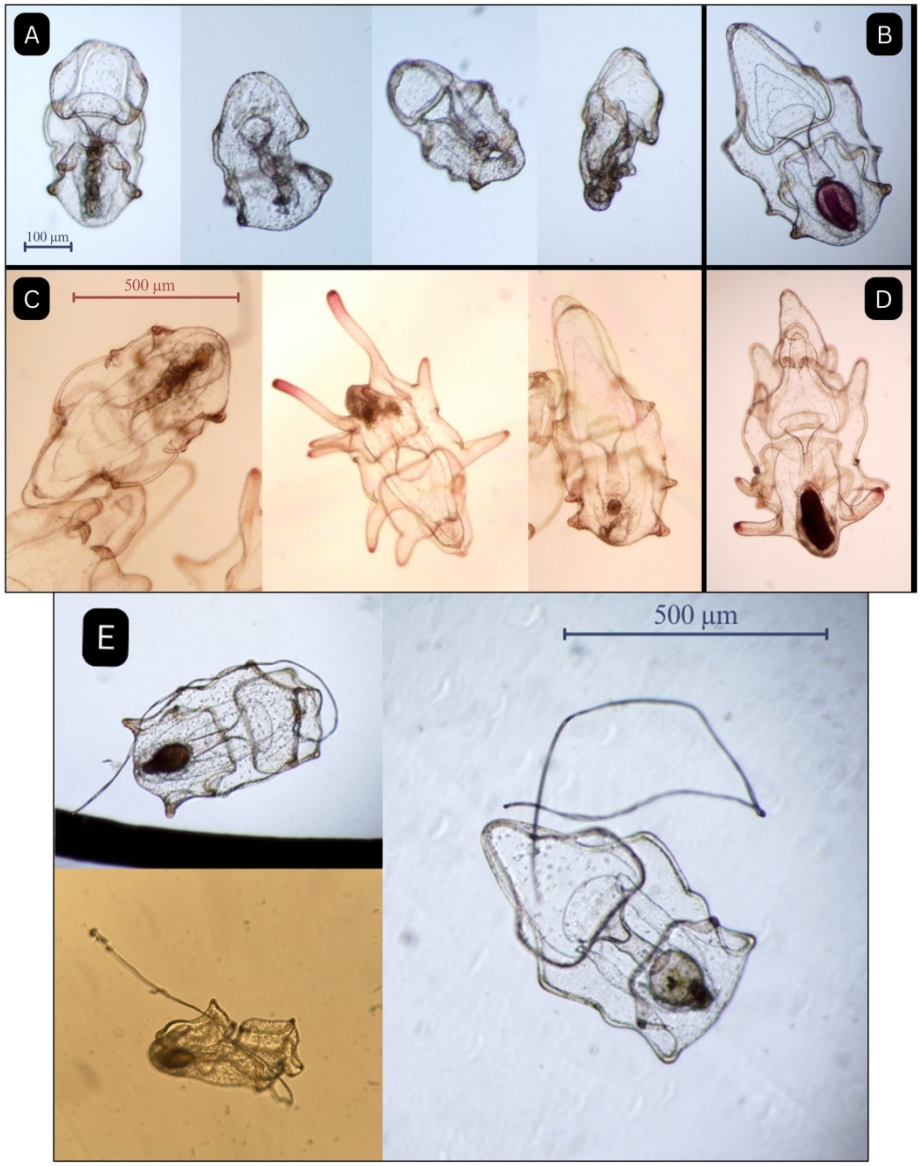

Figure 5

190x241mm (300 x 300 DPI)

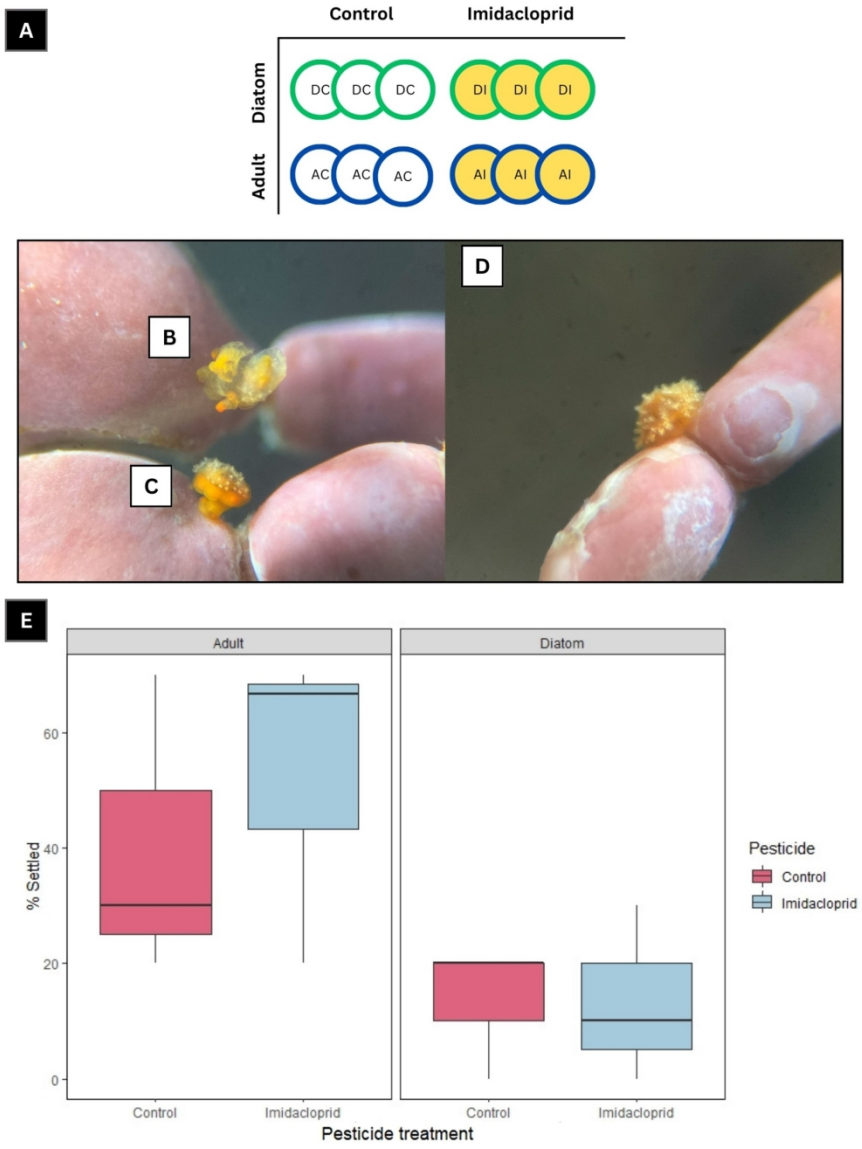

Figure 6

190x260mm (300 x 300 DPI)