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Title: Of Mysid and Menidia: Sublethal Effects of Micro and Nanoplastics on Estuarine Indicator Species.

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Abstract

Microplastics are widely distributed in aquatic environments. The term 'microplastics' encompasses a wide array of particles with unique polymer constituents, morphologies, and sources, such as automobile tire tread. Tire wear particles (TWP) can end up in waterways near densely populated municipalities, where they can interact with aquatic biota. Studying the impact of commonly detected synthetic particles such as TWP, across micro and nanoscopic sizes, on estuarine indicator species may tell us how these anthropogenic contaminants affect the entire ecosystem. This study investigated ingestion, growth, and behavioral responses of inland silversides (*Menidia beryllina*) and mysid shrimp (*Americamysis bahia*) when exposed to three concentrations of TWP (60, 6000, and 60,000 particles/mL which is equivalent to 0.0038, 0.0378 and 3.778 mg/L in mass concentration for micro size particles) at micro and nano size fractions with TWP leachate across 5-25 practical salinity unit (PSU) salinity gradient. Following exposures, *M. beryllina* and *A. bahia* had significantly altered swimming behaviors, such as freezing, in zone duration and total distance moved, that could lead to an increased risk of predation and foraging challenges in the wild. Both *A. bahia* and *M. beryllina* growth was reduced in a concentration dependent manner when exposed to micro TWP, whereas *M. beryllina* demonstrated reduced growth only when exposed to nano TWP. TWP internalization was observed in both taxa at 15 PSU. *A. bahia* and *M. beryllina* had significantly higher particle ingestion at 15 PSU following micro TWP exposure. The presence of adverse effects in *M. beryllina* (growth at micro TWP and behavior) and *A. bahia* (behavior) indicate that even at current environmental levels, which are expected to continue to increase, aquatic ecosystems are likely experiencing negative impacts from TWP pollution.

Of Mysid and Menidia: Sublethal Effects of Micro and Nanoplastics on Estuarine Indicator Species.

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

John Dickens, Author

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Chapter 1
General introduction

Marine debris is a ubiquitous environmental problem, particularly in pelagic and coastal marine environments. An estimated 4.4 – 12.7 million metric tons of plastic waste enter the ocean annually (Rochman et al., 2016a). Through photodegradation and other weathering processes, these synthetic polymers fragment and disperse throughout the ocean (Barnes et al., 2009a). Due to their slow decay, plastics have accumulated in every marine ecosystem from the equator to the poles (Thompson et al., 2004), including a record maximum depth of 10,898 meters in the Mariana Trench (Chiba et al., 2018). Microplastics (MPs), polymers that have fragmented into particles < 5mm in at least one dimension, include synthetics of many unique polymer constituents that have variable effects depending on particle size, shape, chemistry, and concentration (Lithner et al., 2011; Rochman et al., 2019a)

1.1 Sources of microplastics

MP are derived from materials composed of diverse polymer types, each containing a backbone of repeating monomers forming countless unique chemical substances for a multitude of purposes. Additives, plasticizers, colorants, flame retardants, and stabilizers add to the chemical complexity of each particle. MPs that enter waterways often come from terrestrial origins, including primary sources such as agriculture materials, paints, adhesives, and industrial abrasives and from secondary sources including food packaging, textiles, and automobile tires (Rochman et al., 2019a).

1.2 Impacts to marine organisms

Because of their small size, these tiny particles become available to ingest by organisms at lower trophic levels. When ingested, MP have been shown to impact the physiology of over 580 species of zooplankton, corals, bivalves, crabs, sea turtles, cetaceans, seabirds, and many species of fish (Gall and Thompson, 2015); Brander et al. 2021 in review). For example, MPs can affect zooplankton through impairment of larval development and growth (Gandara e Silva et al., 2016) accumulation in tissues, (Woods et al., 2020) and altered feeding and swimming behavior (Cole et al., 2015). Also affected are feeding rates and filtering activity in bivalves and corals (Chapron et al., 2018; Xu et al., 2020). Further, finfish liver congestion and altered intestinal morphology have been observed as well as increasing MP ingestion in seabirds, cetaceans and, pinnipeds (Fossi et al., 2016; Hernandez-Milian et al., 2019; Tanaka and Takada, 2016; Yin et al., 2019a, 2018a). MPs can alter gene expression, directly impact cells and tissues, and less commonly, cause death in organisms at every level of biological organization (Rochman et al., 2016b)

1.3 Nanoplastics

Through weathering, UV radiation, and biodegradation, MPs can further break down into nanoplastics (NP, 1 nm – 100 nm) (Jambeck et al., 2015). Nanoscale particles differ significantly in strength, conductivity, and reactivity from micro-sized particles (Klaine et al., 2012; Mattsson et al., 2015). As particle size decreases, biological reactivity increases due to the increase in surface area (Ferreira et al., 2019). NPs can be ingested by marine organisms and are known to enter the circulatory system and cross the blood brain barrier in many species of invertebrates and fish (Shen et al., 2019).

1.4 Tire wear particles included in microplastic definition

Synthetic rubber emissions from automobile traffic, now broadly considered a common type of microplastic (Rochman et al., 2019b), are a threat to the health of marine ecosystems, especially in metropolitan areas close to estuaries (Gray et al., 2018a; Tian et al., 2021a). Modern tire materials, products of fossil fuels, are composed of complex mixtures of synthetic polymers, natural rubbers, carbon black, polyester and nylon fiber, steel, chemical additives, oils, and pigments (W and M, 1998). These mixtures shed tire wear particles (TWP), characterized as airborne and non-airborne particles generated by the rolling shear of tread against a surface (Rogge et al., 1993a). Once produced, TWP can aggregate with other auto-related particles from brake dust, pavement, and atmospheric deposition (Charters et al., 2015a). These particles can result in consequential ecological problems. Changes in cell morphology and DNA damage due to inhalation of tire particles is known to occur in humans (Gualtieri et al., 2008a). Several laboratory studies have confirmed that TWP leachate is known to be toxic to aquatic organisms across different taxonomic orders (Day et al., 1993; Hartwell et al., 2000a; Tian et al., 2021a; U.S. Bureau of Reclamation et al., 1994). TWP leachate is known to contain high levels of zinc, a vulcanization agent, and is a toxic agent for daphnids, insects, fish, algae, and bacteria (Wagner et al., 2018a). It is estimated that 60% of the total zinc present in the lower San Francisco Bay is derived from TWP (Kontchou et al., 2021).

1.5 Microplastics in estuarine ecosystems

Coastal estuaries are particularly susceptible to micro and nanoplastic (MNP) pollution. Due to riverine and oceanic inputs as well as proximity to land and human activities, these water bodies may act as a MNP sinks (Jiwarungrueangkul et al., 2021; Vermeiren et al., 2016). Estuaries provide rich habitat and ecosystem services for a diversity of recreationally and economically significant species (Barbier et al., 2011a; Worm et al., 2006a) including carbon sequestration, nutrient cycling, and filtering contaminants (Tomas et al., 2015a). Estuaries at latitudes across the U.S. Pacific Northwest within the California Current System (CCS) are highly productive ecosystems that experience seasonal wind-driven offshore upwelling that delivers abundant nutrients to the PNW region (Hickey and Banas, 2003a). These waterbodies receive fresh water from inland rivers, which deliver nutrients and runoff and may harbor agricultural chemicals and microplastics (Le Roux, 2005a). The occurrence and distribution of MP in estuaries can be variable. In Charleston Harbor, 221 - 415 particles/m^2 are reported in sediment and 6 – 40 particles/L in the sea surface microlayer (Gray et al., 2018a). In the Chesapeake Bay MP concentrations in sediment have been measured up to 250,000 particles/ km^2 (Yonkos et al., 2014). In the Tamar Estuary in England, 2.8 x 10^{-5} particles/L have been measured in the water column (Sadri and Thompson, 2014). In the Pearl River Estuary in Hong Kong an average of 149 particles/L sediment and 5,595 particles/m^2 is reported (Fok and Cheung, 2015).

Once in an estuary, low-density microplastics will remain buoyant (Barnes et al., 2009b) and become available to planktonic organisms who may ingest fragments and particles (Cole et al., 2013). As prey organisms are consumed by predators, those particles are susceptible to trophic transfer in estuarine food webs (Athey et al., 2020a; Au et al., 2015a). Results from several recent studies have indicated that estuarine species such as shore crabs, oysters, shrimp, fish, and clams will ingest microplastics that can result in uptake through gill tissue and soft tissues and eventually mortality (Bessa et al., 2018a; Davidson and Dudas, 2016a; Gray and Weinstein, 2017a; Lu et al., 2016; Van Cauwenberghe and Janssen, 2014a; Watts et al., 2016a).

Additionally, organisms inhabiting estuaries are exposed to a wide range of salinities, typically from 0 PSU (freshwater at the river mouth) to 30 PSU (saltwater where the estuary meets the ocean). As estuaries experience daily and seasonal changes in salinity, these dynamics may alter the impacts of pollutants. Estuarine grass shrimp are known to exhibit increased mortality when exposed to pesticides for 96 hours at 30 ppt salinity (DeLorenzo et al., 2009). Pacific oysters (*C. gigas)* will accumulate perfluorochemicals with increasing salinity (Jeon et al., 2010). The presence of MNP and the resulting interactions with euryhaline biota, organisms that can tolerate a wide range of salinity, may be variable because of the influence of different salinities. Decreasing concentrations of MP in water but increasing concentrations of MP in ice have been detected at higher salinities, potentially reducing the interaction of MP and marine organisms (Hoffmann et al., 2020). Increasing concentrations of polymers in green mussels (*Perna viridis*) have been observed with increasing salinities presumably due to higher agglomeration (Khoironi et al., 2018). Another cause for concern is the transport of persistent organic pollutants by MNP. When salinity increases, water solubility of hydrophobic chemicals is reduced (Goff et al., 2017; Hutton et al., 2021a) and facilitates adsorption to nonpolar MNP surfaces (Chen et al., 2019). Testing MNP toxicity across salinities is important because as global ocean temperatures warm, salinity is expected to increase (Durack et al., 2012a; Helm et al., 2010a). This may increase the risk of MNP harboring toxic chemicals to estuarine species exposed to elevated salinities.

1.6 Objectives

The goal of this study was to conduct to a risk assessment of MNP as aquatic contaminants to two important estuarine species. Specifically, the objectives were to investigate the sublethal impact of micro and nano sized TWP concentrations across a salinity gradient, mimicking estuarine conditions these organisms may encounter in the wild. This study aimed to produce concentration dependent dose responses to detect subtle changes in ingestion, growth, and behavior. Chapter two provides an overview of the study and includes experimental methods, analysis, results, and discussion. Chapter three highlights how the findings of this study fit into broader implications, details on its limitations, and recommendations for future research.

Chapter 2

The internalization and sublethal effects of tire wear micro and nanoparticles in two estuarine indicator species

2.1 Introduction

An estimated 4.4 – 12.7 million metric tons of marine debris enter the ocean annually, presenting a threat to pelagic, benthic, and coastal environments (Rochman et al., 2016a). Through photodegradation and weathering processes, these synthetic polymers fragment and disperse throughout the ocean, often concentrating in coastal areas (Barnes et al., 2009a). Synthetic rubber emissions from automobile tires, now broadly considered a common type of microplastic, are a likely threat to the health of marine ecosystems, especially in estuaries, rivers, and streams located near metropolitan areas and busy roadways (Brahney et al., 2021; Gray et al., 2018b; Klöckner et al., 2019; Rochman et al., 2019a; Tian et al., 2021b; Wagner et al., 2018b). Another pathway of tire wear particles (TWP) is stormwater runoff as reported by recent study at 12 sites within the San Francisco Bay estuary, where fibers and TWP (black rubbery fragments) contributed to ~85% of total particles sampled (Werbowski et al., 2021). Modern tire materials, products of fossil fuels, are composed of complex mixtures of synthetic polymers, natural rubbers, carbon black, polyester and nylon fiber, chemical additives, petroleum, and pigments (Baumann and Ismeier, 1998). These mixtures are shed as TWP, characterized as airborne and road wear particles, generated by the rolling shear of tread against a surface (Rogge et al., 1993b). Once produced, TWP can aggregate with other auto-related particles from brake dust, pavement, and atmospheric deposition (Charters et al., 2015b). The presence of these particles in aquatic environments may result in impacts to wildlife and humans. For example, changes in cell morphology and DNA damage due to inhalation of tire particles are known to occur in humans (Gualtieri et al., 2008b). Additionally, tires can leach constituents known to be toxic to aquatic organisms across different taxonomic orders (Hartwell et al., 2000b; Nelson et al., 1994).

Coastal estuaries are susceptible to micro and nano plastic pollution from terrestrial sources, including automobile tires. Estuaries provide rich habitat and ecosystem services for a diversity of recreationally and economically significant species, including carbon sequestration, nutrient cycling, and filtering contaminants (Barbier et al., 2011b; Tomas et al., 2015b; Worm et al., 2006b). For example, estuaries at latitudes across the U.S. Pacific Northwest (PNW) within the Northern California Current System are highly productive ecosystems that experience seasonal wind-driven offshore upwelling that delivers abundant nutrients to the PNW region (Hickey and Banas, 2003b). These water bodies receive freshwater from inland rivers, which deliver nutrients and runoff that may harbor agricultural chemicals and microplastics (Le Roux, 2005b). An automobile tire is designed to last for 40,000 km until it is worn down, and throughout its lifetime, about 30% of its tread erodes and enters the environment (Dannis, 1974; Piotrowska et al., 2019). It is estimated that coastal rivers in Europe transport an annual load of 1.2 kt of TWP to the Atlantic Ocean (Siegfried et al., 2017). Knowledge on the distribution and concentration of TWP in coastal areas is limited (Unice et al., 2019). In Charleston Harbor, TWP were found in all layers (intertidal sediment, subtidal sediment, and sea surface micro layer) with a maximum concentration identified in the intertidal sediments of the Ashley River (203 mg/Kg ww) (Gray et al., 2018b). Another study report predicted average coastal European surface water concentrations to contain 0.03-17.9 mg/L and measured 0.09-6.4 mg/L of TWP (Wik and Dave, 2009), which is in range of this study mass concentration (0.0038-3.778 mg/L ww).

Once in an estuary, low-density microplastics including TWP may remain buoyant for a period of time and become available to planktonic organisms which may ingest these fragmented particles (Barnes et al., 2009a). As predators consume prey organisms, those particles are susceptible to trophic transfer in estuarine food webs (Athey et al., 2020b; Au et al., 2017). Several recent studies have indicated that estuarine species such as shore crabs, oysters, shrimp, fish, and clams will internalize microplastics through ingestion and uptake through gill tissue and soft tissues (Bessa et al., 2018b; Davidson and Dudas, 2016b; Gray and Weinstein, 2017b; Van Cauwenberghe and Janssen, 2014b; Watts et al., 2014). At the same time, organisms inhabiting estuaries are exposed to a wide range of salinities, which may alter the impacts of pollutants as freshwater transitions to saltwater. Testing across salinities is important because as global ocean temperatures warm, salinity is evidenced to increase (Durack et al., 2012b; Helm et al., 2010b). This increase in salinity may alter or potentiate the effects of pollutants, including micro and nanoplastics (MNPs), to estuarine organisms (Hutton et al., 2021b; Shupe et al., 2021).

Americamysis bahia and *Menidia beryllina* are model estuarine organisms used across a range of salinities following guidelines developed by the EPA for whole effluent toxicity testing (Brander et al., 2012; Pillard et al., 1999; Vlaming et al., 2000). The Inland Silverside (*Menidia beryllina*) is a euryhaline teleost endemic to shallow water estuaries along the Atlantic coast from Massachusetts Bay on Cape Cod to the

Gulf of Mexico in Vera Cruz, Mexico (Weinstein, 1986). Introduced populations occur in San Francisco Bay estuary and other California rivers (Brander et al., 2013; Fluker et al., 2011). *M. beryllina* are known to frequent tidal salt marshes, seagrass meadows, and shore zones and generally prefer lower salinities, although they may be found seasonally across the entire salinity gradient and are tolerant of salinities up to 35 PSU (Bengtson, 1984; Weinstein, 1986, p. 197). *M. beryllina* feed on a variety of zooplankton, including copepods, amphipods, and mysid shrimp. *M. beryllina* have been developed as a model species used in ecotoxicology research (Brander et al., 2016, 2012; DeCourten and Brander, 2017; Major et al., 2020). Their wide range of environmental tolerances and apparent "intermediate" sensitivity of *M. beryllina* to toxic contaminants in estuaries make it a suitable indicator species for the study of pollution-induced stress. Mysid shrimp (*Americamysis bahia*) are small crustaceans inhabiting coastal estuarine benthos from Narragansett Bay, Rhode Island to the Gulf of Mexico that are commonly used for toxicity testing (Verslycke et al., 2007, 2003, 2005). *A. bahia* feed on zooplankton and are regarded as important links in aquatic food webs in estuaries on every continent and contribute to biogeochemical mixing, bioturbation, and nutrient cycling (Mauchline et al., 1980; Mees and Jones, 1998; Roast et al., 2004). Due to their ecological role and abundance in a myriad of different environmental conditions, *A. bahia* have often been used as a model organism for evaluating the toxicity of endocrine-disrupting compounds (Verslycke et al., 2004).

Changes in organism behavior result from various cellular, biochemical, and neural processes (Døving, 1991; Little, 1990) that are critical to organism survival as well as fitness, thus a sensitive endpoint for use in toxicity testing (USEPA, 1994).

Numerous studies have drawn links between the biogeochemical and ecological consequences of environmental contamination by demonstrating that subtle changes in fish behavior indicate stress (Beitinger, 1990; Little, 1990; Sprague, 1971). Swimming and feeding behavior, frequency of activity, and velocity have been established as reliable measures of sublethal toxicity stress in fish (Grillitsch et al., 1999; Little and Finger, 1990; Newman and Jagoe, 1996). Recent studies on aquatic organisms exposure to MP have measured behavioral responses using EthoVision software, a video tracking system specifically developed for analyzing activity and movement in fish and invertebrates (Chen et al., 2017; Choi et al., 2018; Noldus et al., 2001). For example, larval zebrafish exhibited hypoactive locomotion and reduced total distance moved (cm) during Ethovision behavioral analysis following chronic nanoplastic exposures (Chen et al. 2017). The current experiment synthesizes methods of early and recent studies to measure several of these historically documented stress responses as well as growth in *M. beryllina* and *A. bahia* using periodic light and dark cycles as introduced stimuli (Pannetier et al., 2020; Romney et al., 2019).

Micro and nanoplastic exposures can cause adverse effects on the growth and development of larval aquatic organisms primarily through ingestion (Athey et al., 2020b; Lo and Chan, 2018). Inhibited growth may reduce probability of attack because of inconspicuousness, but in the long term may increase failure to escape as a result of less developed sensory and locomotion abilities (Fuiman and Magurran, 1994). Further, reduced growth and stunted development increases the amount of time a larval organism spends in a specific stage or size class, impacting cumulative predation mortality rate (Shepherd and Cushing, 1980). Additionally, several studies have reported the influence of salinity on growth and development in larval estuarine organisms. For example, striped bass (*Morone* saxitilis) growth is optimal at 5 PSU when compared to 0 and 10 PSU, sea bream (*Sparus aurata*) develops most efficiently at 25 PSU, and southern flounder (*Paralichthis lethostigma*) develop optimally between 5-30 PSU (Peterson et al., 1996; Smith et al., 1999; Tandler et al., 1995). In *M.* beryllina, optimal growth in laboratory conditions has been documented at 15 PSU while Mysid species was 30 PSU (Middaugh et al., 1987). Therefore, measuring growth in larval individuals following a period of salinity stress may yield unique insight into the effects of TWP across different salinities on developing organisms. Particularly considering that salinity regimes are already being altered by global climate change (DeCourten et al., 2019).

This study aims to investigate the sublethal effects (behavior and growth) of TWP exposure across a salinity gradient similar to that found in estuarine conditions. Subtle changes in behavior are essential to document because they may increase predation risk and population-level effects. Recently studies identified an acute toxicity came from the TWP and 6PPD-quinone was identified as a driver of toxicity in Coho salmon (Tian et al., 2021b). Following such event this study will investigate the effects of environmentally relevant concentrations of micro-and nano sized TWP and its leachate on behavior in the early life stages of indicator species *A. bahia* and *M. beryllina*. As data on TWP pollution and effects are rare, this study fills critical knowledge gaps on uptake and internalization, growth impacts, and stress responses to an emerging microplastic pollutant by species that may act as proxies for threatened or endangered species and ecosystems sensitive to anthropogenic pollution.

2.2 Methods

2.2.1 Chemicals

Suwanee River Natural organic matter (NOM) - 2R101N used to create suspensions of MNPs in exposure wells was purchased from International Humic Substance Society, St. Paul, MN. Tissue-Clearing Reagent CUBIC-R+ [for Animals] (T3741) and Tissue-Clearing Reagent CUBIC-L [for Animals] (T3740) for visualization of particles within organisms following exposures were purchased from Tokyo Chemical Industry Co., Ltd.

2.2.2 Microplastics preparation

Detailed TWP preparation protocol has been provided in SI. Briefly, TWP from tire tread was prepared by cryomill process in a ceramic chamber (Retsch CryoMill, Haan, Germany). After milling, 3 g tire particles were combined with 300 ml of solution in a flask containing 50 mg/L Suwanee River NOM prepared in Milli-Q water then filtered through a 0.2 mm filter. The solution is then run through a coarse strainer to remove the glass beads and strained through a 20 µm standard mesh sieve, producing a resulting solution with particles <20 µm in at least one dimension. Then, using a 47 mm syringe filter holder containing a 1 µm mixed cellulose ester (Advantec) filter the solution is further filtered to produce a suspension of nanoparticles <1 µm in at least one dimension. The filter holder is then backflushed with clean NOM suspension, and the backflushed solution is collected to produce a suspension of tire particles in the range of 1-20 µm. A portion of the prepared nano TWP fraction was further filtered using a 30K MWCO centrifugal filter (Corning Spin-X #431489) ran at 7800 rpm for 5 minutes to rinse particles and to produce TWP leachate. The solution particle counts are determined separately for each fraction of the suspension. The micron (1-20 µm) sample particle count is determined by triplicate sampling of the suspension and the particle count analysis by flow cytometry (Acurri C6 Flow Cytometer, BD Biosciences, San Jose, CA). The nanoscale (<1 µm) sample particle count is also determined in triplicate by Nanoparticle Tracking Analysis (NTA) on a NanoSight instrument (NanoSight NS500, Malvern Instruments, Westborough, MA).

2.2.3 Model organisms, their sources and experimental setup

Americamysis bahia larvae were purchased from Aquatic Biosystems in Fort Collins, Colorado and reared in three tanks at 15, 20, and 25 PSU salinities with filtered artificial seawater prepared (AFSW). Following EPA protocol 833-C-09-001 (USEPA, 2009). when adult *A. bahia* reproduced, larvae were moved to additional tanks of the same salinity and reared for seven days. Micro and nano TWP exposures with mysids were initiated at seven days post fertilization (dpf) (n=9) under static renewal condition for seven days. *Menidia beryllina* embryos were harvested from Hatfield Marine Science Center into three acclimation aquaria of 5, 15, and 25 PSU salinities with filtered AFSW following modified methods from(Middaugh et al., 1987) as done in previous studies in the Brander lab (DeCourten et al., 2020; Hutton et al., 2021b). Larvae were placed into exposure vessels at 6 ±1 days post fertilization (dpf) (n=6) and maintained under static renewal conditions for 96 h.

Each model species was exposed to a total of 26 treatments: each containing water control, NOM control with four TWP concentration treatments (micro and nano with 60, 6000, and 60000 particles/mL which is equivalent to 0.0038, 0.378 and 3.778 mg/L in mass concentration for micro size particles; 0.014% TWP leachate) across three salinities per species as described above. Nominal water concentrations with detailed QA/QC are provided in SI table 1. Water quality parameters were measured daily over the exposure period at the time of 80% water renewal. Cumulative hatching and mortality were recorded daily. *A. bahia* were fed concentrated brine shrimp (*Artemia franciscana*) ad libitum, and *M. beryllina* were fed Gemma Microdiet 0.2 mg/beaker/day (Skretting, Westbrook, Maine). Both organisms were fed daily and allowed to feed for at least two hours before water changes. Table SI 2 and 3 provides water quality parameters maintained throughout the experiment. A control filter was setup to measure background contamination.

2.2.4 Behavioral assays

Following MNP exposures of 7d (*A. bahia*) and 96 h (*M. beryllina*), behavioral assays were performed using a DanioVision Observation Chamber (Noldus, Wageningen, the Netherlands) for the Dark: Light cycle (Mundy et al., 2021; Segarra et al., 2021) Briefly, *A. bahia* and *M. beryllina* larvae were randomized and placed in individual 10 ml beaker 12 well plates in the Ethovision Observation Chamber (EOB) to observe natural photo motor response. Larvae were allowed to acclimatize for at least 1 hour before placing into the EOB. After acclimatization outside, another 5-minute acclimatization time was provided inside the dark chamber, followed by three 2-minute intervals of dark stimuli and three 2-minute intervals of light stimuli.

Behavior and activity were recorded and tracked by a Basler Gen 1 Camera using Ethovision XT15 software. Velocity thresholds were determined for swimming parameters between 0.5 cm/s (freezing) – 2.0 cm/s (moving) (Segarra et al., 2021). A virtual center zone (1.6 cm diameter) was established to measure the time that larvae spent in the center of the 2.2 cm diameter in the beaker. All behavioral tests were conducted between 09:00 and 18:00 h. The resolution was set at 1280 x 960, light cycles were programmed at 10,000 lux and the frame rate was set at 25/s. A total of seven variables were analyzed in this study which included in Table 1. Following behavioral analysis, organisms were euthanized humanely, Silversides per IACUC protocol #0035, and fixed in paraformaldehyde (PFA) to preserve tissues for examination of MP internalization.

2.2.5 Growth and TWP internalization

At least three individuals from each species per treatment were collected for growth. Length and width measurements were collected via dissecting scope equipped with Moticam visual software and particle uptake was visualized on a Zeiss Axio Observer inverted microscope (Carl Zeiss, White Plains, NY). Growth data were assessed by creating a growth index with the following formula:

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

Where W is the Width of the organism, L is the length, and d is the number of days the organism is exposed to the TWP. This relationship provides the index used to plot the final growth curve. Organisms were then cleared using a protocol adapted for larval organisms with CUBIC^{TM} clearing reagents (Ohnuma et al., 2017; Susaki et al., 2015). Briefly, to remove pigmentation and allow visualization of internalized microplastics (1-20 um), individual organisms fixed in 3% PFA were washed in 5 ml phosphate-buffered saline (PBS) for 30 minutes and incubated in 5 ml CUBIC-L at 37 ° C for seven days to encourage lipid removal. Following this step, organisms were washed again in 5 ml PBS for an additional two hours and then transferred to CUBIC-R + for an additional seven days to clear the remaining tissue.

2.2.6 Statistical analysis

Statistical analysis was performed using RStudio Version 1.0.153. Dose-response curves were generated to evaluate larval swimming behavior and growth effects across concentration treatments. The growth data were analyzed using a maximum likelihood estimate (MLE) approach was used to evaluate whether non-monotonic curves were a better fit to the data than a null (intercept-only) model. Five different concentration-effect curves (linear regression, quadratic, sigmoidal, 5-parameter unimodal, and 6-parameter unimodal) were tested to fit responses of all three concentrations and control. A maximum likelihood ratio test was used to examine whether each curve provided a better fit than an intercept-only null model with a significance level of $\alpha < 0.05$. All calculations for the concentration−effect curves were performed using mean behavior variables, re-scaled between 0 and 1 within each cycle to facilitate comparison between salinity. R scripts used for data preparation, statistical analysis, and graphing can be found at https://github.com/jwilsonwhite/DRcurves.git, and examples using the same package are published in other studies (Brander et al., 2016; Frank et al., 2019; Mundy et al., 2020) Concentration dependent dose response curves (C-DRC) for behavioral data were prepared by drm function in r using DRC package by Ritz et al (2010) (Ritz, 2010). A 3-4 parameter using nonlinear regression approach was used to prepare the model at each salinity and combined using ggplot2 function in R. Analysis of Variance (ANOVA) was used to evaluate differences among treatment groups. A Tukey HSD post-hoc test was used to compare particle concentrations between treatments, and a Dunnett's post-hoc test was used to compare leachate treatments to controls. Differences were considered statistically significant at $p < 0.05$.

2.3 Results and discussion

2.3.1 Behavioral impact

Average *A. bahia* larvae survival for control and exposure treatments was 98 ± 2% and 90 ± 3 %, respectively, with no significant difference across the treatments (ANOVA (Normal distribution, Tukey HSD post-hoc, $p < 0.05$). Out of seven behavioral responses analyzed in response to micro and nano TWP exposure, half of them were significantly different from the control group in at least one concentration in both light and dark cycle at some salinity (except lowest nano TWP exposure group) (Fig. 1C & D). In the micro and nano TWP exposed group of *A. bahia* turn angle, freezing, movement and in zone duration were most significantly affected at each salinity. In leachate-exposed *A. bahia,* four of the seven variables demonstrated significant alterations from the control group (Fig1B). When compared between dark and light cycle, *A. bahia* demonstrated increased distance and meander in the light cycle at highest salinity whereas increasing freezing frequency and time spent in the zone at lowest salinity. When compared between TWP sizes, nano TWP caused hyperactivity in *A. bahia* reflected by their swimming distances significantly increasing. C-DRC for the selected variables in dark and light cycle micro TWP demonstrated about 70% behavioral alterations whereas in nano TWP exposure group about 80% behavioral alterations in both dark and light cycle (Fig. 2). In terms of salinity, behavior alterations in both nano and micro TWP exposures were significantly higher at higher salinity (20 and 25 PSU). This suggests nano TWP affected mysids more at higher concentration as reported by other studies (Kögel et al., 2020; Lee et al., 2013; Rist et al., 2017). When nano (1-9 µm) and micro (>10 µm) plastics were exposed to *Daphnia magna*, nanoplastic was reported to effect 16% higher on feeding rates and reproduction compared to microplastic exposure (Rist et al., 2017). Other studies reported hyperactive behavior in zebrafish (*Danio rerio*) exposed to PS and sticklebacks (*Gasterosteus aculeatus*) exposed to PE (Bour et al., 2020; Chen et al., 2020). Moreover, hyperactivity has been reported in F1 offspring of zebrafish exposed to polyvinyl chloride (PVC) and high-density polyethylene (HDPE) (Cormier, 2020).

Average *M. beryllina* larvae survival for control and exposure treatments was 97 ± 3% and 91 ± 2 %, respectively, with no significant difference across the treatments (Normal distribution, Tukey HSD post-hoc, $p < 0.05$). In *M. beryllina,* all the behavioral variables demonstrated significant variation from the control group in at least one salinity (Fig. 3C & D). In both dark and light cycles, *M. beryllina* spent an increased time in the zone (the beaker center part) compared to control with increased turn angle. Dark and light cycle behavioral observation showed a similar pattern except *M. beryllina* meandered more compared to the dark cycle at all the exposure concentrations, with at least one salinity condition. Following TWP leachate exposure concentration more than half of the variables demonstrated a significant change in behavior from control at both dark and light cycle in at least one salinity condition (Fig. 3B). Similar behavior changes were observed in delta smelt (*Hypomesus transpacificus*) when exposed to pesticides as a response to contaminants stress.(Mundy et al., 2021, 2020) C-DRC for the selective variables demonstrated 79% behavioral alterations in micro TWP exposed Silversides whereas in nano TWP exposure group about 75% behavioral alterations at both micro and nano exposed TWP Silversides at both dark and light cycle (Fig. 4). In Silverside behavior salinity dependent behavioral changes were not significant at micro TWP exposed group, in contrast to nano TWP where higher salinity seems to effect behavior more. This may be because of the agglomeration nature of nano TWP at higher salinity. Altered swimming behavior, reduced velocity and decreased feeding activity have also been observed in larval zebrafish (*Danio rerio*), larval rockfish (*Sebastes schlegelii*) and sheepshead minnow (*Cyprinodon variegatus*) when exposed to PS and PE microplastics (Chen et al., 2017; Choi et al., 2018; Noldus et al., 2001; Yin et al., 2018b, 2019b).

When compared between *M. beryllina* and *A. bahia* behavioral variable relationship, there were some correlations (Pearson) identified between some variables (Table SI 3). There was a direct correlation observed in *M. beryllina* between distance related to movement, freezing (0.32-Dark, 0.16- Light). In contrast to mysid shrimp, there was an inverse relationship between movement and freezing (-0.54-Dark, -0.59- Light). Freezing demonstrated a weak inverse relationship with velocity for *M.*

beryllina and *A. bahia*. Turn angle and freezing mean also showed weak inverse relationship with movement for *M. beryllina* and *A. bahia*. This suggests random movement that can caused by additional stress due to the TWP. Within different salinities, lowest salinity (15 PSU) in *A.bahia* demonstrated highest variation from control in dark and light cycle when exposed to all concentrations of micro TWP. This was in contrast to nano TWP and leachates, where highest salinity (25 PSU) demonstrated most impact on behavioral variation. Similar results were observed in *M. beryllina*, where in dark and light cycle lowest salinity (5 PSU) shown most variation from control when exposed to micro TWP in contrast to nano TWP and leachate exposure group, where most behavioral variation from control seen in highest salinity (25 PSU). These results are in support of the recent findings that nanoplastics increase agglomeration with increasing salinity (Shupe et al., 2021).

Behavioral changes can be an outcome of physiological changes like respiratory stress (Abdel-Tawwab et al., 2019; Hashemi et al., 2019) that maybe cause by gills and skin changes to oxygen consumption with altered ion regulation (Kolandhasamy et al., 2018; Watts et al., 2016b). Similarly, the ingestion of irregularly sized TWP may also induce irregular behavior (Wang et al., 2016; Wright et al., 2013) (Fig 1 and 2). These particles may also come in contact with the skin, gills, fins, and eyes of the organisms when present in high concentrations, resulting in abnormal swimming behavior (Choi et al., 2018). Several studies have reported that MNPs can cause movement-related neurotoxicity in organisms (Barboza et al., 2018; Lei et al., 2018; Yin et al., 2018b). Swimming behavior is crucial for defensive, food searching and social activity (Colwill and Creton, 2011) that all require motor as well as sensory systems (Roberts et al., 2011; Wong et al., 2010) to work in concert to avoid being easily preyed upon. *M. beryllina* are known to occur in schools and exhibit diel migrations following zooplankton prey, often displaying high school densities during the nocturnal period, presumably to reduce predation (Wurtsbaugh and Li, 1985). The presence of high TWP concentrations may alter migration or shoaling patterns and limit population ranges, although environmentally relevant TWP concentrations in larger water bodies may not present significant risk at this time. *A. bahia* exposed to nano TWP concentrations (60 and 6,000 p/ml) at 15 PSU and 25 PSU in dark cycles exhibited further total distance moved while organisms exposed to TWP leachate in light cycles exhibited shorter total distance moved at 25 PSU salinity. Increased activity from TWP exposure in nocturnal periods may not present high risk to the diurnally benthic *A. bahia,* which becomes planktonic at night to forage for food and engage in reproductive activity (Wortham-Neal and Price, 2002). However, decreased activity from TWP leachate in *A. bahia* during diurnal periods may increase susceptibility to predation by fish or crustaceans who forage during the day, or cause reduced food intake.

M. beryllina exposed to both micro and nano TWP exhibited increased duration of time spent in central habitat across all concentrations, salinities, except individuals exposed to nano TWP in the highest concentration and TWP leachate, both at lowest salinity in dark cycles. Similarly, *A. bahia* exhibited increased zone duration across all TWP concentrations, salinities, and light-dark cycles, including individuals exposed to TWP leachate. Occupancy of the boundaries of a novel environment is thoroughly documented to indicate a stress response in fish, rodents, and humans (Kallai et al., 2007; Schnörr et al., 2012; Sharma et al., 2009; Treit and Fundytus, 1988). An increase in central habitat occupancy that is significantly different from control organisms may indicate increased exploration or indiscriminate feeding behavior. Previous studies observed impaired swimming competence and reduced exploratory behavior in *N. japonica* exposed to PS microbeads (Wang et al., 2020). The uninhibited exploration behavior we observed may lead to an increased risk of predation in these highly susceptible larval fish.

2.3.2 Growth and Ingestion

A. bahia growth demonstrated a significant concentration-dependent decrease in both the highest salinities (Normal distribution, Post-hoc Tukey's test, ANOVA, $p < 0.05$) micro TWP exposure (Fig. 3). When compared between the salinities, *A. bahia* demonstrated comparatively better growth at the highest salinity, which was reduced significantly at the highest TWP concentration (Tukey HSD post-hoc, ANOVA, $p < 0.05$). There was no significant growth reduction demonstrated in nano TWP-exposed *A. bahia* over all concentrations. However, the highest salinity demonstrated better growth compared to both lower salinities. The appearance of ingested TWP was concentration-dependent in *A. bahia* as shown in Fig. 5A and 6A. TWP ingestion is also documented in other benthic invertebrates.(Khan et al., 2019; Redondo-Hasselerharm et al., 2018) In the case of amphipod crustacean (*Hyallela* azteca) gut retention time of 24–48 h were observed in ingested TWP with a significant impact on net growth when exposed to 500–2000 p/ml (Khan et al., 2019).

M. beryllina demonstrated significant concentration-dependent reduced growth at both micro and nano TWP exposed groups at all salinities except in nano TWP group at lowest salinity (Tukey HSD post-hoc ANOVA, $p < 0.05$; Fig. 4). This is true in the case of ingested micro TWP also, *M. beryllina* where ingested particles were observed at two highest concentrations with the highest number of ingested TWP at middle salinity (Fig. 5B and 6B). This is consistent with a recent study that traced TWP in the gut of 14% of individuals across all five fish species surveyed in urbanized estuarine conditions (Parker et al., 2020).

Ingestion of 1 – 20 mm micro TWP was observed in *A. bahia* and *M. beryllina* at 6000 p/ml and 60,000 p/ml. A generalized linear model (GLM) was run for particles count at all three concentrations (micro TWP) at different salinities for both the model species (Fig. 5A and B). The GLM for *A. bahia* suggested concentration dependent ingestion at all the salinities ($\chi2 = 18.12$, df = 3, $p < 0.005$). Results also suggested salinity dependent ingestion over concentration ($\chi2 = 4.16$, df = 6, $p<0.05$), and concentration dependent ingestion at the lowest salinities (5 and 15 PSU) ($\chi2 = 2.55$, df = 3, $p<0.005$). However, 15 PSU demonstrated increasing ingestion compared to 5 and 25 PSU salinity group ($\chi2 = 1.04$, df = 6, $p<0.005$). Results suggest that ingestion may likely be the most common interaction nondiscriminatory feeding fish larvae and zooplankton have with TWP. Previous studies confirm that *M. beryllina* will ingest microplastic at high concentrations when exposed to zooplankton internalized with TWP, although most particles were observed to be egested within 24 hours of internalization (Athey et al., 2020b). Gut retention time for TWP in *A. bahia* is unknown, however, and maybe dependent on particle size and shape. TWP are irregular in shape and may contribute to varying retention times. Egestion of 10 um polystyrene microspheres in other mysid species (*N. integer*) has been observed to occur within 12 hours of ingestion (Setälä et al., 2014). Microplastic can also agglomerate with increasing salinity, leading to longer retention times in estuarine species closer to marine environments (Ogonowski et al., 2016). In a study comparing the physiological toxicity of polystyrene and carboxylated polystyrene (PS-COOH) in mysid shrimp, both plastics were observed to reduce feeding efficiency in these organisms.

We observed reduced growth in *M. beryllina* across all micro TWP and two nano TWP (6000 and 60,000 p/ml) concentrations with increasing salinity, except for individuals exposed to nano TWP at 5 PSU. Other studies demonstrated growth inhibition of larval fish due to ingestion and accumulation of microplastics in gut content (Athey et al., 2020b; Santos et al., 2020). *A. bahia* appeared to be less sensitive to growth restriction by TWP and exhibited a reduction in growth only in micro concentrations at 20 PSU and 25 PSU. Nano TWP did not elicit a significant response in growth reduction in *A. bahia.* Inhibited growth in another species, *N. japonica,* has been observed as a result of chronic polystyrene exposure (Lee et al., 2021). Several studies investigated the effect that microplastics have on the growth of small aquatic organisms. Though some studies found that microplastic, particularly polyethylene, exposure did not affect growth, (Malinich et al., 2018; Mazurais et al., 2015) others noted detrimental effects of microplastics on growth (Athey et al., 2020b; Lee et al., 2021). These contrasting reports may be attributed to the wide variety of microplastic compositions, shapes, and sizes, as well as the lengths of exposure. While no studies could be found in the literature on the effects of TWP on mysid or Silverside growth, the findings of this study are in line with toxicity assessments for other microplastics. For example, exposure of mysids (*N. japonica*) to polystyrene (PS) and PS-COOH resulted in growth inhibition in a dose-dependent manner in which increasing concentrations resulted in decreasing growth (Wang et al., 2020). Additionally, mysid (*N. awatschensis*) showed impaired growth when exposed to melamine resin microparticles over four weeks (Lee et al., 2021). For amphipod (*H. Azteca),* chronic exposure to polyethylene microplastic particles and acute exposure to polypropylene microplastic fibers significantly decreased growth (Au et al., 2015b). Similarly, growth inhibition in larval fish has been documented as an effect of exposure to micro polyvinyl chloride, (Xia et al., 2020) polystyrene, (Lönnstedt and Eklöv, 2016) low density polyethylene, (Athey et al., 2020b) and microplastic mixtures (Naidoo and Glassom, 2019; Pannetier et al., 2020). Furthermore, in a meta-analysis of the literature (Foley et al., 2018) found that overall, exposure of zooplankton to microplastics decreases growth, and food dilution is thought to be one of the major mechanisms of MP toxicity to aquatic organisms in general.

2.3.3 Discussion

In aquatic environments, TWP are influenced by tidal processes, currents, and waves, and may disperse throughout the estuarine system. At lower salinities closer to the river mouth, TWP may remain suspended or float. This may make TWP more available to organisms that feed in the water column. TWP and other particulates will agglomerate at higher salinities and biofouling may occur, increasing the potential for higher density particles to settle out into benthic environments. As mysids are indiscriminate feeding epibenthic organisms, this settling out may increase the likelihood that mysid shrimp occurring at higher salinities will encounter and ingest TWP. Additionally, mysid shrimp are confirmed to ingest MP by preying on copepods

(Setälä et al., 2014). TWP are likely to follow the same fate of planktonic trophic transfer. Inland Silversides typically feed in the water column on copepods, mysids, and other zooplankton, although bottom feeding has been observed (Weinstein., 1986). In this respect, inland Silversides may ingest TWP in both the demersal and benthic environments. Future studies should further investigate the estuarine processes that affect TWP circulation and transport and how this will impact aquatic species.

Chapter 3.

General Conclusion

Recently the role of behavioral ecotoxicology in environmental conservation has been discussed by various scholars (Ford et al., 2021). This includes lab-based research that can help to study more about the individual, population, and ecosystem processes. This research demonstrated the occurrence of significant behavioral changes in response to the environmentally relevant concentrations (60 particles/ml = 0.0038 mg/L) of TWP as well as to higher potential future concentrations and leachates under various salinities found in the estuarine environment. These behaviors represent ecologically important stimulus responses in field conditions including activity (movement, velocity, freezing), boldness (in zone duration and frequency), and exploration (meander, turn angle, distance moved). Behavioral responses connect directly to population fitness and ecosystem-level impacts, therefore carry high relevance to be considered by policymakers. Additionally, growth and TWP ingestion data represent the significant impacts of micro and nano TWP on both the model species that may have population-level implications. Specifically, in estuarine conditions over different salinity gradients, that can help in risk assessment over wider environmental ranges. Future studies should further investigate the estuarine processes that affect TWP circulation and transport and how this will impact aquatic species. TWP leachate toxicity should also be considered with respect to aquatic species whose ecological role disproportionately outweighs biomass. Although automobiles are here to stay, limiting TWP from entering the environment is paramount if we wish to preserve sensitive aquatic ecosystems and fisheries. Possible actions to take in order to achieve this goal may include providing incentives for citizen awareness of and participation in waste reduction, (Eriksen et al., 2014, p. 201; Rochman et al., 2021) redesigning tire constituents with biopolymers and materials for circularity, (Karan et al., 2019) extending tire producer responsibility for end of life products, (Leal Filho et al., 2019) improving wastewater treatment technology, (Edo et al., 2020; Katyal et al., 2020), passing legislation to ban certain synthetic materials, and increasing use of public transportation rather than single vehicle use (Deng et al., 2020).

References

Abdel-Tawwab, M., Monier, M.N., Hoseinifar, S.H., Faggio, C., 2019. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. Fish Physiol. Biochem. 45, 997–1013. https://doi.org/10.1007/s10695-019-00614-9

Athey, S.N., Albotra, S.D., Gordon, C.A., Monteleone, B., Seaton, P., Andrady, A.L., Taylor, A.R., Brander, S.M., 2020a. Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. Limnol. Oceanogr. Lett. 5, 154–162. https://doi.org/10.1002/lol2.10130

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015b. Responses of Hyalella azteca to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572.

Au, S.Y., Lee, C.M., Weinstein, J.E., Hurk, P. van den, Klaine, S.J., 2017. Trophic transfer of microplastics in aquatic ecosystems: Identifying critical research needs. Integr. Environ. Assess. Manag. 13, 505–509. https://doi.org/10.1002/ieam.1907

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011b. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81, 169–193. https://doi.org/10.1890/10-1510.1

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57. https://doi.org/10.1016/j.aquatox.2017.12.008

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009a. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Baumann, W., Ismeier, M., 1998. Emissions from tires in dedicated use. Kautsch. Gummi Kunststoffe 51, 182–186.

Beitinger, T.L., 1990. Behavioral Reactions for the Assessment of Stress in Fishes. J. Gt. Lakes Res., Fish Community Health: Monitoring and Assessment in Large Lakes 16, 495–528. https://doi.org/10.1016/S0380-1330(90)71443-8

Bengtson, D., 1984. Resource partitioning by Menidia menidia and Menidia beryllina (Osteichthyes: Atherinidae). Mar. Ecol. Prog. Ser. 18, 21–30.

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018b. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044

Bour, A., Sturve, J., Höjesjö, J., Carney Almroth, B., 2020. Microplastic Vector Effects: Are Fish at Risk When Exposed via the Trophic Chain? Front. Environ. Sci. 8. https://doi.org/10.3389/fenvs.2020.00090

Brahney, J., Mahowald, N., Prank, M., Cornwell, G., Klimont, Z., Matsui, H., Prather, K.A., 2021. Constraining the atmospheric limb of the plastic cycle. Proc. Natl. Acad. Sci. 118, e2020719118. https://doi.org/10.1073/pnas.2020719118

Brander, S.M., Cole, B.J., Cherr, G.N., 2012. An approach to detecting estrogenic endocrine disruption via choriogenin expression in an estuarine model fish species. Ecotoxicology 21, 1272–1280. https://doi.org/10.1007/s10646-012-0879-2

Brander, S.M., Connon, R.E., He, G., Hobbs, J.A., Smalling, K.L., Teh, S.J., White, J.W., Werner, I., Denison, M.S., Cherr, G.N., 2013. From 'Omics to Otoliths: Responses of an Estuarine Fish to Endocrine Disrupting Compounds across Biological Scales. PLOS ONE 8, e74251. https://doi.org/10.1371/journal.pone.0074251

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

Chapron, L., Peru, E., Engler, A., Ghiglione, J.F., Meistertzheim, A.L., Pruski, A.M., Purser, A., Vétion, G., Galand, P.E., Lartaud, F., 2018. Macro- and microplastics affect cold-water corals growth, feeding and behaviour. Sci. Rep. 8, 15299. https://doi.org/10.1038/s41598-018-33683-6

Charters, F.J., Cochrane, T.A., O'Sullivan, A.D., 2015b. Particle size distribution variance in untreated urban runoff and its implication on treatment selection. Water Res. 85, 337–345. https://doi.org/10.1016/j.watres.2015.08.029

Chen, C., Chen, L., Yao, Y., Artigas, F., Huang, Q., Zhang, W., 2019. Organotin Release from Polyvinyl Chloride Microplastics and Concurrent Photodegradation in Water: Impacts from Salinity, Dissolved Organic Matter, and Light Exposure. Environ. Sci. Technol. 53, 10741–10752. https://doi.org/10.1021/acs.est.9b03428

Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584–585, 1022–1031. https://doi.org/10.1016/j.scitotenv.2017.01.156

Chen, Q., Lackmann, C., Wang, W., Seiler, T.-B., Hollert, H., Shi, H., 2020. Microplastics Lead to Hyperactive Swimming Behaviour in Adult Zebrafish. Aquat. Toxicol. 224, 105521. https://doi.org/10.1016/j.aquatox.2020.105521

Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., Ogido, M., Fujikura, K., 2018. Human footprint in the abyss: 30 year records of deep-sea plastic debris. Mar. Policy 96, 204–212. https://doi.org/10.1016/j.marpol.2018.03.022

Choi, J.S., Jung, Y.-J., Hong, N.-H., Hong, S.H., Park, J.-W., 2018. Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (Cyprinodon variegatus). Mar. Pollut. Bull. 129, 231–240. https://doi.org/10.1016/j.marpolbul.2018.02.039

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The Impact of Polystyrene Microplastics on Feeding, Function and Fecundity in the Marine Copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic Ingestion by Zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f

Colwill, R.M., Creton, R., 2011. Locomotor behaviors in zebrafish (Danio rerio) larvae. Behav. Processes 86, 222–229. https://doi.org/10.1016/j.beproc.2010.12.003
Cormier, B., 2020. Microplastic toxicity for fish : beyond simple vectors for pollutants? (phdthesis). Université de Bordeaux.
Dannis, M.L., 1974. Rubber Dust from the Normal Wear of Tires. Rubber Chem. Technol. 47, 1011–1037. https://doi.org/10.5254/1.3540458
Davidson, K., Dudas, S.E., 2016a. Microplastic Ingestion by Wild and Cultured Manila Clams (Venerupis philippinarum) from Baynes Sound, British Columbia. Arch. Environ. Contam. Toxicol. 71, 147–156. https://doi.org/10.1007/s00244-016-0286-4
Day, K.E., Holtze, K.E., Metcalfe-Smith, J.L., Bishop, C.T., Dutka, B.J., 1993. Toxicity of leachate from automobile tires to aquatic biota. Chemosphere 27, 665–675. https://doi.org/10.1016/0045-6535(93)90100-J
DeCourten, B., Romney, A., Brander, S., 2019. The Heat Is On: Complexities of Aquatic Endocrine Disruption in a Changing Global Climate, in: Separation Science and Technology. Elsevier, pp. 13–49. https://doi.org/10.1016/B978-0-12-815730-5.00002-8
DeCourten, B.M., Brander, S.M., 2017. Combined effects of increased temperature and endocrine disrupting pollutants on sex determination, survival, and development across generations. Sci. Rep. 7, 9310. https://doi.org/10.1038/s41598-017-09631-1
DeCourten, B.M., Forbes, J.P., Roark, H.K., Burns, N.P., Major, K.M., White, J.W., Li, J., Mehinto, A.C., Connon, R.E., Brander, S.M., 2020. Multigenerational and Transgenerational Effects of Environmentally Relevant Concentrations of Endocrine Disruptors in an Estuarine Fish Model. Environ. Sci. Technol. 54, 13849–13860. https://doi.org/10.1021/acs.est.0c02892
DeLorenzo, M.E., Wallace, S.C., Danese, L.E., Baird, T.D., 2009. Temperature and salinity effects on the toxicity of common pesticides to the grass shrimp, *Palaemonetes pugio*. J. Environ. Sci. Health Part B 44, 455–460. https://doi.org/10.1080/03601230902935121
Deng, H., Wei, R., Luo, W., Hu, L., Li, B., Di, Y., Shi, H., 2020. Microplastic pollution in water and sediment in a textile industrial area. Environ. Pollut. 258, 113658. https://doi.org/10.1016/j.envpol.2019.113658
Døving, K.B., 1991. Assessment of animal behaviour as a method to indicate environmental toxicity. Comp. Biochem. Physiol. Part C Comp. Pharmacol. 100, 247–252. https://doi.org/10.1016/0742-8413(91)90162-M
Durack, P.J., Wijffels, S.E., Matear, R.J., 2012a. Ocean Salinities Reveal Strong Global Water Cycle Intensification During 1950 to 2000. Science 336, 455–458. https://doi.org/10.1126/science.1212222
Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020. Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. Environ. Pollut. 259, 113837. https://doi.org/10.1016/j.envpol.2019.113837
Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's

Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE 9, e111913. https://doi.org/10.1371/journal.pone.0111913

Ferreira, I., Venâncio, C., Lopes, I., Oliveira, M., 2019. Nanoplastics and marine organisms: What has been studied? Environ. Toxicol. Pharmacol. 67, 1–7. https://doi.org/10.1016/j.etap.2019.01.006

Fluker, B.L., Pezold, F., Minton, R.L., 2011. Molecular and morphological divergence in the inland silverside (Menidia beryllina) along a freshwater-estuarine interface. Environ. Biol. Fishes 91, 311. https://doi.org/10.1007/s10641-011-9786-2

Fok, L., Cheung, P.K., 2015. Hong Kong at the Pearl River Estuary: A hotspot of microplastic pollution. Mar. Pollut. Bull. 99, 112–118. https://doi.org/10.1016/j.marpolbul.2015.07.050

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631, 550–559.

Ford, A.T., Ågerstrand, M., Brooks, B.W., Allen, J., Bertram, M.G., Brodin, T., Dang, Z., Duquesne, S., Sahm, R., Hoffmann, F., Hollert, H., Jacob, S., Klüver, N., Lazorchak, J.M., Ledesma, M., Melvin, S.D., Mohr, S., Padilla, S., Pyle, G.G., Scholz, S., Saaristo, M., Smit, E., Steevens, J.A., van den Berg, S., Kloas, W., Wong, B.B.M., Ziegler, M., Maack, G., 2021. The Role of Behavioral Ecotoxicology in Environmental Protection. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.0c06493

Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios. Environ. Pollut. 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022

Fuiman, L.A., Magurran, A.E., 1994. Development of predator defences in fishes. Rev. Fish Biol. Fish. 4, 145–183. https://doi.org/10.1007/BF00044127

Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92, 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041

Gandara e Silva, P.P., Nobre, C.R., Resaffe, P., Pereira, C.D.S., Gusmão, F., 2016. Leachate from microplastics impairs larval development in brown mussels. Water Res. 106, 364–370. https://doi.org/10.1016/j.watres.2016.10.016

Goff, A.D., Saranjampour, P., Ryan, L.M., Hladik, M.L., Covi, J.A., Armbrust, K.L., Brander, S.M., 2017. The effects of fipronil and the photodegradation product fipronil desulfinyl on growth and gene expression in juvenile blue crabs, Callinectes sapidus, at different salinities. Aquat. Toxicol. 186, 96–104. https://doi.org/10.1016/j.aquatox.2017.02.027

Gray, A.D., Weinstein, J.E., 2017b. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). Environ. Toxicol. Chem. 36, 3074–3080. https://doi.org/10.1002/etc.3881

Gray, A.D., Wertz, H., Leads, R.R., Weinstein, J.E., 2018a. Microplastic in two South Carolina Estuaries: Occurrence, distribution, and composition. Mar. Pollut. Bull. 128, 223–233. https://doi.org/10.1016/j.marpolbul.2018.01.030

Grillitsch, B., Vogl, C., Wytek, R., 1999. Qualification of spontaneous undirected locomotor behavior of fish for sublethal toxicity testing. Part II. Variability of measurement parameters under toxicant-induced stress. Environ. Toxicol. Chem. 18, 2743–2750. https://doi.org/10.1002/etc.5620181214

Gualtieri, M., Mantecca, P., Cetta, F., Camatini, M., 2008a. Organic compounds in tire particle induce reactive oxygen species and heat-shock proteins in the human alveolar cell line A549. Environ. Int. 34, 437–442. https://doi.org/10.1016/j.envint.2007.09.010

Gualtieri, M., Mantecca, P., Cetta, F., Camatini, M., 2008b. Organic compounds in tire particle induce reactive oxygen species and heat-shock proteins in the human alveolar cell line A549. Environ. Int. 34, 437–442. https://doi.org/10.1016/j.envint.2007.09.010

Hartwell, I., Jordahl, D., Dawson, C., 2000a. The Effect of Salinity on Tire Leachate Toxicity. Water. Air. Soil Pollut. 121, 119–131. https://doi.org/10.1023/A:1005282201554

Hartwell, I., Jordahl, D., Dawson, C., 2000b. The Effect of Salinity on Tire Leachate Toxicity. Water. Air. Soil Pollut. 121, 119–131. https://doi.org/10.1023/A:1005282201554

Hashemi, S.A.R., Stara, A., Faggio, C., 2019. Biological Characteristics, Growth Parameters and Mortality Rate of Carassius auratus in the Shadegan Wetland (Iran). Int. J. Environ. Res. 13, 457–464. https://doi.org/10.1007/s41742-019-00186-9

Helm, K.P., Bindoff, N.L., Church, J.A., 2010a. Changes in the global hydrological-cycle inferred from ocean salinity. Geophys. Res. Lett. 37. https://doi.org/10.1029/2010GL044222

Hernandez-Milian, G., Lusher, A., MacGabban, S., Rogan, E., 2019. Microplastics in grey seal (Halichoerus grypus) intestines: Are they associated with parasite aggregations? Mar. Pollut. Bull. 146, 349–354. https://doi.org/10.1016/j.marpolbul.2019.06.014

Hickey, B.M., Banas, N.S., 2003b. Oceanography of the U.S. Pacific Northwest Coastal Ocean and estuaries with application to coastal ecology. Estuaries 26, 1010–1031. https://doi.org/10.1007/BF02803360

Hoffmann, L., Eggers, S.L., Allhusen, E., Katlein, C., Peeken, I., 2020. Interactions between the ice algae Fragillariopsis cylindrus and microplastics in sea ice. Environ. Int. 139, 105697. https://doi.org/10.1016/j.envint.2020.105697

Hutton, S.J., St. Romain, S.J., Pedersen, E.I., Siddiqui, S., Chappell, P.E., White, J.W., Armbrust, K.L., Brander, S.M., 2021b. Salinity Alters Toxicity of Commonly Used Pesticides in a Model Euryhaline Fish Species (Menidia beryllina). Toxics 9, 114. https://doi.org/10.3390/toxics9050114

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1126/science.1260352

Jeon, J., Kannan, K., Lim, H.K., Moon, H.B., Ra, J.S., Kim, S.D., 2010. Bioaccumulation of Perfluorochemicals in Pacific Oyster under Different Salinity Gradients. Environ. Sci. Technol. 44, 2695–2701. https://doi.org/10.1021/es100151r

Jiwarungrueangkul, T., Phaksopa, J., Sompongchaiyakul, P., Tipmanee, D., 2021. Seasonal microplastic variations in estuarine sediments from urban canal on the west coast of Thailand: A case study in Phuket province. Mar. Pollut. Bull. 168, 112452. https://doi.org/10.1016/j.marpolbul.2021.112452

Kallai, J., Makany, T., Csatho, A., Karadi, K., Horvath, D., Kovacs-Labadi, B., Jarai, R., Nadel, L., Jacobs, J.W., 2007. Cognitive and affective aspects of thigmotaxis strategy in humans. Behav. Neurosci. 121, 21–30. https://doi.org/10.1037/0735-7044.121.1.21

Karan, H., Funk, C., Grabert, M., Oey, M., Hankamer, B., 2019. Green Bioplastics as Part of a Circular Bioeconomy. Trends Plant Sci. 24, 237–249. https://doi.org/10.1016/j.tplants.2018.11.010

Katyal, D., Kong, E., Villanueva, J., 2020. Microplastics in the environment: impact on human health and future mitigation strategies. Environ. Health Rev. https://doi.org/10.5864/d2020-005

Khan, F., Ahmed, W., Najmi, A., 2019. Understanding consumers' behavior intentions towards dealing with the plastic waste: Perspective of a developing country. Resour. Conserv. Recycl. 142, 49–58. https://doi.org/10.1016/j.resconrec.2018.11.020

Khoironi, A., Anggoro, S., Sudarno, 2018. The existence of microplastic in Asian green mussels. IOP Conf. Ser. Earth Environ. Sci. 131, 012050. https://doi.org/10.1088/1755-1315/131/1/012050

Klaine, S.J., Koelmans, A.A., Horne, N., Carley, S., Handy, R.D., Kapustka, L., Nowack, B., Kammer, F. von der, 2012. Paradigms to assess the environmental impact of manufactured nanomaterials. Environ. Toxicol. Chem. 31, 3–14. https://doi.org/10.1002/etc.733

Klöckner, P., Reemtsma, T., Eisentraut, P., Braun, U., Ruhl, A.S., Wagner, S., 2019. Tire and road wear particles in road environment – Quantification and assessment of particle dynamics by Zn determination after density separation. Chemosphere 222, 714–721. https://doi.org/10.1016/j.chemosphere.2019.01.176

Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A.M., Sanden, M., 2020. Micro- and nanoplastic toxicity on aquatic life: Determining factors. Sci. Total Environ. 709, 136050. https://doi.org/10.1016/j.scitotenv.2019.136050

Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610–611, 635–640. https://doi.org/10.1016/j.scitotenv.2017.08.053

Kontchou, J.A., Nachev, M., Sures, B., 2021. Ecotoxicological effects of traffic-related metal sediment pollution in Lumbriculus variegatus and Gammarus sp. Environ. Pollut. 268, 115884. https://doi.org/10.1016/j.envpol.2020.115884

Le Roux, J.P., 2005a. Grains in motion: A review. Sediment. Geol. 178, 285–313. https://doi.org/10.1016/j.sedgeo.2005.05.009

Leal Filho, W., Saari, U., Fedoruk, M., Iital, A., Moora, H., Klöga, M., Voronova, V., 2019. An overview of the problems posed by plastic products and the role of extended producer responsibility in Europe. J. Clean. Prod. 214, 550–558. https://doi.org/10.1016/j.jclepro.2018.12.256

Lee, D.-H., Lee, S., Rhee, J.-S., 2021. Consistent exposure to microplastics induces age-specific physiological and biochemical changes in a marine mysid. Mar. Pollut. Bull. 162, 111850. https://doi.org/10.1016/j.marpolbul.2020.111850

Lee, K.-W., Shim, W.J., Kwon, O.Y., Kang, J.-H., 2013. Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod *Tigriopus japonicus*. Environ. Sci. Technol. 47, 11278–11283. https://doi.org/10.1021/es401932b

Lei, L., Liu, M., Song, Y., Lu, S., Hu, J., Cao, C., Xie, B., Shi, H., He, D., 2018. Polystyrene (nano)microplastics cause size-dependent neurotoxicity, oxidative damage and other adverse effects in *Caenorhabditis elegans*. Environ. Sci. Nano 5, 2009–2020. https://doi.org/10.1039/C8EN00412A

Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409, 3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038

Little, E.E., 1990. Behavioral toxicology: Stimulating challenges for a growing discipline. Environ. Toxicol. Chem. 9, 1–2. https://doi.org/10.1002/etc.5620090101

Little, E.E., Finger, S.E., 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environ. Toxicol. Chem. 9, 13–19. https://doi.org/10.1002/etc.5620090103

Lo, H.K.A., Chan, K.Y.K., 2018. Negative effects of microplastic exposure on growth and development of Crepidula onyx. Environ. Pollut. 233, 588–595. https://doi.org/10.1016/j.envpol.2017.10.095

Lönnstedt, O.M., Eklöv, P., 2016. Environmentally relevant concentrations of microplastic particles influence larval fish ecology. Science 352, 1213–1216.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Response to Comment on “Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (*Danio rerio*) and Toxic Effects in Liver.” Environ. Sci. Technol. 50, 12523–12524. https://doi.org/10.1021/acs.est.6b04379

Major, K.M., DeCourten, B.M., Li, J., Britton, M., Settles, M.L., Mehinto, A.C., Connon, R.E., Brander, S.M., 2020. Early Life Exposure to Environmentally Relevant Levels of Endocrine Disruptors Drive Multigenerational and Transgenerational Epigenetic Changes in a Fish Model. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.00471

Malinich, T.D., Chou, N., Sepúlveda, M.S., Höök, T.O., 2018. No evidence of microplastic impacts on consumption or growth of larval Pimephales promelas. Environ. Toxicol. Chem. 37, 2912–2918.

Mattsson, K., Ekvall, M.T., Hansson, L.-A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered Behavior, Physiology, and Metabolism in Fish Exposed to Polystyrene Nanoparticles. Environ. Sci. Technol. 49, 553–561. https://doi.org/10.1021/es5053655

Mauchline, J., ED, B.J., ED, R.F., ED, Yonge M., ED, YONGE M., 1980. THE BIOLOGY OF MYSIDS AND EUPHAUSIIDS. Biol. MYSIDS EUPHAUSIIDS.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., Zambonino-Infante, J.,

2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Mar. Environ. Res. 112, 78–85. https://doi.org/10.1016/j.marenvres.2015.09.009

Mees, J., Jones, M.B., 1998. The hyperbenthos. Oceanogr. Lit. Rev. 7, 1175–1176.

Middaugh, D.P., Hemmer, M.J., Goodman, L.R., 1987. Methods for spawning, culturing and conducting toxicity-tests with early life stages of four atherinid fishes: the inland silverside, Menidia beryllina, Atlantic silverside, M. menidia, tidewater silverside, M. peninsulae and California grunion, Leuresthes tenuis. Office of Research and Development, U.S. Environmental Protection Agency, Washington DC 20460.

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

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

Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar. Pollut. Bull. 145, 254–259. https://doi.org/10.1016/j.marpolbul.2019.02.037

Nelson, S., Mueller, G., Hemphill, D., Hemphill, D., 1994. Identification of tire leachate toxicants and a risk assessment of water quality effects using tire reefs in canals. Bull. Environ. Contam. Toxicol. 52, 574–581.

Newman, M.C., Jagoe, C.H., 1996. Ecotoxicology: A Hierarchical Treatment. CRC Press.

Noldus, L.P.J.J., Spink, A.J., Tegelenbosch, R.A.J., 2001. EthoVision: A versatile video tracking system for automation of behavioral experiments. Behav. Res. Methods Instrum. Comput. 33, 398–414. https://doi.org/10.3758/BF03195394

Ogonowski, M., Schür, C., Jarsén, Å., Gorokhova, E., 2016. The Effects of Natural and Anthropogenic Microparticles on Individual Fitness in Daphnia magna. PLOS ONE 11, e0155063. https://doi.org/10.1371/journal.pone.0155063

Ohnuma, S., Katagiri, T., Maita, M., Endo, M., Futami, K., 2017. Application of Tissue Clearing Techniques to 3D Study of Infectious Disease Pathology in Fish. Fish Pathol. 52, 96–99. https://doi.org/10.3147/jsfp.52.96

Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K., Danion, M., Cachot, J., 2020. Environmental samples of microplastics induce significant toxic effects in fish larvae. Environ. Int. 134, 105047. https://doi.org/10.1016/j.envint.2019.105047

Parker, B.W., Beckingham, B.A., Ingram, B.C., Ballenger, J.C., Weinstein, J.E., Sancho, G., 2020. Microplastic and tire wear particle occurrence in fishes from an urban estuary: Influence of feeding characteristics on exposure risk.

Mar. Pollut. Bull. 160, 111539. https://doi.org/10.1016/j.marpolbul.2020.111539

Peterson, R.H., Martin-Robichaud, D.J., Berge, O., 1996. Influence of temperature and salinity on length and yolk utilization of striped bass larvae. Aquac. Int. 4, 89–103. https://doi.org/10.1007/BF00140591

Pillard, D.A., Dufresne, D.L., Tietge, J.E., Evans, J.M., 1999. Response of mysid shrimp (Mysidopsis bahia), sheepshead minnow (Cyprinodon variegatus), and inland silverside minnow (Menidia beryllina) to changes in artificial seawater salinity. Environ. Toxicol. Chem. 18, 430–435. https://doi.org/10.1002/etc.5620180310

Piotrowska, K., Kruszelnicka, W., Bałdowska-Witos, P., Kasner, R., Rudnicki, J., Tomporowski, A., Flizikowski, J., Opielak, M., 2019. Assessment of the Environmental Impact of a Car Tire throughout Its Lifecycle Using the LCA Method. Materials 12, 4177. https://doi.org/10.3390/ma12244177

Redondo-Hasselerharm, P.E., de Ruijter, V.N., Mintenig, S.M., Verschoor, A., Koelmans, A.A., 2018. Ingestion and Chronic Effects of Car Tire Tread Particles on Freshwater Benthic Macroinvertebrates. Environ. Sci. Technol. 52, 13986–13994. https://doi.org/10.1021/acs.est.8b05035

Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in Daphnia magna – Quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398–407. https://doi.org/10.1016/j.envpol.2017.05.048

Ritz, C., 2010. Toward a unified approach to dose-response modeling in ecotoxicology. Environ. Toxicol. Chem. 29, 220–229.

Roast, S., Widdows, J., Pope, N., Jones, M., 2004. Sediment-biota interactions: mysid feeding activity enhances water turbidity and sediment erodability. Mar. Ecol. Prog. Ser. 281, 145–154. https://doi.org/10.3354/meps281145

Roberts, A.C., Reichl, J., Song, M.Y., Dearinger, A.D., Moridzadeh, N., Lu, E.D., Pearce, K., Esdin, J., Glanzman, D.L., 2011. Habituation of the C-Start Response in Larval Zebrafish Exhibits Several Distinct Phases and Sensitivity to NMDA Receptor Blockade. PLoS ONE 6, e29132. https://doi.org/10.1371/journal.pone.0029132

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H.D., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019a. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4371

Rochman, C.M., Browne, M.A., Underwood, A.J., van Franeker, J.A., Thompson, R.C., Amaral-Zettler, L.A., 2016a. The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. Ecology 97, 302–312. https://doi.org/10.1890/14-2070.1

Rochman, C.M., Munno, K., Box, C., Cummins, A., Zhu, X., Sutton, R., 2021. Think Global, Act Local: Local Knowledge Is Critical to Inform Positive Change

When It Comes to Microplastics. Environ. Sci. Technol. 55, 4–6. https://doi.org/10.1021/acs.est.0c05746
Rogge, W.F., Hildemann, L.M., Mazurek, M.A., Cass, G.R., Simoneit, B.R.T., 1993a. Sources of fine organic aerosol. 3. Road dust, tire debris, and organometallic brake lining dust: roads as sources and sinks. Environ. Sci. Technol. 27, 1892–1904. https://doi.org/10.1021/es00046a019
Romney, A.L.T., Yanagitsuru, Y.R., Mundy, P.C., Fangue, N.A., Hung, T.-C., Brander, S.M., Connon, R.E., 2019. Developmental staging and salinity tolerance in embryos of the delta smelt, Hypomesus transpacificus. Aquaculture 511, 634191. https://doi.org/10.1016/j.aquaculture.2019.06.005
Sadri, S.S., Thompson, R.C., 2014. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. Mar. Pollut. Bull. 81, 55–60. https://doi.org/10.1016/j.marpolbul.2014.02.020
Santos, D., Félix, L., Luzio, A., Parra, S., Cabecinha, E., Bellas, J., Monteiro, S.M., 2020. Toxicological effects induced on early life stages of zebrafish (Danio rerio) after an acute exposure to microplastics alone or co-exposed with copper. Chemosphere 261, 127748. https://doi.org/10.1016/j.chemosphere.2020.127748
Schnörr, S.J., Steenbergen, P.J., Richardson, M.K., Champagne, D.L., 2012. Measuring thigmotaxis in larval zebrafish. Behav. Brain Res. 228, 367–374. https://doi.org/10.1016/j.bbr.2011.12.016
Segarra, A., Mauduit, F., Amer, N.R., Biefel, F., Hladik, M.L., Connon, R.E., Brander, S.M., 2021. Salinity Changes the Dynamics of Pyrethroid Toxicity in Terms of Behavioral Effects on Newly Hatched Delta Smelt Larvae. Toxics 9, 40. https://doi.org/10.3390/toxics9020040
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013
Sharma, S., Coombs, S., Patton, P., de Perera, T.B., 2009. The function of wall-following behaviors in the Mexican blind cavefish and a sighted relative, the Mexican tetra (Astyanax). J. Comp. Physiol. A 195, 225–240. https://doi.org/10.1007/s00359-008-0400-9
Shen, M., Zhang, Y., Zhu, Y., Song, B., Zeng, G., Hu, D., Wen, X., Ren, X., 2019. Recent advances in toxicological research of nanoplastics in the environment: A review. Environ. Pollut. 252, 511–521. https://doi.org/10.1016/j.envpol.2019.05.102
Shepherd, J.G., Cushing, D.H., 1980. A mechanism for density-dependent survival of larval fish as the basis of a stock-recruitment relationship. ICES J. Mar. Sci. 39, 160–167. https://doi.org/10.1093/icesjms/39.2.160
Shupe, H.J., Boenisch, K.M., Harper, B.J., Brander, S.M., Harper, S.L., 2021. Effect of Nanoplastic Type and Surface Chemistry on Particle Agglomeration over a Salinity Gradient. Environ. Toxicol. Chem. etc.5030. https://doi.org/10.1002/etc.5030
Siegfried, M., Koelmans, A.A., Besseling, E., Kroeze, C., 2017. Export of microplastics from land to sea. A modelling approach. Water Res. 127, 249–257. https://doi.org/10.1016/j.watres.2017.10.011

Smith, T.I.J., Denson, M.R., Heyward, L.D., Jenkins, W.E., Carter, L.M., 1999. Salinity Effects on Early Life Stages of Southern Flounder Paralichthys lethostigma. J. World Aquac. Soc. 30, 236–244. https://doi.org/10.1111/j.1749-7345.1999.tb00870.x

Sprague, J.B., 1971. Measurement of pollutant toxicity to fish—III: Sublethal effects and “safe” concentrations. Water Res. 5, 245–266. https://doi.org/10.1016/0043-1354(71)90171-0

Susaki, E.A., Tainaka, K., Perrin, D., Yukinaga, H., Kuno, A., Ueda, H.R., 2015. Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging. Nat. Protoc. 10, 1709–1727. https://doi.org/10.1038/nprot.2015.085

Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6, 34351. https://doi.org/10.1038/srep34351

Tandler, A., Anav, F.A., Choshniak, I., 1995. The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, Sparus aurata, larvae. Aquaculture 135, 343–353. https://doi.org/10.1016/0044-8486(95)01029-7

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science 304, 838–838. https://doi.org/10.1126/science.1094559

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., Kolodziej, E.P., 2021b. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science 371, 185–189. https://doi.org/10.1126/science.abd6951

Tomas, F., Martínez-Crego, B., Hernán, G., Santos, R., 2015b. Responses of seagrass to anthropogenic and natural disturbances do not equally translate to its consumers. Glob. Change Biol. 21, 4021–4030. https://doi.org/10.1111/gcb.13024

Treit, D., Fundytus, M., 1988. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol. Biochem. Behav. 31, 959–962. https://doi.org/10.1016/0091-3057(88)90413-3

Unice, K.M., Weeber, M.P., Abramson, M.M., Reid, R.C.D., van Gils, J.A.G., Markus, A.A., Vethaak, A.D., Panko, J.M., 2019. Characterizing export of land-based microplastics to the estuary - Part I: Application of integrated geospatial microplastic transport models to assess tire and road wear particles in the Seine watershed. Sci. Total Environ. 646, 1639–1649. https://doi.org/10.1016/j.scitotenv.2018.07.368

U.S. Bureau of Reclamation, Nelson, S., Mueller, G., Hemphill, D., 1994. Identification of tire leachate toxicants and a risk assessment of water quality effects using tire reefs in canals. Bull. Environ. Contam. Toxicol. 52, 574–581.

USEPA, 2009. Culturing Americamysis bahia Supplement to Training Video- EPA 833-C-09-001.

USEPA, 1994. Risk Assessment Guidance for Superfund, Volume II: Environmental Evaluation Manual (EPA/540-1-89/001).
Van Cauwenberghe, L., Janssen, C.R., 2014a. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010
Vermeiren, P., Muñoz, C.C., Ikejima, K., 2016. Sources and sinks of plastic debris in estuaries: A conceptual model integrating biological, physical and chemical distribution mechanisms. Mar. Pollut. Bull. 113, 7–16. https://doi.org/10.1016/j.marpolbul.2016.10.002
Verslycke, T., Ghekiere, A., Raimondo, S., Janssen, C., 2007. Mysid crustaceans as standard models for the screening and testing of endocrine-disrupting chemicals. Ecotoxicology 16, 205. https://doi.org/10.1007/s10646-006-0122-0
Verslycke, T., Vangheluwe, M., Heijerick, D., De Schamphelaere, K., Van Sprang, P., Janssen, C.R., 2003. The toxicity of metal mixtures to the estuarine mysid Neomysis integer (Crustacea: Mysidacea) under changing salinity. Aquat. Toxicol. 64, 307–315. https://doi.org/10.1016/S0166-445X(03)00061-4
Verslycke, T.A., Fockedey, N., McKenney, C.L., Roast, S.D., Jones, M.B., Mees, J., Janssen, C.R., 2004. Mysid crustaceans as potential test organisms for the evaluation of environmental endocrine disruption: A review. Environ. Toxicol. Chem. 23, 1219–1234. https://doi.org/10.1897/03-332
Verslycke, T.A., Vethaak, A.D., Arijs, K., Janssen, C.R., 2005. Flame retardants, surfactants and organotins in sediment and mysid shrimp of the Scheldt estuary (The Netherlands). Environ. Pollut. 136, 19–31. https://doi.org/10.1016/j.envpol.2004.12.008
Vlaming, V. de, Connor, V., DiGiorgio, C., Bailey, H.C., Deanovic, L.A., Hinton, D.E., 2000. Application of whole effluent toxicity test procedures to ambient water quality assessment. Environ. Toxicol. Chem. 19, 42–62. https://doi.org/10.1002/etc.5620190106
W, B., M, I., 1998. Emissions from tires in dedicated use. Kautsch. Gummi Kunststoffe 51, 182–186.
Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., Reemtsma, T., 2018b. Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. Water Res. 139, 83–100. https://doi.org/10.1016/j.watres.2018.03.051
Wang, X., Liu, L., Zheng, H., Wang, M., Fu, Y., Luo, X., Li, F., Wang, Z., 2020. Polystyrene microplastics impaired the feeding and swimming behavior of mysid shrimp Neomysis japonica. Mar. Pollut. Bull. 150, 110660. https://doi.org/10.1016/j.marpolbul.2019.110660
Wang, Z., Yin, L., Zhao, J., Xing, B., 2016. Trophic transfer and accumulation of TiO2 nanoparticles from clamworm (Perinereis aibuhitensis) to juvenile turbot (Scophthalmus maximus) along a marine benthic food chain. Water Res. 95, 250–259. https://doi.org/10.1016/j.watres.2016.03.027
Watts, A.J., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T.S., 2014. Uptake and retention of microplastics by the shore crab Carcinus maenas. Environ. Sci. Technol. 48, 8823–8830.

Watts, A.J.R., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016a. Effect of Microplastic on the Gills of the Shore Crab *Carcinus maenas*. Environ. Sci. Technol. 50, 5364–5369. https://doi.org/10.1021/acs.est.6b01187

Watts, A.J.R., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016b. Effect of Microplastic on the Gills of the Shore Crab *Carcinus maenas*. Environ. Sci. Technol. 50, 5364–5369. https://doi.org/10.1021/acs.est.6b01187

Weinstein, M.P., 1986. Habitat Suitability Index Models: Inland silverside. National Wetlands Research Center.

Werbowski, L.M., Gilbreath, A.N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M.D., Deshpande, A.D., Rochman, C.M., 2021. Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. ACS EST Water acsestwater.1c00017. https://doi.org/10.1021/acsestwater.1c00017

Wik, A., Dave, G., 2009. Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. Environ. Pollut. 157, 1–11. https://doi.org/10.1016/j.envpol.2008.09.028

Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., Goodspeed, J., Suciu, C., Tan, J., Grimes, C., Chung, A., Rosenberg, M., Gaikwad, S., Denmark, A., Jackson, A., Kadri, F., Chung, K.M., Stewart, A., Gilder, T., Beeson, E., Zapolsky, I., Wu, N., Cachat, J., Kalueff, A.V., 2010. Analyzing habituation responses to novelty in zebrafish (Danio rerio). Behav. Brain Res. 208, 450–457. https://doi.org/10.1016/j.bbr.2009.12.023

Woods, M.N., Hong, T.J., Baughman, D., Andrews, G., Fields, D.M., Matrai, P.A., 2020. Accumulation and effects of microplastic fibers in American lobster larvae (Homarus americanus). Mar. Pollut. Bull. 157, 111280. https://doi.org/10.1016/j.marpolbul.2020.111280

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., Watson, R., 2006a. Impacts of Biodiversity Loss on Ocean Ecosystem Services. Science 314, 787–790. https://doi.org/10.1126/science.1132294

Wortham-Neal, J.L., Price, W.W., 2002. Marsupial Developmental Stages in Americamysis Bahia (Mysida: Mysidae). J. Crustac. Biol. 22, 98–112. https://doi.org/10.1163/20021975-99990213

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068

Wurtsbaugh, W., Li, H., 1985. Diel migrations of a zooplanktivorous fish (Menidia beryllina) in relation to the distribution of its prey in a large eutrophic lake1. Limnol. Oceanogr. 30, 565–576. https://doi.org/10.4319/lo.1985.30.3.0565

Xia, X., Sun, M., Zhou, M., Chang, Z., Li, L., 2020. Polyvinyl chloride microplastics induce growth inhibition and oxidative stress in Cyprinus carpio var. larvae. Sci. Total Environ. 716, 136479.

Xu, Q., Xing, R., Sun, M., Gao, Y., An, L., 2020. Microplastics in sediments from an interconnected river-estuary region. Sci. Total Environ. 729, 139025. https://doi.org/10.1016/j.scitotenv.2020.139025

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018a. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). J. Hazard. Mater. 360, 97–105. https://doi.org/10.1016/j.jhazmat.2018.07.110

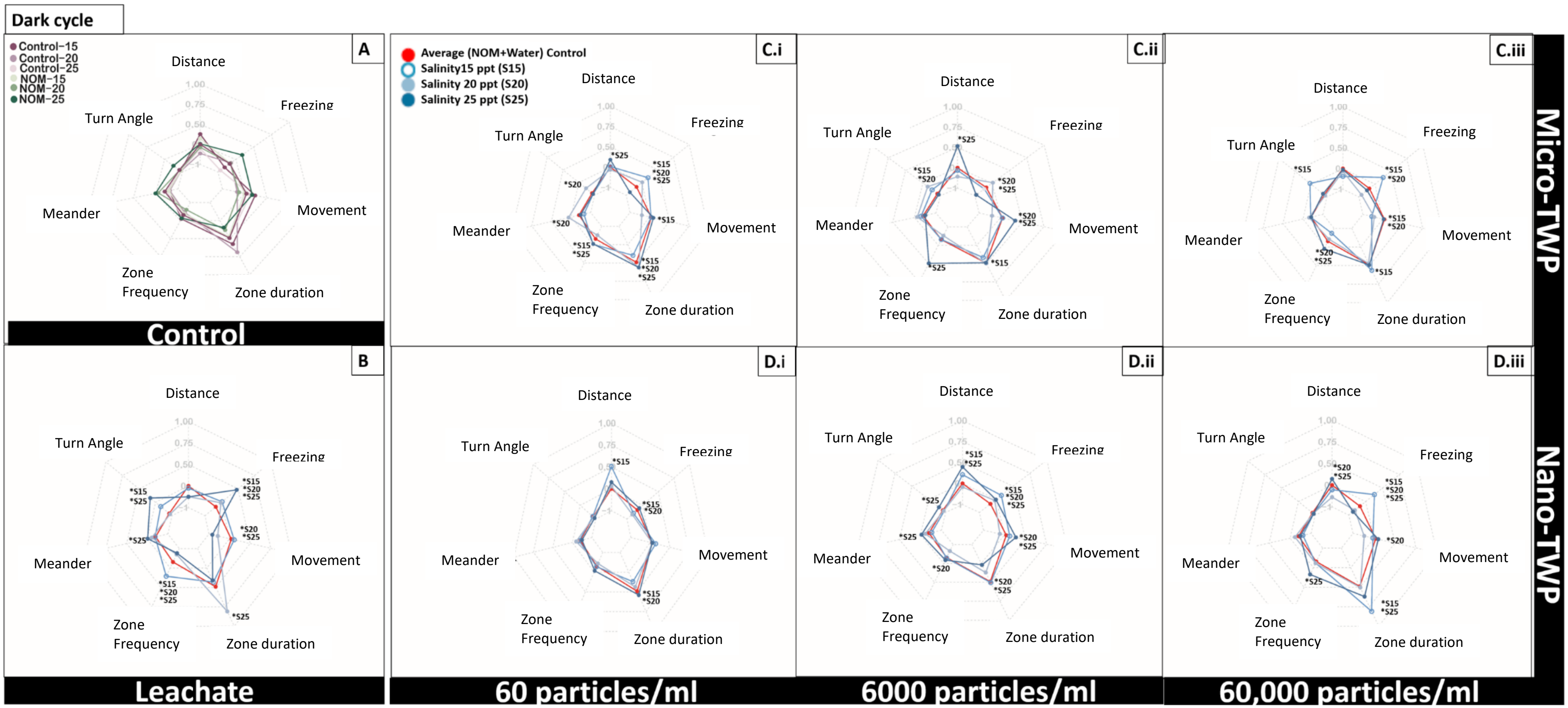
Yin, L., Liu, H., Cui, H., Chen, B., Li, L., Wu, F., 2019a. Impacts of polystyrene microplastics on the behavior and metabolism in a marine demersal teleost, black rockfish (Sebastes schlegelii). J. Hazard. Mater. 380, 120861. https://doi.org/10.1016/j.jhazmat.2019.120861

Yonkos, L.T., Friedel, E.A., Perez-Reyes, A.C., Ghosal, S., Arthur, C.D., 2014. Microplastics in Four Estuarine Rivers in the Chesapeake Bay, U.S.A. Environ. Sci. Technol. 48, 14195–14202. https://doi.org/10.1021/es5036317

TABLES AND FIGURES

Table 1 Behavioral variables from Noldus EthoVision software used in this study to analyze Mysid shrimp (*A. bahia*) larvae and Silverside (*M. beryllina*) larvae behavioral response

Variable	**Unit**	**Description**
Distance (Total)	cm	Total distance moved inside the well throughout the video recording time.
Freezing	S	The mean of total time fish moving less than 2 seconds
Movement	S	Duration for which the selected body point was changing location with respect to center
In Zone duration	S	The total time spent in the beaker center part (zone)
In Zone Frequency		The number of time fish spent time in zone
Meander	Deg/cm	Turning in animals moving at different speed.
Turn Angle	degree	Difference in heading between two samples.



Dark cycle
A
Control-15
Control-20
Control-25
NOM-15
NOM-20
NOM-25
Distance
Freezing
Movement
Zone duration
Zone Frequency
Meander
Turn Angle
Control
B
Leachate
Average (NOM+Water) Control
Salinity15 ppt (S15)
Salinity 20 ppt (S20)
Salinity 25 ppt (S25)
C.i
C.ii
C.iii
Micro-TWP
D.i
D.ii
D.iii
Nano-TWP
60 particles/ml
6000 particles/ml
60,000 particles/ml

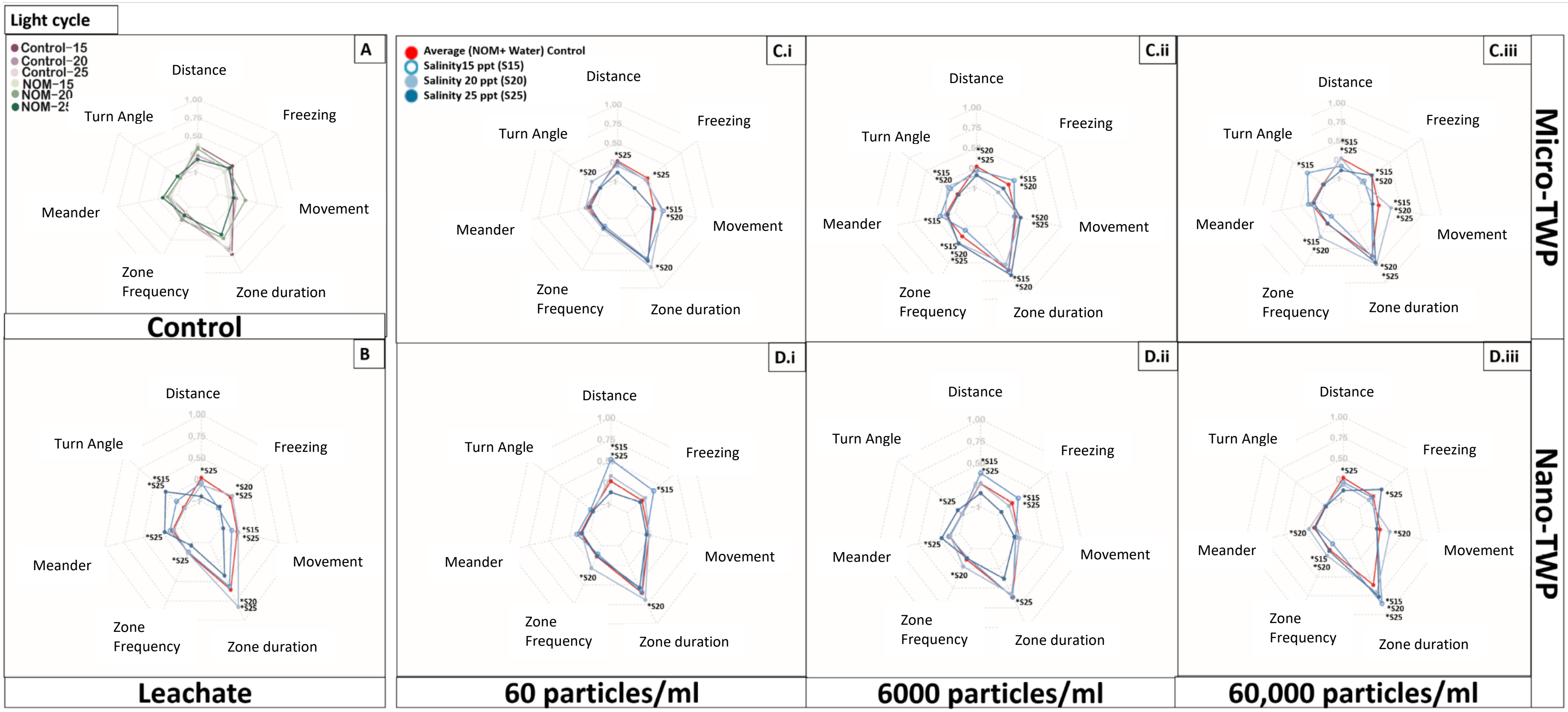


Figure 1 Mysid shrimp (*A. bahia*) behavioral response represented as radar plot after 7 days exposure to micro and nano TWP in combined average dark and light cycles with leachate exposed at both the cycles across a salinity gradient 15psu – 25psu. A) Control in water and NOM; B) Leachate; C) Micro TWP exposed group with average water and NOM control; D) Nano TWP Exposed group with average water and NOM control. Data normalized to 0-1 scale. * $p < 0.05$ ANOVA test followed by Dunnet's test, comparing all concentrations to their respective salinity NOM control within each cycle per salinity.

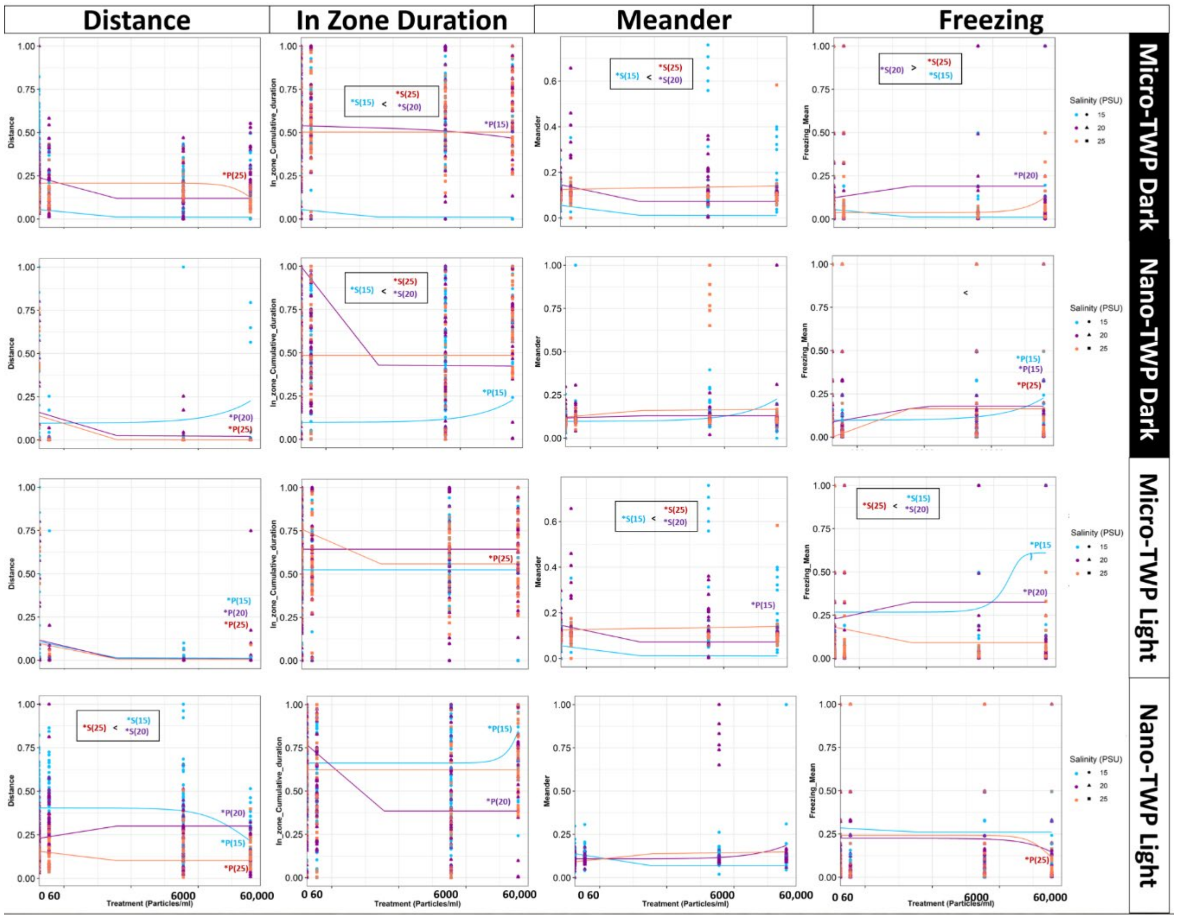
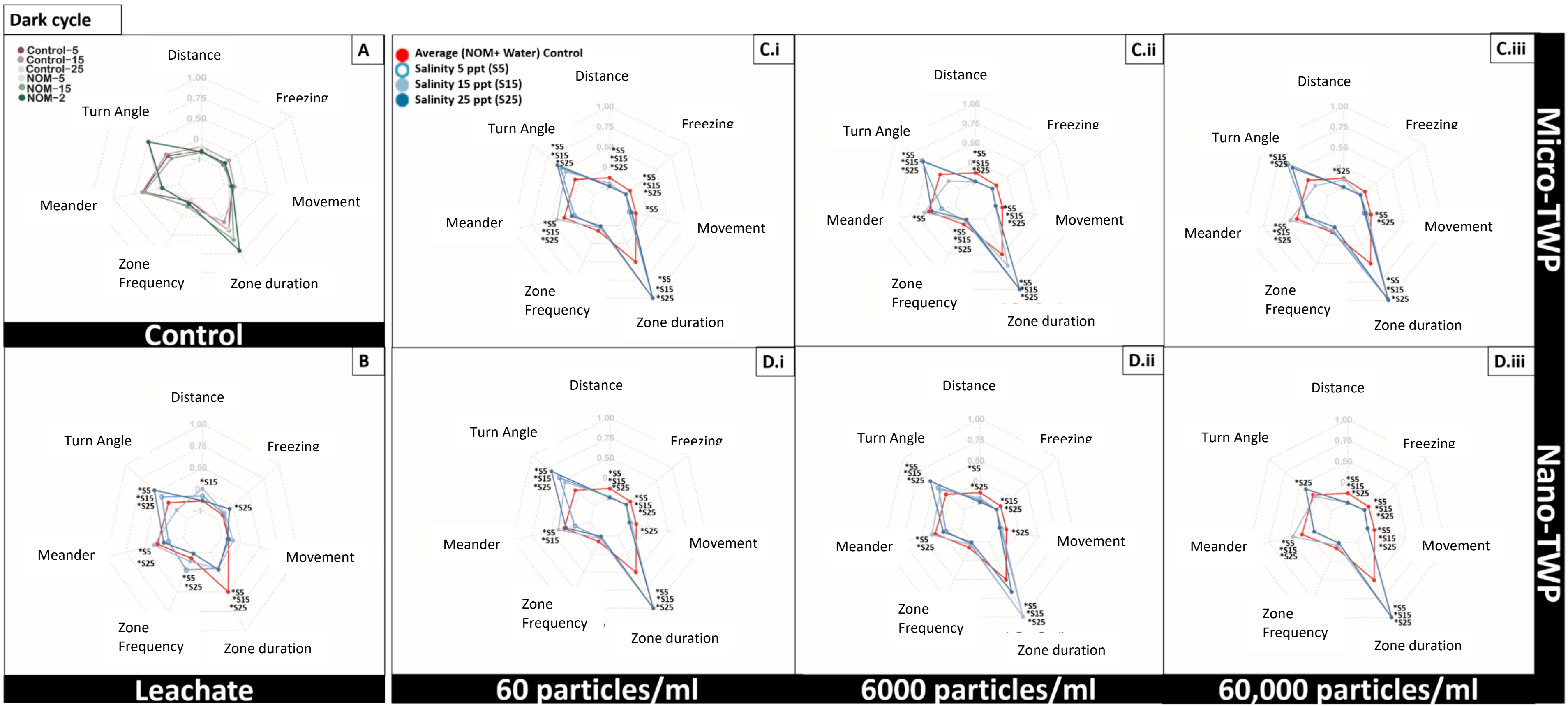


Figure 2 Mysid shrimp (*A. bahia*) behavioral dose response curves after 7 days exposure to micro and nano TWP in combined average dark and light cycles across a salinity gradient 15psu – 25psu. "P" represents particle count and "S" salinity. Data normalized to 0-1 scale. * $p < 0.05$ ANOVA test followed by Dunnet's test, comparing all concentrations to their respective salinity NOM control within each cycle per salinity



Dark cycle
A
Control-5
Control-15
Control-25
NOM-5
NOM-15
NOM-2
Distance
Freezing
Movement
Zone duration
Zone Frequency
Meander
Turn Angle
Control
B
Leachate
C.i
Average (NOM+ Water) Control
Salinity 5 ppt (S5)
Salinity 15 ppt (S15)
Salinity 25 ppt (S25)
C.ii
C.iii
Micro-TWP
D.i
D.ii
D.iii
Nano-TWP
60 particles/ml
6000 particles/ml
60,000 particles/ml

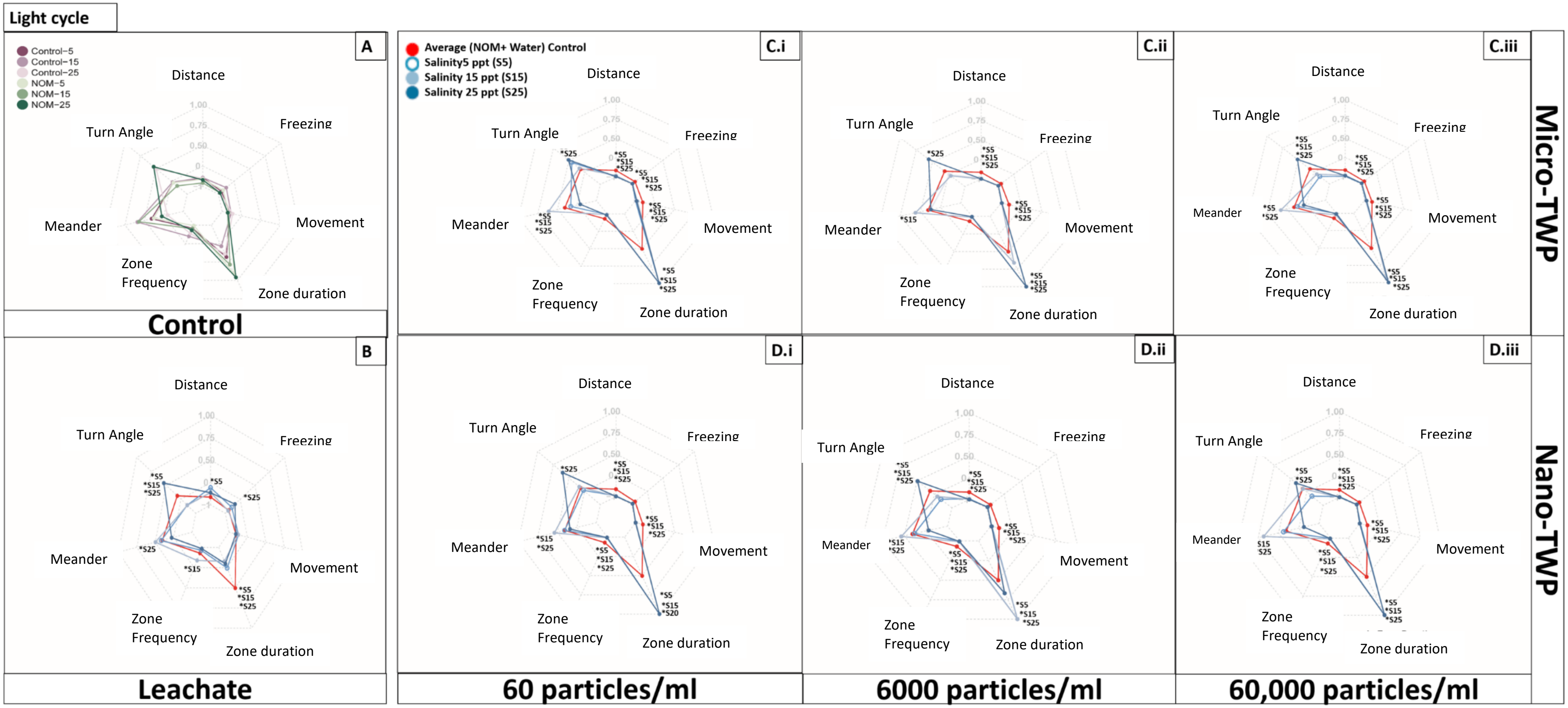


Figure 3. Silverside (*M. beryllina*) larvae behavioral response represented as radar plot after 7 days exposure to micro and nano TWP in combined average dark and light cycles with leachate exposed at both the cycles across a salinity gradient 5 PSU – 25 PSU. A) Control in water and NOM; B) Leachate; C) Micro TWP exposed group with average water and NOM control; D) Nano TWP Exposed group with average water and NOM control. Data normalized to 0-1 scale. * $p < 0.05$ ANOVA test followed by Dunnet's test, comparing all concentrations to their respective NOM salinity control within each cycle per salinity.

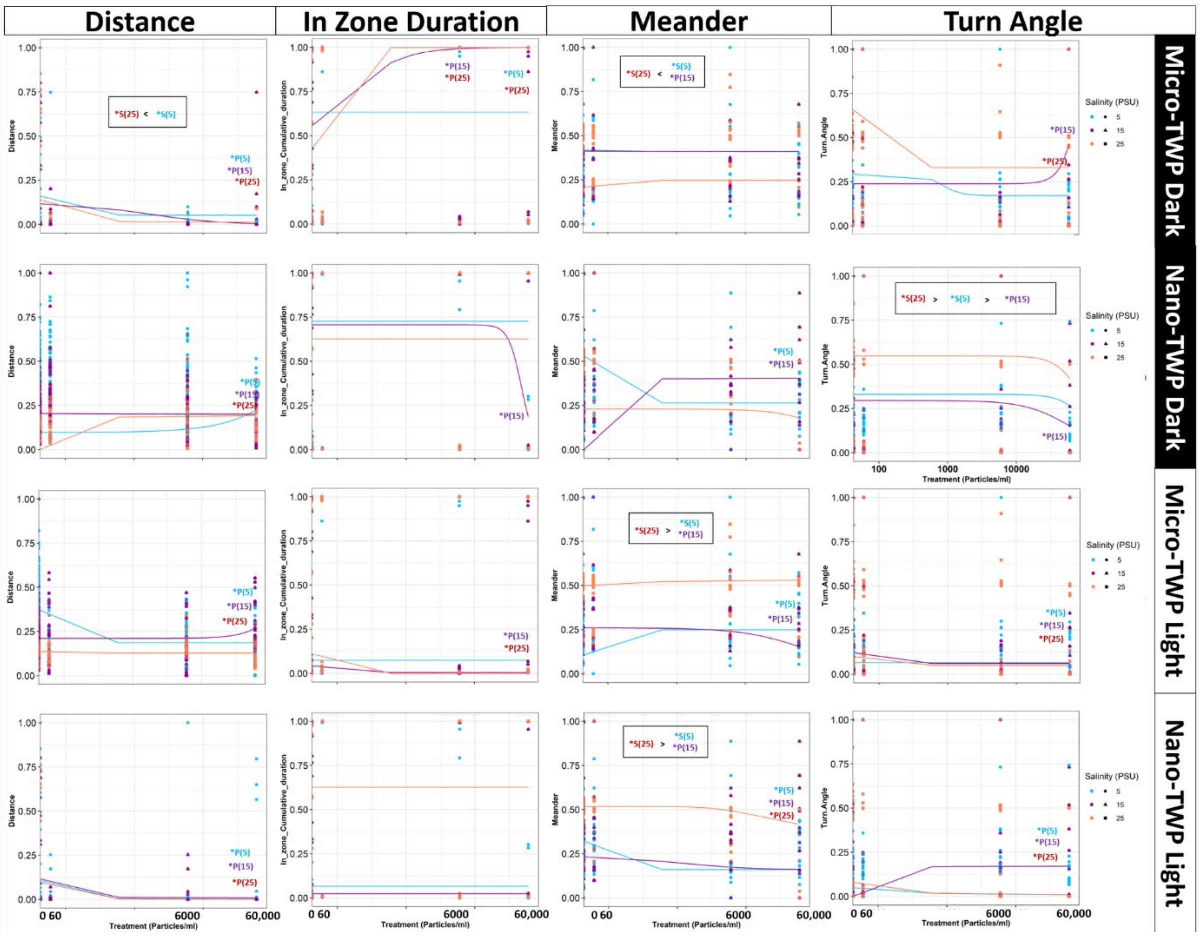


Figure 4. Silverside (*M. beryllina*) larvae behavioral dose response curves after 7 days exposure to micro and nano TWP in combined average dark and light cycles across a salinity gradient 15 PSU – 25 PSU. "P" represents particle count and "S" salinity. Data normalized to 0-1 scale. * $p < 0.05$ ANOVA test followed by Dunnet's test, comparing all concentrations to their respective salinity NOM control within each cycle per salinity.

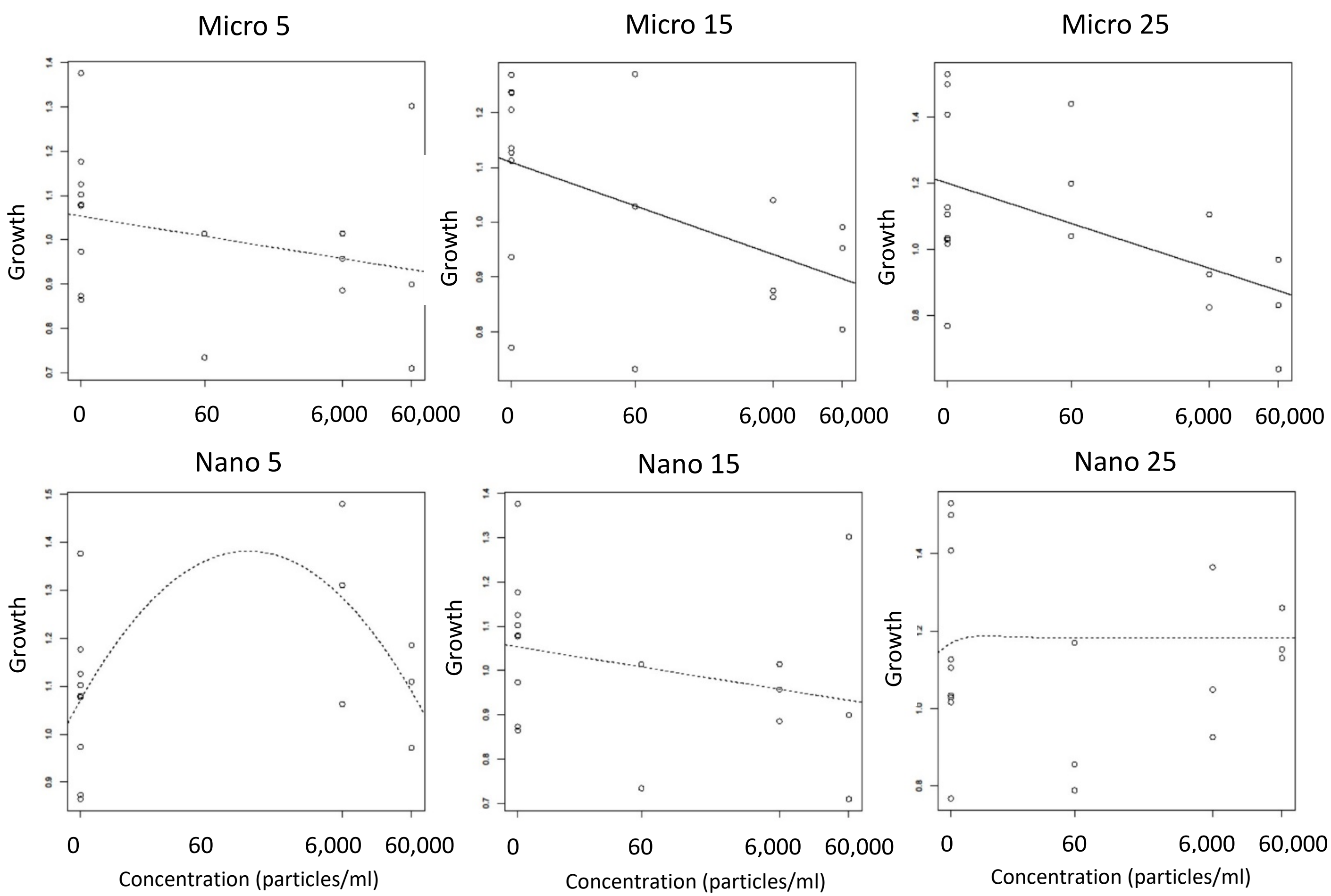


Figure 5. Dose response growth curve of mysid shrimp (*A. bahia*) larvae exposed to micro and nano TWP across 15-25 PSU salinity gradient. Each circle represents the rescaled growth index mean of one larva (n=9). Data are presented on a log10 X+ 0.05 axis. Curves shown as a solid line are significantly better fits than a null intercept-only model (p<0.05), curves shown as a dashed line are the best-fit of the five-curve option (lowest p-value), but not significantly better than the null model.

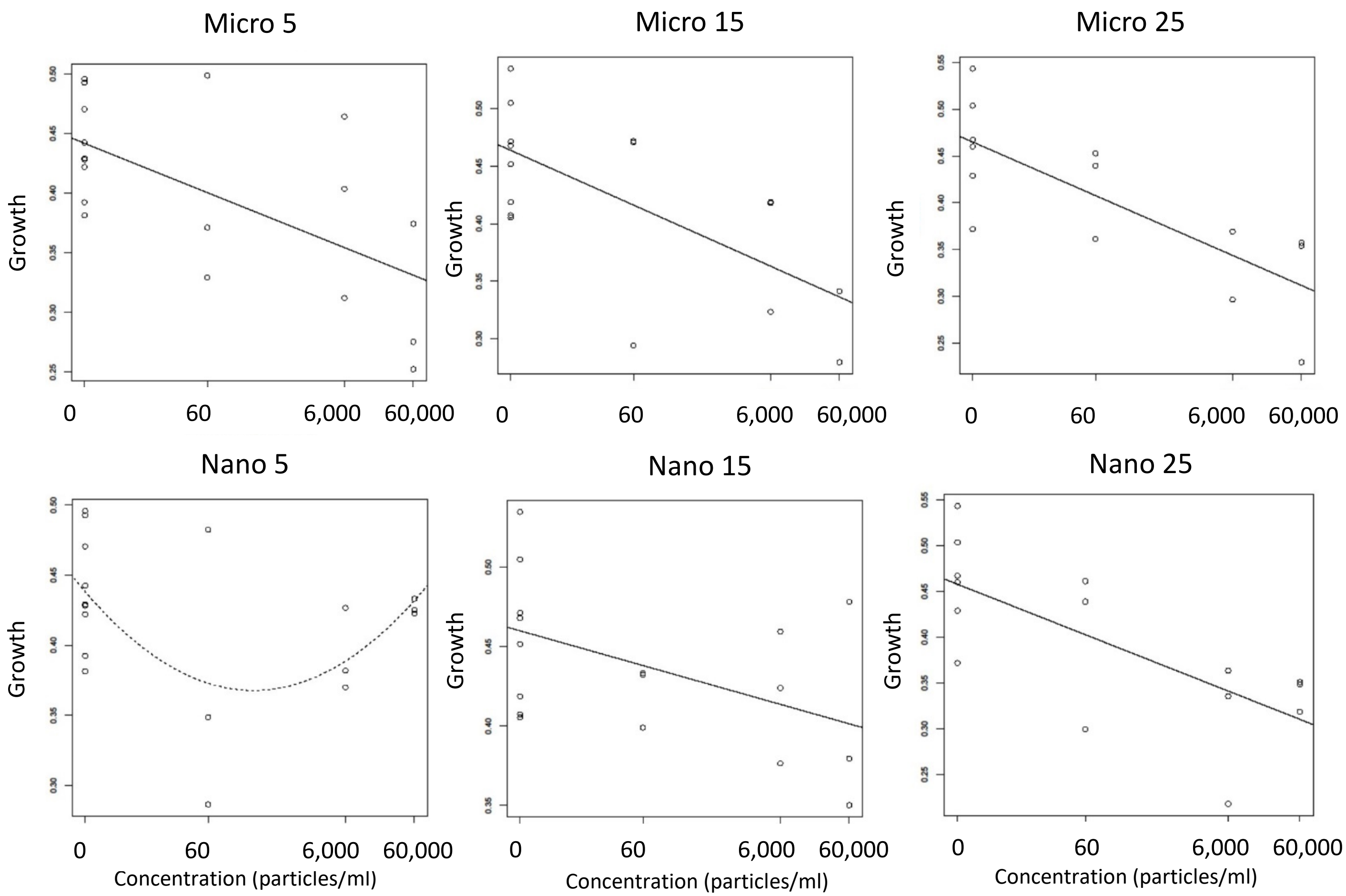
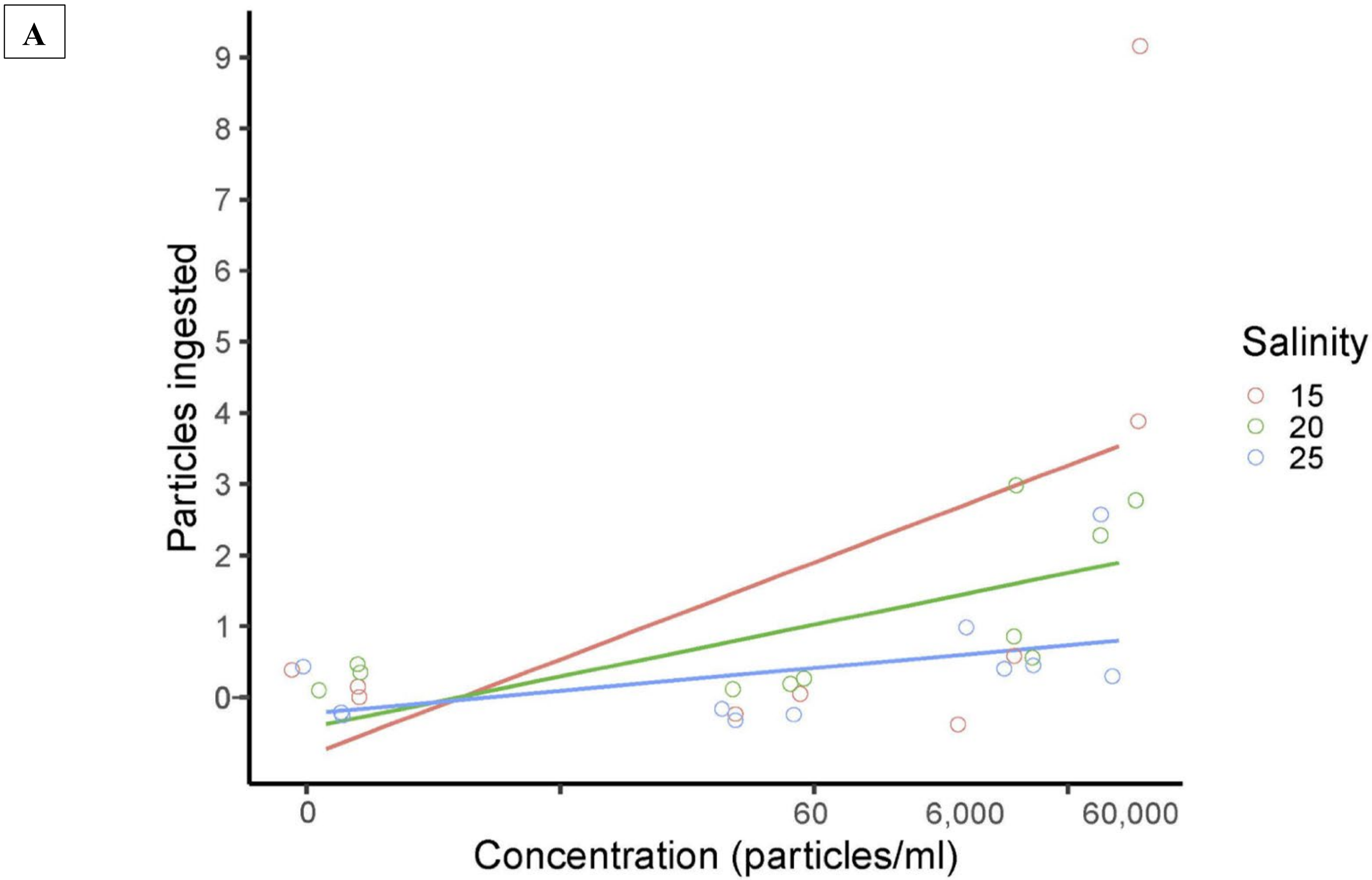


Figure 6. Dose response growth curve of silverside (*M. beryllina*) yolk-sac larvae exposed to micro and nano TWP across 5-25 PSU salinity gradient. Each circle represents the rescaled growth index mean of one larva (n=6). Data are presented on a log10 X+ 0.05 axis. Curves shown as a solid line are significantly better fits than a null intercept-only model ($p<0.05$), curves shown as a dashed line are the best-fit of the five-curve option (lowest p-value), but not significantly better than the null model.


A
Particles ingested
9
8
7
6
5
4
3
2
1
0
0
60
6,000
60,000
Concentration (particles/ml)
Salinity
15
20
25

B

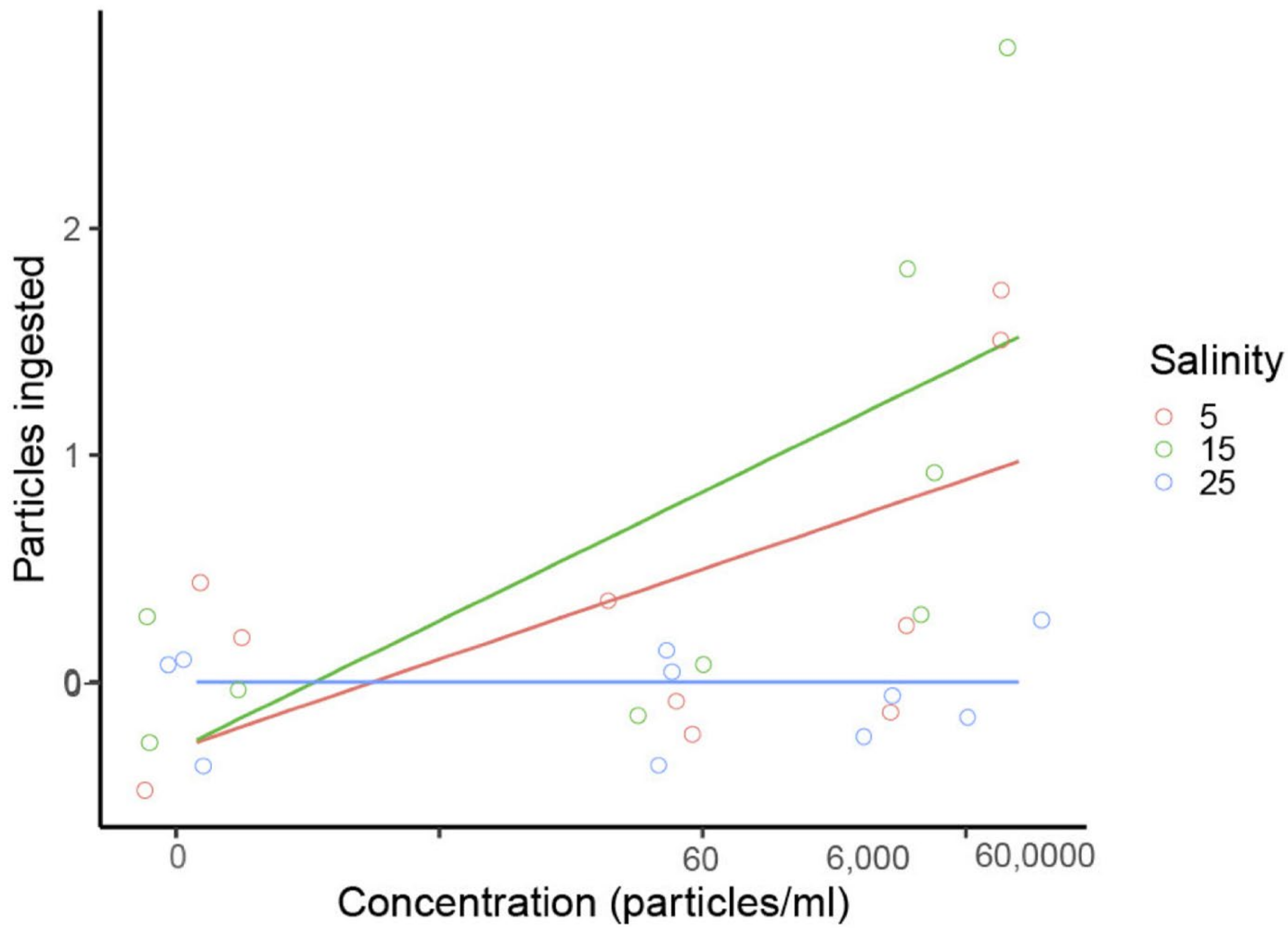
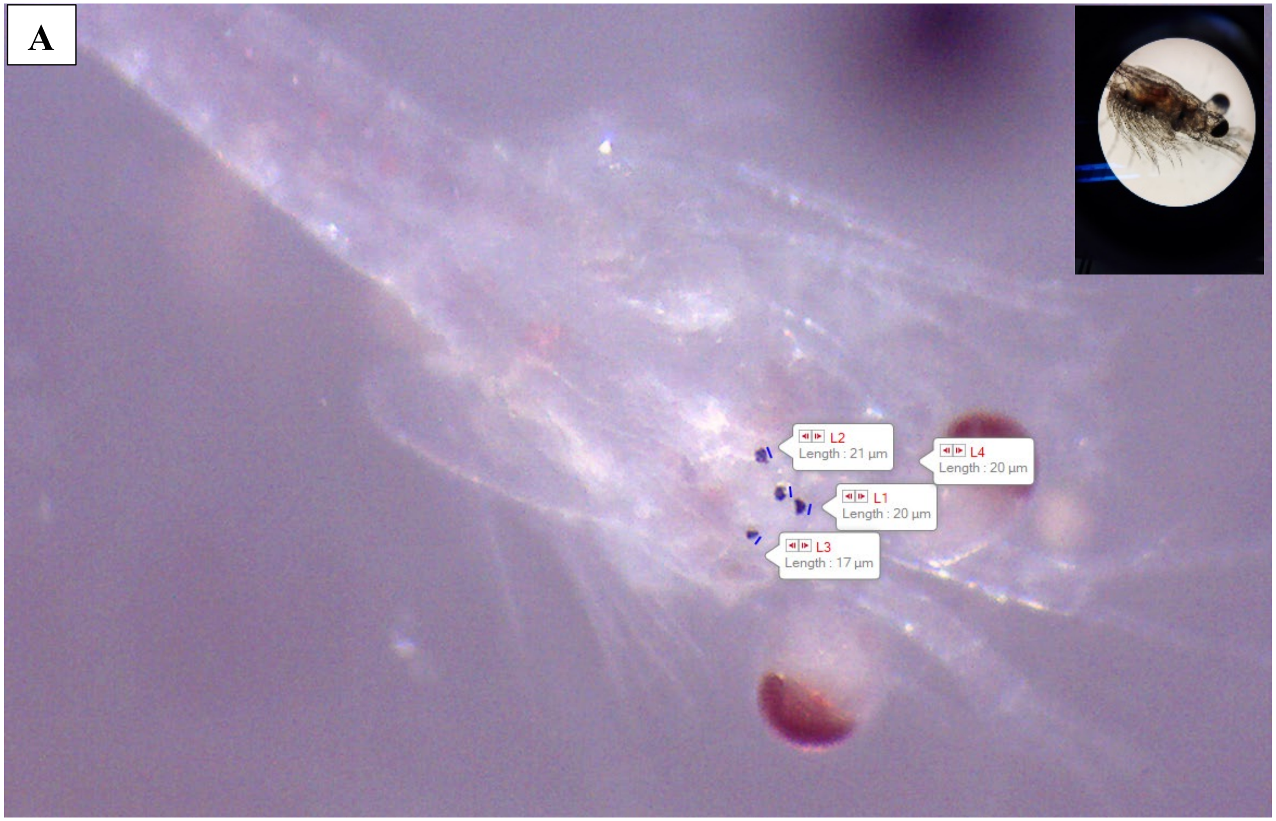


Figure 7. Generalized linear model of TWP ingested by A) mysid shrimp (*A. bahia*) and B) silverside (*M. beryllina*) yolk sac larvae from micro TWP concentration at 5-25 PSU salinity gradient range.


A
L2
Length : 21 µm
L4
Length : 20 µm
L1
Length : 20 µm
L3
Length : 17 µm

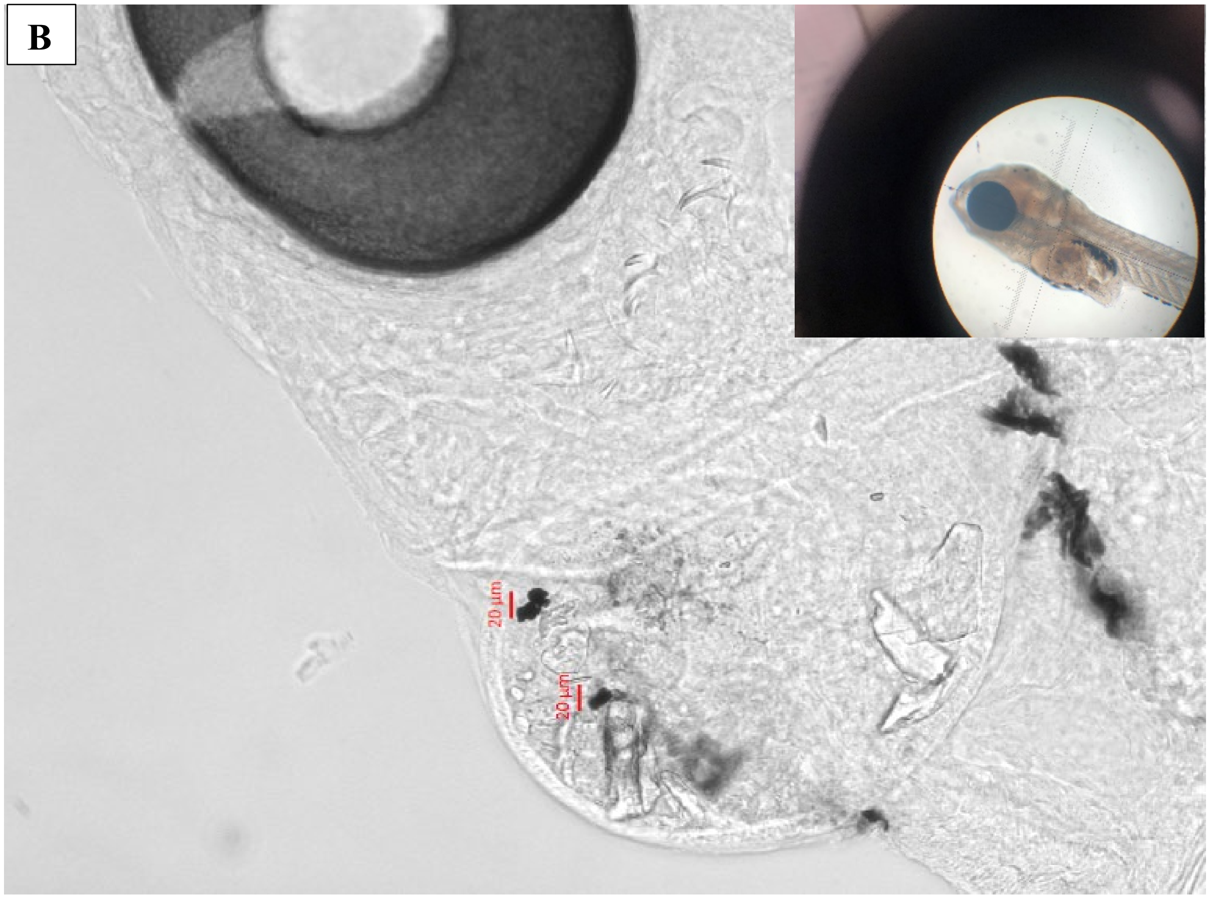


Figure 8. A) mysid shrimp (*A. bahia*) imaging at 15 PSU salinity with highest micro TWP concentrations at 35x. and B) silverside (*M. beryllina*) yolk sac larvae image from Zeiss microscope at 10x exposed to highest micro TWP concentration at 15 PSU salinity. Insight images are showing control organisms.

DEDICATION

I wish to dedicate this document to the memory of Michael Wayne Dickens, who shared with me his love for the ocean and the life it has given us all.